Title: Efficacy of interventions to reduce *Salmonella* on fresh pork products to improve food safety **- NPB #17 – 047**

revised

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Industry Summary:
This project served as a valuable investigation into the implementation and utilization of organic acid applications in pork processing to minimize pathogens, reduce total bacteria, and maximize pork shelf life. Organic acid applications are widely used in the beef and poultry industries within the U.S., but less work has been done to validate effectiveness in the pork industry. With three key objectives in this area, this project was conducted in three distinct phases:

- Phase I: This first phase served as a proof of concept. Pork cheek meat and warm pork skin inoculated with high concentrations of pathogenic *Salmonella* to evaluate the reduction effectiveness using 10 different intervention treatments in a simulated pork processing facility.

- Phase II: Building from phase I, phase II worked with whole carcasses to study these applications in functioning pork facilities. Whole pork carcasses in a small pork harvest facility, and large commercial facility were inoculated with a non-pathogenic *E. coli* to evaluate the effective reduction from selected intervention treatments.

- Phase III: The best performing interventions from phase II were sprayed on pork trim, prior to grinding to evaluate effect on retail ground pork shelf-life.

Table 1. List of all ten treatments used in phase I and then designation for the interventions used in each subsequent phase of the project

Treatment	Phase I	Phase II. Part 1	Phase II. Part 2	Phase III.
Control	Yes	Yes	-	Yes
Water	Yes	Yes	-	-
Hypobromous acid	Yes	Yes	-	-
Lactic acid (3%)	Yes	Yes	Yes	Yes
Lauramide arginine ethyl ester	Yes	Yes	-	-
Peracetic acid	Yes	-	-	-
Peracetic acid + acetic acid (2%)	Yes	Yes	Yes	Yes
Peracetic acid + sulfuric acid and sodium sulfate (Titon™)	Yes	Yes	Yes	Yes
Sulfuric acid and sodium sulfate (Titon™)	Yes	Yes	Yes	-
Citric acid (1.3%)	Yes	-	-	-

The first phase aimed to address the objective of organic acid application to reduce bacterial presence, especially pathogenic bacteria on pork cheek meat and warm pork skin. In this phase pork cheek meat and warm pork skin pieces were intentionally inoculated with pathogenic *Salmonella* at a high concentration and then treatments were applied using a CHAD cabinet to spray the intervention on the inoculated pieces. Microbiological analysis was done to enumerate, or count, the *Salmonella* present before intervention treatment, one hour after intervention treatment, and 24 hours after intervention treatment. With this information the results of phase I were the effectiveness, or the reduction of *Salmonella* on pork skin and cheek meat from the intervention application.

The second phase built off of this proof of concept from phase I, and the most effective six interventions, plus a water and control treatment, were utilized to evaluate the effectiveness in reducing non-pathogenic *E. coli* from whole pork carcasses in a functioning pork harvest facility. The *E. coli* cocktail, of high concentration, was sprayed on the ham and shoulder of pork carcasses. An attachment time was allowed for the bacteria to adhere to the carcass surface, and then the appropriate intervention was sprayed over the entire carcass. Swabs were taken to determine reduction after intervention application and chilling. As a follow-up to this first portion, a secondary study in phase II was done in a large commercial pork harvest facility using the four most effective organic acid applications. Similar to phase I, microbiological analysis was done to enumerate, or count the *E. coli* present before intervention treatment, one hour after intervention treatment, and 24 hours after intervention treatment.

After the work done in phase I and II the most effective organic acid interventions were able to be identified. These leading interventions demonstrated significant reduction of both pathogenic *Salmonella* in phase I, and non-pathogenic *E. coli* in phase II. Phase III addressed the effect of using these effective interventions from carcasses and cheek meat, on pork trim prior to grinding. In Phase III pork trim was divided into four 50lb batches. Each batch was assigned a treatment (see Table 1), intervention treatment was sprayed evenly on the pork trim (a control treatment was also included which had no chemical intervention applied), and then pork trim as mixed before grinding using a coarse blade and regrinding using a fine blade. The finely ground pork was vacuum packaged in roll stock vacuum packages and with eight packages from each treatment pulled on each of the sampling days (0d, 7d, 14d, 21d, and 28d after treatment/grinding/packaging). To understand the shelf-life up to 28d after preparing the ground pork microbiological analysis was done for generic aerobic bacteria and psychrotrophic (cold environment

preferred) bacteria. Instrumental color readings when the package was first opened, and at 10min and 20min after opening, were taken to understand if product color was influenced by usage of the selected interventions. A lab procedure was done to identify if oxidation was occurring in the ground product, as oxidation creates negative odors and tastes in aged product. Additionally, an odor panel was conducted to detect off-odor formation, especially for acidic, oxidized, sweaty, or sour odors.

Overall phase I demonstrated effectiveness of implementing interventions to address pathogenic *Salmonella* control during the harvest process, prior to pork carcass chilling. Phase II demonstrated effective reduction of *E. coli* inoculated carcasses in a small-scale traditional chilled pork harvest facility, and in a large-scale commercial pork facility with a blast chiller. Phase III demonstrated no dramatic negative organoleptic changes to pork trim when treated with selected organic acid interventions prior to grinding. Additionally, phase III highlighted an opportunity for further work to address the most efficient use of intervention application in pork trim for extended shelf-life and maintained product quality. Across all applications utilized in this study (warm skin pieces, chilled cheek pieces, whole hog carcasses, and pork trim), 3% lactic acid, PAA acidified with acetic acid, and PAA acidified with Titon™ were found to consistently be comparable in effectiveness against pathogenic and surrogate bacterial loads.

Keywords: pork, carcass, harvest safety, intervention, organic acids

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Scientific Abstract:

Introduction.

Salmonella is a leading cause of foodborne illness in the United States, and is one of the leading pathogens found on pork products. There is a need to find an effective control for *Salmonella* and other pathogens. Thus, the objectives of this project were (1) Determine the efficacy of various interventions on the reduction of pork head meat and carcass pieces inoculated with *Salmonella* in pathogen processing area. (2) Determine the efficacy of the reduction of surrogate organisms in a pork production facility to validate the reduction on pork carcasses. (3) Determine the impact of intervention treatments on the shelf life of pork trim.

Materials and Methods.

Phase 1: Fresh pork cheek meat and hot pork skin, was inoculated with a 5-strain cocktail of *Salmonella* strains. Interventions tested in this study included: 1)**Titon-**Sulfuric acid and sodium sulfate (pH 1.3), 2)**PAA-**Peracetic acid (350 ppm), 3)**LA-**Lactic acid (3%), 4)**CA-**Citric acid (1.3%), 5)**HBR-**Hypobromous acid (300 ppm), 6)**LAE-**Lauramide arginine ethyl ester (200 ppm), 7)**PAA+Acetic-**Peracetic acid (400 ppm) with 2% acetic acid, 8)**PAA+Titon™** Sulfuric acid and sodium sulfate (pH 1.3) combined with peracetic acid (350 ppm), 9)**Water**, and 10)**Control**-no treatment. A CHAD cabinet was used to apply individual treatments at ambient temperature. *Salmonella* on the pork pieces after inoculation and 24hrs after treatment was enumerated to determine the reduction from each treatment.

Phase 2: Pork carcasses in two different facilities with two different chill systems were utilized and carcasses were inoculated with non-pathogenic *Escherichia coli*. Carcasses in portion one were treated with the following: 1)**Titon™** , 2)**PAA**, 3)**LA**, 4)**CA,** 5)**HBR**, 6)**LAE**,7)**PAA+Acetic**, 8)**PAA+Titon™** . Carcasses in the second portion of phase II, in the large commercial facility with

an industrial carcass sprayer and blast chiller, were treated with the following treatments: 1) **control**, 2) **PAA + Acetic**, 3) **PAA + Titon™** , 4) **LA**. Phase II determined aerobic plate count and psychrotrophic bacteria reductions 24hrs after intervention and chilling.
Phase 3: Pork trimmings were divided into 50-lb batches for each individual treatment (n=4 batches). Treatments included, control (no intervention), PAA+Titon™ , PAA+Acetic, and LA. Shelf-life measurements taken included instrumental color (at 0min, 10min, and 20min after package opening), pH, proximate analysis, TBARS, raw product odor acceptability, aerobic plate count and psychrotrophic plate count bacterial enumeration.

Results

On warm pork skin, organic acid applications demonstrated pathogenic *Salmonella* reduction of 1.56 to 2.06 log CFU/cm^2. On pork cheek meat pathogenic *Salmonella* reduction at 24-hours after chilling ranged from 1.37 to 3.98 log CFU/cm^2. In the small-scale facility, with a traditional hot box cooler, intervention treatments showed a 1.74 to 4.91 log CFU/100cm^2 reduction of non-pathogenic *E. coli* on pork carcasses from inoculation to 22hrs post chilling. In the commercial pork facility, with the large sprayer cabinet and a blast chiller, significant reductions of 2.96 to 4.80 log CFU/100cm^2 reduction of *E. coli* was achieved ($p<0.05$). On uninoculated pork carcasses in the commercial pork facility with a blast chiller, aerobic plate counts of bacteria were reduced 2.14 to 3.58 log CFU/100cm^2 by intervention application. Odor acceptability was significantly impacted by day but not by treatment group ($p>0.05$) in the shelf-life study of ground pork. Ground pork color was also minimally impacted by treatment of pork trim.

Conclusions

Overall phase I demonstrated efficacy of selected interventions to address pathogenic *Salmonella* control during the harvest process, prior to pork carcass chilling. Phase II demonstrated effective reduction of *E. coli* inoculated carcasses in a small-scale traditional chilled pork harvest facility, in addition to a large-scale commercial pork facility with a blast chiller. Phase III demonstrated no significant negative organoleptic changes to pork trim when treated with selected organic acid interventions prior to grinding. Additionally, phase III highlighted an opportunity for further work to address the most efficient use of intervention application in pork trim for extended shelf-life and maintained product quality. Across all applications utilized in this study (warm skin pieces, cheek pieces, whole hog carcasses, and pork trim), the intervention treatments found to be most successful were 3% lactic acid, PAA acidified with acetic acid, and PAA acidified with Titon™.

Introduction:

There are on average 9.4 million cases of foodborne illness in the United States each year. *Salmonella* continues to be a leading cause of morbidity in the United States, accounting for 11% of the total annual foodborne illness cases (> 1 million) as well as 35% of hospitalizations and 28% of deaths related to foodborne disease (Scallan et al., 2011). Source attribution data show that the pair *Salmonella enterica*/pork ranks 13th among the most common food/pathogen combinations with an annual cost of illness of $218 million, nearly 68,000 illness cases, over 1,200 hospitalizations, and 25 deaths per year (Batz et al., 2012). Overall, foodborne diseases implicating contaminated pork products rank second among all food categories and cost over $1,900 million per year (Batz et al., 2012). More recent estimates indicate that *Salmonella* accounts for 24% of the total annual economic burden for the leading 15 foodborne pathogens in the U.S., the highest of all pathogens studied (Hoffmann et al., 2015).

Microbiological baselines studies conducted by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) have determined the most recent prevalence of *Salmonella* in market hogs to be 69.64% (1,365/1,960) at pre-evisceration and 2.70% (53/1,960) at post-chill, with an estimated national prevalence of 1.66% (UDSA-FSIS, 2011). An exploratory sampling program for pork products was initiated by USDA-FSIS in 2015 in which intact, non-intact, and comminuted pork products are being sampled and tested for *Salmonella* and other foodborne pathogens and indicators. However, these data are not yet available. An overall prevalence of *Salmonella* of 3.7% was reported by Schmidt et al. (2012) with samples collected after chilling at two large commercial pork processing plants in the U.S. Although the prevalence is low, it is important to keep in mind the large number of swine that are harvested each year in the U.S. and their potential contribution to foodborne diseases (Baer et al., 2013).

Salmonella contamination of fresh pork is affected by many factors, beginning on the farm and continuing through fabrication (Buncic and Sofos, 2012). Given that the risk of *Salmonella* contamination from pork carcasses to products cannot be eliminated given our current technological limitations, the use of physical, chemical, and even biological interventions has been evaluated to minimize the prevalence of *Salmonella* along the pork production chain and to reduce the risk of foodborne salmonellosis in the general population (Buncic and Sofos, 2012).

Typically, organic acids – particularly lactic acid at 2.0-2.5%– and water (ca. 82 °C or as steam) have been used as strategies decontamination of pork carcasses (Buncic and Sofos, 2012). However, the reduction in frequency and levels of contamination, as well as the effect on quality parameters, are not well established. Considering that pork and pork products are widely consumed in the U.S. and around the world, it is essential for producers, packers, and processors to be aware of decontamination strategies for the control of foodborne zoonoses potential transmitted via consumption of contaminated pork products (Baer et al., 2013).

It is generally recognized that in industrialized countries, processing procedures in place at swine abattoirs result in decreased prevalence of *Salmonella* as the carcasses move from harvest toward the cooler (O'Connor et al., 2012). However, the possibility of a breach in process control may lead to product with a frequency and/or concentration of microbial pathogens, of which *Salmonella* is of major concern. Recently, Totton et al. (2016) summarized change in *Salmonella* prevalence and/or quantity associated with pathogen reduction treatments (washes, sprays, steam) on pork carcasses or skin-on carcass parts in comparative designs (natural or artificial contamination). The results of the systematic review indicated that there was no strong evidence that any one

intervention protocol (acid temperature, acid concentration, water temperature) was clearly superior to others for *Salmonella* control (Totton et al., 2016).

Given the current limitations for the control of *Salmonella*, the search for alternative, cost-effective, decontamination strategies is active by evaluating interventions using technologies that are successful in other products such as poultry and beef. One such strategy is the use of an aqueous solution of sulfuric acid and sodium sulfate (SSS). The product is intended for use as a spray, wash, or dip at a pH range of 1.0 to 2.2. The product is considered Generally Recognized as Safe – GRAS by the U.S. Food and Drug Administration (notice GRN 000408). Per the USDA-FSIS, the mix is approved as an acidifier or surface antimicrobial in meat and poultry products (Directive 7120.1). The antimicrobial efficacy of the mix has been demonstrated by Scott et al. (2015) in whole chilled chicken wings inoculated with *Salmonella*. Immersion of samples for 10 or 20 s in SSS resulted in pathogen reductions of 0.8 to 0.9 and 1.1 to 1.2 log CFU/ml, respectively. The authors concluded that the blend applied at pH 1.1 for 20 s was an effective antimicrobial intervention to reduce *Salmonella* contamination on chicken wings (Scott et al., 2015). The antimicrobial efficacy of the mix against foodborne pathogens in other meat matrices has not been yet reported in the scientific literature.

Given the need for intervention technologies in the pork industry, we evaluated existing and novel interventions to address *Salmonella* on pork carcasses and in head meat under simulated industry conditions and using surrogates in a processing plant environment. We also evaluated shelf life and quality changes of the product.

Objectives:

1. Determine the efficacy of various interventions on the reduction of pork head meat and carcass pieces inoculated with *Salmonella* in pathogen processing area.
2. Determine the efficacy of the reduction of surrogate organisms in a pork production facility to validate the reduction on pork carcasses.
3. Determine the impact of intervention treatments on the shelf life of pork products collected from pork carcasses.

Materials & Methods:

Phase I. Overview:

Phase I served as a proof of concept challenging pork cheek meat and skin pieces inoculated with pathogenic *Salmonella* and challenged with organic acid interventions to determine effectiveness. Warm pork skin was inoculated with a 5-strain *Salmonella* cocktail prior to challenge with an organic acid treatment. Organic acid was applied using a CHAD spraying cabinet.

Phase I. Methodology

Sample Preparation

Pork Skins

Fresh pork skin was sourced from a commercial pork processing facility. Upon receiving the fresh product, the skins were trimmed to uniform squares to be an appropriate size for sampling (slightly larger than the 25 cm^2 swab template). Portioned pork skins were stored overnight (8-12hrs) in deep stainless-steel sheet pans in an incubator set to 37°C to simulate hot carcass temperatures. Skins were maintained warm (37 ±5°C) until inoculation and processing.

Fresh Cheek Meat

Fresh cheek meat was sourced from a commercial pork processing facility. Upon receiving the fresh product, the cheeks were portioned to the appropriate size for sampling (slightly larger than the 25 cm^2 swab template). Portioned meat was

stored overnight under refrigeration (4 ±3°C). Chilled temperature was maintained until product processing.

Preparation of Pathogen Cocktails

Five strains of *Salmonella* isolated from pork or associated with human illness were selected from the Texas Tech University Food Microbiology strain collection (Lubbock, Texas). For a previous internal Texas Tech food safety lab project, stock strains isolated (strains listed in Table 12) were subjected to a passage process to isolate serotypes with resistance to the antibiotic rifampicin and isolates were cataloged in the strain collection then kept in frozen storage for use on future projects (*8*). Frozen cultures of the five rifampicin resistant *Salmonella* strains were used to prepare a stock cocktail (9 $\log_{10}$ CFU/mL). For each individual serotype, 1 µL of the frozen stock culture was added to 100 mL of tryptic soy broth (TSB, Millipore, Darmstadt, Germany) and grown for 24 H at 37 °C. Cultures were then centrifuged at 10,000 rpm for 20 min (Eppendorf, Hamburg, Germany) and the supernatant was discarded, and the cell pellets were resuspended in sterile TSB. Equal volumes of the five *Salmonella* cultures were combined and mixed thoroughly to create a stock cocktail. To determine the concentration of the cocktail, serial dilutions were made in 9 mL buffered peptone water (BPW, Millipore, Darmstadt, Germany) and plated onto tryptic soy agar (TSA, Millipore, Darmstadt, Germany) modified with rifampicin solution (Rif, Biosynth, Itasca, IL, United States) to have a concentration of 100 mcg/mL of rifampicin within the agar solution, and incubated for 24 H at 37°C then counted to confirm that 9 $\log_{10}$ CFU/mL concentration was reached. To generate a bulk volume of the stock cocktail, 1 mL of the stock cocktail was transferred to 100 mL of tryptic soy broth (TSB, Millipore, Darmstadt, Germany). The bulk cocktail was then diluted to 7 $\log_{10}$ CFU/mL in BPW in the Texas Tech Pathogen Laboratory prior to inoculating pork samples.

Sample Inoculation

A BPW concentrate was prepared with 2 L of deionized water and 126.225g of buffered peptone water media to make a concentrate of 4.95 L, which would later be diluted with sterile water. Sterile water for diluting the BPW concentrate was prepared by autoclaving deionized water in jugs with spigots. For inoculation, 50 mL of bulk cocktail was diluted with one bottle of BPW concentrate and 2.95 L of sterile water in a deep stainless-steel pan to make a batch of inoculum (7 $\log_{10}$ CFU/mL).

The mixed inoculum was poured in a sterile stainless-steel pan for inoculation. Pieces (cheek meat and skins) were dip inoculated into the inoculum for 2 minutes to achieve a 5 $\log_{10}$ CFU/mL adhesion. After the inoculation period, the product was then placed on stainless steel trays lined with baking racks. Inoculated skin pieces were allowed to rest on the racks for 15 mins at room temperature (approximately 21° C) for cell attachment prior to applying spray intervention treatments. Fresh cheek meat samples were held at 4°C for 15 minutes during cell adhesion to maintain chilled temperatures.

Application of treatments

Treatments were applied using a Chad Sanitizing Cabinet (CHAD Equipment LLC., Olathe, KS., United Sates) that was custom made for the Texas Tech food safety pathogen laboratory. The machine runs at a rate of 1 foot per 2.5 seconds at 40 lb./in2, and 40 psi. In between treatments, equipment was cleaned and sanitized using procedures established for the pathogen laboratory proven to eliminate pathogens when present on multiple types of material.

Table 2. All treatments used by formal name, abbreviated name, preparation instructions, and concentration used

Treatment	Common name for reference throughout report	Preparation of Treatment	Concentration of Treatment
Water	Water	Standard tap water at ambient temperature (21° C) was placed into the treatment tank of the CHAD cabinet and applied at 40 psi.	n/a
Sulfuric acid and sodium sulfate	Titon™	Titon™ (Zoetis, Parsippany-Troy Hills, NJ, United States) was provided to Texas Tech by Zoetis. The Titon™ solution was prepared by mixing ~43 mL of concentrate per gallon of ambient temperature water.	A pH meter was used to confirm the desired 1.3 pH and was adjusted as needed.
Peracetic Acid	PAA	A peracetic acid concentrate, Actrol (Zoetis, Parsippany-Troy Hills, NJ, United States), was provided to Texas Tech by Zoetis. ~6.1 mL of the Actrol concentrate was used per gallon of water.	To verify mixing, a Peracetic Acid titration kit was used to determine desired 350 ppm and was adjusted as needed.
Peracetic Acid with 2% Acetic Acid Solution	PAA + Acetic	A peracetic acid concentrate, Actrol (Zoetis, Parsippany-Troy Hills, NJ, United States), was provided to Texas Tech by Zoetis. The Actrol solution was mixed with Acetic Acid (Fisher Scientific, Geel, Belgium) by mixing ~76 mL of Acetic Acid with 7 mL of Actrol per gallon of water.	A Peracetic Acid titration kit was used to determine desired 400 ppm and was adjusted as needed.
Peracetic Acid with Sulfuric acid and sodium sulfate	PAA + Titon™	The Titon™ solution was prepared by mixing ~43 mL of concentrate per gallon of ambient water. A peracetic acid concentrate, Actrol (Zoetis, Parsippany-Troy Hills, NJ, United States) of ~7 mL of the Actrol concentrate was added to the Titon™ mixture.	A pH meter was used to confirm the desired 1.3 pH and was adjusted as needed. To verify mixing, a Peracetic Acid titration kit was used to determine desired 350 ppm and was adjusted as needed.
Lactic Acid 3%	LA	An 88% lactic acid concentrate solution (Birko Corp, Henderson, CO, United States) was diluted by measuring 50 mL of concentrate per gallon of water to reach desired 3% concentration of lactic acid.	To verify mixing, a Lactic Acid Test Kit (ChemWorld, Taylor, MI, United States) was used to determine desired percentage (3%) of Lactic Acid had been reached and was adjusted as needed.

Hypobromous Acid	HBR	A small-scale micro-feeder device, BoviBrom (Passport Food Safety Solutions, West Des Moines, IA, United States), that dispersed hypobromous acid was loaned to Texas Tech for the duration of this project. The micro-feeder was attached to a water source and allowed to run for up to 2 H, flushing high concentrated amounts of the BoviBrom product from the system. Water inputs and BoviBrom output settings were adjusted per the manufacturers guidelines to reach desired 300ppm of hypobromous acid.	To check that the desired ppm was reached a Hanna Portable Photometer (Hanna Instruments, Woonsocket, RI, United States) measured the solution, per Passport suggested calibrations and calculations. When the machine began to output 300 ppm, the BoviBrom Solution was collected in clean jugs to be transported to the ICFIE Pathogen Laboratory.
Lauramide arginine ethyl ester	LAE	A sample of a lauric agrinate concentrate, CytoGuard LA (A&B Ingredients, Fairfield, NJ, United States) was provided to Texas Tech.	Per manufacturer directions and calculation formulas, the 4 mL of concentrate was mixed with 4 L of water to reach the desired 200 ppm.
Citric Acid	CA	Citric Acid (Fisher Scientific, Geel, Belgium) was prepared by mixing ~20 g of Citric Acid per gallon of water.	To verify mixing, a Citric Acid Test Kit (ChemWorld, Taylor, MI, United States) was used to confirm that 1.3% had been reached.

Enumeration of Pathogens

Pork Skin and cheek meat

Swabs pre-moistened with buffered peptone water (BPW) (10 ml) were used to sample a 25 cm^2 area of the product and samples were collected pre-treatment (1 swab per treatment group), 5 minutes post treatment (3 swabs per treatment group) and 24 H post treatment (3 swabs per treatment group). After sample collection, swabs were transported to the TTU Food Microbiology Laboratory for microbial analysis. The sponges were processed in a stomacher (Model 400 circulator, Seward, West Sussex, United Kingdom) at 230 rpm for 2 minutes. Using 9mL BPW tubes, serial dilutions were made, and then spread plated on TSA+rif plates using 100µL aliquots. Plates were incubated for 18 to 24 H at 37°C. After incubation period, plates were counted and recorded as CFU/cm^2. For samples below countable range, a BAX rapid detection was run to confirm the presence or absence of *Salmonella*.

Phase II. Overview:

Phase II was conducted in two-parts, using two different facilities with differing chill methods. A five-strain cocktail of non-pathogenic *Escherichia coli* was inoculated on pork carcasses prior to intervention application and chilling to determine effectiveness. In the first facility, over two weeks, four replications were completed using the 8 treatments from Phase I. The second portion of phase II was conducted across three days over separate weeks in a commercial abattoir utilizing three of the most effective treatments from the first portion of Phase II.

Sample Preparation

The first portion of phase II was conducted at the Texas Tech University Meat Laboratory over two different days. Split carcasses (n=8) were railed off for inclusion in the study after final inspection, but prior to entering the hot box.

The second portion of phase II was conducted in a commercial pork abattoir located in Guymon, OK. Seventeen carcasses were railed off immediately before the final wash cabinet in two replications. Each replication was conducted on different days of production. After the carcasses were inoculated the carcasses were put back on the line to be treated with the designated treatment application. Carcasses were kept at the end of the production line to ensure they remained separated from the normal production carcasses.

Preparation of Pathogen Cocktails

Non-pathogenic ATCC surrogate strains of *Escherichia coli* (BAA 1427, 1428, 1429, 1430 and 1431) were utilized during an in-plant challenge trial to assess the efficacy of treatments on the reduction of surrogates that mimic *Salmonella* behavior. The surrogate ATCC organisms were independently propagated in a BSL-I laboratory on the Texas Tech University campus to formulate cocktail inoculums for the in-plant challenge trial to target an estimated 10^5 CFU/cm^2 on the carcass surfaces. Individual ATCC surrogate frozen isolates were inoculated into individual, labeled, 10 ml tubes of sterile Buffered Peptone Water (BPW), for incubation 18-24 hours at 37 °C. Individual tubes were used to inoculate 5 sterile 1 L bottles of BPW for incubation 12 ± 2 hours at 37 °C. Each surrogate bottle was screened for *Salmonella, E. coli* O157:H7 and *Listeria monocytogenes* using BAX real-time PCR prior to use in the plant.

Sample Inoculation

Texas Tech Meat Lab

Background swabs were collected on each day to represent carcass surface prior to inoculation. Each carcass was sprayed with the *E. coli* surrogate cocktail, on the ham and the shoulder of the carcass side, and allowed 30 minutes for cell attachment, while hanging at ambient temperature. After attachment a 100cm^2 area on the shoulder and ham were swabbed using a sterile swab pre-moistened with 25 ml BPW. After intervention application, carcasses were allowed to sit in the hot box holding cooler (4± 2 °C) for one hour before a 1hr intervention effectiveness swab was taken at each sampling site (shoulder and ham). After 24hrs held in the hot box holding cooler a 24hr swab was taken from each location (shoulder and ham).

Commercial Plant

Prior to inoculation a 100cm^2 area on the right shoulder of each pork carcasses was swabbed using a sterile swab pre-moistened with 25 ml BPW to determine the aerobic plate count. Carcasses were then inoculated on the left shoulder with the *E. coli* surrogate cocktail, and attachment allowed for 30-40 minutes at ambient temperature.

Application of treatments

Texas Tech Meat Lab

Interventions, as assigned, were sprayed onto the carcass surface for 20 seconds, using a handheld sprayer (Chapin 1-Gallon Plastic Tank Sprayer) located 6-10 inches from the carcass surface. Treatments were prepared as described in Table 2.
Treatments utilized include the following:

1. ***Control***
2. ***Water***
3. ***Titon™***
4. ***PAA + Acetic***
5. **PAA + Titon™**
6. ***LA***
7. ***HBR***
8. ***LAE***

Commercial Plant

For intervention application carcasses were put back on the rail and traveled through a commercial spray cabinet. The spray cabinet consisted of 4 spray bars (6' length) constructed of ¾" PVC (schedule 80) pipe, two spray bars at the entrance of the cabinet and 2 spray bars at the exit with five FloodJet spray nozzles per bar separated by 14"; 0.2 gpm flow rate nozzles (Spraying Systems #1/8K-PVC2).

Dosatron Pump set up: (14 gpm water driven flow proportional pumps).

- 0.5-5% Dosatron pump used for acids (Dosatron # D14MZ5AFII)
- 0.03-0.3% Dosatron pump for PAA (Dosatron # D14MZ3000VFIIK)

Pump mixing order was acid then PAA set up in series followed by a mixing chamber then to spray bars. Flow rate was measured with a 10 gpm rotameter set up on the incoming water before the pumps.

Treatments utilized include the following:

1. **Lactic Acid 3% (LA):**
   Flow at 5gpm, pressure at 25 psi. Chemical measurements: 3.0% LA (measured with a lactic acid test kit), 2.0 pH (all pH measurements were taken with a

Mettler Toledo model # SG2 SevenGo portable pH meter; calibrated with a pH 2 buffer each day before use), water temperature of 18°C.

2. **PAA + Titon™:**
   Flow at 5gpm, pressure at 25 psi. Acid pump set at 1.2%, PAA pump set at 0.18%. Chemical measurements: 405ppm PAA (measured with a PAA test kit), 1.2 pH, water temperature of 18°C.

3. **PAA + Acetic:**
   Flow at 5gpm, Pressure at 25 psi. Acid pump set at 2.4% to get a true 2.0% Acetic Acid concentration in water, PAA pump set at 0.18%. Chemical measurements: 405ppm PAA (measured with a PAA test kit), 2.8 pH, water temp of 18°C.

Sample collection and processing

Sampling time-point were as follows: 0h (after 30-minute *E. coli* attachment,) 1h (immediately after intervention application, but prior to encountering any chilling), and 15-22hr (after intervention application and traditional industry chilling period). In the small pork harvest facility carcasses were railed off for inclusion in the study. A pre-determined area of 100 cm^2 on both the ham and shoulder area of both sides of each carcass were swabbed. In the large commercial facility, at each time-point, *E. coli* swabs were taken from the left shoulder, and APC swabs taken from the right shoulder. All swab areas were marked with 100 cm^2 stamped area to ensure same area was not sampled repeatedly (see appendix 1).

Microbiological enumeration was completed in the Experimental Sciences building at Texas Tech University. Swabs were stomached 2 minutes at 230 RPM prior to dilutions performed with 9 ml BPW tubes. For *E. coli* enumeration, spiral plating was performed on MacConkey Agar plates with a thin layer of Tryptic Soy Agar (TSA) for recovery of injured cells. Aerobic plate counts were obtained from spiral plating onto TSA plates. All samples were plated in duplicate. After incubation 18 hours at 37 °C plates were read using the Q-Count Spiral plate software system. Duplicate plates were averaged from most appropriate dilution and transformed to $\log_{10}$ for statistical analysis.

Phase III. Overview

Phase III existed to determine effect of organic acid application applied to pork trim pieces on the shelf-life of ground pork. Shelf-life measurements taken included instrumental color (at 0min, 10min, and 20min after package opening), pH, proximate analysis, TBARS, raw product odor acceptability, aerobic plate count bacterial enumeration, and psychrotrophic plate count bacterial enumeration. Phase III consisted of four treatments used.

Sample Preparation

Pork trimmings was received in 50-lb cases from the Texas Tech meat laboratory under refrigerated conditions. Pork trimmings were divided into 50-lb batches for each individual treatment (n=4 batches). One batch was held as the control, and the remaining lots were treated with the designated intervention treatments, see Table 3. After application of each designated treatment, trimmings were ground (coarse and then fine ground) and packaged into 454-g vacuum packaged rollstock packaging. Each package was then stored in dark storage for 28 days at 2-4°C. Once each package had reached their designated aging period, packages were removed from storage and sampled.

Table 3. Number of packages from each treatment assigned to each sampling day for phase III shelf-life portion

Treatment	lb	0-d	7-d	14-d	21-d	28-d
Control	50	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb
Lactic Acid 3%	50	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb
PAA+Titon™	50	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb
PAA+Acetic	50	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb	n=8; 1-lb

Application of treatments
Interventions, were sprayed onto the trim surface prior to grinding for 20 seconds, using a handheld sprayer (Chapin 1-Gallon Plastic Tank Sprayer) ensuring to cover the entire trim surface. Treatments were prepared as described in Phase I (Table 2). Treatments utilized:

1. *Control – no organic acid application*
2. *Lactic Acid 3%*
3. *PAA + Titon™*
4. *PAA + Acetic*

Instrumental Color
Surface color was measured on the surface of each 1-pound ground pork sample, in three different locations using a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates, Reston, VA, USA). Color measurements specially L*, a*, and b* were taken immediately after the package was opened (time 0), then again at 15 min, and 30 min to measure color changes during bloom.

pH
Three 10 g samples taken from the composite sample per treatment (n=3 per treatment on each sampling day) were blended with 100 ml of deionized water and mixed for 30 s, the pH values were obtained by using a bench top pH meter.

Proximate Analysis
Samples for compositional analysis (protein, moisture, and fat) were obtained after bloom measurements. Analysis was conducted using an AOAC-approved (Official Method 2007.04) near-infrared spectrophotometer (FOSS Food Scan™). Compositional values are reported on a percent (%) basis.

TBARS
Approximately 100 g of sample from each individual treatment after each aging period was powdered using liquid nitrogen to ensure uniformity of sample analyzed. Lipid oxidation by-products, a measurement of oxidative rancidity, using a modified thiobarbituric acid (TBA) reactive substances (TBARS) originally developed by Buege & Aust (1978), with modifications described in Martin (2014), 4.0 mL of a 15% trichloroacetic acid (TCA) and 20 mM TBA stock solution and 100 µL of 10% butylated hydroxyanisole solution were added to 2 mL of blended and centrifuged (3,000 rpm for

10 minutes) samples. Samples were then heated (100°C water bath for 15 minutes), cooled (ice/water bath for 15 minutes), and centrifuged (3,000 rpm for 10 minutes), prior to spectrophotometric analysis. Sample absorbance was measured twice at 531 nm using a spectrophotometer (Genesys 20, ThermoScientific, Inc.).

Odor Panels

Untrained panelists (n= 6-8) evaluated the odor of a sample removed from their packaging at each sampling interval. Briefly, 60-g meat samples were allotted to capped glass jars and kept under refrigerated conditions until a 15-minute acclimation period directly before evaluation by panelists. Jars were coded with a three-digit code and presented to individual panelists in isolated booths under red lights. Each panelist (n= 6-8) evaluated samples by sniffing the container headspace and scoring samples for overall odor acceptability and intensity of four off odor characteristics on a 5-point scale (1= no off-odor, 5= extreme off odor).

Off odor attributes to be evaluated:

- Acid
    - Anchor: sour cream-5
- Oxidized
    - Anchor: Microwaved vegetable oil – 5
- Sweaty
    - Anchor: Distilled water with Cat food (75:25 ratio) – 4/5
- Sour/spoiled
    - Anchor: Buttermilk 5

Enumeration of Pathogens

Microbiological enumeration was completed in the Experimental Sciences building at Texas Tech University. From each sample (n=8) a 25 g sample was transferred into a filter bag, and 225 ml of BPW was added. Samples were placed on an orbital mixer for 10 min at 230 rpm prior to dilutions in 9 ml BPW tubes. Aerobic plate counts (APC) and psychrotrophic bacteria were obtained by spiral plating on plate count agar (PCA). All samples were plated in duplicate. PCA were incubated for 18 hours at 37°C and psychrotorophic bacteria were incubated for 7 days at 4°C. Plates were read using the Q-Count Spiral plate software system. Duplicate plates were averaged from most appropriate dilution and transformed to $\log_{10}$ for statistical analysis.

Statistical Analysis

All data were analyzed using SAS (SAS Inst. Inc., Version 9.4, Cary, NC). All bacterial enumeration was converted into $\log_{10}$ for statistical analysis relative to CFU/g or CFU/ cm^2. The data were set up in a randomized complete block design and were analyzed using a student's T-test by the way of PROC MIXED procedure in SAS (SAS Inst. Inc., Version 9.4, Cary, NC). In this analysis, treatment served as a fixed effect and replicate as the variable effect. This analysis was used to determine the least square means for each of the treatments. Differences between least squared means were determined using pairwise comparisons (pdiff/LSD method) and these were compared to determine significant differences using a P-value of 0.05. Odor acceptability was determined using a PROC FREQ to obtain frequency of acceptance across treatment and ground pork age.

Results:

PHASE I. RESULTS:

Table 4. Least squares means for warm pork skin pieces inoculated with pathogenic *Salmonella* challenged with various chemical carcass interventions during phase I. Results reported as log CFU/cm^2

	Org	TRT	0 post attach (Log_{10})	1 post intervention (Log_{10})	24hr post chill (Log_{10})	Log_{10} Reduction (0hr to 24hr)
SKIN	Sal	Control	5.98 ^{ab}	6.63 ^{a}	5.29 ^{b}	0.69
		Water	5.93	6.12	5.31	0.62
		PAA	6.75	6.24	5.18	1.56
		PAA + Acetic	6.69	5.97	4.88	1.81
		Titon™	6.97	6.29	5.16	1.81
		PAA + Titon™	6.32 ^{a}	6.01 ^{a}	4.59 ^{b}	1.73
		LA	6.31	5.90	4.82	1.49
		LAE	7.08	5.76	5.51	1.56
		CA	6.92	5.52	5.41	1.51
		HBR	7.04 ^{a}	5.38 ^{ab}	4.98 ^{b}	2.06

^{abc} Different superscripts in the same row indicate significant differences (p<0.001 for all products). For each treatment at each sampling time

Table 5. Least squares means for pork cheek pieces inoculated with pathogenic *Salmonella* and challenged to various carcass chemical interventions during phase I. Results reported as log CFU/cm^2

	Org	TRT	0 post attach (Log_{10})	1 post intervention (Log_{10})	24hr post chill (Log_{10})	Log_{10} Reduction (0hr to 24hr)
CHEEK	Sal	Control	5.21 ^{a}	5.40 ^{a}	4.01 ^{b}	1.20
		Water	5.32 ^{a}	4.9 ^{a}	3.26 ^{b}	2.06
		PAA	5.41 ^{a}	4.80 ^{a}	1.99 ^{b}	3.43
		PAA+ Acetic	3.95	4.09	2.57	1.37
		Titon™	5.04 ^{a}	4.62 ^{a}	2.21 ^{b}	2.83
		PAA + Titon™	5.55 ^{a}	3.83 ^{ab}	1.56 ^{b}	3.98
		LA	5.16 ^{a}	4.14 ^{ab}	2.10 ^{b}	3.06
		LAE	5.16 ^{a}	4.62 ^{a}	3.46 ^{b}	1.71
		CA	5.04 ^{a}	5.02 ^{a}	3.39 ^{b}	1.64
		HBR	5.15^{a}	4.83 ^{a}	3.45 ^{b}	1.69

^{abc} Different superscripts in the same row indicate significant differences (p<0.001 for all products). For each treatment at each sampling time

PHASE II. Part 1 RESULTS:

Table 6. Least squares means of *E. coli* log_{10} CFU/100 cm^2 of pork carcasses inoculated with *E. coli* surrogate in small pork facility.

Org	TRT	0hr (Log_{10})	1hr (Log_{10})	22hr (Log_{10})	Log_{10} Reduction (0hr to 24hr)
EC	PAA + Acetic	6.86[a]	4.74[a]	1.95[b]	4.91
	LA	6.74[a]	4.54[b]	2.35[c]	4.39
	PAA + Titon™	6.77[a]	5.14[b]	2.55[c]	4.22
	Titon™	6.70[a]	5.20[b]	3.78[c]	2.92
	HBR	6.58[a]	5.38[b]	3.75[c]	2.83
	Control	6.91[a]	6.28[a]	4.71[b]	2.20
	Water	6.99[a]	5.83[b]	5.03[b]	1.96
	LAE	6.45[a]	5.88[ab]	4.71[b]	1.74

[abc] Different superscripts in the same row indicate significant differences ($p<0.001$ for all products). For each treatment at each sampling time n=8. There were 8 carcasses, over two days, so total of 16 carcasses/32 sides. Each side treated as individual treated sample.

PHASE II. Part 2 RESULTS:

E. coli surrogate cocktail mean was 8.80 (sd=0.12) log_{10} CFU/50 cm^2 across all six sampling events.

Table 7. Least squares means of *E. coli* log_{10} CFU/100 cm^2 of pork carcasses inoculated with *E. coli* surrogate in large commercial pork facility

Org	TRT	0hr (Log_{10})	1hr (Log_{10})	22hr (Log_{10})	Log_{10} Reduction (0hr to 22hr)
EC	LA	6.53[a]	5.37[b]	3.56[c]	2.96
	PAA + Acetic	7.26[a]	4.55[b]	2.46[c]	4.80
	PAA + Titon™	6.80[a]	3.93[b]	3.75[b]	3.06

[ab]Different superscripts in the same row indicate significant differences ($p\leq0.001$ for all products). For each treatment at each sampling time n=32.

Table 8. Least squares means of APC (non-innoculated, generic plate aerobic bacterial counts) log_{10} CFU/100 cm^2 of pork carcasses in commercial pork facility

Org	TRT	0hr (Log_{10})	1hr (Log_{10})	22hr (Log_{10})	Log_{10} Reduction (0hr to 22hr)
APC	LA	4.74[a]	2.43[b]	1.15[c]	3.58
	PAA + Acetic	4.42[a]	1.57[b]	1.44[b c]	2.98
	PAA +Titon™	3.96[a]	2.05[b]	1.82[b]	2.14

[ab]Different superscripts in the same row indicate significant differences ($p\leq0.001$ for all products). For each treatment at each sampling time n=32.

PHASE III. RESULTS:

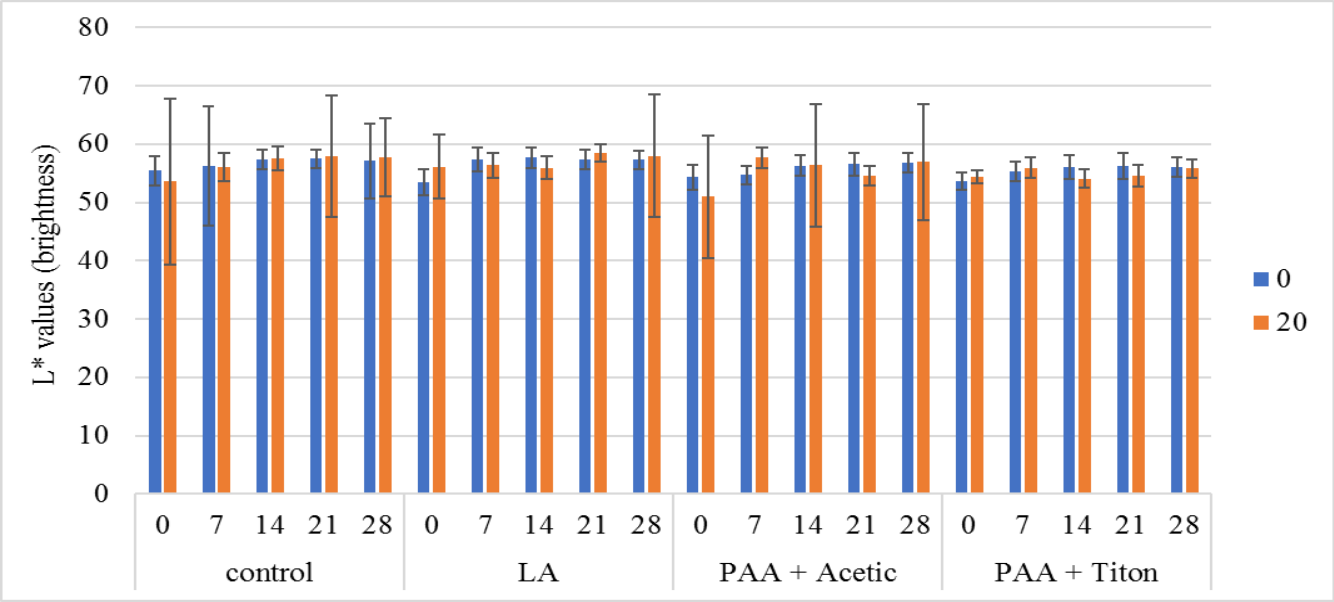


Figure 1. Mean L* values (brightness) from pork packages across aging days at 0min and 20min after package opening. Error bars indicate standard deviation.

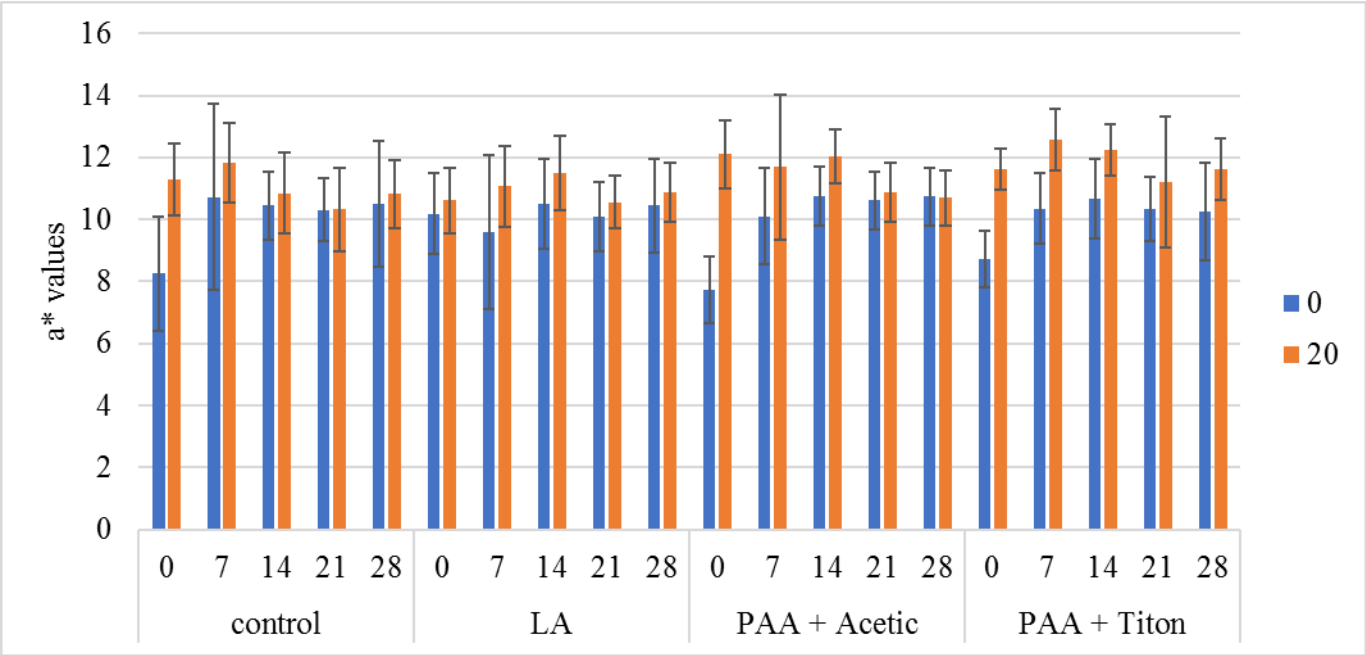


Figure 2. Mean a* values (redness) from ground pork across aging days at 0min and 20min. Error bars indicate standard deviation.

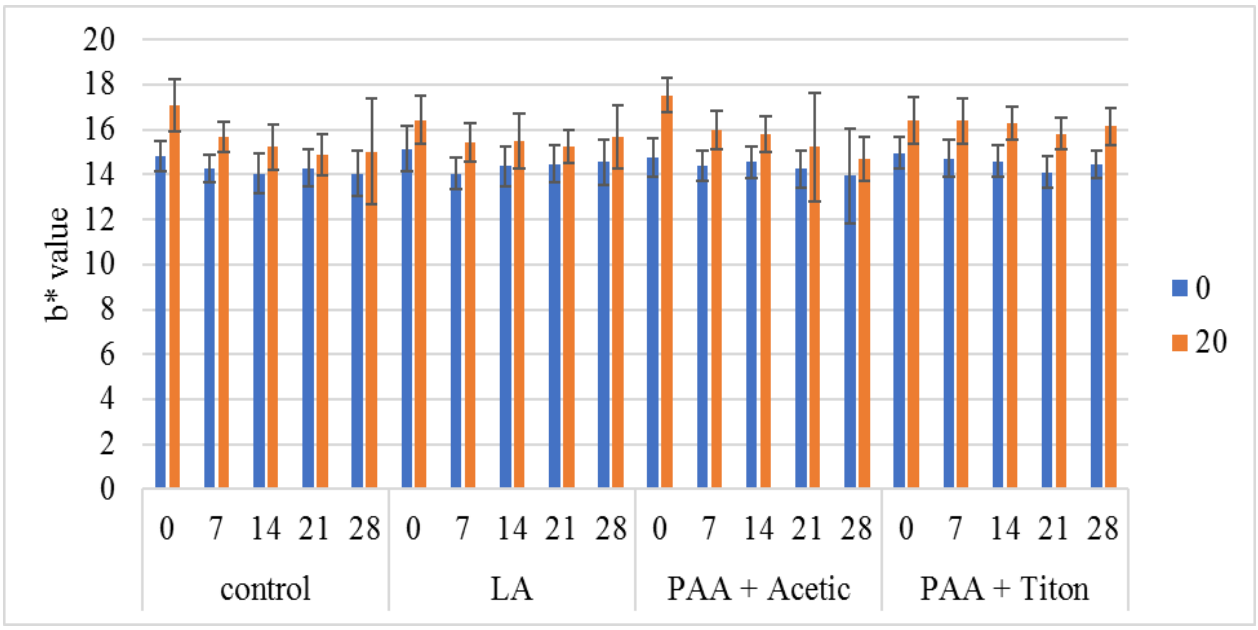


Figure 3. Mean b* values (yellowness) from ground pork across aging days at 0min and 20min. Error bars indicate standard deviation.

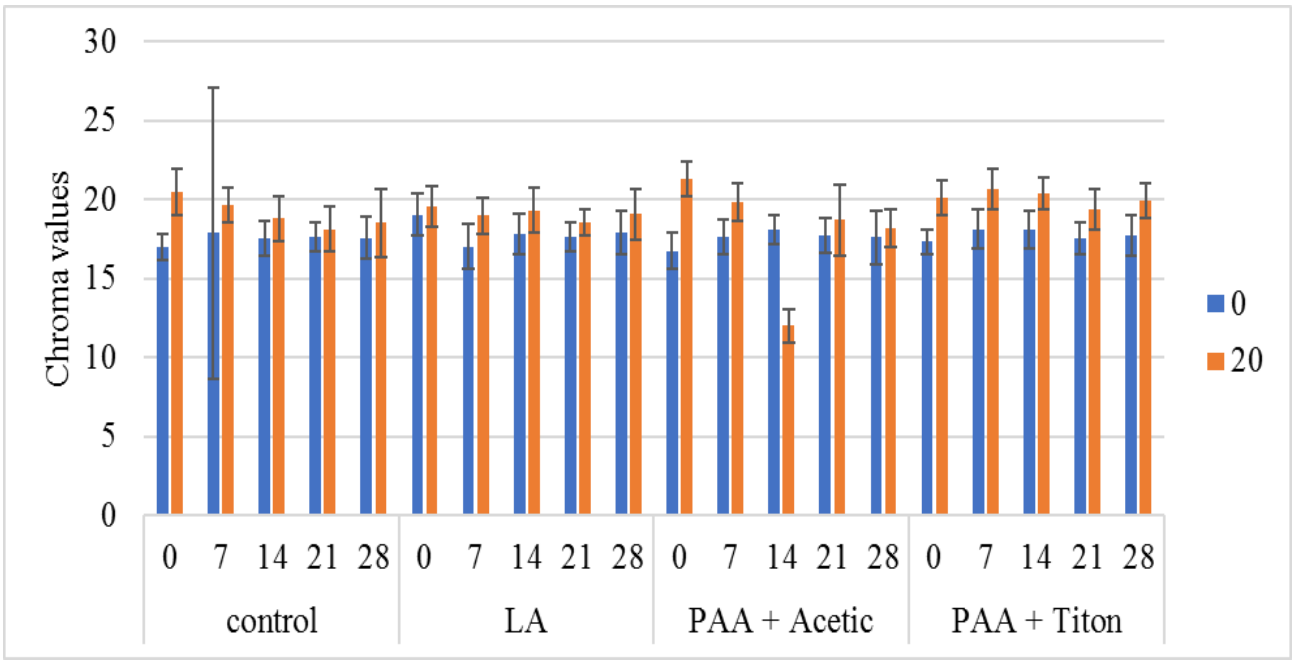


Figure 4. Mean chroma values from ground pork across aging days at 0min and 20min after package opening. Error bars indicate standard deviation.

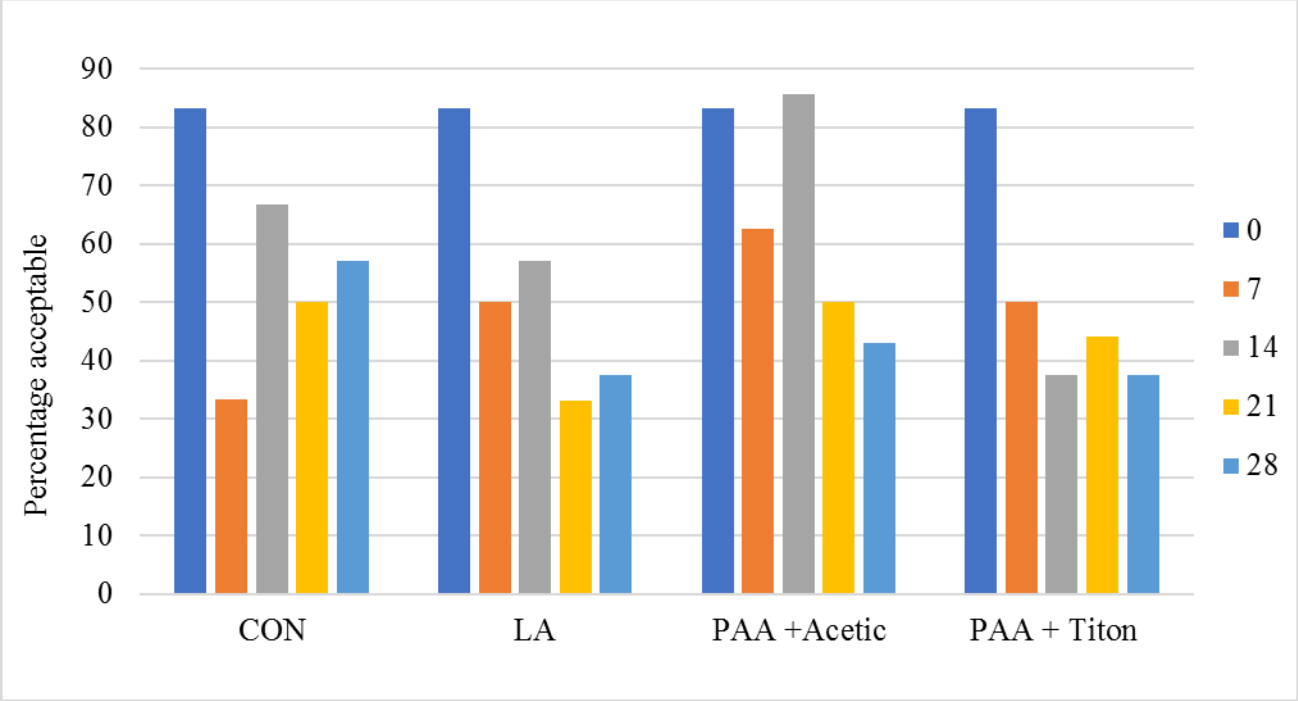


Figure 5. Acceptability of ground pork by percentage of panelists scoring product as acceptable pork odor (n=7-8 panelists on each sampling day)

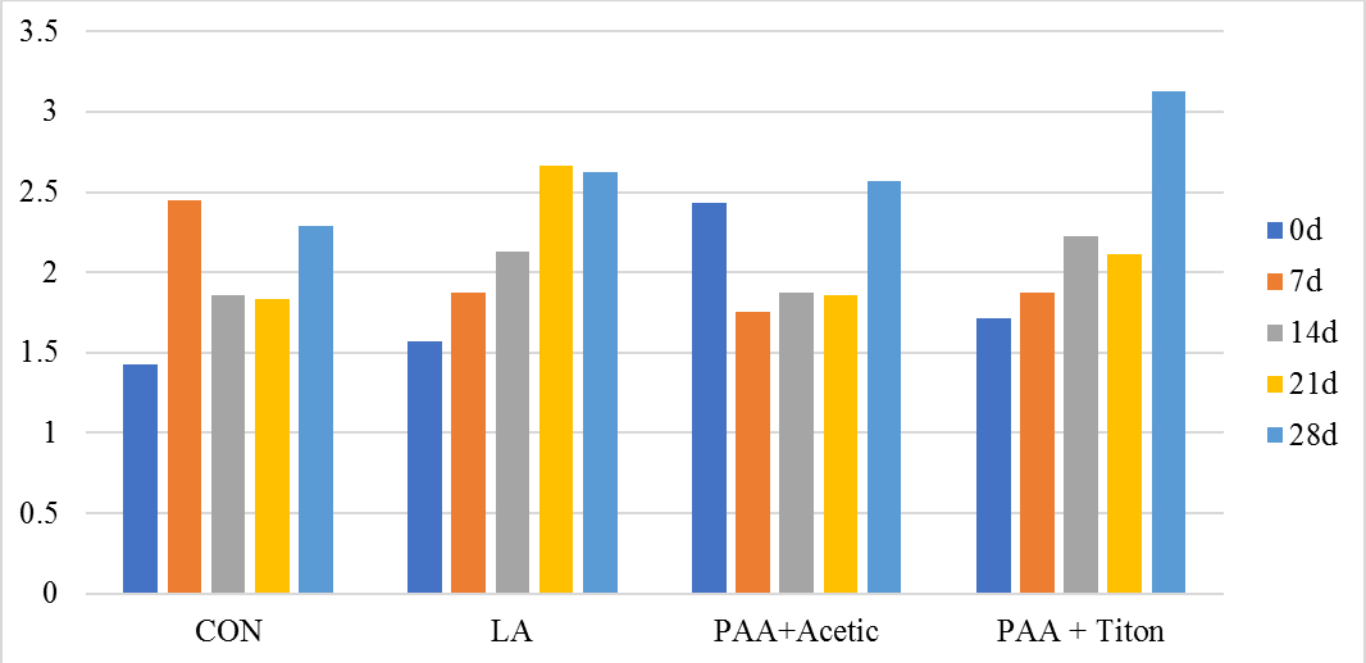


Figure 6. Mean scoring of acidic off-odor in ground pork by treatment and sampling day from 1 (no acidic off-odor) to 5 (strong acidic off-odor)

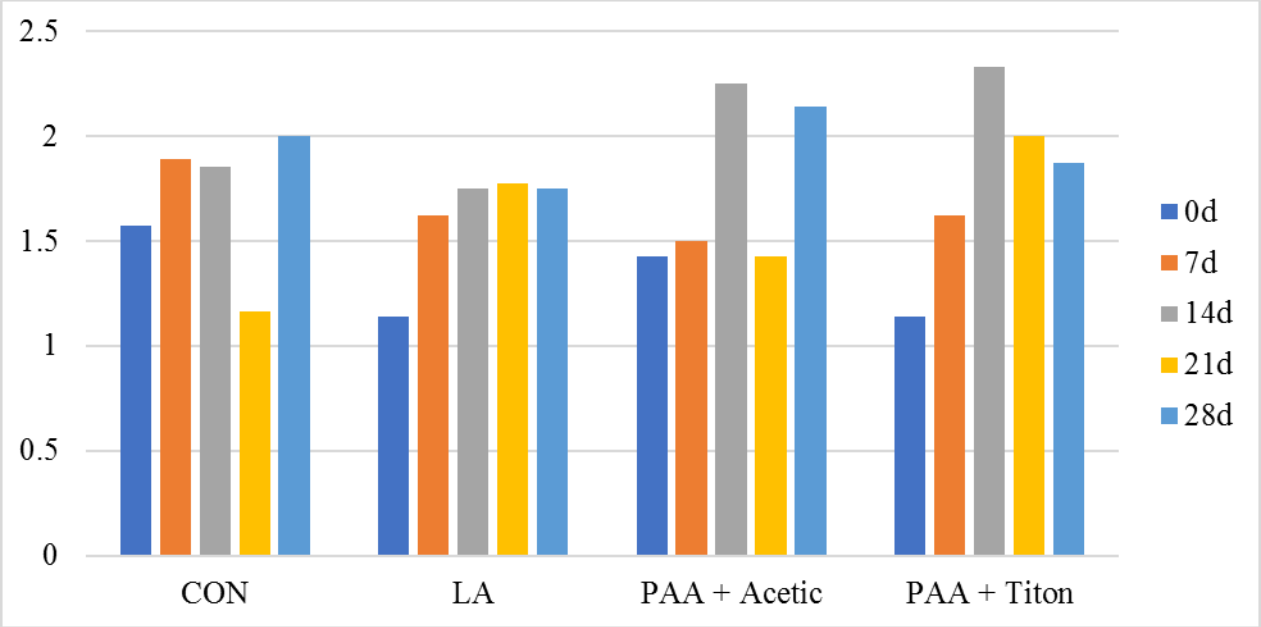


Figure 7. Mean scoring of oxidized off-odor in ground pork by treatment and sampling day from 1 (no oxidized off-odor) to 5 (strong oxidized off-odor)

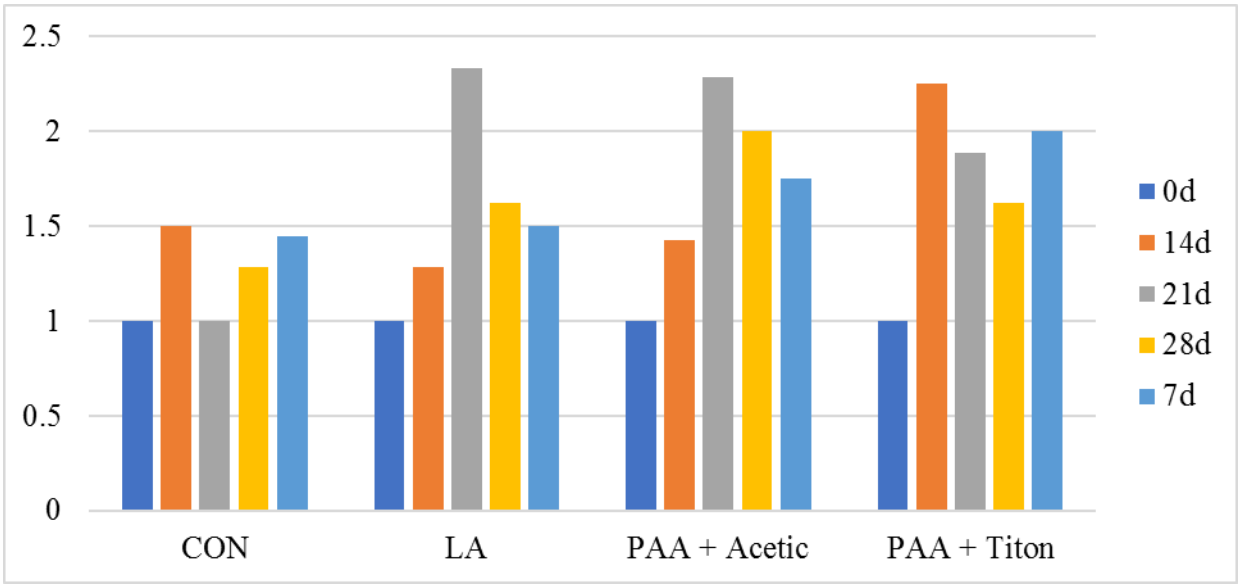


Figure 8. Mean scoring of sweaty off-odor in ground pork by treatment and sampling day from 1 (no sweaty off-odor) to 5 (strong sweaty off-odor)

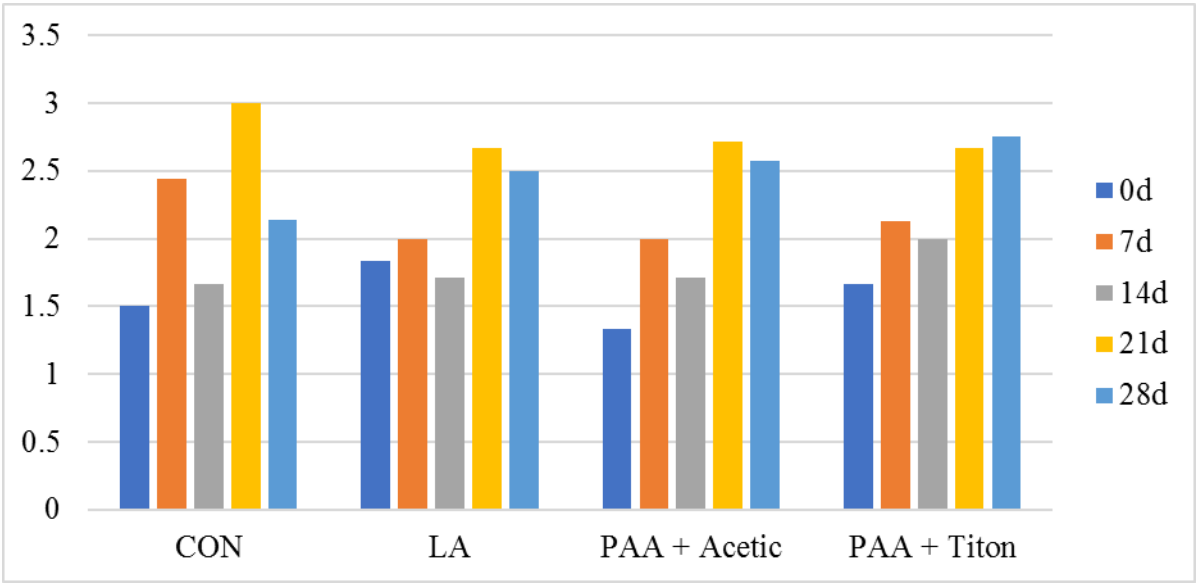


Figure 9. Mean scoring of sour off-odor in ground pork by treatment and sampling day from 1 (no sour off-odor) to 5 (strong sour off-odor)

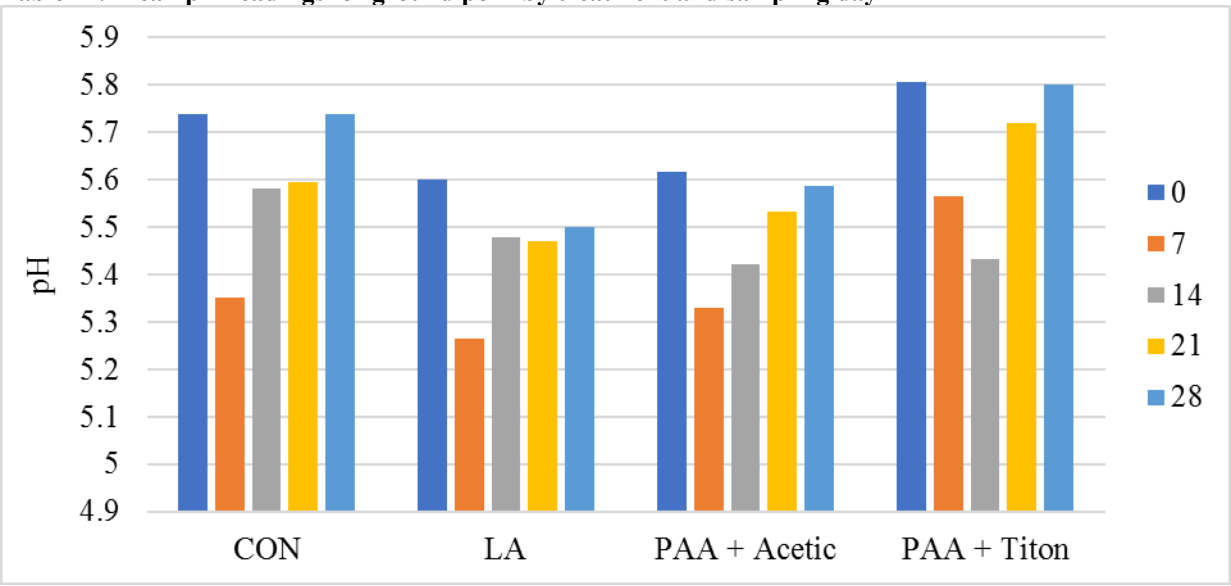
Table 9. Least Square means aerobic plate count (APC) enumeration by treatment and sampling day (Log_{10} CFU/g)

	0d	7d	14d	21d	28d
CON	6.3 [a]	7.2 [b]	6.8 [b]	6.8 [b]	6.7 [ab]
LA	7.0 [a]	7.2 [a]	6.8 [a]	6.9 [a]	6.7 [a]
PAA + Acetic	5.7 [a]	7.4 [b]	6.7 [b c]	6.7 [b]	7.0 [b d]
PAA+ Titon™	5.8 [a]	7.2 [b]	6.9 [b]	7.0 [b]	6.5 [c]
[abcd] Different letters across each row indicate significance ($p< 0.05$)					

Table 10. Least square means for psychrotrophic bacterial enumeration by treatment and sampling day (Log10 CFU/g)

	0d	7d	14d	21d	28d
CON	6.6 [a]	8.2 [a]	9.9 [b]	7.9 [a]	7.7 [a]
LA	6.1 [a]	8.2 [bc]	7.0 [ac]	8.0 [bc]	7.5 [ac]
PAA + Acetic	8.4	8.4	7.2	7.9	7.7
PAA+ Titon™	8.6	8.3	7.0	8.1	7.5
[abc] Different letters across each row indicate significance ($p< 0.05$); n=8 packages/trt on each sampling day.					

Table 11. Mean pH readings for ground pork by treatment and sampling day



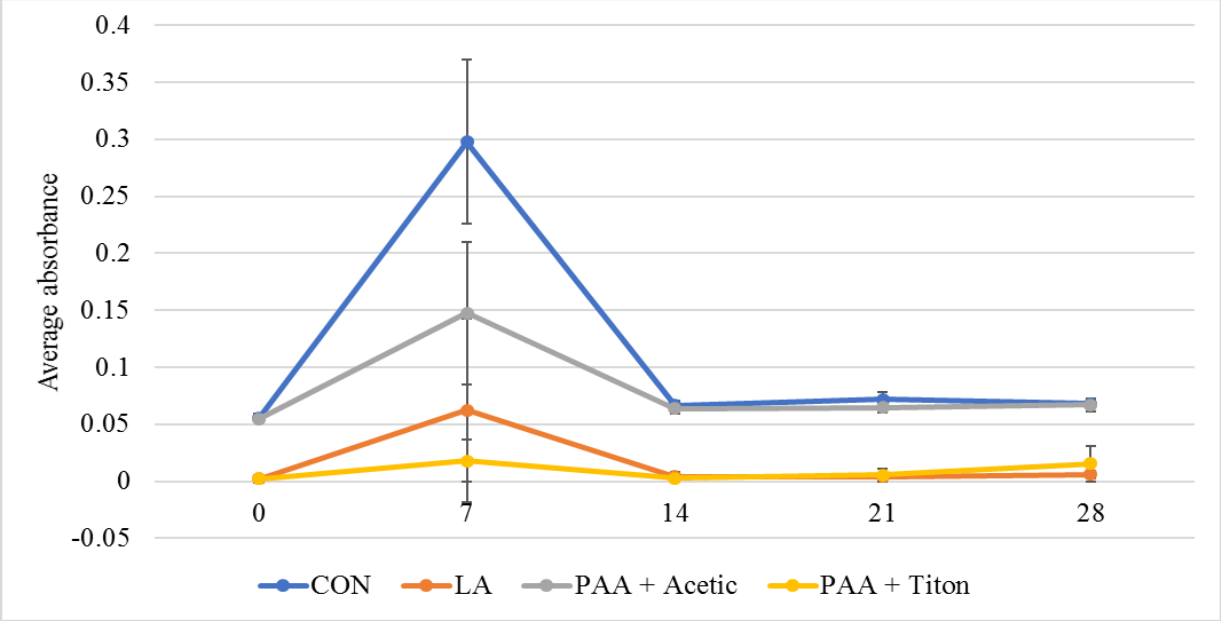


Figure 10 Average absorbance from TBARS for indication of lipid oxidation in ground pork by treatment across sampling days

Discussion:

Explain your research results and include a summary of the results that is of immediate or future benefit to pork producers.

This project provided some valuable information regarding the effectiveness of implementing organic acid applications in commercial pork processing facilities in order to reduce microbial growth, control pathogens of concern, and potential extend pork trim shelf-life. Phase one served as a proof of concept to demonstrate effectiveness on warm pork skin, simulation of intervention application during the pork harvest process, prior to chilling. On warm pork skin, organic acid applications demonstrated 24-hour reduction of 1.56 to 2.06 log CFU/cm^2 of pathogenic *Salmonella* (Table 4). On pork cheek meat, a product with potential for contamination during the harvest process, pathogenic *Salmonella* reduction at 24-hours after chilling ranged from 1.37 to

3.98 log CFU/cm^2 (Table 5). Both these studies provide evidence of effective organic acid interventions that could be utilized in the pork harvest process, prior to chilling, as a method of reducing and/or controlling pathogenic *Salmonella.*

During phase II the most effective organic acid interventions were applied in a commercial pork facility, using a pre-existing spray cabinet, to demonstrate effectiveness of these interventions if applied after final trim on the harvest floor, and before product chilling. With this portion of the study a non-pathogenic *E. coli* surrogate cocktail was used to mimic high contamination in a commercial facility during normal operating procedures. In the small-scale facility, with a traditional hot box cooler, intervention application showed a 1.74 to 4.91 log CFU/100cm^2 reduction of *E. coli* on pork carcasses from inoculation to 22hrs post chilling (Table 6). In the commercial pork facility, with the large sprayer cabinet and a blast chiller, a 2.96 to 4.80 log CFU/100cm^2 reduction of *E. coli* was achieved (Table 7). On uninoculated pork carcasses in the commercial pork facility with a blast chiller, aerobic plate counts of bacteria were reduced 2.14 to 3.58 log CFU/100cm^2 by intervention application (Table 8). The work done in phase II provides valuable information for the effectiveness of selected interventions if implemented in the pork harvest process prior to chilling, for reduction of *E. coli* and aerobic bacteria.

Phase III provided some valuable information regarding the potential for implementation of organic acid application on pork trim to maintain safety and shelf-life stability of ground pork. While the information gathered from this portion of the study is promising, further work needs to be done in the future to identify the most effective application strategy for lengthening shelf-life and reducing microbial load.

In phase III pork color was not negatively impacted by organic acid application on pork trim prior to grinding. Overall L* values, indication of brightness, significantly differed by treatment and product age ($p< 0.0001$), but without a treatment x age interaction ($p>0.05$) (Figure 1). Indication of redness, a* value significantly differed by treatment, day, and minutes post package opening with a day x minute interaction ($p< 0.0001$), and a day x treatment x minute three-way interaction ($p= 0.04$) (Figure 2). The b* color values had significant differences by treatment, day, and minute of package opening ($p< 0.05$) (Figure 3). Calculated chroma (chroma=$(a^2 + b^2)^{1/2}$) also differed significantly by treatment, age, and minute of package opening ($p< 0.05$) (Figure 4).

Overall pork odor acceptability differed significantly by aging day ($p=0.02$), but not by treatment (Figure 5). Trends in acceptability and off-odor development can be seen in figures 1 - 5 above. From these results, it can be hypothesized that PAA acidified with acetic acid could contribute to maintained odor acceptability in ground pork compared to some alternative organic acid applications. Acidic off-odor differed by age ($p=0.002$), but not by treatment ($p> 0.05$) (Figure 6). Overall oxidation off-odor did not differ significantly by treatment or aging($p> 0.05$) (Figure 7). Sweaty off-odor development differed significantly by aging day ($p=0.01$) but not by treatment (Figure 8). An increasing sour off-odor development differed significantly by ground pork age ($p= 0.001$), but not by treatment (Figure 9).

For aerobic plate counts (APC), in the ground pork product, a treatment by day interaction occurred ($p=0.007$) (Table 9). Psychrotrophic bacterial counts did not differ significantly by treatment or sampling day ($p>0.05$) (Table 10). A trend was observed in the control of psychrotrophic bacterial growth by all three organic acid treatments on day 14 compared to the control treatment. Ground pork pH differed by day ($p<0.0001$) and treatment ($p<0.0001$), with a significant day x treatment interaction ($p=0.042$). At 0d mean pH for Titon™ was highest (5.8), followed by the control product (5.7), and PAA+ Acetic acid (5.6) (Table 11). Pork trim used in phase III, across all treatments, was 20% fat and 80% lean, with 16.7% protein. PAA + Titon™ and LA had decreased lipid oxidation compared to PAA + Acetic and control pork samples over the 28 d

of storage. Lipid oxidation was consistent for all 4 treatments on days 0, 14, 21, and 28 however, there was an unexplainable spike in lipid oxidation for samples on day 7.

Overall phase I demonstrated effectiveness of implementing interventions to address pathogenic *Salmonella* control during the harvest process, prior to pork carcass chilling. Phase II demonstrated effective reduction of *E. coli* inoculated carcasses in a small-scale traditional chilled pork harvest facility, and in a large-scale commercial pork facility with a blast chiller. Phase III demonstrated no dramatic negative organoleptic changes to pork trim when treated with selected organic acid interventions prior to grinding. Additionally, phase III highlighted an opportunity for further work to address the most efficient use of intervention application in pork trim for extended shelf-life and maintained product quality. Across all applications utilized in this study (warm skin pieces, cheek pieces, whole hog carcasses, and pork trim), 3% lactic acid, PAA acidified with acetic acid, and PAA acidified with Titon™ were found to consistently be comparable in effectiveness against pathogenic and surrogate bacterial loads.

- END -

Appendix.

Table 12. List of *Salmonella* isolates used to create pathogenic *Salmonella* cocktail for phase I.

Salmonella strain	Original source of isolate
S. Newport T1-473	Human
S. Typhimurium CSU FSL R1-089	Human
S. Enteriditis T1-496	Human
S. Montevideo 11TTU382B	Cattle lymph node
S. Anatum 11TTU158B	Cattle lymph node

Figure 11. Pork carcass with 100cm^2 swab template stamped for swab location assignment in phase II.

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