1 Evaluation of alkaline electrolyzed water to replace traditional phosphate enhancement
2 solutions: Effects on water holding capacity, tenderness, and sensory characteristics

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4 Macc Rigdon¹, Yen-Con Hung², Alexander M. Stelzleni^{1*}

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6 ¹Animal and Dairy Sciences Department, University of Georgia, 425 River Rd. Athens,
7 Ga 30602, USA. ²Departement of Food Science and Technology, University of Georgia,
8 1109 Experiment St. Griffin, Ga 30223, USA.

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10 *Corresponding Author. Email address: astelz@uga.edu Address: 425 River Rd. Athens,
11 Ga 30602, USA

Abstract

Sixty-four pork loins were randomly assigned to one of 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 (targeting 110%) and 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 for industry standard solutions.

Keywords: water holding capacity, enhancement, pork, water, alkaline

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, Jensen, Prestat, & Zhu, 2002; Hayes, Desmond, Troy, Buckley, & Mehra, 2006). Typical enhancement solutions 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 alkaline salts and phosphates have on the protein charges (Baublits, Mehaffey, Saha, Meullenet, & Sawyer, 2006; Offer & Trinick, 1983).

In the United States approximately 57% of fresh pork is enhanced (*Anonymous*, 2014) and of these fresh enhanced products 79.5% are enhanced by needle injection (Muth, Ball, & Coglaiti, 2012). Although salt and phosphate enhancement solutions have been widely used and have been successful in binding additional water (Ranken, 1976), the use of salt or phosphate is not always desirable (e.g. low sodium products, clean label products, and phosphates not being accepted by some countries in meat enhancement solutions). Ingredient and labeling concerns has led others to investigate substituting salt, or phosphates with other alkaline solutions including sodium hydroxide (NaOH) and

ammonium hydroxide (NH_4OH ; AH) in an attempt to raise the pH of the meat system for increased water holding capacity or ingredient replacement (Kingwascharapong & Benjakul, 2016; Knipe, 1982; Moiseev & Cornforth, 1997). As the consuming public becomes more circumspect of chemicals used in their foods and listed ingredients, even if they have a functional role (Brewer, 1998) and demand and willingness to pay for cleaner-label items increases (Zurawicki, 2015) the meat industry must respond to maintain viability. However, replacement ingredients must be tested and be able to retain the functionality of those they are replacing. Alkaline electrolyzed water (AEW), when produced with sodium chloride (NaCl), forms a dilute sodium hydroxide (NaOH) solution and may hold promise as a replacement of salts and phosphates in enhancement solutions by increasing the meat system pH. Alkaline electrolyzed water, which must be labeled as sodium hydroxide, is generally recognized as safe (GRAS; 21 CFR 184.1763, 21 CFR 184.1631, 9 CFR 424.21) and can be used “as a pH control agent in water used in poultry and red meat processing” at a level “sufficient for purpose” (USDA-FSIS Directive 7120.1).

Electrolyzed water is produced by passing a dilute salt solution through a membrane with an electrical current flowing across the membrane producing both acidic electrically oxidized water ($\text{pH} \approx 2.5$) and AEW with an approximate pH of 10.8 (Huang, Hung, Hsu, Huang, & Hwang, 2008). Acidic and alkaline electrolyzed water have shown to possess bactericidal properties when used in equipment (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999) and meat (Ding, Rahman, Purev, & Oh, 2010) applications. An additional benefit of electrolyzed water is that it is easy and economical to produce and use (Kim, Hung, & Brackett, 2000). If a processor were to invest in electrolyzed water as

an antimicrobial, they could potentially use both phases by incorporating the AEW phase in other areas of facility operations including enhancement solutions. Although NaOH has been successfully used to increase the pH and water holding capacity of a meat system when used in conjunction with salts or phosphates, the research is limiting on the use of NaOH alone as a total replacement of salt and phosphates. 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.

2. Materials and methods

2.1. Pork Loin Procurement and Enhancement

Sixty-four Institutional Meat Purchase Specifications 413 whole boneless pork loins (longissimus thoracis et lumborum) that were considered red, firm, and normal by plant personnel were procured 2 d postmortem from a multi-national pork supplier (daily capacity >12,000 head) 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 ($1 \pm 2^\circ\text{C}$), 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, $5.75 \times 10^{-3} \text{ M NaOH}$), 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).

Electrolyzed reduced water was produced using an electrolyzed water generator (ROX-20TA-U, Hoshizaki Electric, Japan) according to Park, Hung, and Brackett (2002) where deionized water and a dilute salt (6% NaCl) solution were simultaneously passed through a generator at approximately 18 amps and 10 volts. Alkaline electrolyzed water was collected from the cathode side of the generator with an approximate pH and ORP of 11.76 and -187, respectively.

After treatment randomization whole loins were enhanced using a multi-needle injector (Injectamatic PI21, Koch Equipment LLC, Kansas City, MO, USA) to a target of 110% of raw weight followed by a 15 min rest period. The multi-needle injector was calibrated with test loins prior to each treatment application. Weights were recorded for loins prior to enhancement, 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, and cation concentration. 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 cation, 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.

2.2. Cation Analysis

Samples for cation analysis were thawed ($0 \pm 2^\circ\text{C}$) for 24 hr and then the samples were digested following the EPA Method 3052 (USEPA, 1995). Briefly, approximately 1 g of sample were weighed and placed in fluorocarbon polymer microwave vessels and had 10 mL of concentrated HNO₃ added to each vessel before sealing. The sealed vessels were placed in a microwave digester (CEM Mars 6 Microwave, Matthews, NC, USA) and heated at 200°C for 30 min. The digests were quantitatively transferred into volumetric flasks and brought to 100 mL with deionized water. The samples were analyzed for various cations following EPA Method 200.8 (Creed, Brockhoff, & Martin, 1994) by Inductively Coupled Plasma – Optical Emission Spectroscopy (Spectro Acros FHS16, Kleve, Germany).

2.3. 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 $\omega_1 = 100$; $\omega_2 = 100 - [\text{Free Drip on Percent Basis (FDPB)}]$; $\omega_3 = \omega_2 -$

[expressible juice loss due to vacuum packaging on percent basis (EJVPB)]; $\omega_4 = \omega_3 -$
[expressible juice loss due to gravimetric force on percent basis (EJGPB)].

2.3.1. 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 cooler ($1 \pm 2^\circ\text{C}$) for 24 h and reweighed. This is reported as free drip using the following equations:

Free Drip = (enhanced weight – drip weight) / enhanced weight

FDPB = Free Drip

Chops were then vacuum packaged, held for 4 d in dark, cold storage ($1 \pm 2^\circ\text{C}$) to simulate distribution and storage periods, and then removed from storage on their respective days of shelf life (0, 5, 10, 15, 20, 25, 30 d).

2.3.2 Expressible Juice (EJ):

On their respective day vacuum packaged chops were unpackaged and weighed. Expressible juice due to vacuum (EJV) was calculated following:

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

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

Following calculations of EJV, expressible juice due to gravimetric force (EJG) was 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 x 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:

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

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

2.3.3. 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, Harris, and Shorthose (1972). After the drying period samples were removed from the oven and a final weight was recorded. Bound water was measured by calculating dry loss using the equation:

$\text{Dry Loss} = (\text{EJG weight} - \text{dried weight}) / \text{EJG weight}$

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

2.4. Cookery, Warner-Bratzler Shear Force (WBSF), and Sensory Attributes

Twenty-four hours prior to cooking, chops were allowed to thaw in a cooler ($1 \pm 2^\circ\text{C}$). 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 and internal temperatures were monitored using copper-constantan thermal couplers (Omega Engineering, Stamford, CT, USA) placed in the approximate geometric center of each chop. For WBSF, chops weights were recorded for thaw loss and cook loss and the chops were chilled in a cooler ($1 \pm 2^\circ\text{C}$) 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 with the exception of chilling. 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. Sample cubes (1.27 cm x 1.27 cm x chop thickness) 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 unsiced 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 h 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.

2.5. 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. 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 without affecting model strength. The multiple value recordings for sensory analysis (panelist) and WBSF (core) from each chop were not averaged before statistical analysis to account for within sample variation. Recorded off-flavors were analyzed using the Pearson's chi-squared option of Proc Freq (SAS). 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. Results and Discussion

3.1 Whole Loin Water Loss

After loin treatment randomization there was no difference in raw loin weights ($P > 0.05$; Table 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 raw loins were similar ($P > 0.29$) across all treatments, as well as the pH of loins post enhancement ($P > 0.30$). The pH of loins treated with EOH had a slight numeric decrease (0.04 units) after enhancement while loins treated with EOK had a slight numeric increase pH (0.09) when compared with their respective initial pH ($P > 0.05$). The numeric decrease in pH for EOH loins was not expected as others have shown an increase in pH with the addition of NaOH to meat systems (Knipe, 1982). Loins enhanced with AEW based solutions had less than $\frac{1}{4}$ of the sodium ($P < 0.01$; Table 1) present in IS loins. However, even though the EOH and EOK loins had a dilute NaOH solution added their total sodium content was similar ($P < 0.05$) to the CON loins. Likewise, EOK and IS enhanced loins had more than double the potassium content ($P < 0.05$) compared to CON and EOH enhanced loins.

Similar changes in pH have been reported where loins injected with an alkaline food grade ammonium hydroxide (AH) solution had a lower pH, and lost more water after enhancement (Cerruto-Noya, Vanoverbeke, & DeWitt, 2009). However, Hamling and Calkins (2008), Parsons, VanOverbeke, Goad, and DeWitt (2011), and Naveena, Kiran, Reddy, Ramakrishna, Vaithiyanathan, and Devatkal (2011) all reported an increase in meat pH after enhancement with various solutions containing AH in beef and buffalo. The mechanism for a numerically lower pH in the current study is not fully understood, however, 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; Hayashi & Kameda, 1980). The pH value of the IS solution (6.78) may relate to more of the functional groups on individual amino acids remaining protonated due to their pKa (Campbell & Farrell, 2012). With EOH and EOK solutions having pH values beyond the pKa of most amino acids the surplus of hydroxide ions may allow for potential attacks on intramolecular bonds between residues (Campbell & Farrell, 2012). Hydrating these bonds could cleave proteins into smaller segments (Campbell & Farrell, 2012), thereby increasing their buffering capacity. Additionally, potential causes include protein denaturation by reversible dissociation of secondary and tertiary structures, or destruction of amino acid side chains and subsequent loss of protein functionality as described by Hayashi and Kameda (1980).

3.2. Chop Water Loss:

When free drip was analyzed chops treated with EOH lost the most water, followed by EOK-treated chops (Table 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 EJv and EJg (Figure 1a and 1b respectively). For EJv, regardless of day, IS had the lowest 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 storage. 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 2 and 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 2a, 2b, 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 2 and 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 necessary 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; additionally, the 100% basis will allow for comparisons between loins that had numerical differences in starting weight. 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, inside retail over wrap packages 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 assay, found that EOH increased the amount of free water prevalent in the chop. Cerruto-Noya et al. (2009) reported similar findings when an AH enhancement solution was compared to a salt/phosphate enhancement solution in beef strip loins. Industry standard solutions, although enhanced to 110% of their raw 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 and becoming more stable (Hamm, 1960) due to the increased

ionic strength of the solution. 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 AH to salt/phosphate solutions did not enhance their water holding 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). This has been supported by Knipe (1982) who showed that the addition of NaOH to salt and/or phosphate increased meat pH and water holding capacity over that of salt and phosphate alone. However it is clear that enhancement solution pH is not the sole factor in determining the WHC of meat products (Bertram, Meyer, Wu, Zhou, & Andersen, 2008). In fact, others (Hamling & Calkins, 2008; Parsons et al., 2011;), as previously mentioned, reported an increase in meat pH with the use of AH, but also reported an increase in purge during shelf life. Contrarily, Naveena et al. (2011) reported an increase in WHC of buffalo meat when AH (0.1, 0.5, and 1.0%) was utilized as a marinade, however, the WHC test employed utilized NaCl so the results could reflect an interaction of AH and NaCl and not the effects of AH alone. Other factors that can influence water holding capacity include initial meat pH, solution ionic strength (salt and phosphate type and concentration), and water source (pH and mineral concentration). However, Kristinsson and Hultin (2003) reported that solutions with low ionic strength had greater water holding capacity, water retention, and gel

formation than high ionic strength solutions containing salts when evaluating chicken breast muscle. Kristinsson and Hultin (2003) concluded that the number of negatively charged proteins and subsequent increase in pH was a controlling factor in water uptake and retention. The effects of pH on water holding capacity described by Bertram et al. (2008), parallels the results found in the current study where chops treated with the EOH solution, which had the greatest pH, lost the most water over time. Bertram et al. (2008) went on to say that an elevated pH could give rise to protein denaturation. Sodium hydroxide, such as what is produced during the electrolysis process when using a sodium chloride based solution, and other strong alkalis are known to be able to cause denaturation of secondary and tertiary protein structures, and subsequently a loss of functionality (Hayashi & Kameda, 1980).

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 ($P < 0.01$) than CON, EOH, and EOK by 82.15, 86.67, and 83.51%, respectively (Table 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, yet similar ($P > 0.05$) to each other. The thaw and cook loss findings of the current study align with the findings of other reports utilizing AH as a replacement in enhancement solutions, even when the AH increased the meat system pH (Hamling & Calkins, 2008; and Cerruto-Noya et al., 2009). However, Naveena et al.

(2011) reported an increase in cook yields for marinated buffalo when comparing AH based solutions to a distilled water marinated control.

Shear force (Table 4) was lower for 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 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 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. Pearson's chi-squared analysis indicated that the off-flavors detected were not by chance ($P < 0.04$). However, over 80% of the cells had expected counts less than 5, which leaves the test open for committing a Type II Error due to the low number of samples actually having off-flavors detected (Figure 3). Therefore, the data given is for descriptive purposes to show the frequency of off-flavors detected within each treatment. Of the 128 observations for each treatment (16

samples and 8 panelists) the percentage of samples with off-flavors categorized were 7.03, 7.03, 13.28, and 21.88% for CON, EOH, EOK, and IS, respectively. Sample off-flavors for CON and EOH were fairly equally distributed across the lexicon while EOK samples had more pronounced off-flavors categorized as acidic, metallic, and salty. The most predominant off-flavor for the IS treatment, which had the most off-flavors categorized, was salty, followed by metallic, acidic, and soapy.

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 et al., 2003; Jones-Hamlow et al., 2015; 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). However, Naveena et al. (2011) found that increasing AH content in meat marinades led to a decrease in shear force, which was related to an increase in the soluble collagen fraction. Furthermore, Hamling and Calkins (2008) also reported a decrease in shear force, and an increase in sensory tenderness and juiciness as AH content increased in beef enhancement solutions. Flavor results of the present 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, the current study showed that the addition of potassium lactate increased 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, their association with off-flavors (Brewer et al., 1991; Jensen et al., 2003). The off-flavors detected in the EOK and IS samples are in line with what would be expected given the differences in sodium and potassium content in the current study compared to CON and EOH enhanced loins. Sodium and potassium are both known to increase salty sensations, and excess potassium has also been associated with bitter, astringent, and metallic sensations (Brewer, Gills, & Vega, 1995).

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 additional functional additives is not recommended.

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1 **Figure 1:**
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Figure 2:

A.

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C.

14 **Figure 3:**

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Figure 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 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 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 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 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 ($P < 0.05$).

Figure 3: Sensory off-flavor descriptor Chi-squared frequency distribution. Out of 128 observed samples for each treatment there were 9, 9, 17, and 28 off-flavor detections for CON, EOH, EOK, and IS, respectively.

Table 1

Least squares means for loin enhancement characteristics.

Traits ²	Treatments ¹				SEM	<i>P</i> - value
	CON	EOH	EOK	IS		
Raw 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 raw	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
Cation ³ , mg/kg						
Sodium	426.29 ^a	454.20 ^a	472.23 ^a	1971.32 ^b	38.07	<0.01
Potassium	3899.49 ^b	3303.70 ^a	8695.69 ^c	8908.18 ^c	191.50	<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.

²Raw 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 raw: pH of the loin pre-enhancement; pH enh; pH of the loins post-enhancement.

³Cation values are from post rest, uncooked chops.

Table 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 24 h; EJV: Expressible juice, vacuum packaging, or moisture lost under vacuum pressure; EJG: Expressible juice, gravimetric, or moisture lost due to gravimetric force; 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, EJV, EJG, and dry loss.

Table 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

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

19 ¹Free Drip: Moisture loss after hanging for 24 h; EJV: Expressible juice, vacuum packaging, or moisture lost under vacuum pressure;
20 EJG: Expressible juice, gravimetric, or moisture lost due to gravimetric force; Dry Loss: moisture lost after EJG samples are place in
21 drying oven for 24 h; Total Loss: Total moisture lost, or the sum of free drip, [EJV](#), [EJG](#), and dry loss.
22 ²Due to free drip measurements starting simultaneous and only running 24 h no day effect was analyzed.

Table 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 scored on an 1 – 8 scale for tenderness, pork flavor, and juiciness (1 – extremely tough, extremely bland, extremely dry, and 8 – extremely tender, extremely intense, extremely juicy) while off flavor was scored on an 1 – 6 scale (1 – none detected, and 6 – extreme off-flavor).