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Effects of pre-rigor injection of sodium citrate or acetate, or post-rigor injection of phosphate plus salt on post-mortem glycolysis, pH, and pork quality attributes ☆

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Abstract

Forty pork carcass sides were assigned to one of four treatments: pre-rigor citrate (CIT) or acetate injection (ACE); post-rigor phosphate and salt injection (PHOS); and non-injected control (CON). Loins in 20 sides were injected at 50 min post-mortem with 4% solutions of CIT or ACE to approximately 110% of projected loin weights, and 10 loins were injected at 24 h post-mortem to 106.6% with a solution of 4.4% PHOS and 2.2% salt. Although CIT increased pH ($P < 0.05$), neither CIT nor ACE altered ($P > 0.05$) glycolytic metabolite concentrations. The pH increase in muscles from the CIT treatment was most likely due to its buffering ability rather than to its glycolytic inhibition. Pre-rigor CIT injection improved tenderness without the detrimental effects on color or flavor found with PHOS, but neither CIT nor ACE altered glycolytic metabolites or improved firmness, wetness, or fresh visual color over CON. Poor flavor attributes of the ACE treatment will hinder its use as an ingredient for pork enhancement solutions.

Keywords: Pork; Pre-rigor injection; Citrate; Acetate; Phosphate

1. Introduction

Improving pork quality traits, such as tenderness, juiciness, and flavor, is a common goal in the pork industry. Great strides have been made to improve handling conditions and to alter genetics to remove stress susceptibility, but pork quality defects cost the industry an average of $2.13 per carcass (Stetzer & McKeith, 2003). It is now common practice to 'enhance' pork with solutions of phosphate, salt, and various other ingredients. Although these solutions improve tenderness and juiciness (Smith, Simmons, McKeith, Bechtel, & Brady, 1984), they concomitantly induce some negative consequences in flavor and consumer acceptability (Brewer, Jensen, Prestat, & Zhu, 2002).

Pork quality is highly dependent on the relationship of pH and temperature early post-mortem (Sayre & Briskey, 1963). Anaerobic glycolysis is responsible for pH decline in post-mortem muscle. The lack of oxygen and absence of a circulatory system from exsanguination leads to post-mortem myocellular accumulation of lactate and hydrogen ions. If glycolysis occurs at an accelerated rate, pH declines too rapidly, and muscle proteins denature due to the combination of low pH and high temperature.

Citrate is recognized for its glycolysis-inhibiting properties by inhibiting the glycolytic enzyme, phosphofructokinase (PFK; Newsholme, Sugden, & Williams, 1977). This enzyme regulates the transfer of a phosphate from adenosine triphosphate (ATP) to fructose-6 phosphate (F6P), producing adenosine diphosphate (ADP) and fructose-1,6 bisphosphate (F16BP). Phosphofructokinase has been

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identified as a key regulatory enzyme of glycolysis in post-mortem muscle (Dalrymple & Hamm, 1975; Rhoades et al., 2005).

Citrate inhibits PFK directly, because it binds to the enzyme and reduces its affinity for its substrate, F6P, and its activator, ADP (Kemp & Krebs, 1967). Citrate binding also increases the enzyme's affinity for ATP, its second inhibitor (Kemp & Krebs, 1967). The net product of the glycolysis reaction is ATP; therefore, excess ATP in the cell indicates a surplus of energy. Consequently, ATP inhibits PFK and slows the glycolysis reaction, in addition to acting as the enzyme's source for phosphate. Acetate is converted to citrate in the body by citrate synthase, resulting in similar effects (Fushimi et al., 2001).

Killefer (2004) injected pork loins at 1 h post-mortem with a solution of citrate, phosphate, and salt. He reported increased ultimate pH values, improved color, less cook loss, and decreased shear force values compared with controls injected with phosphate and salt. Sodium citrate has been used as a glycolytic inhibitor in beef muscle to improve tenderness (Streitel, Ockerman, & Cahill, 1977; Perversi, Calkins, & Velazco, 2003). They hypothesized that the increase in pH due to glycolytic inhibition created an environment in which the protein-denaturing calpains would be more active. Perversi et al. (2003) stated that citrate addition to beef muscle was not detrimental to flavor attributes.

Phosphate injection was originally developed to help lower the sodium content of processed meats, such as ham, but has been incorporated into fresh meats to improve tenderness and juiciness (Smith et al., 1984). Phosphate 'enhancement' is now commonly used in the pork industry to increase pH and improve pork quality attributes. Although phosphate injection may increase salty flavor and decrease display life (Robbins et al., 2002), the routine use of this technology in industry makes it imperative to include a phosphate-injected treatment to compare to new technologies for improving pork quality.

The objective of our study was to determine the effectiveness of pre-rigor injection of pork carcasses with sodium citrate or acetate, or post-rigor injection of phosphate plus salt on post-mortem glycolysis, pH decline, and pork quality attributes, including display life and sensory-panel scores.

2. Materials and methods

2.1. Pigs and treatments

Two replicates of 10 pigs ($n = 20$) were fed finishing diets containing ractopamine for at least 14 d before harvest. Pigs were weighed and assigned to pairs of similar weights. The four sides from each pair of pigs were assigned to one of four treatments as follows: pre-rigor citrate injection (CIT), pre-rigor acetate injection (ACE), post-rigor phosphate plus salt injection (PHOS), and non-injected control (CON).

2.2. Harvest

Twenty hours before harvest, pigs were fasted and transported to the Kansas State University Meats Laboratory. Pairs of pigs were harvested in random order. Pigs were stunned by using both an electric stunning wand and a captive bolt stunner. After stunning, pigs were exsanguinated and harvested according to normal procedures; the procedure was approved by the Institutional Animal Care and Use Committee. After the carcasses were split and washed, each side was weighed.

2.3. Pre-rigor injection

At approximately 50 min post-mortem, loins from sides assigned to CIT and ACE treatments were injected with a 4% solution of sodium citrate or sodium acetate in distilled water. A hand-held injector fitted with five 10-cm injection needles was used to inject the solutions. Before injection, the skin was sliced perpendicular to the length of the loin at approximately 3-cm increments to allow the injection needles to penetrate the skin and into the *longissimus* muscle. The loins were injected from a point in the muscle opposite the last lumbar vertebrae to a point immediately posterior to the scapula. The solutions were injected at room temperature, and injection-solution temperature was recorded for each side to ensure uniformity. Sides were weighed again, and pump percentage was calculated. It was assumed that the loin was 20% of the total side weight, and that only the loin absorbed the solution. The approximated injection percentage of the loins injected pre-rigor was calculated to be 10%.

To monitor temperature decline, a temperature logger (Hobo® XT, Onset Computer Corporation) was placed in the *longissimus* muscle of each side. A slice was made at the sirloin-loin juncture, and the temperature probe was inserted into the muscle at least 7 cm, at a 45° angle to the skin surface. After injection (approximately 50 min post-mortem), a muscle sample was removed from the anterior portion of the injected *longissimus* muscle from both sides of the carcasses. The muscle samples were cubed, quick-frozen in liquid nitrogen, packaged, temporarily stored on dry ice, and stored at −80 °C for pH and glycolytic-metabolite analysis. Additional muscle samples were removed from the *longissimus* muscle of each side at 3, 6, 12, and 24 h post-mortem and were frozen in liquid nitrogen as described previously. At least 3 cm of muscle was maintained between muscle sample locations, to minimize the effects of chilling rate on the cut surfaces.

2.4. Pre-24 h pH analysis

Frozen muscle samples were pulverized into a powder with a Waring Blender and stored at −80 °C until analysis for pH and glycolytic metabolites. Duplicate 5-g samples of powdered muscle were mixed with 50 ml of a solution of iodoacetate in 150 mM potassium chloride (Bendall,

1973). The mixture was covered with Parafilm and allowed to acclimate to room temperature for no less than 4 h. Then, the solution was remixed, and each duplicate was read twice by using two Accumet glass electrode pH probes with a portable meter (model AP61; Fisher Scientific; Fairlawn, NJ). The four readings were averaged for statistical analysis.

2.5. Glycolytic metabolites

Samples from 50 min, 3, and 12 h were analyzed for glucose-6 phosphate (G6P), fructose-6 phosphate (F6P), fructose-1,6 bisphosphate (F16BP), a combination of glyceraldehyde-3 phosphate and dihydroxyacetone phosphate (GAPDAP), and lactate according to the methods of Bergmeyer (1974) and S. B. Smith (2005, personal communication). Approximately 1 g of frozen, pulverized muscle tissue was deproteinated with perchloric acid prepared for glycolytic metabolite analysis as described by Bergmeyer (1974), and stored at 1 °C for no more than 4 weeks until analysis.

Determination of G6P and F6P was carried out according to procedures described by Bergmeyer (1974), with minor modification (Rhoades et al., 2005). Determination of F16BP and GAPDAP was completed according to Bergmeyer (1974). Concentrations of DAP are generally quite low in muscle (Bergmeyer, 1974; S. B. Smith, personal communication), so GAP and DAP were measured in concert.

Lactate concentration was determined according to the procedure of S. B. Smith (2005, personal communication). Buffer was prepared with 3.8 g glycine, 4.0 ml 85% hydrazine hydrate, 76 mg EDTA, 8 ml 1 N sodium hydroxide, and distilled, deonized water to make about 90 ml of solution. The pH was adjusted to 9.4 with sodium hydroxide, and the final volume was brought to 100 ml with water. Cuvettes were prepared with 1.95 ml buffer, 2 mg NAD in 0.2 ml water, 0.9 ml water, and 0.05 ml sample extract. Absorbance was read at 340 nm, lactic dehydrogenase (1.55 U/cuvette) was added, and the reaction occurred for 90 min. A second absorbance reading was made to determine lactate concentrations. Absorbance increased with increasing NADH production, and one NADH was produced for each lactate molecule present.

2.6. Fabrication and post-rigor injection

At 24 h post-mortem, sides were fabricated into loins (IMPS 410), and the anterior section, from which the muscle samples were removed, was discarded. Loins assigned to CIT, ACE, and CON treatments were vacuum packaged and stored at 1 °C. Loins assigned to post-rigor PHOS treatment were injected with a solution containing 4.4% sodium tripolyphosphate (Prayon, Inc.) and 2.2% sodium chloride with the hand-held injector used for the CIT and ACE treatments. After two rounds of PHOS injection, the loins had absorbed 6.6% of their pre-injected weight, and were not injected again to keep the treatments as uniform as possible. After injection, the loins assigned to PHOS were vacuum packaged and stored overnight at 1 °C. The loins were re-weighed after vacuum storage and retained 104.6% of their pre-injected weight.

2.7. Chop removal, initial pork color, firmness, and wetness

At 2 d post-mortem, loin sections were de-boned, and three 2.54-cm chops were removed from the posterior section of the *longissimus*. One chop, for analysis by a trained sensory panel, was vacuum packaged and stored at 1 °C for 8 d before freezing at −20 °C. An additional chop was vacuum packaged and stored at 1 °C overnight for pH and expressible-moisture analysis. The third chop was allowed to bloom for no less than 30 min and was evaluated by a three-member, trained visual panel for color, firmness, and wetness. Color was evaluated by using the official NPPC color standard cards (1 = lightest and 6 = darkest; NPPC, 1999). Firmness and wetness were evaluated separately by using three-point scales (1 = softest or wettest and 3 = firmest or driest).

2.8. Display color

Chops for visual evaluation were packaged in white foam trays with absorbent pads, over-wrapped with PVC film (MAP-PAC M – 23,250 cc $O_2/m^2/24$ h gauge), and placed in an open-top display case under continuous fluorescent lighting (1614 lux, 3000 K). A trained visual panel of no less than six persons evaluated color each day for 7 d of display. Panelists scored each chop for color on a six-point scale (1 = extremely bright pink, 2 = bright pink, 3 = dull pink, 4 = slightly dark pink or tan, 4.5 = borderline unacceptable, 5 = moderately dark pink or tan, 6 = dark pink or tan) and discoloration on a seven-point scale (1 = no discoloration (0%), 2 = slight discoloration (1–19%), 3 = small discoloration (20–39%), 4 = modest discoloration (40–59%), 5 = moderate discoloration (60–79%), 6 = extensive discoloration (80–99%), 7 = total discoloration (100%)). The panelists' scores for each day were averaged for analysis.

2.9. Display loss

After display, packages were weighed and chops were removed, dabbed with a paper towel, allowed to dry for 5 min, and weighed again to calculate display loss, calculated as follows: ((pre-display weight − post-display weight)/pre-display weight) × 100.

2.10. Instrumental color

Each day of display, a HunterLab Miniscan XE Plus spectrophotometer (Model D/8-S, 1.4 cm aperture, Hunter Associates Laboratory) was used to obtain L^*, a^*, and b^* values on the PVC over-wrapped chops. Each chop was measured twice by using a 3.2-cm aperture, a 10° observer, and illuminant D_{65}. Readings were averaged for analysis.

2.11. Expressible moisture

At 3 d post-mortem, chops assigned to expressible-moisture evaluation were removed from their vacuum bags. Expressible moisture was determined using the methods of Dhanda, Pegg, Janz, Aalhus, and Shand (2002). Duplicate samples (2–3 g) were removed from the interior of the chop, parallel to the muscle fiber direction, with a scalpel and tweezers. The remainder of the chop was vacuum packaged and stored at 1 °C until pH analysis. Samples were weighed and placed in a 50-ml centrifuge tube fitted with one piece of Whatman No. 3 filter paper folded around one piece of Whatman No. 50 filter paper. The tubes were capped and centrifuged at 2100 rpm for 10 min at 750*g*. After centrifugation, samples were weighed again, and expressible moisture was calculated as: ((pre-centrifuge weight − post-centrifuge weight)/pre-centrifuge weight) × 100.

2.12. Ultimate pH analysis

Duplicate samples (10 g each) were minced with a scalpel. Samples were placed in a filtered stomacher bag with 100 ml of distilled water and were stomached for 2 min. After samples were stomached, pH was measured with a portable Accumet probe and meter (model AP61; Fisher Scientific). Data from duplicate samples were averaged for analysis.

2.13. Sensory panel evaluations

Chops for analysis by the trained sensory panel were stored frozen for 3 months and thawed overnight at 4 °C. Chops were cooked to 70 °C in a Blodgett dual-air flow convection oven (model DFG-201, G.S. Blodgett Co., Inc.), and temperature was monitored with 30-gauge, type-T thermocouples inserted into the geometric centers of the chops and attached to a Doric temperature recorder (model 205, Vas Engineering). After cooking, the outer connective tissue was removed, and the chops were cut into cubes (1.27 cm × 1.27 cm × chop thickness) and held in preheated double broilers. No fewer than 6 trained panelists were seated in an environmentally controlled room, at approximately 21 °C and 55% relative humidity, in individual booths under adjustable red-plus-green lighting that was less than 110 lumen combined. Each panelist was given two warm-up samples from a non-injected chop to orient themselves before each panel. A panel leader discussed the warm-up samples with the panelists. Then, two cubes from each chop were served to panelists in a statistically randomized order, and a score was determined by using an eight-point scale to the nearest 0.5 (AMSA, 1995).

2.14. Statistical analysis

Muscle temperature data were analyzed as an incomplete block with the repeated measure of time, with individual pig as the block. Pre-rigor pH and glycolytic-metabolite data were analyzed in a split-plot design, with injection treatment as the whole plot and time post-mortem as the sub plot. Pig was used as the block in the whole plot. Visual color, firmness, wetness, expressible moisture, and ultimate pH were analyzed in an incomplete block, with pig as the block. Visual- and instrumental-display data were analyzed in an incomplete-block design, with the repeated measure of time, and pig as the block. Data from the trained sensory panel were analyzed in an incomplete-block design, blocking on pig and panelist. Injection treatment and time post-mortem were treated as fixed effects, while pig and panelist were treated as random effects. Data were analyzed with PROC MIXED in the Statistical Analysis System (Version 8.0, SAS Institute), and least squares means were separated by using the PDIFF test when $P < 0.05$. For repeated-measures analysis, the repeated-measures command was used with the autoregressive option.

3. Results and discussion

3.1. Temperature

All treatments followed an exponential decline in temperature to −1 °C. At 1 h post-mortem, *longissimus* muscles from CON and PHOS treatments, which received no pre-rigor injections, were warmer ($P < 0.05$) than those from CIT and ACE treatments, and those from the ACE treatment were warmer ($P < 0.05$) than those from the CIT treatment. Nevertheless, muscle temperatures among treatments were similar ($P > 0.05$) for measurements taken in 1-h increments afterwards. It is probable that the temperature of the injection solution (approximately 17 °C) lowered the temperature of the muscle in the first few minutes after injection, but did not affect chill rate after 1 h.

3.2. Pre-24 h pH

There was no time × treatment interaction ($P > 0.05$) for pre-24 h pH (Table 1). *Longissimus* muscles from the CIT treatment had the highest ($P < 0.05$) pre-24 h pH values, whereas those from the ACE treatment did not differ ($P > 0.05$) from CON and PHOS treatments. Jerez, Calkins, and Velazco (2003) reported increased pH values at 24- and 72-h post mortem for beef muscles injected with CIT and ACE, but they did not report pre-rigor pH. In our study, pH was highest ($P < 0.05$) at 50 min post-mortem, and values at 3, 6, 12, and 24 h were similar ($P > 0.05$), indicating that the majority of pH decline occurred during the first 3 h.

3.3. Glycolytic metabolites

A time × injection treatment interaction ($P < 0.05$) was found for G6P concentration (Fig. 1). All four treatments resulted in similar ($P > 0.05$) G6P concentrations at 50 min post-mortem. However, values for G6P in CON muscles increased ($P < 0.05$) with post-mortem time, and were higher ($P < 0.05$) than for the CIT and ACE treatments

Table 1
Mean values for pre-24 h pH of *longissimus* muscle from carcasses injected 50 min post-mortem with sodium citrate or sodium acetate, non-injected control carcasses, and carcasses injected with phosphate + salt at 24 h post-mortem

Time	Pre-rigor injection		Non-injected control	Post-rigor phosphate + salt	Mean[A]
	Acetate	Citrate			
50 min	5.87	5.96	6.00	5.90	5.93[a]
3 h	5.45	5.58	5.49	5.49	5.51[b]
6 h	5.52	5.56	5.49	5.47	5.51[b]
12 h	5.49	5.52	5.46	5.44	5.48[b]
24 h	5.50	5.55	5.47	5.48	5.50[b]
Mean	5.57[b]	5.63[a]	5.58[b]	5.56[b]	

[ab] Means for times and treatments lacking common superscript letters differ ($P < 0.05$).
[A] Standard error for all means = 0.02.

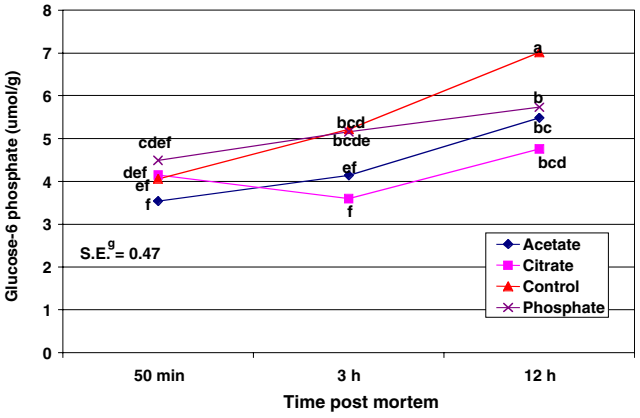

Fig. 1. Mean concentrations of glucose-6 phosphate in *longissimus* muscle at 50 min, 3 h, and 12 h post mortem from carcasses injected pre-rigor with acetate or citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. [a–f]Means lacking common superscript letters differ ($P < 0.05$). [g]Largest interaction mean standard error.

at 3 and 12 h post-mortem. In muscles from the CIT treatment, G6P concentrations were lowest ($P < 0.05$) at 3 h, but did not increase ($P > 0.05$) from 50 min to 12 h. Concentrations of G6P in *longissimus* muscles from the ACE treatment increased ($P < 0.05$) with time, but were always similar to the CIT-treated muscles.

Muscles from the CON and PHOS treatments had higher concentrations ($P < 0.05$) of F6P than those from the ACE and CIT treatments (Fig. 2). Concentrations of F6P were similar ($P > 0.05$) at 50 min and 3 h, but were higher ($P < 0.05$) at 12 h. No interaction existed for F6P values ($P > 0.05$).

Glucose-6 phosphate is the precursor to F6P, which is a substrate for PFK. Successful inhibition of PFK by CIT and ACE treatments should have resulted in elevated concentrations of G6P and F6P. Nevertheless, our G6P and F6P concentrations were highest ($P < 0.05$) for CON and PHOS treatments, indicating that CIT and ACE activated PFK activity rather than inhibited it. Kemp and Krebs (1967) stated that, in combination with low concentrations

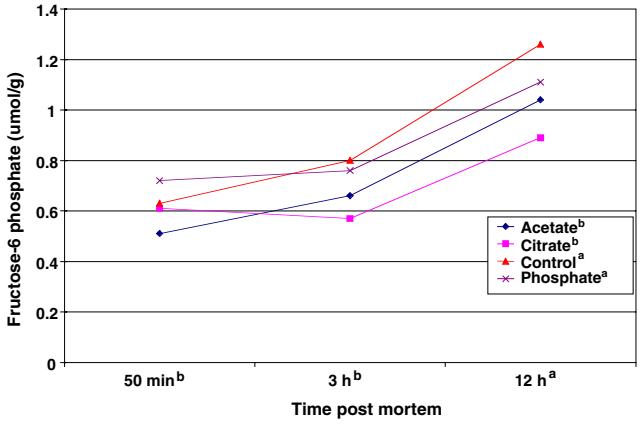

Fig. 2. Mean concentrations of fructose-6 phosphate in *longissimus* muscle at 50 min, 3 h, and 12 h post mortem from carcasses injected pre-rigor with acetate or citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. [ab]Main effect means lacking common superscript letters differ ($P < 0.05$), standard error for ante-mortem treatment main effects = 0.06, standard error for time main effects = 0.05.

of ATP, CIT may act as an activator of PFK. Citrate increases the enzyme's affinity for ATP at the substrate site and activates the reaction. Reactions of rigor take place due to a drop in ATP concentration (Hamm, 1977). Therefore, pre-rigor CIT injection, in combination with low ATP levels associated with rigor, may have actually activated PFK. Although previous researchers have not experienced this phenomenon in post-mortem beef muscle (Jerez et al., 2003; Perversi et al., 2003; Streitel et al., 1977), pork is inherently more glycolytic than beef is and goes into the rigor state earlier post-mortem. It is possible that the approximately 10% addition of water diluted G6P and F6P concentrations in the ACE and CIT *longissimus* muscles, but this dilution effect was not evidenced in other metabolites.

Concentrations of F16BP decreased with time for all four treatments (Fig. 3). A time × treatment interaction ($P < 0.05$) was found for F16BP values. For CON, PHOS, and ACE treatments, the 50-min concentrations were higher ($P < 0.05$) than at 3 and 12 h. However, for the CIT treatment, concentrations at 50-min and 3-h were similar ($P > 0.05$), and were greater than that at 12 h ($P < 0.05$), indicating that the rate of decline in F16BP in the CIT-treated muscles may have been slower than the other treatments.

There was a time × treatment interaction ($P < 0.05$) for GAPDAP concentrations (Fig. 4). Both CIT and ACE were similar ($P > 0.05$) to CON. All treatments were similar at 3 and 12 h post-mortem. The 50-min concentrations were highest ($P < 0.05$), and the 3 and 12 h concentrations were similar ($P > 0.05$) for all treatments.

The product of PFK is F16BP, and inhibition of PFK should have resulted in decreased concentrations of F16BP for CIT and ACE treatments. Maintenance of high levels of F16BP at 3 h for CIT could indicate that F16BP levels were being replenished by PFK as they were used,

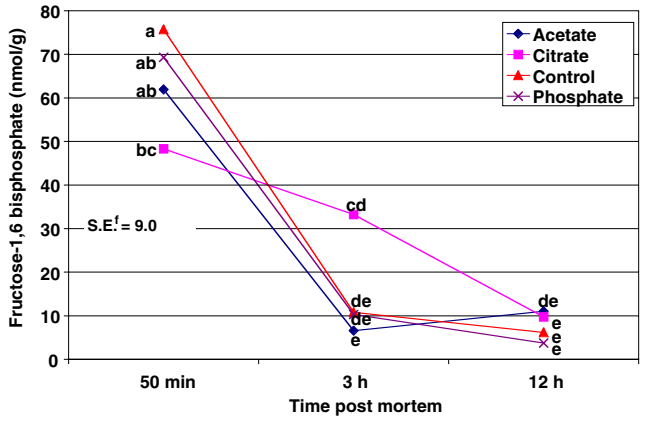

Fig. 3. Mean concentrations of fructose-1,6 bisphosphate in *longissimus* muscle at 50 min, 3 h, and 12 h post mortem from carcasses injected pre-rigor with acetate or citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. [a–e]Means lacking common superscript letters differ ($P < 0.05$). [f]Largest standard error for interaction means.

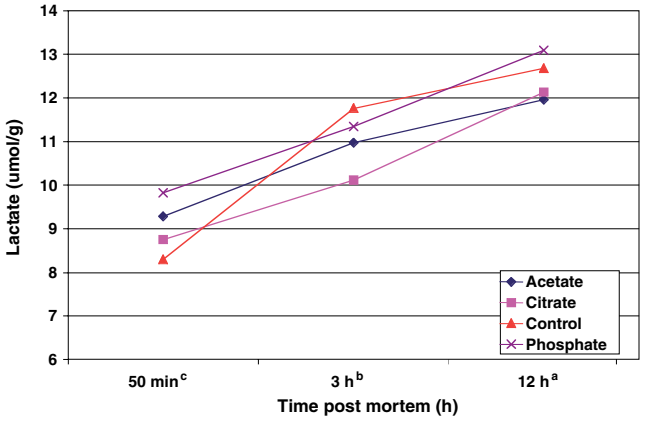

Fig. 5. Mean concentrations of lactate in *longissimus* muscle at 50 min, 3 h, and 12 h post mortem from carcasses injected pre-rigor with acetate or citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. [a–c]Main effect means lacking common superscript letters differ ($P < 0.05$); standard error for ante-mortem treatment main effect = 0.30; standard error for time main effect = 0.26.

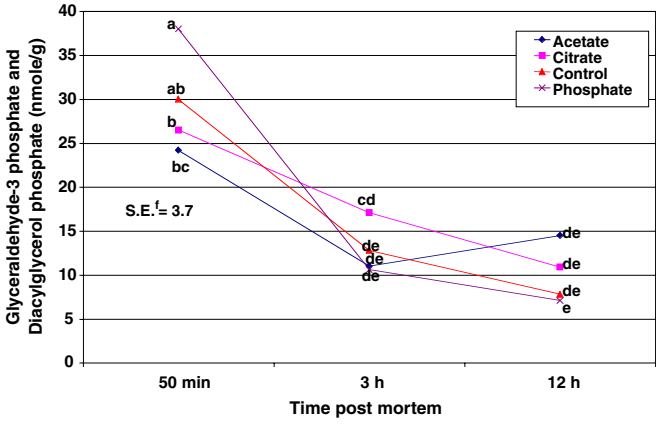

Fig. 4. Mean concentrations for a combination of glyceraldehyde-3 phosphate and dihydroxyacetone phosphate in *longissimus* muscle at 50 min, 3 h, and 12 h post mortem from carcasses injected pre-rigor with acetate or citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. [a–e]Means lacking common superscript letters differ ($P < 0.05$). [f]Largest standard error for interaction means.

and that PFK was activated rather than inhibited. The F16BP concentrations in muscles from CON, PHOS, and ACE treatments were not being replenished. Aldolase, the enzyme that cleaves F16BP to form GAP and DAP, operates continuously in the presence of its substrate, F16BP (Voet, Voet, & Pratt, 2002). Therefore, GAPDAP concentrations indicate PFK activity. Our data indicate that GAPDAP concentrations were not being replenished by PFK for any treatment.

Muscles from the CIT treatment had lower ($P < 0.05$) lactate concentrations than those designated for 24-h PHOS injection (Fig. 5), but those from CON and ACE treatments were not different ($P > 0.05$) in lactate concentration than those from CIT or PHOS treatments. There was no interaction ($P > 0.05$) for lactate concentrations. Lactate concentrations increased ($P < 0.05$) as post-mortem time increased, which was expected because lactate accumulates with time (Hamm, 1977).

Cross-over diagrams represent ratios of glycolytic metabolite concentrations at different times post-mortem: 3 h/50 min (Fig. 6), 12 h/3 h (Fig. 7), and 12 h/50 min (Fig. 8). These cross-over diagrams represent changes in metabolites over time. The values on the *y*-axis are ratios of metabolite levels at two times post-mortem multiplied by 100. The glycolytic metabolites of G6P, F6P, F16BP, GAPDAP, and lactate indicate rate-limiting enzymes in pathways, and are presented from left to right on the *x*-axis in the order in which they occur in glycolysis. Ratios greater than 100 indicate that the metabolite is accumulating over time, whereas values less than 100 indicate that the metabolite is decreasing over time. If the line between two

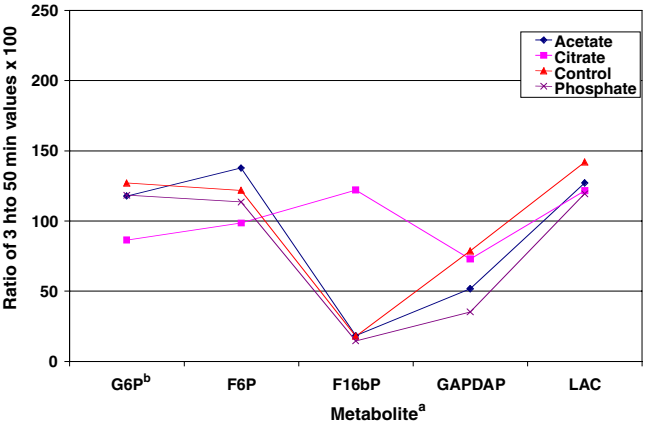

Fig. 6. Cross-over diagram for glycolytic metabolites of pork *longissimus* from carcasses injected pre-rigor with citrate or acetate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. Data points are ratios of [(mean at 3 h)/(mean at 50 min)] × 100. [a]Means for ratios within metabolites were not different ($P > 0.05$). [b]Largest standard error for G6P = 12.0, F6P = 13.2, F16BP = 34.2, GAPDAP = 16.2, lactate = 10.3.

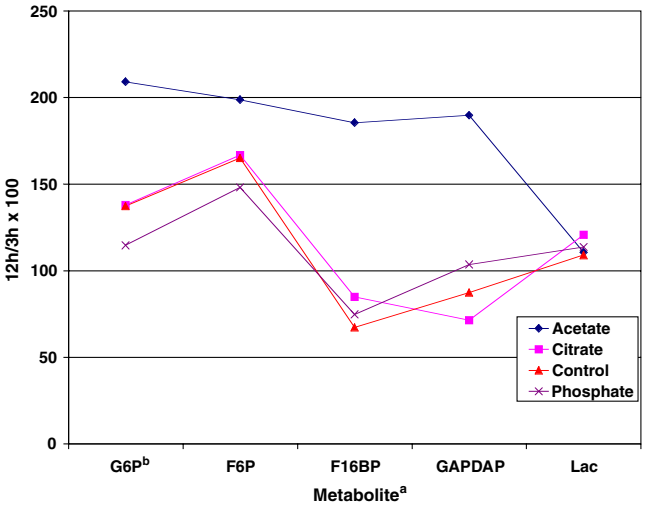

Fig. 7. Cross-over diagram for glycolytic metabolites of pork *longissimus* from carcasses injected pre-rigor with citrate or acetate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt. Data points are ratios of [(mean at 12 h)/(mean at 3 h)] × 100. [a]Means for ratios within metabolites were not different ($P > 0.05$). [b]Largest standard error for G6P = 42.3, F6P = 28.1, F16BP = 35.4, GAPDAP = 50.0, lactate = 6.3.

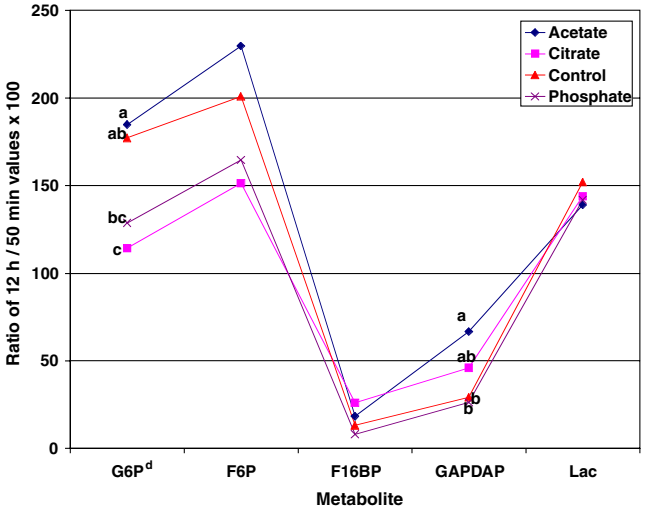

Fig. 8. Cross-over diagram for glycolytic metabolites of pork *longissimus* from carcasses injected pre-rigor with citrate or acetate, non-injected control carcasses, and carcasses designated for injection post-rigor with phosphate + salt. Data points are ratios of [(mean at 12 h)/(mean at 50 min)] × 100. [a–c]Means, within a metabolite, lacking common superscript letters differ ($P < 0.05$). [d]Largest standard error for G6P = 17.8, F6P = 20.2, F16BP = 4.9, GAPDAP = 8.1, lactate = 11.2.

metabolites crosses over 100, then the enzyme between them is rate-limiting.

For 3 h/50 min (Fig. 6), ratios of PFK-substrate compounds (G6P and F6P) were greater than 100 for samples from CON, PHOS, and ACE treatments, whereas those from the CIT treatment were less than 100. For the post-PFK compounds (F16BP and GAPDAP), ratios were less than 100 for CON, PHOS, and ACE, but ratios of F16BP for CIT were greater than 100. This pattern of ratios indicated that a rate-limiting step existed for CON, PHOS, and ACE treatments for the reaction catalyzing the change from F6P to F16BP, or PFK. But, the rate-limitation of PFK in the CIT treatment from 50 min to 3 h is questionable. For the CIT treatment, ratios of pre-PFK metabolites were less than 100, indicating that these metabolites did not accumulate between 50 min and 3 h. If G6P and F6P did not accumulate, they were most likely being used by PFK, so PFK was not limiting glycolysis. The post-PFK metabolite, F16BP, did not decrease from 50 min to 3 h as was expected, indicating that the pool of F16BP was being replenished by an uninhibited-PFK enzyme. Ratios for lactate greater than 100 indicate that this compound accumulated over time in all treatments.

Ratios of 12 h/3 h concentrations of G6P and F6P were greater than 100 for all treatments (Fig. 7), but F16BP and GAPDAP ratios were less than 100 for CON, PHOS, and CIT treatments, indicating that PFK was the rate-limiting factor between 3 and 12 h. It is likely that PFK had become inactivated after 3 h in the CIT-treated muscles. Muscles in the ACE treatment had 12 h/3 h ratios greater than 100 for the post-PFK compounds, indicating a lack of rate limitation by PFK during this time period. From 3 h to 12 h, the pre-PFK metabolites were increasing in all treatments, and the post-PFK metabolites were decreasing in the CON, CIT, and PHOS-treated muscles. In the ACE-treated muscles, the F16BP and GAPDAP concentrations were not decreasing during this time period, indicating that the enzymes after PFK in glycolysis had also become inactive. Lactate ratios were also greater than 100, but were not as elevated as the 3 h/50 min ratios, indicating that lactate levels did not increase as much between 3 and 12 h as they did between 50 min and 3 h.

From 50 min to 12 h, all four treatments exhibited a pattern indicative of a rate-limiting step at PFK (Fig. 8). Pre-PFK ratios were greater than 100, and post-PFK ratios were less than 100. Lactate ratios indicated an accumulation of that compound.

Glycolytic metabolite data and cross-over diagrams indicate that CIT and ACE were ineffective as glycolytic inhibitors when injected into pork muscle, even though muscles injected with CIT had higher pH values. The CIT solution likely increased muscle pH due to its buffering capacity and multiple negative charges on the citrate ion. Jerez et al. (2003) found that pre-rigor injection of beef muscles with CIT inhibited glycolysis, as evidenced by increased muscle pH and glycogen levels. Glycogen levels were not measured in our study. Allison, Bates, Booren, Johnson, and Doumit (2003) claimed that PFK was inactivated within 20 min post-mortem and, therefore, does not affect pork quality attributes. Nevertheless, Rhoades et al. (2005) and Dalrymple and Hamm (1975) stated that PFK is the main rate-limiting enzyme in post-mortem muscle glycolysis. Cross-over diagrams indicated that PFK was still active in the muscle after 50 min post-mortem when the CIT and ACE solutions were introduced into the muscle system, because the glycolytic

metabolites were still changing after 50 min post-mortem. Concentrations of ATP may have been at a non-saturated state early post-mortem, and PFK may have been activated by CIT as discussed earlier. Killefer (2004) also found CIT to be inhibitory in pork, but his injection solution included phosphate and salt, which would have drastically affected muscle pH. Enzyme activities are altered at higher pH. Furthermore, the increase in ionic strength, due to the phosphate and salt, may have affected the PFK activity in his study.

3.4. Pork quality attributes

Mean values for visual color, firmness, and wetness, as well as expressible moisture, ultimate pH, and display loss are presented in Table 2. According to visual panelists, chops from the ACE and CIT treatments were less firm ($P < 0.05$) and wetter ($P < 0.05$) than those from CON and PHOS. These inferiorities were not surprising inasmuch as the CIT and ACE treatments added approximately 10% water to the *longissimus* muscle. Chops from the PHOS treatment also had added water, but the percentage was lower than for CIT and ACE. The PHOS treatment also greatly increased ($P < 0.05$) muscle pH. Chops from the PHOS treatment had the highest ($P < 0.05$) ultimate pH values, and chops from the CIT treatment had higher ($P < 0.05$) ultimate pH values than those from CON or ACE treatments. Chops from the CIT and ACE treatments had greater ($P < 0.05$) display losses than those from PHOS and CON treatments. Chops from the PHOS treatment had the least ($P < 0.05$) display loss. Visual color and expressible moisture were not affected by injection treatment.

Killefer (2004) used CIT in conjunction with phosphate and salt injection in pork and found increased ultimate pH compared to that of controls injected with phosphate. He also reported increased visual color, and a tendency ($P = 0.06$) for increased firmness in chops injected with CIT. Jerez et al. (2003) reported increased pH at 24 and 72 h in beef muscles injected with CIT and ACE.

3.5. Display evaluations

Visual color and discoloration scores throughout display are presented in Fig. 9. Visual color scores increased ($P < 0.05$) throughout display for all four treatments, indicating a deterioration of color during display. Chops from the PHOS treatment had the highest (darkest; $P < 0.05$) visual scores each day of display compared with those of other treatments. Chops from the PHOS treatment were also considered unacceptable (color scores greater than 4.5) by the panelists after 5 d of display, whereas no other treatment reached that mark. Chops from the ACE and CIT treatments were similar ($P > 0.05$) to those from CON each day of display. Chops from the PHOS treatment were more discolored ($P < 0.05$) than those from other treatments throughout the display period (Fig. 9). Discoloration scores of chops from the ACE treatment were similar ($P > 0.05$) to those from CON throughout display. For the first 6 d of display, chops from the CIT treatment were similar ($P > 0.05$) to those from CON, but the

Table 2
Visual evaluations, expressible moisture, ultimate pH, and display loss of *longissimus* chops from pork carcasses injected pre-rigor with sodium acetate or sodium citrate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt

Item	Pre-rigor injection		Non-injected control	Post-rigor phosphate + salt	SE[c]
	Acetate	Citrate			
Color[a]	3.26	3.16	4.48	4.33	0.53
Firmness[b]	1.95^{y}	2.14^{y}	2.36^{z}	2.49^{z}	0.15
Wetness[b]	1.93^{y}	1.96^{y}	2.46^{z}	2.41^{z}	0.18
Expressible moisture	18.9	20.0	20.1	18.6	1.35
Ultimate pH	5.51^{x}	5.63^{y}	5.48^{x}	5.99^{z}	0.03
Display loss	9.36^{z}	9.71^{z}	7.45^{y}	4.73^{x}	0.58

^{xyz} Means, within a row, lacking common superscript letters differ ($P < 0.05$).

[a] Color was evaluated on a 6-point scale using official color standards from the National Pork Producers Council (1 = lightest and 6 = darkest).

[b] Firmness and wetness were evaluated separately on 3-point scales (1 = softest and wettest and 3 = firmest and driest).

[c] Standard error of the mean.

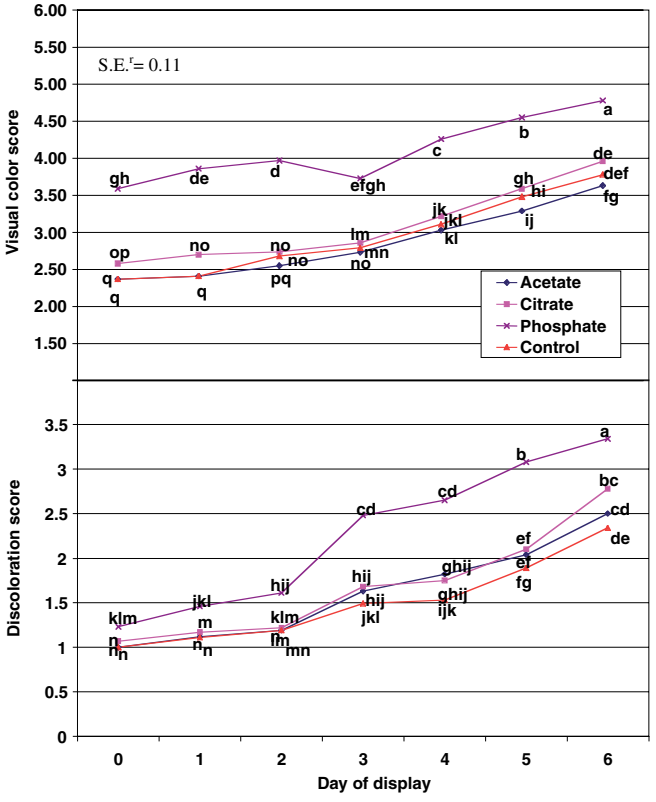

Fig. 9. Visual color and discoloration scores for *longissimus* chops from pre-rigor acetate or citrate injection, non-injected control carcasses, and carcasses injected post-rigor with phosphate and salt, over 7 d of display. [a–q]Means lacking common superscript letters differ ($P < 0.05$). [r]Standard error of the interaction means.

discoloration scores were higher ($P < 0.05$) on the final day of display for chops from the CIT treatment than those from CON.

Chops from PHOS loins were darkest (lowest L^*; $P < 0.05$) throughout display (Fig. 10). Although chops from the ACE treatment were similar ($P > 0.05$) to those from CON for the first 2 d of display, they were lighter ($P < 0.05$) than those from CON for the last 5 d of display. Chops from the CIT treatment were similar ($P > 0.05$) to those from CON in L^* value throughout the display period. Chops from the CIT treatment did not change ($P > 0.05$) throughout display, whereas L^* values for chops from PHOS and CON peaked ($P < 0.05$) after 1 d of display. Jerez et al. (2003) stated that L^* values for CIT-injected beef samples were similar to CON, but ACE-injected samples were lighter than CON. Killefer (2004) reported lower L^* values for CIT-injected pork loins than for CON, both initially and after 14 d in modified atmosphere packaging, but their CON and CIT solutions both contained phosphate and salt.

Chops from the PHOS treatment were less red (lower a^*; $P < 0.05$) than those from CON and ACE each day of display (Fig. 11). Chops from the CIT treatment were similar ($P > 0.05$) to those from PHOS on d 0 and on the final 2 d of display. Chops from the CON treatment were similar to those from the ACE treatment the first 2 d of display, and similar to chops from the CIT treatment after 1 d, but they had the highest a^* values ($P < 0.05$) on the final 5 d of display. Chops from the ACE treatment were redder ($P < 0.05$) than those from the CIT treatment on d 0 and 2, but they were similar ($P > 0.05$) throughout the rest of display. Killefer (2004) found that pork chops from CIT-injected loins had higher a^* values than did controls injected with PHOS, but his CIT treatment also included PHOS. Samples from the CIT and ACE treatments were less red than non-injected controls in beef (Jerez et al., 2003).

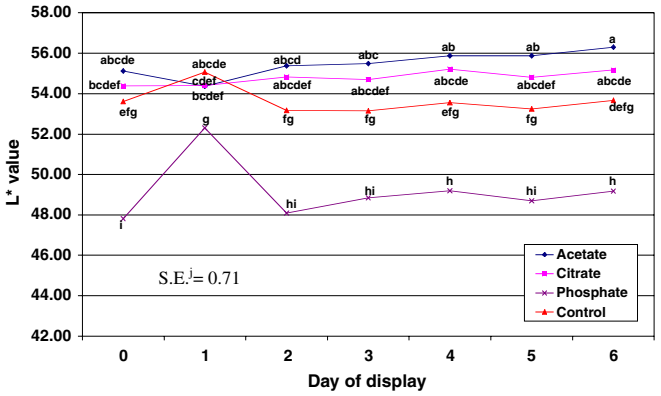

Fig. 10. Values for L^* for chops from acetate or citrate pre-rigor injection at 50 min post-mortem, non-injected control carcasses, and carcasses designated for injection post-rigor with phosphate + salt over 7 d of display. [a–i]Means lacking common superscript letters differ ($P < 0.05$). [j]Standard error of the interaction means.

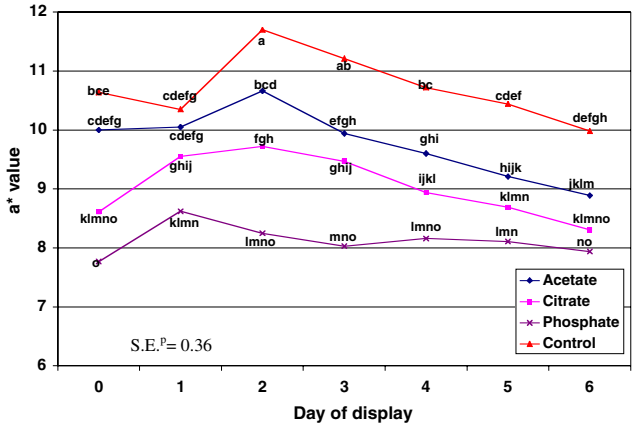

Fig. 11. Values for a^* for *longissimus* chops from acetate or citrate injection at 50 min post-mortem, non-injected control carcasses, and carcasses designated for injection post-rigor with phosphate + salt, over 7 d of display. [a–o]Means lacking common superscript letters differ ($P < 0.05$). [p]Standard error of the interactions means.

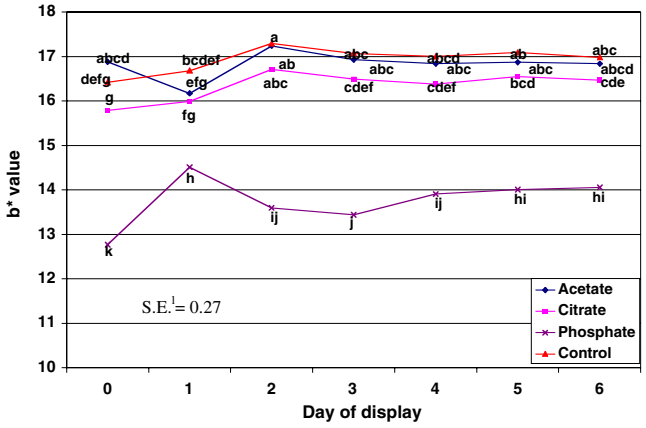

Fig. 12. Values for b^* for *longissimus* chops from acetate or citrate pre-rigor injection at 50 min post-mortem, non-injected control carcasses, and carcasses designated for injection post-rigor with phosphate + salt, over 7 d of display. [a–k]Means lacking common superscript letters differ ($P < 0.05$). [l]Standard error of the interaction means.

Chops from the PHOS treatment had the lowest b^* values (least yellow; $P < 0.05$) throughout display (Fig. 12), whereas chops from CIT and ACE treatments were similar ($P > 0.05$) to those from CON throughout display. Chops from the PHOS treatment had the lowest b^* values on d 0, whereas values for b^* did not notably change ($P > 0.05$) over time for any of the other treatments. Previous research in beef found that samples injected with CIT were less yellow than controls, whereas samples from the ACE treatment were similar to controls (Jerez et al., 2003).

3.6. Sensory attributes

Values for attributes evaluated by the trained sensory-panel are displayed in Table 3. Control chops were toughest ($P < 0.05$), chops from the PHOS treatment were most tender ($P < 0.05$), and the CIT and ACE treatments, which

Table 3
Mean values and standard errors for traits evaluated by a trained sensory panel for *longissimus* chops from carcasses injected pre-rigor with sodium citrate or sodium acetate, non-injected control carcasses, and carcasses injected post-rigor with phosphate + salt

Item	Pre-rigor injection		Non-injected control	Phosphate + salt	SE[f]
	Acetate	Citrate			
Myofibrillar tenderness[a]	5.78[y]	5.83[y]	4.81[z]	6.34[x]	0.19
Juiciness[b]	5.12[z]	4.99[z]	4.83[z]	6.17[y]	0.15
Pork flavor intensity[c]	4.56[z]	5.22[y]	5.19[y]	4.83[z]	0.22
Off flavor[d]	5.42[z]	7.52[y]	7.09[y]	5.30[z]	0.24
Connective tissue[e]	7.34[y]	7.38[y]	6.94[z]	7.42[y]	0.14
Overall tenderness	6.02[y]	6.13[y]	5.10[z]	6.61[x]	0.18

[xyz] Means within a row lacking common superscript letters differ ($P < 0.05$).
[a] Myofibrillar tenderness and overall tenderness were evaluated on an 8-point scale (1 = extremely tough and 8 = extremely tender).
[b] Juiciness was evaluated on an 8-point scale (1 = extremely dry and 8 = extremely juicy).
[c] Pork flavor intensity was evaluated on an 8-point scale (1 = extremely bland and 8 = extremely intense pork flavor).
[d] Off flavor was evaluated on an 8-point scale (1 = abundant and 8 = none).
[e] Connective tissue amount was evaluated on an 8-point scale (1 = abundant and 8 = none).
[f] Standard error of the mean.

were injected pre-rigor, were intermediate in both myofibrillar and overall tenderness. Control chops also had the lowest ($P < 0.05$) connective tissue scores, indicating a greater perception of detectable connective tissue. The increase in tenderness of chops from the PHOS treatment may have been partly due to the swelling of myofibrils caused by phosphate and salt and to the dilution of the proteins by the injection solutions (Patterson, Parrish, & Stromer, 1988). In pork, chops from loins injected with CIT had lower shear force values than controls (Killefer, 2004). Citrate has been injected into pre-rigor beef muscle to improve tenderness (Jerez et al., 2003; Perversi et al., 2003; Streitel et al., 1977). These researchers theorized that citrate inhibited post-mortem glycolysis, and the muscle-tenderizing calpain enzymes were more active due to the increased pH in muscles injected with CIT. Nevertheless, ACE was not effective at improving beef tenderness, even though pH was increased compared with CON (Jerez et al., 2003).

Chops from the PHOS treatment were also juiciest ($P < 0.05$), whereas chops from CIT and ACE treatments were similar ($P > 0.05$) to those from CON. The increase in ultimate pH, resulting in improved water-holding capacity, by the PHOS injection likely was responsible for the improved juiciness of that treatment.

Chops from CON and CIT treatments had higher ($P < 0.05$) pork flavor intensity scores and less incidence ($P < 0.05$) of off-flavors than those from PHOS and ACE treatments. It is likely that the off-flavors associated with the PHOS and ACE treatments masked the pork flavor of the chops. The most common off-flavor descriptor for chops from the PHOS treatment was salty. Other off-flavor descriptors of soapy, metallic, rancid, and acidic were sometimes used to describe the chops from the PHOS treatment. Chops from the ACE treatment were most commonly described as sweet or sugary, as well as acidic, lemony, or vinegary. Other infrequent off-flavor descriptors included chemical, soapy, salty, metallic, cleaner fluid, and Tabasco. Although chops from the CIT and CON treatments had less incidence ($P < 0.05$) of off-flavors than those from ACE and PHOS, some descriptors were provided. Chops from the CIT treatment were infrequently described as acidic, metallic, salty, bitter, and rancid, whereas those from CON were described as acidic, bitter, salty, and metallic.

Our results are in agreement with those of Perversi et al. (2003), who stated that beef injected with CIT was similar to controls in beef desirability ratings. Phosphate injection has long been known to improve tenderness and juiciness in pork (Smith et al., 1984), and increase saltiness (Brewer et al., 2004), and PHOS is used widely in industry to improve yields and consumer satisfaction. Nevertheless, the PHOS treatment resulted in increased off-flavors in our study.

4. Conclusions

Glycolytic metabolite data indicated that the increase in pH in muscle injected with CIT was not due to an inhibition of glycolysis post-mortem. The pH increase in the muscle was likely due to the relatively high pH of the citrate solution. The highly glycolytic conditions of pork muscle and the low ATP concentrations during rigor may have overwhelmed CIT's ability to inhibit glycolysis. Glycolytic metabolite data reinforced the rate-limiting affects of phosphofructokinase in post-mortem muscle.

Although pre-rigor CIT injection increased pH and improved tenderness, compared with CON, visual firmness and wetness were decreased with CIT injection. Chops from the CIT treatment were similar to those from CON in pork-flavor intensity, and there were no excessive off-flavors. Perhaps using CIT in conjunction with a phosphate and salt solution would allow for improved muscle water-holding capacity, and the water-soluble CIT in the injection-solution would be more accessible to react with PFK. The addition of the CIT may allow for a lower concentration of phosphate to be added to the product and might alleviate the off-flavors associated with the phosphate.

Chops from the ACE treatment were superior to those from CON in tenderness, but glycolytic metabolite and pH data indicated that ACE did not inhibit post-mortem glycolysis. Furthermore, the decreased pork-flavor intensity and objectionable off-flavors of ACE injection likely will discourage use of this compound at this concentration in the future in fresh meat injection solutions.

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