Introduction

Enhancement solutions containing salt and phosphates have been used by the pork industry to improve overall quality (Baublits et al., 2006; Miller, 1998). Functional non-meat ingredients in commercial pork products are used for their ability to improve and stabilize color, as well as increase shelf life (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). Muscle pH has a direct influence on meat color, with higher meat system pH values resulting in a darker color (Baublits et al., 2005; Fernández-López et al., 2004). The darker color of high pH meat products is a result of the meat’s ability to bind more water, causing the muscle to swell and absorb more light (Romans et al., 1994; Lawrie, 1991).

Meat color is the main factor in consumer acceptance of meat products and is also the primary determinant of freshness and quality at the point of sale (Damodaran et al., 2008; Brewer et al., 2002). The majority of fresh pork in the US is enhanced using phosphate solutions (USDA-FSIS, 2014) because of their ability to improve water retention (Long et al., 2014).
Although ingredients such as phosphates have high functionality in meat enhancement systems, consumers have become weary of additives in their food, regardless of their role in safety and a satisfactory eating experience (Baublits et al., 2006; Brewer, 1998). Alkaline electrolyzed water (AEW) may hold promise as a replacement for traditional non-meat ingredients for ensuring a satisfactory eating experience due to its elevated pH, low oxidation reduction potential (ORP), and sodium hydroxide (NaOH) content. Sodium hydroxide has been used in conjunction with salt and phosphates in meat enhancement systems (Kingswarcharapong and Benjakul, 2016; Moiseev and Cornforth, 1997; Knipe, 1982). However, information is scarce on the use of NaOH as a standalone enhancement solution (Rigdon et al., 2017).

Additionally, most commercially enhanced pork is displayed and sold in vacuum style packaging. However, most published pork color data is in reference to product that has been exposed to oxygen so the meat would bloom. Given the emphasis that consumers place on color at the point of purchase, it is important to understand how enhancement solutions can impact color while on display. Therefore, the objective of this study was to determine the effects of AEW on enhanced pork loin color stability and lipid oxidation under vacuum packaged conditions.

Materials and Methods

Institutional Animal Care and Use Committee approval was not required for this research.

Pork loin procurement and enhancement

The samples for this study were collected from the loins utilized in Rigdon et al. (2017), which describes in detail pork loin selection, handling, and enhancement. Briefly, 64 Institutional Meat Purchase Specifications 413 whole boneless pork loins (longissimus thoracis et lumbrorum) were procured 2 d postmortem from a multinational pork supplier (daily slaughter capacity > 12,000 head) across 2 replications (32 loins per replication). The pork loins for each replicate were randomly selected from the morning’s fabrication line, vacuum packaged, and boxed according to plant standard operating procedures. Loins were then transported (0 ± 2°C) 575 km to the University of Georgia Meat Science Technology Center (Athens, GA). Upon arrival, the pork loins were placed in cold (1 ± 2°C) dark storage until 4 d postmortem. On d 4 the loins were randomly assigned to 1 of 4 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 4 treatments included: 1) alkaline electrolyzed reduced water (EOH; pH ≈ 11.5, ORP ≈ -187 mV, 5.75 × 10⁻³ M NaOH), 2) alkaline electrolyzed reduced water with 2.5% potassium lactate (EOK; Hawkins, Minneapolis, MN; pH ≈ 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 ≈ 6.78), and 4) no enhancement (CON).

After treatment randomization, whole loins where enhanced (except CON) to a target of 110% of raw weight with a multi-needle injector (Injectamatic PI21, Koch Equipment LLC, Kansas City, MO). The enhanced loins were allowed to rest and purge for 15 min before fabrication (Rigdon et al., 2017).

After the post enhancement rest period, the whole loins were cut into 2.54-cm chops. Starting from the anterior end, 2 chops were removed for Warner-Bratzler shear force and 7 chops were removed for moisture, mineral, and pH analysis as reported by Rigdon et al. (2017). The following 7 chops (2.54 cm) were cut, vacuum packaged (B-620 series; 30-50 ml O2/m2 per 24 h; 101,325 Pa; 23ºC; Cryovac Sealed Air Corporation, Duncan, SC), and randomly assigned to 0, 5, 10, 15, 20, 25, and 30 d of shelf life. After day of display was assigned, chops were boxed and placed in cold dark storage (1 ± 2°C) for 4 d to simulate transportation and storage time prior to retail display.

Objective color

After transportation simulation, vacuum-packaged chops were placed in a coffin style retail display case (3 ± 2°C, with 2 defrost cycles every 24-h; M1X-E, Hussmann, Bridgeton, MO) with 24-h continuous florescent lighting (Octron/ECO; 30,000K; F032/830/ECO; Sylvania Company, Versailles, KY) at a range of 1400 to 1900 lux for 30 d. Chop objective color was recorded on d 0, 5, 10, 15, 20, 25, and 30 of display using a Hunter-Lab MiniScan EZ (Hunter Associates Laboratory, Reston, VA) with illuminant A and 10° viewing angle, standardized using white and black tiles as recommended by the manufacturer before each use. Commission Internationale de l’Eclairage 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, respectively. Reflectance readings were recorded as arctangent (b*/a*) and chroma C* = (a*² + b*²)¹/₂.
from 400 to 700 nm to calculate redness (630:580) and Kubelka-Monk (K/S) ratios, indicating the proportion of myoglobin that was in the deoxymyoglobin (DMb: K/S\textsubscript{474}:K/S\textsubscript{525}), oxymyoglobin (OMb: K/S\textsubscript{610}:K/S\textsubscript{525}), and metmyoglobin (MMb: K/S\textsubscript{572}:K/S\textsubscript{525}) state according to American Meat Science Association (2012). Lower K/S ratios indicated more myoglobin in the given state.

**Subjective color**

Subjective color characteristics were evaluated by a 5-member trained panel consisting of University of Georgia personnel. All panelists were subjected to the Farnsworth-Munsell 100 hue color test to determine the panelist’s ability to detect hue, or differences in color, and had a total error score of less than 40. On d 0, 5, 10, 15, 20, 25, and 30 of simulated retail display, panelists evaluated chops (± 2 h) used for instrumental color analysis for average color, percent discoloration, muscle darkening, and purge characterization. Average color and percent discoloration were measured on an 8 point scale (where 8 = extremely dark purplish-pink, 96 to 100% discoloration; 7 = dark purplish-pink, 80 to 95% discoloration; 6 = moderate purplish-pink, 60 to 79% discoloration; 5 = slight dark purplish-pink, 40 to 59% discoloration; 4 = slight purplish-pink, 20 to 39% discoloration; 3 = moderately bright purplish-pink, 19 to 19% discoloration; 2 = bright purplish-pink, 0 to 4% discoloration; 1 = extremely bright purplish-pink, 0% discoloration) modified from American Meat Science Association (1991) and Stivarius et al. (2002). Muscle darkening was evaluated on a 7 point scale (where 7 = very dark; 6 = moderately dark; 5 = slightly dark; 4 = no darkening) as described in American Meat Science Association (2012). Purge discoloration was evaluated on a 6 point scale (where 6 = dark brown; 5 = light brown; 4 = dark purplish-red; 3 = red; 2 = pink; 1 = clear) modified from American Meat Science Association (2012).

**Lipid oxidation**

Lipid oxidation was preformed using the thiobarbituric acid reactive substances method outlined by Ahn et al. (1998) with modifications. After simulated retail display, chops were removed from the retail case on their respective day and placed into the freezer (−20°C) until further analysis. Chops were removed from the freezer 24 h prior to analysis and placed into a cooler (0 ± 2°C) for 18 to 24 h. The chops were removed from vacuum packages, and the subcutaneous fat and epimysial connective tissue was removed. Chops were minced thoroughly and mixed by hand to form a representative sample. Subsequently, a 5-g sample was placed into a 50-mL conical screw cap centrifuge tube. Fifteen milliliters of distilled water were added to the tubes and homogenized (IKA T18 Ultra Turrax, Wilmington, NC) on high for 30 s. The tubes were centrifuged (CR 312, Jouan INC., Winchester, VA) at 1850 × g for 10 min at room temperature. Two milliliters of supernatant were removed and added in duplicate to 13 × 100 mL culture tubes with 100 μL butylated hydroxyl toluene (7.2%) and 4 mL of thiobarbituric acid/trichloroacetic acid 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 (25°C) for 10 min. Samples were centrifuged at 3,000 × g (CR 312, Jouan INC.) for 15 min at room temperature 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 of tissue.

**Statistical analysis**

Data were analyzed using Proc Mixed of SAS (version 9.3; SAS Inst. Inc., Cary, NC) 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. For subjective color analysis, panelist was included as a covariate; however, panelist was not a significant factor adding variation (P > 0.08). Color data were analyzed as repeated measures with an autoregressive (1) covariance structure (Liu et al., 2007). When an enhancement by day of display interaction was detected (objective and subjective color) the slice option was used to identify differences within day of display. 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 α = 0.05.

**Results and Discussion**

**Objective color**

There was not a treatment by shelf life day interaction for L* (P = 0.25). However, L* values for EOH-treated chops were the lightest (P < 0.05), IS chops were the darkest (P < 0.05), and CON and EOK were inter-
mediate and similar to each other ($P > 0.05$; Table 1), yet different ($P < 0.05$) than both EOH and IS chops. As days of display increased, all chops became lighter in color ($P < 0.05$) except for d 20, which was lighter ($P < 0.05$) than d 30 but similar ($P > 0.05$) to d 25 (Table 2).

All chops were similar ($P > 0.05$) for $a^*$ on d 0 through d 25 (Fig. 1A). However, after 25 d of display, IS chops had greater ($P < 0.05$) $a^*$ values than EOH, while EOK and EOH chops remained similar ($P > 0.05$). Values for $b^*$ were similar to each other on d 0 and d 5 of display; however, $b^*$ was greater ($P < 0.05$) for EOH chops compared to IS chops on d 10 of display (Fig. 1B). 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 chop $b^*$ values were greater ($P < 0.05$) than EOH and CON chops, with IS chops similar ($P > 0.05$) to all treatments. Overall, chroma values (Fig. 1C) were similar ($P > 0.05$) for vacuum packaged chops throughout display, with the exception of d 20 where CON chops were more vivid than IS and EOH chops which were similar ($P > 0.05$) to each other. Chops from EOH and EOK were similar ($P > 0.05$) and had greater hue values (were less red; $P < 0.05$) than both CON and IS chops across all days of display (Table 1). As expected, hue values increased ($P < 0.05$) with time in display (Table 2). In relation to reflectance data, it was noted that redness (630nm:580nm) followed a similar trend to hue angle, where IS and CON chops exhibited more red color ($P < 0.05$) than EOH chops regardless of day (Fig. 2A). Additionally, CON and EOK chops were similar ($P > 0.05$) to each other for redness during the entire display period. The EOH chops were

Table 1. Least squares means for the main effect of pork loin enhancement on vacuum packaged loin chop objective color, subjective color, and lipid oxidation characteristics across 30 d of refrigerated retail display.

<table>
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<tr>
<th>Traits</th>
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$a^* \text{Means within a row that do not have common superscripts are different ($P < 0.05$).}$

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

$^2$Oxymyoglobin is represented as the reflectance values K/S$_{525}$/K/S$_{550}$ from the Kubelka-Monk equations. Lower values indicate a greater proportion of oxymyoglobin.

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

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

$^5$TBARS = Thiobarbituric acid reactive species, a measure of lipid oxidation, reported as mg malondialdehyde/kg of tissue.
similar \( (P > 0.05) \) to EOK chops through 20 d of display, after which they were the least red \( (P < 0.05) \) of the 4 treatments. Differences in redness were not due to the proportion of myoglobin remaining in the oxygenated state as treatment did not affect OMb proportions (Table 1). Day of display influenced OMb proportions \( (P < 0.05) \). However, the largest difference in OMb was 0.02 units indicating the relative differences were very small in magnitude (Table 2). The OMb data show that oxygen was effectively excluded from the packages due to the steady state of values across treatments and time and the relatively high values obtained when compared to other reports (Li et al., 2012; Gonzalez et al., 2009; McKenna et al., 2005). Although there were statistical differences \( (P < 0.01) \) for the Kubelka-Munk ratios for MMb, all treatment values were large, stable, and close to each other indicating very little MMb formation throughout the 30 d of display (Fig. 2B). Values for DMb (Fig. 2C) indicate that all chops across days of display had high DMb formation (low \( K/S_{474}/K/S_{525} \)) throughout the display.

Figure 1. Least squares means for the effect of treatment by day of display on objective color measurements for pork loin chops. CON = Control; EOH = Alkaline electrolyzed water; EOK = Alkaline electrolyzed water with potassium lactate; IS = Industry standard enhancement solutions. a–c Means within each day that do not have common superscripts are different \( (P < 0.05) \). A) \( a^* \); treatment effect \( P = 0.18 \), day of display effect \( P < 0.01 \), treatment by day effect \( P < 0.01 \). Standard error of the mean = 0.29. B) \( b^* \); treatment effect \( P = 0.16 \), day of display effect \( P < 0.01 \), treatment by day effect \( P < 0.01 \). Standard error of the mean = 0.22. C) Chroma; treatment effect \( P = 0.49 \), day of display effect \( P < 0.01 \), treatment by day effect \( P < 0.01 \). Standard error of the mean = 0.31.
period. Interestingly, the IS chops had greater $K/S_{474}:K/S_{525}$ values (indicating less DMb formation) than all other treatments ($P < 0.01$), while EOH chops exhibited the most DMb formation ($P < 0.01$) on all days. Although the differences between IS and EOH chops is relatively small when evaluating MMb and DMb (0.02 to 0.04 units), the lower proportion of MMb and DMb in IS chops helps explain the greater redness noted for IS chops when evaluating $a^*$, hue, and 630nm:580nm reflectance.

Provided the pH of the EOH and EOK solutions (11.50 and 10.92, respectively), it was hypothesized that the free hydroxide from AEW would increase the pH of the meat system, resulting in increased water holding capacity and a darker, redder chop that would be similar to traditionally enhanced pork loin chops. However, as reported by Rigdon et al. (2017), the loin pH for CON, EOH, EOK, and IS treatments were similar to each other pre- (5.80, 5.75, 5.66, and 5.85, respectively) and post-enhancement (5.80, 5.71, 5.75, and 5.86, respectively). Even though post-enhancement pH was similar among treatments, the EOH enhanced chops exuded more moisture than IS enhanced chops due to vacuum pressure.
during packaging and display across all days of display. The EOH enhanced chops exuded more moisture than EOK and CON chops after 10 d of display (Rigdon et al., 2017). The increased exudate from EOH is likely responsible for the lighter color (greater L*) of EOH chops when compared to EOK, CON, and IS chops via removal of the water-soluble myoglobin, a primary determinate of color. Similar to the current study, when beef steaks were enhanced with a strong alkali solution (ammonium hydroxide), they were lighter and less red than salt and phosphate enhanced steaks (Cerruto-Noya et al., 2009), attributed to the increased water holding capacity and pH of the phosphate enhanced steaks. In addition to their role in pH and increased water retention, phosphates also improve fresh meat color and stability through their antioxidant properties (Long et al., 2014; Romans et al., 1994). Although AEW has a low oxidation reduction potential (Huang et al., 2008) which has been associated with color stability (Ahn and Nam, 2004), the low ORP of the EOH treatment did not compare to IS treated chops in redness. Lactate inclusion can improve meat color via its effect on enzyme and metabolic activity post-mortem and antioxidant properties (Nair et al., 2014), primarily by replenishment of NADH and the subsequent reduction of MMb to OMb or DMb (Kim et al., 2006). The inclusion of k-lactate in the EOK and IS treatments likely helped maintain some color stability and redness compared to the EOH chops, as noted by the differences in 630nm:580nm readings and DMb content as shelf life progressed. Although there were differences among the treatments for myoglobin state ratios, overall the differences were small in magnitude, largely attributed to the use of vacuum packaging which aids in color retention and reduces MMb formation (Damodaran et al., 2008).

Subjective color

There was a treatment by day of display interaction (P = 0.02) for subjective average color scores (Fig. 3A). When evaluated by day of display panelist rated IS chops as being darker purplish-pink (P < 0.05) than all other treatments for all days. Additionally, EOH chops were rated as being brighter purplish-pink (P < 0.05) than all other treatments. Across all days of display, CON and EOK chops were similar to each other (P > 0.05) and rated as moderately bright to bright purplish-pink, between IS and EOH chops. Chop darkening due to enhancement did not exhibit a treatment by day interaction (P = 0.68). Similar to overall color scores and reported L* values, IS chops were darker (P < 0.05) than all other chops, while EOH chops were lighter (P < 0.05; Table 1) than both CON and EOK chops. Control and EOK chops were similar (P > 0.05). Contrary to L* values, panelists found that chop surfaces became darker as day on display increased (P < 0.01; Table 2). There was no difference for the percentage of surface discoloration between the treatments (P = 0.11) or days of display (P = 0.32).

When evaluating enhanced products in a vacuum package, the color of the moisture, or purge, trapped in the package is evaluated by the consumer along with the meat product itself. Therefore, when evaluating color, it is also important to evaluate purge color. Subjective purge color scores exhibited a treatment by day interaction (P < 0.01; Fig. 3B). Through 5 d of display all treatments had similar (P > 0.05) purge color scores. However, after d 10, IS chop purge was lighter in color (P < 0.05) than purge from all other treatments. Control, EOH, and EOK had similar (P > 0.05) purge color on d 10 and d 15. However, after d 20, EOH and EOK chop purge became browner (P < 0.05). Average color as rated by panelist showed that EOH chops were lighter purplish-pink than all other treatments, following the same trends of L* values of lightness. Muscle darkening over time was contrary to L* values but aligned with chroma, where chops became less vivid as shelf life increased. It is plausible that as chops became less vivid, they appeared to be duller and darker to the panelists. When evaluating fresh pork (not vacuum packaged), Brewer and McKeith (1999) reported that there was clear consumer discrimination against chops that were very light pink. Although the chops in the current study were vacuum packaged, the lightness of the EOH chops could negatively impact purchase intent based on the findings of Brewer and McKeith (1999). Cerruto-Noya et al. (2009) reported similar findings to those of the current study where panelists reported phosphate enhanced chops maintained color stability longer than those enhanced with an ammonium hydroxide solution. The increased browning of the purge in EOH packages was attributed to the excessive purge in the package (Rigdon et al., 2017), and subsequently, myoglobin in the purge potentially oxidizing (Aberle et al., 2001). It was also noted by several panelists that the purge in the EOH chop packages became cloudy and opaque over time. 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.

Lipid oxidation

There was not a treatment by day interaction (P = 0.59) for lipid oxidation. Even with the low ORP of AEW the EOH chops exhibited more oxidation (P <
0.05; Table 1) than all other treatments. However, day of display did not affect lipid oxidation \( (P = 0.67; \text{Table 2}) \). Although the differences in lipid oxidation due to enhancement solutions are likely of little concern biologically, they are interesting in that it was thought the low ORP of AEW would protect EOH chops from lipid peroxidation. However, EOH chops, even under vacuum storage, exhibited almost twice the malondialdehyde content compared to the other treatments. In addition to the AEW of the EOH treated chops, the inclusion of lactate (Knock et al., 2006) and phosphates (Pokorny et al., 2001) can both act as protectants against lipid oxidation. However, the low peroxidation levels in the current study were attributed to the proper vacuum seal, as noted by the overall low malondialdehyde levels and the reflectance data for OMb, MMb, and DMb. Even after 30 d of display, all chops were below the lipid oxidation threshold for fresh pork (0.5 mg/kg malondialdehyde) noted by Lanari et al. (1995).

**Conclusions**

Solution enhancement of meat products is a widely utilized method to improve the quality, consistency, value, and eye appeal to the consumer. However, as consumer demand shifts to the desire for products with lower sodium and a cleaner label, the industry must continue to seek ingredients that can maintain the functionality and consumer expected properties as those presently in use that incorporate salts and phosphates. As novel enhancement solutions are being tested, it is important to evaluate meat color, as color is a key attribute to the consumer indicating the quality and freshness of a product. Although the color of a product may be stable, if it is not comparable to what the consumer is familiar with, the overall perception may be diminished. In the current study non-enhanced loins and those enhanced with an industry standard enhancement solutions allow for comparison to the typical pork loin chop colors that consumers may be accustomed to. Although pork loin chop color was stable.

![Figure 3](image-url)
when enhanced only with alkaline electrolyzed water, both objective and subjective color measurements indicated that alkaline electrolyzed water treater chops were lighter in color than both IS and CON treatments. The inclusion of potassium-lactate to the alkaline electrolyzed water solution created chops that were similar in color to the non-enhanced chops; however, they were still lighter than the industry standard enhanced chops. Additionally, the chops enhanced with alkaline electrolyzed water had a less stable purge color that turned brown and opaque over the course of the retail display. The use of alkaline electrolyzed water is not currently recommended for use in pork enhancement solutions.

**Literature Cited**


Knipe, C. L. 1982. Effects of inorganic polyphosphates on reduced sodium and conventional meat emulsion characteristics. Dissertation, Iowa State University, Ames, IA.


