

I. **Project Title and NPPC project identification number:** "Optimization of electrolyzed oxidizing water and comparison with other antimicrobial compounds to reduce pathogens on fresh or further processed pork products"  
NPPC # 01-070

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II. **Abstract:**

Electrolyzed oxidizing (EO) water is a newly recognized disinfecting compound that has the potential to be used for inactivation of pathogenic and/or spoilage microorganisms associated with vegetables, fresh meat or further processed meat surfaces. The generation of EO water occurs when a current is passed a salt-water solution through to produce an acidic solution containing dilute hypochlorous acid, high oxidation reduction potential (ORP) of approximately 1150 mV, and 10-80 mg/l chlorine. A basic solution is also produced containing sodium hydroxide and a negative ORP. Studies were performed to evaluate the effectiveness of EO water against pathogens in cell suspensions and associated with fresh and ready-to-eat (RTE) pork surfaces. In the first study, the stability of EO water was evaluated under different storage temperatures (4 and 25°C) and its effectiveness was determined for reducing cell suspensions of *Salmonella* Typhimurium and *Listeria monocytogenes* at 0, 1, 5, 10, and 15 min. The results demonstrated that the free chlorine concentration of acidic EO water increased after 24 h when stored at 4°C. "Aged" acidic EO water and acidic EO water made immediately prior to treatment were shown to effectively reduce both cell suspensions of *S. Typhimurium* (> 8 log<sub>10</sub> CFU/ml for both temperatures) and *L. monocytogenes* (8 and 6.5 log<sub>10</sub> CFU/ml for 25 and 4°C, respectively) when treated up to 15 min. In the second study, parameters were optimized for reduction of *L. monocytogenes* on RTE meat surfaces (i.e. frankfurters and ham). Preliminary studies indicated that when dipped for 15 min at 25°C with EO water produced at 14 or 19 amperage, the most significant reduction of *L. monocytogenes* was observed with water produced at 19 amperage. Acidic EO water, basic EO water, 2% acetic acid, and 10% TSP sprays were also evaluated for reducing *L. monocytogenes* on frankfurters; none of the treatments significantly reduced the pathogen. Furthermore, a combination of basic EO water spray followed by acidic EO water spray applied to experimentally inoculated frankfurter surfaces significantly reduced *L. monocytogenes* immediately after treatment 0.6 log<sub>10</sub> CFU/g. However, the reduction was not maintained after 7 days of refrigerated storage (0.25 log<sub>10</sub> CFU/g). Conversely, inoculated ham surfaces treated with acidic and basic EO water alone and in combination resulted in significant reductions of approximately 0.77, 1.04, and 0.7 CFU/g at 0, 3, and 7 days refrigerated storage, respectively. In the final study, the effectiveness of EO water was compared with chlorinated water and lactic acid against populations of *S. Typhimurium*, *L. monocytogenes*, and *Campylobacter coli* on fresh pork surfaces stored up to 7 days at 4°C. Acidic EO water significantly reduced *S. Typhimurium* and *L. monocytogenes* across all three sample days. *C. coli* was significantly reduced immediately following treatment with acidic EO water, but the reduction was not maintained following storage up to 7 days. These studies have demonstrated the effectiveness of EO water against pathogens associated with fresh and RTE pork surfaces. The results from these studies suggest that EO water may provide meat processors with an additional antimicrobial regimen for reducing pathogens associated with meat surfaces.

III. **Introduction**

Organic acids, chlorine dioxide, trisodium phosphate, heat, steam, or hot water are generally recognized as safe (GRAS) interventions and are used extensively by the meat and poultry industries to reduce bacterial contamination on meat surfaces. These interventions have been found to be effective for immediately reducing foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*

attached to meat or poultry surfaces. Other novel compounds that have been or are currently being investigated to reduce microbes on meat and poultry under commercial conditions include hydrogen peroxide, as well as acidified sodium chlorite (Alcide™). Despite the availability of these compounds and potential effectiveness of these interventions, researchers are continually investigating the use of other novel, antimicrobial agents or preservatives to reduce or inhibit pathogenic or spoilage organisms associated with fresh meat and further processed meats and to do it more effectively and economically.

Electrolyzed oxidizing (EO) water is a newly recognized disinfecting compound that has the potential to be used for inactivation of pathogenic microorganisms associated with fresh or further processed pork surfaces. The generation of EO water involves reactions in a cell containing inert positively charged and negatively charged electrodes, respectively, separated by a membrane, and through which a very dilute salt water solution passes (see Figure below). By subjecting the electrodes to direct current voltage, two types of water possessing different characteristics are generated: an electrolyzed basic aqueous solution (pH 11.4) and oxidation-reduction potential [ORP] of -795 mV containing dilute sodium hydroxide (NaOH) is produced by the cathode and an electrolyzed acidic solution containing dilute hydrochloric acid (HCl) is produced from the anode side and has a high ORP of about 1,150 mV (Kim et al., 2000). Small amounts of oxygen and hydrogen gas are also produced during this reaction. Hypochlorous acid (HOCl) is also produced during electrolysis of saline-added water, with the amount of HOCl increasing in response to the amount of sodium chloride present. The resulting water exhibits a pH of approximately 2.6 with a residual chlorine level of 10-60 mg/liter. The antimicrobial activity of EO water does not appear to be due to the type(s) of water generated, the ORP, or presence of chlorine, but the combination of all of these.

Due to the easy process used to derive the water and the less adverse impact on the environment, this technology has been well received by consumers in Japan. Additionally, Japanese consumers can purchase EO water machines for less than \$1000 to purify water or sanitize food surfaces within their homes. Recent studies conducted by researchers have indicated that *Escherichia coli* O157:H7 and *Listeria monocytogenes* exposed to EO water for two minutes were reduced by  $\geq 7 \log_{10}$  CFU/ml or 99.99999%. Reductions of  $>5 \log_{10}$  CFU/100 cm<sup>2</sup> or 99.999% against *E. coli* O157:H7 and *L. monocytogenes* were observed when cutting boards were treated with EO water for 10 minutes.

Additional studies conducted in our laboratories indicate that EO water is more effective than chlorine, ozone, or water, and as effective as acetic acid and trisodium phosphate for reducing *Salmonella* spp., *E. coli* biotype 1, or coliforms on chilled, treated poultry carcasses immediately after treatments and up to 7 days of refrigerated storage, respectively. While these studies clearly illustrate that EO water is an effective antimicrobial for reducing foodborne pathogens in water, on cutting boards, and on poultry carcasses, no comparison studies have been conducted to determine EO water application to reduce or inhibit pathogens associated with fresh or processed pork surfaces.

#### IV. Objectives:

1. To optimize the parameters needed to apply electrolyzed oxidizing water to effectively inhibit populations of *Escherichia coli* biotype 1, coliforms, *Campylobacter coli*, *Listeria monocytogenes*, or *Salmonella* Typhimurium on fresh or ready-to-eat pork products
2. To compare the effectiveness of electrolyzed oxidizing water with commercially available antimicrobial compounds against populations of *E. coli* biotype 1, coliforms, *E. coli* O157:H7, *L. monocytogenes*, or *S. Typhimurium* associated with fresh or ready-to-eat pork products

#### V. Procedures:

##### Bacterial cultures and media

*Campylobacter coli* ATCC 33559 (American Type Culture Collection, Manassas, VA), *Salmonella* Typhimurium ATCC 13311, and *Listeria monocytogenes* Scott A were obtained from the Food Microbiology Culture Collection at Pennsylvania State University. *L. monocytogenes* and *S. Typhimurium* were grown aerobically at 35°C for 24 h in trypticase soy broth (TSB, Difco) and stored at -76°C in TSB containing 10% glycerol (Difco). Frozen

stock cultures of *C. coli* were stored at  $-76^{\circ}\text{C}$  in Brucella broth (BB, Difco) containing 10% glycerol and propagated at  $42^{\circ}\text{C}$  under microaerophilic conditions for 48 h using anaerobic jars (Becton Dickinson, Microbiology Systems, BDMS, Cockeysville, MD) and CampyPak Plus generating envelopes (BDMS). Prior to experiments, *S. Typhimurium* and *L. monocytogenes* were propagated aerobically in TSB at  $35^{\circ}\text{C}$  for 18 h, while *C. coli* was propagated microaerophilically in BB at  $42^{\circ}\text{C}$  for 48 h.

### **Electrolyzed oxidizing water generation**

EO water was generated by passing a salt (12% NaCl) solution across a charged bipolar membrane composed of vinylidene polyfluoride. The salt solution and deionized water were pumped into the EO water generator (ROX Water Electrolyzer, Hoshizaki America, Inc., Peachtree City, GA). According to the manufacturer, by subjecting the platinum electrodes to direct voltage (19 amperage), two types of water are generated. From the cathode side an electrolyzed basic solution [pH 11.6, ORP of  $-795$  mV, containing dilute sodium hydroxide (NaOH)] and from the anode side an electrolyzed acidic solution (pH 2.6, ORP of 1150, and approximately 50 ppm free chlorine) containing dilute hypochlorous acid (HOCl). Small amounts of oxygen and hydrogen gas were also produced during this reaction. Acidic EO water was made immediately prior to treatment and referred to as EOA. "Aged" acidic EO water (AEOA) was made 24 h prior to treatment and stored at  $4^{\circ}\text{C}$  in an airtight bottle.

### **Free Chlorine Determination**

Free chlorine content of the CL and EO water treatments was measured using Hach DPD-FEAS digital titrator method (Hach Company, Loveland, CO) as described by the manufacturer. Briefly, a 25 ml sample, diluted 10-fold with sterile distilled water, was transferred into an Erlenmeyer flask. A DPD Free Chlorine Powder Pillow was added to the sample and swirled to mix. The sample was titrated using 0.00564 N Ferrous Ethylenediammonium Sulfate (FEAS) to a colorless endpoint. Free chlorine was calculated from the number obtained following titration, inclusive of the dilution factor (1:10).

### **Total Chlorine Determination**

Total chlorine measurements of the CL and EO water treatments were measured using Hach DPD-FEAS digital titrator method (Hach Company, Loveland, CO) following manufacturer's directions. Briefly, 10 ml of sample was transferred to a 250 ml Erlenmeyer flask. One Dissolved Oxygen 3 Powder Pillow and one Potassium Iodide Powder Pillow were added and swirled to mix. One dropper (1 ml) of starch indicator was added and swirled to mix resulting in a dark blue color. The solution was titrated using 0.113 N Sodium Thiosulfate to a colorless endpoint. Total chlorine content (ppm) was calculated using the number obtained following titration and the digit multiplier supplied by Hach.

### **Determination of pH and ORP**

Measurements of pH were taken from the antimicrobial solutions using a Corning pH meter (Corning Inc., Corning, NY). ORP measurements of antimicrobial solutions were obtained using Corning pH meter with Orion ion electrode (Orion Research Inc., Beverly, MA).

### **Stability of EO water experiment**

Acidic and basic EO water were generated at 19 amperage. Five hundred milliliter aliquots were produced and stored in Pyrex bottles (no. 1395) at either  $4$  or  $25^{\circ}\text{C}$ . Measurements of free and total chlorine, pH and ORP were taken on days 0, 1, 2, and 3. Initial experiments were conducted to determine the combination of time and temperature of storage deemed most detrimental to bacterial populations and used in subsequent cell suspension experiments.

### **Cell suspension experiment**

One ml of an overnight culture of *L. monocytogenes* or *S. Typhimurium* was added to 9 ml of test solution [distilled water (DI; control), acidic EO water (EOA), basic EO water (EOB), acidic EO water "aged" 24 h at  $4^{\circ}\text{C}$

(AEOA; 100 ppm free chlorine; pH 2.2, 1170 mV ORP), chlorinated water (CL; 30 ppm free chlorine, pH 9), acidified chlorinated water (CLA; 30 ppm free chlorine, pH 4.5). Chlorinated water was acidified using 0.1 N hydrochloric acid (HCl; Sigma). Each treatment was carried out at 25 or 4°C. Aliquots were taken from each treatment after 0, 5, 10, and 15 min exposure, and samples serially diluted in BPW. Dilutions were spread plated on TSA in duplicate and incubated at 35°C for 24-48 h.

To ensure detection of low levels of *S. Typhimurium* following treatment, 1 ml sample was also pre-enriched in 9 ml of lactose broth (Difco Laboratories) and incubated for 24 h at 35°C. After 24 h of pre-enrichment, 1 ml of lactose broth was transferred to 9 ml of selenite cystine broth (SC; Difco Laboratories), another 1 ml was transferred to 9 ml of tetrathionate broth (TT; Difco Laboratories) and all tubes incubated for 24 h at 35°C. After incubation, samples were taken from SC and TT, streaked for isolation onto xylose lysine decarboxylase (XLD; Difco) agar plates, and incubated for 48 h at 35°C. Typical colony morphology was identified on XLD (black colonies) and verified serologically using the Oxoid *Salmonella* latex test (Oxoid, Inc, Ogdensburg, NY). To ensure detection of low levels of *L. monocytogenes* following treatment, 1 ml of sample was enriched in 9 ml Fraser broth (Difco Laboratories) for 24 h at 35°C. Following incubation, samples were streaked for isolation onto Oxford agar plates and incubated at 35°C for 48 h. Typical *L. monocytogenes* morphology (black colonies on Oxford) was verified serologically using Visual Immunoassay for *Listeria* (TECRA Diagnostics, Roseville, Australia).

### Optimization study

Frankfurters and ham ends were purchased from a local grocery store. All RTE meats were stored at 4°C until needed. Samples were removed aseptically from packaging immediately prior to treatment. For all experiments, frankfurters or ham surfaces were surface treated with UV light in a biological safety hood on sterile bacon racks. Surfaces were exposed evenly by turning every 10 min for up to 30 min. The pathogen was inoculated onto UV-treated meat surfaces by spray inoculation with a hand-held spray bottle under a biological safety hood. The external surfaces of the ham samples were inoculated with *L. monocytogenes* by spray inoculation as described above. The bacterial culture was allowed to attach to external meat surfaces for 15 minutes prior to any treatments. Using this procedure, approximately 4 log<sub>10</sub> CFU/g of pathogen was obtained on meat surfaces.

Acidic EO water (EOA) was generated at 14 amps [pH of 2.34, 1130 mV oxidation-reduction potential (ORP), 36 ppm free chlorine, and 44 ppm total chlorine] and 19 amps (pH of 2.3, 1154 mV ORP, 45 ppm free chlorine and 50 ppm total chlorine). After inoculation, frankfurters were dipped in 500 ml of either solution for 1, 5, 10, 15, or 30 min at 4°C or 25°C. EO water was chilled to 4°C by surrounding the beaker with ice. Following treatments, 25 g samples of inoculated and treated product were stomached (Stomacher 400, Tekmar, Cincinnati, OH) with 25 ml BPW for 2 minutes at 230 rpm in a filtered stomacher bag (Spiral Biotech, Norwood, MA). Samples were serially diluted in BPW prior to plating on Oxford agar plates (Difco) using the Autoplate 4000 (Advanced Instruments, Norwood, MA). Plates were incubated at 35°C for 48 h and counted manually. To ensure detection of low levels of the pathogen, 1 ml of stomached samples was incubated for 24-48 h at 35°C in Fraser broth (Difco). Enriched samples were then plated on Oxford media to determine the presence/absence of *L. monocytogenes*. Typical *L. monocytogenes* colonies were verified using Visual Immunoassay for *Listeria monocytogenes* (TECRA Diagnostics, Roseville, Australia) according to the manufacturer's instructions. Based on these preliminary experiments, EO water produced at 19 amps was determined most effective against *L. monocytogenes* associated with meat at 25°C for a minimum of 15 min.

### Shelf life study

Experimentally inoculated frankfurters were dipped in 500 ml of EOA for 15 min at room temperature (25°C) and allowed to drip for 60 sec. Following treatments, frankfurters were individually vacuum-packaged (Koch Supplies; 3-Mil vacuum pouch), stored at 4°C and sampled at days 0, 1, 7, 14, 21, and 28. Sampling and microbiological analyses were performed as previously described.

## Treatment comparison study

Experimentally inoculated frankfurters were sprayed manually with approximately 2 ml of either 2% acetic acid (AA), 10% trisodium phosphate (TSP), EOA or EOB. Frankfurters remained undisturbed for 60 sec prior to sampling or vacuum packaging. Sampling was done as described above on days 0, 3, 7, and 14. Frankfurters were stored aerobically at 4°C. Since EO water is thought to “bleach” surfaces due to the presence of chlorine, colorimetric determination of frankfurters was accomplished using Hunter L\*a\*b\* measurements with a Minolta colorimeter (Minolta Co., Ramsey, NJ).

## Multiple intervention study

Experimentally inoculated chicken frankfurters were treated by spraying approximately 2 ml of EOB followed by spraying 2 ml of EOA. Surfaces remained undisturbed for 2 minutes in between treatments. Frankfurters were either sampled immediately (day 0) or individually vacuum packaged as described previously and sampled after 7 days of refrigerated (4°C) storage. Sampling and microbiological analyses were performed as described above. Hunter L\*a\*b\* values were measured as described above.

## Ham study

Smoked ham surfaces were spray inoculated with approximately 5 log<sub>10</sub> CFU/ml of *L. monocytogenes* as described previously. Inoculated ham surfaces were allowed to attach for 15 minutes on sterile trays in a biological safety hood. Following attachment, ham surfaces were sprayed with approximately 2 ml of the antimicrobial of interest (EOA, EOB, or a combination of EOB followed by EOA). Samples remained undisturbed for 1 min at 25°C after treatment application. Sampling was performed immediately (day 0) after treatment or following refrigerated, vacuum packaged storage at days 3 and 7. Hunter L\*a\*b\* values were also measured as each day as previously described. For each sampling, 25 g of the treated sample was stomached for 2 minutes at 230 rpm with 25 ml of BPW in filtered stomacher bag as described previously. Plating and enumeration were conducted as previously described.

## Spray washing experiment

Prior to inoculation in porcine feces, 10 ml of each pathogen (*S. Typhimurium* and *L. monocytogenes*, *C. coli*) were centrifuged (Beckman Instruments Inc., model J2-21, Palo Alto, CA) at 12,000 x g for 15 min at 4°C (*S. Typhimurium* and *L. monocytogenes*) or at 8,000 x g for 10 min at 4°C (*C. coli*) to harvest the cells. After centrifugation, the pellets were resuspended in 10 ml of buffered peptone water (BPW) and serially diluted (1:10) in BPW to obtain approximately 5 log<sub>10</sub> cells/ml for all pathogens.

Pork bellies were obtained from hogs slaughtered in a local commercial establishment that used hot water as a microbiological intervention. After chilling for 24 h at 4°C, skin-on intact surface samples were obtained from the belly section. Freshly defecated porcine feces were obtained from gestating sows housed in the facilities of the Swine Center at Pennsylvania State University.

Prior to inoculation, 10 g feces was stomached (Stomacher 400, Tekmar, Cincinnati, OH) for 2 min with 90 ml BPW in a filtered stomacher bag (Spiral Biotech, Norwood, MA) containing diluted cultures of *S. Typhimurium*, *L. monocytogenes*, and *C. coli* to obtain pathogen levels of approximately 7 log<sub>10</sub> CFU/g of feces. Pork surfaces were inoculated as described below.

Each pork belly was divided into 4 even sections. Each section was pre-marked with edible ink using a sterile, cotton-tipped swab (Hardwood Products, Co., Guilford, MN) and a sterile 25-cm<sup>2</sup> stainless steel template. The pork bellies were surface treated using UV light in a biological safety hood. Surfaces were evenly exposed to UV light by turning sections every ten minutes for a total time of no more than 30 minutes (Cutter and Siragusa, 1994). UV-treated surfaces were inoculated with approximately 8 ml of porcine fecal suspension containing approximately 7 log<sub>10</sub> *S. Typhimurium*, *L. monocytogenes*, and *C. coli* using a sterile spray bottle. Bacteria on inoculated surfaces were allowed to attach for 15 minutes at room temperature under a biological safety hood, to obtain pathogen levels of approximately 6 log<sub>10</sub> CFU/cm<sup>2</sup>.

Following inoculation and attachment, the pork belly sections were hung vertically on a stainless steel rack in a biological safety hood and sprayed with the antimicrobial of interest [distilled water (control), EO water (pH 2.4-2.7, 1,150 mV ORP, 50 ppm free chlorine), "aged" EO water (pH 2.3, 1,150 mV ORP, 100 ppm free chlorine), 2% lactic acid, or 20 ppm sodium hypochlorite] for 15 sec using a food-grade handheld garden sprayer (Hudson, Hastings, MN; Model 67220). Once sprayed with antimicrobials, samples were held horizontally undisturbed for 1 h at 4°C prior to sampling. Untreated control samples were excised prior to spray treatments. Following spray treatments, samples were obtained by aseptically excising a 25 cm<sup>2</sup> x 0.5 cm thick piece with a sterile scalpel (Sakura, Torrence, CA) and forceps as previously described. All excised samples were diluted in 25 ml of BPW and stomached for 2 min in a filtered stomacher bag. Samples were serially diluted in BPW prior to plating onto respective media. For enumeration of mesophilic aerobic plate counts (APC), diluted stomachates were spiral plated in duplicate on trypticase soy agar (TSA; Difco) using the Autoplate 4000 (Advanced Instruments, Norwood, MA) and incubated at 35°C for 36 h. For enumeration of *E. coli* biotype 1 and coliforms, 1 ml of stomachates were plated in duplicate onto 3M *E. coli*/coliform Petrifilm™ (3M, ST. Paul, MN) according to the manufacturer's instructions and incubated for 48 h at 35°C. Enumeration of *S. Typhimurium* was performed by spiral plating in duplicate onto xylose lysine deoxycholate agar (XLD; Difco) and incubating for 24-48 h at 35°C. *L. monocytogenes* was enumerated by spiral plating in duplicate onto Oxford agar (OX; Difco) and incubated for 24-48 h at 35°C. Enumeration of *C. coli* was performed by spiral plating onto modified CCDA-Preston agar (mCCDA; Oxoid, Ogdensburg, NY) and incubating at 42°C under microaerophilic conditions using anaerobic jars (BDMS) and CampyPak Plus generating envelopes (BDMS) for 48 h. All plates were enumerated manually or with the Q-count image analyzer (Advanced Instruments). The lowest level of detection of organisms was 1.30 log<sub>10</sub> CFU/cm<sup>2</sup> of stomachate using spiral plating procedures.

To ensure detection of low levels of pathogens following treatments, samples were enriched as described previously. For *C. coli*, 1 ml sample was added to 9 ml of *Campylobacter* enrichment broth (Bolton's formula, Oxoid) with lysed horse blood and antibiotic supplement and incubated for 4 h at 35°C, followed by 20 h at 42°C under microaerophilic conditions. Enriched samples were streaked onto mCCDA agar and incubated for 24 h at 42°C under microaerophilic conditions. Typical *C. coli* colonies were verified serologically using *Campylobacter* agglutination test kit (Oxoid).

Following excision of day 0 samples, the remaining portion of each belly was placed loosely in individual polypropylene bags (Seward, London, UK) to prevent cross contamination and stored aerobically at 4°C until sampled on day 2. On day 2, two samples were aseptically excised from each treated and untreated belly. One sample was subjected to enumeration procedures described above, while the other sample was individually vacuum packaged (Koch Supplies) and stored at 4°C for 5 days. Following storage, the vacuum packaged sample was enumerated as described above.

## Statistical analysis

Means of bacterial populations (log<sub>10</sub> CFU/ml) from each treatment were calculated from three replications for each experiment. Data were analyzed using SPSS statistical package (SPSS Inc., Chicago, IL). General Linear Model with repeated measures (holding time constant) was performed to note differences ( $P < 0.05$ ) among means for treatments. Comparisons of means were done using Tukey's HSD multiple comparison test.

## VI. Results

### Objective 1:

#### Stability of EO water experiment

When both acidic and basic EO water (EOA and EOB) characteristics were monitored over time, it was determined that EO water stored at 4°C was more stable. The pH of both EOA and EOB remained fairly consistent over the 3 days. The ORP began to increase for EOB after day 0, while the ORP of EOA remained relatively unchanged. Free chlorine levels for EOA stored at 25°C were consistent until day 3 when levels decreased. Total chlorine for EOA was high at 106.9 ppm, increased to 113.2 ppm after 24 h of storage, but consistently decreased

thereafter. EOA stored at 4°C demonstrated an increase in free chlorine over the 3 days of storage; the total chlorine concentration of EOA stored at 4°C increased after 24 h of storage. ORP, free and total chlorine of EOA stored at 4°C for 24 h was consistently higher than the same water stored at 25°C.

### Cell suspension experiment

Cell suspensions of *S. Typhimurium* were treated at 25 or 4°C for 0, 5, 10, or 15 min with chlorine (CL), chlorinated water acidified to pH 4.5 (CLA), basic EO water (EOB), acidic EO water (EOA) and acidic EO water that was “aged” at 4°C for 24 h (AEOA). At 25°C, EOA and AEOA significantly reduced bacterial populations for all times examined. Immediately following the addition of the test solutions, EOA and AEOA afforded a 3.21 and 3.78 log<sub>10</sub> CFU/ml reduction, respectively. After 5 minutes of treatment, AEOA treatment afforded the greatest reduction of 7.6 log<sub>10</sub> CFU/ml. Cells treated with EOA were rapidly inactivated after 10 minutes of treatment. DI, CL, CLA, and EOB treatments were not reduced significantly for any of the times examined.

When the treatments for *S. Typhimurium* were carried out at 4°C, remaining populations following EOA and AEOA treatments were significantly different from the control treatments. AEOA was most effective at reducing populations of *S. Typhimurium* with reductions of 5.01, 6.36, 7.79, and 8.0 log<sub>10</sub> CFU/ml for 0, 5, 10, and 15 min of exposure, respectively. EOA effectively reduced over 5 log<sub>10</sub> CFU/ml of the pathogen after 15 min of treatment. As demonstrated previously, DI, CL, CLA, and EOB were not effective treatments for reducing *S. Typhimurium* in cell suspensions.

When *L. monocytogenes* was treated at 25 or 4°C for 0, 5, 10, or 15 min remaining populations following treatments with DI, CL, CLA, and EOB were not statistically significant from each other at either temperature. For treatments carried out at 25°C, AEOA was the most effective at time 0 with a 6.72 log<sub>10</sub> CFU/ml reduction. Conversely, EOA resulted in only a 3.65 log<sub>10</sub> CFU/ml reduction of the pathogen; however, both treatments were statistically significant. After 5 minutes of treatment, EOA significantly reduced the pathogen greater than 6 log<sub>10</sub> CFU/ml. The decline in viable cells continued following treatments with EOA such that after 10 minutes, no cells were detected following enrichments. The decline in viable *L. monocytogenes* cells following treatment with AEOA was greatest initially (6.72 log<sub>10</sub> CFU/ml), with a continued decline of 6.85, 7.60, and 8.17 log<sub>10</sub> CFU/ml for 5, 10, and 15 min, respectively.

When treatments were carried out at 4°C, the most significant reductions (> 5 log<sub>10</sub> CFU/ml) were observed with AEOA, regardless of time. EOA also reduced levels of *L. monocytogenes* greater than 4 log<sub>10</sub> CFU/ml up to 15 min post-treatment and was statistically different from remaining populations treated with DI, CL, CLA or EOB.

The statistical analysis identified time as a linear response for all treatments at all temperatures, indicating that time increased, the amount of the pathogen decreased accordingly.

### Optimization study

Acidic EO water (EOA) produced at 19 amps reduced populations of *L. monocytogenes* to the greatest extent (1.5 log<sub>10</sub> CFU/g) when the frankfurters were dipped for 15 min at 25°C. Based on these preliminary data, additional experiments utilized EO water produced at 19 amps and the treatments were carried out at 25°C.

### Shelf life study

Immediately following treatment, EOA reduced *L. monocytogenes* by nearly 2 log<sub>10</sub> CFU/g. This reduction was maintained for up to seven days. Over time, the reduction diminished as bacterial populations increased; however, acidic EO water-treated frankfurters had significantly lower populations of *L. monocytogenes* as compared to untreated samples on all days, except day 21. The effectiveness diminished over time presumably because *L. monocytogenes* can recover and grow at refrigeration temperatures. It is possible that the diminished effectiveness may also be due to loss of residual antimicrobial over time.

### Objective 2:

### Treatment comparison study

No significant differences were noted for all treatments across both sampling days for aerobic plate counts. Despite this observation, EOA afforded a 0.6 log<sub>10</sub> CFU/g reduction of APC immediately following treatment and 1.07 log<sub>10</sub> CFU/g reduction after 7 days of refrigerated storage. The greatest reduction (0.86 log<sub>10</sub> CFU/g) of APC was observed following immediate application of EOB. EOB was nearly as effective as EOA after 7 days against APC with a 1.05 log<sub>10</sub> CFU/g reduction.

The most significant treatment against populations of *L. monocytogenes* was EOA with a 0.39 and 0.50 log<sub>10</sub> CFU/g reduction for days 0 and 7, respectively. Against *L. monocytogenes*, EOB performed nearly as well as EOA with a 0.33 and 0.30 log<sub>10</sub> CFU/g reduction after 0 and 7 days, respectively. The greatest reduction immediately following treatment was observed with TSP with a 0.44 log<sub>10</sub> CFU/g reduction, while EOA afforded the greatest reduction over time. The observed differences of all the treatments may be attributed to the short contact time. Previous experiments have demonstrated that a prolonged contact time significantly enhances activity against pathogens.

Hunter L\* a\* b\* values were measured to determine if the EO water would significantly oxidize the pigments on the meat surfaces. No significant difference was noted for any of the treatments for L\*, a\* and b\* values as compared to untreated controls.

### Multiple intervention study

After determining that individual sprays of EOA and EOB afforded only slight reductions in *L. monocytogenes*, a multi-hurdle approach was conducted. When EOB and EOA water solutions were applied sequentially, a significant reduction was observed (0.6 log<sub>10</sub> CFU/g). By day 7, only a slight reduction in *L. monocytogenes* was observed.

There was no statistical difference between treatments for L\* values for both sampling days with the sequential treatment. However, a\* and b\* values from EOB/EOA treated samples were significantly different than untreated samples. Higher a\* values are indicative of more red coloration. Immediately following treatment, the b\* values, which indicate yellow coloration, were significantly different for EOB/EOA treatments as compared to untreated samples. However, by day 7 of refrigerated storage, differences in L\*, a\* or b\* values between treatments were not observed.

### Ham study

Based on preliminary results from the previous studies, *L. monocytogenes* associated with ham surfaces was treated with acidic EO water (EOA), basic EO water (EOB), alone or in combination. Immediately following treatment with EOA, a significant reduction (0.47 log<sub>10</sub> CFU/g) in the pathogen was observed. The reduction was maintained after aerobic refrigerated storage with a 0.39 and 0.55 log<sub>10</sub> CFU/g reduction on day 0 and 7, respectively. Although EOB afforded a slight reduction in *L. monocytogenes* on ham surfaces immediately following treatment, the significance was not as great as EOA or the combination of both EOA and EOB (EOB/EOA). The most significant reduction, 0.77, 1.04, 0.7 log<sub>10</sub> CFU/g in *L. monocytogenes* occurred when the ham surfaces were treated with the combination of EOB followed by EOA (EOB/EOA) for days 0, 3, and 7, respectively. Hunter L\*a\*b\* values were not significantly different among the treatments for 0 and 7 days.

These studies have demonstrated that with increased contact time, *L. monocytogenes* populations associated with ready-to-eat meat surfaces could be reduced using EO water. It is important to realize that processors may not have more than a few seconds to treat the meat surfaces following cooking and prior to packaging. Of the treatments examined, it appears that a multi-hurdle approach using EOA and EOB may be considered for reducing pathogens associated with RTE meat surfaces. Additional studies that can enhance the effectiveness of the compound, be applied in a reasonable timeframe, and be incorporated into RTE meat processing should be evaluated.

Based on the composition of the color molecules present in meat, it was expected that high ORP values may oxidize the pigments, which in turn would impact the overall color and appearance of meats. Despite the extreme

ORP values associated with EO water treatments, the resulting Hunter L\*a\*b\* values from the studies indicate that EO water does not have a detrimental “bleaching” effect on the surface of RTE meats.

A considerable amount of outbreaks and recalls associated with RTE meats in recent years makes post processing decontamination a concern for the RTE meat industry. Currently, the processed meat industry is limited to such interventions as steam, heat and composition of the product (i.e. pH, salts and curing agents) to prevent or eliminate *L. monocytogenes* associated with RTE meats, such as frankfurters. EOA has been previously shown to be effective against *L. monocytogenes* associated with cutting board surfaces and in cell suspensions. The current studies demonstrate that EOB and EOA water, alone or in combination were capable of reducing populations of *L. monocytogenes* on experimentally inoculated frankfurters and ham. The information from this study may be useful in determining additional inexpensive and practical methods to enhance the microbiological safety of RTE meat products.

### Spray washing experiment

Treatments for APC, *E. coli*, total coliforms, *L. monocytogenes* and *S. Typhimurium* were not significantly different from each other when applied to experimentally inoculated pork bellies; however they were significantly different from the untreated samples. Lactic acid (LA) and acidic EO water (EOA) were the only treatments to significantly reduce populations of *C. coli* 1.71 and 1.81 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively, at days 2 and 7.

When populations of *E. coli* biotype 1 were monitored, all the treatments were significantly different from the untreated samples; however the greatest reductions were observed with EOA and AEOA, in which reductions of 1.13 and 1.16 log<sub>10</sub> CFU/cm<sup>2</sup> were observed, respectively. After 7 days of storage, lactic acid afforded the greatest reduction (0.81 log<sub>10</sub> CFU/cm<sup>2</sup>).

Treatments of total coliforms afforded approximately the same reduction as for *E. coli* biotype 1. Initially, only lactic acid, EO water and “aged” EO water afforded the greatest reduction with approximately 1 log<sub>10</sub> CFU/cm<sup>2</sup> reduction for each. After day 2 and 7 of refrigerated storage, there were no significant differences between any of the antimicrobial treatments. Lactic acid afforded the most reduction with a 1.25 and 1.14 log<sub>10</sub> CFU/cm<sup>2</sup> reduction, respectively.

At days 0, 2, or 7, populations of *S. Typhimurium* were not significantly reduced following any of the treatments tested. Of the compounds evaluated, lactic acid was the most effective treatment, resulting in a 1.79, 1.46 and 3.79 log<sub>10</sub> CFU/cm<sup>2</sup> reduction, respectively for day 0, 2 and 7. EO and “aged” EO water each afforded a 1.67 and 1.55 log<sub>10</sub> reduction, respectively for day 0; however, by day 2, *S. Typhimurium* was only reduced by 1.28 and 1.12 log<sub>10</sub> CFU/cm<sup>2</sup>. By day 7, the pathogen was reduced 1.36 and 1.1 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively for EO water and “aged” EO water.

Of the pathogens tested, *L. monocytogenes* was not reduced significantly by the application of the antimicrobials. Initially, “aged” EO water effectively reduced the organism 1.38 log<sub>10</sub> CFU/cm<sup>2</sup>; after 2 days of aerobic storage, treatments with distilled water demonstrated a 1.17 log<sub>10</sub> CFU/cm<sup>2</sup> reduction. By day 7, lactic acid was the most effective antimicrobial for reducing *L. monocytogenes* with a 1.52 log<sub>10</sub> CFU/cm<sup>2</sup> reduction.

When *C. coli* associated with pork surfaces was spray treated, significant differences were observed with treatments of lactic acid and EO water at day 0. Of these compounds, EO water was the most effective with a 1.81 log<sub>10</sub> CFU/cm<sup>2</sup> reduction. Lactic acid afforded the greatest reduction after 2 days of storage with a 1.74 log<sub>10</sub> CFU/cm<sup>2</sup> reduction. By day 7, *C. coli* was only detected through selective enrichment for treatments with distilled water, chlorine, EO water and “aged” EO water.

In the current study, we have demonstrated the potential antimicrobial activity of EO water for use by the pork industry. However, if an increased contact time (>10 min) is necessary to effectively reduce microbial contaminants on poultry surfaces, it may be necessary to determine the feasibility of incorporating such a system into pork processing and as such, additional experiments are warranted.