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The bactericidal activity of acidic electrolyzed oxidizing water against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on raw fish, chicken and beef surfaces

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ABSTRACT

The bactericidal efficacy of acidic electrolyzed oxidizing water (AC-EW) (pH = 2.30, free chlorine = 38 ppm) and sterile distilled water (DW) on three pathogens (*Escherichia coli* O157:H7 *Salmonella* Typhimurium, and *Listeria monocytogenes*) inoculated on raw trout skin, chicken legs and beef meat surfaces was evaluated. The decontaminating effect of AC-EW and DW was tested for 0 (control), 1, 3, 5 and 10 min at 22 °C. AC-EW significantly ($P < 0.05$) reduced the three pathogens in the inoculated samples compared to the control and DW. The level of reduction ranged between ca.1.5–1.6 logs for *E. coli* O157:H7 and *S.* Typhimurium in the inoculated foods. However, AC-EW exhibited less bactericidal effect against *L. monocytogenes* (1.1–1.3 logs reduction). AC-EW elicited about 1.6–2.0 log reduction in the total mesophilic count. Similar treatment with DW reduced pathogens load by ca. 0.2–1.0 log reduction and total mesophiles by ca. 0.5–0.7 logs. No complete elimination of the three pathogens was obtained using AC-EW possibly because of the level of organic matter and blood moving from food samples to the AC-EW solution. This study demonstrates that AC-EW could considerably reduce common foodborne pathogens in fish, chicken and beef products.

1. Introduction

Development of processes to reduce or eliminate pathogenic microorganisms that can be transmitted through foods of animal origin such as *Salmonella* spp, *Listeria monocytogenes* and *Escherichia coli* O157:H7 is crucial. For example, non-typhpoidal *Salmonella* and *L. monocytogenes* accounted for around 47% of the total cases of death from microbial foodborne illnesses (Scallan et al., 2011). In the United Kingdom, 30–40% of chicken brought to the home were found to be contaminated with *Salmonella* (Kramer, Frost, Bolton, & Wareing, 2000). Acidic electrolyzed oxidizing water (AC-EW) has been recognized by the food industry as a promising potential decontamination technique (Hao et al., 2012). AC-EW has several advantages over the use of chlorine containing compounds including: strong antimicrobial activity and cheap raw materials are required to produce AC- EW (water, NaCl or KCl). Overall, AC-EW is more environmentally friendly than other sanitizers yielding minimal wastes and contaminants and can be easily produced on-site and therefore requires minimum handling and storage costs (Huang, Hung, Hsu, Huang, & Hwang, 2008). The combined effect of AC-EW and chitosan was also used successfully to reduce microbial load and to extend the shelf life of American shad fillets stored under refrigeration (Xu et al., 2014). Additionally, when AC-EW was added to wash water used for vegetable produce, it was effective in eliminating *E. coli* O157:H7 and in reducing the possibility of cross-contamination (Vicente, Gomez, Gil, Pupunat, & Allende, 2015).

Other forms of electrolyzed water are effective sanitizers. Neutral electrolyzed water exhibited a remarkable inhibitory effect against sessile cells of *L. monocytogenes* in biofilm adhering to stainless steel (Arevalos-Sanchez et al., 2013). Slightly acidic (pH = 5.7) electrolyzed water significantly reduced the *E. coli* and *Salmonella* spp. in ready-to-eat fresh cut vegetables (Issa-Zacharia, Kamitani, Miwa, Muhimbula, & Iwasaki, 2011) and also it posed a strong bactericidal activity against the seafood borne pathogens; *Vibrio parahaemolyticus* and *Vibrio vulnificus* in artificial broth system; and the effect was more pronounced compared to sodium

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hypochlorite at the same available free chlorine concentration (Quan, Choi, Chung, & Shin, 2010).

Jadeja, Hung, and Bosilevac (2013) demonstrated that AC-EW is equally effective against *E. coli* O157:H7 as well as other non-O157 shiga toxin-producing *E. coli* (STEC). Also, AC-EW was also more effective for inactivating STEC than sodium hypochlorite at the same free chlorine concentration. Nonetheless, in this study, the AC-EW anti-*E. coli* activity was tested in buffered phosphate saline (pH = 7) but not in a real food system.

Combination treatments of AC-EW with acid and heat are also effective. Mild heating (40 °C) of slightly acidic electrolyzed water in combination with fumaric acid (0.5%) resulted in approximately 2.5–3.0 log reduction in *Salmonella* Typhimurium, *E. coli* O157:H7, *L. monocytogenes* and *Staphylococcus aureus* inoculated in fresh pork (Mansur, Tango, Kim, & Oh, 2015). Mildly heated AC-EW (43 °C) was used effectively to elicit a significant reduction of *E. coli* O157:H7 and *S.* Typhimurium from inoculated beef hides (Jadeja & Hung, 2014). However, refrigeration may impede AC-EW effectiveness. AC-EW was not effective against *Morganella morganii* and *L. monocytogenes* inoculated on fish surfaces with growth observed during cold storage. Notwithstanding, applying AC-EW to conveyor belts used for fish processing assisted in reducing microbial load and in the removal of some microbial biofilms (McCarthy & Burkhardt, 2012).

The objective of this study was to examine the efficacy of AC-EW treatment for reducing the total microbial load and certain inoculated foodborne pathogens, namely: *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on the surfaces of fish (trout), chicken and beef.

2. Materials and methods

2.1. Bacterial strains

A cocktail of three strains of *L. monocytogenes* (ATCC 19113, ATCC 19114, and ATCC 7644), *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), and *S.* Typhimurium (ATCC 19585, ATCC 13311, and ATCC 14028), were obtained from the bacterial culture collection of the School of Food Science- Washington State University (Pullman, WA, USA). All the strains were kept refrigerated on Tryptic Soy Agar (TSA: Difco, Chicago, IL, USA) slants and enriched individually prior to the experiment in 9 ml of Tryptic Soy Broth (TSB: Difco, Chicago, IL, USA) at 37 °C for 24 h. Enriched bacterial cultures of each species were compiled aseptically and cells were recovered by centrifugation at 4000 g for 15 min at 4 °C. Bacterial cell pellets were washed three times with 5 ml of buffered peptone water (BPW: Difco) and re-suspended in BPW to produce a culture cocktail of approximately 10^9 CFU/ml.

2.2. Preparation of inoculated samples

Fish (trout) (skin on fillet), beef and chicken legs (skin on) were purchased from a local grocery store. Ten gram samples were dissected using a sterile scalpel and washed with deionized water, drained, and placed on sterile aluminum foil in a laminar flow biosafety hood. Samples were inoculated by applying 100 μl of each of the culture cocktail in a drop-wise manner on the flesh surface. To ensure uniform distribution of the pathogens onto the muscle samples, a sterile L-shaped glass spreader was used. Additionally, to ensure a tight attachment of the pathogens, inoculated samples were air dried for 1 h inside a biosafety cabinet with the fan running at ambient temperature (22 °C). To determine the initial bacterial counts of each of the inoculated pathogens onto fish, chicken or beef; culture cocktails were enumerated by plating onto selective media (refer to Section 2.4).

2.3. Preparation and application of AC-EW water

The AC-EW was generated using 0.1% potassium chloride solution using a Super Oxide Series P electrolyzed water generator (Proton Lab., Portland, OR, USA). The solution collected from the anode chamber (AC-EW) was used in this study. AC-EW was generated within 1 h of the start of an experiment. The AC-EW pH was determined using a pH meter (Corning Instrument, NY, USA) and free available chlorine was measured using a chlorine test kit (detection limit of free chlorine, 5 mg/l; Bio-Lab Co., Decatur, GA, USA). The bactericidal activity of AC-EW against the three pathogens inoculated onto trout, chicken and beef muscle and on total mesophilic bacteria was determined by soaking inoculated samples in 500 ml of sterile distilled water (DW) or in 500 ml of AC-EW for selected treatment times (1, 3, 5 and 10 min) at 22 °C. Inoculated but untreated samples served as a control. After treatment for the assigned time intervals, samples were immediately transferred to a stomacher bag containing 50 ml of D/E Neutralizing Broth (Difco) and homogenized vigorously for 2 min with a Seward stomacher (400 Circulator, Seward, London, UK). One milliliter of homogenized sample was serially diluted in 9 ml of sterile BPW (Difco).

2.4. Bacterial enumeration

Surviving pathogens were enumerated by spread-plating 100 μl of stomached and 10-fold serially diluted samples onto a corresponding selective medium for each of the pathogens. The following media were used: Sorbitol MacConkey agar (SMAC; Difco) to enumerate *E. coli* O157:H7, Xylose Lysine Desoxycholate agar (XLD; Difco) to enumerate *S.* Typhimurium, and modified Oxford Agar Base (OAB; Difco) with antimicrobic supplement (Bacto Oxford Antimicrobic Supplement, Difco) to enumerate *L. monocytogenes*. SMAC and XLD plates were incubated at37 °C for 24 h and OAB was incubated at 37 °C for 48 h. Total mesophilic bacteria were enumerated after spread plating 100 μl onto duplicate plates of TSA (Difco) and incubating at 32 °C for 48 h.

2.5. Data analysis

The experiments were replicated independently three times and standard deviations were determined. Surviving bacterial counts were log transformed and data were analyzed with a computer software package (SAS Institute, Cary, NC) using analysis of variance and Fisher's least significant difference (LSD) test for mean separations ($P < 0.05$).

3. Results and discussion

The bactericidal activity of AC-EW (average pH = 2.30, free available chlorine concentration of 38 ± 2 mg/l) was examined against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in the muscle tested. As shown in Fig. 1, Fig. 2, and Fig. 3, AC-EW exhibited a considerable bactericidal activity against *E. coli* O157:H7 in fish, chicken and beef compared to the control (untreated samples) or compared to the samples treated with DW. About 1.0 and 1.5 logs reduction in *E. coli* O157:H7 was obtained in AC-EW after 5 and 10 min treatment time, respectively, compared to the control (Fig. 1). Also, a significant ($P < 0.05$) reduction (0.5–0.6 logs) of *E. coli* O157:H7 was elicited with AC-EW compared to the DW treatments after 5 and 10 min treatment times. The same pattern of inactivation was observed for chicken and beef (Figs. 2 and 3). Treatment of chicken and beef for 10 min using the AC-EW resulted in about 0.8–1.4 logs more reduction, respectively, compared to the same treatment time using DW and the same

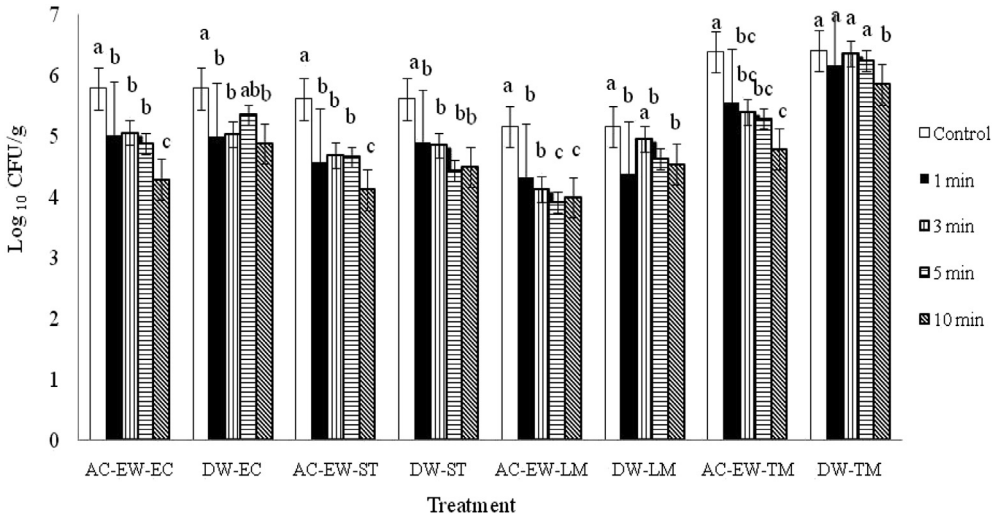

Fig. 1. Survivors of *Escherichia coli* O157:H7 (EC), *Salmonella* Typhimurium (ST), *Listeria moncocytogenes* (LM) and total mesophiles (TM) inoculated on fish surface after treatment with either acidic electrolyzed water (AC-EW) or sterile distilled water (DW) for different periods of times. The error bars indicate 95% confidence intervals. Control is inoculated but untreated samples. Bars with different lowercase letters are significantly different ($P < 0.05$).

treatment led to >1.5 logs reduction in *E. coli* O157:H7 compared to the control.

Figs. 1–3 show surviving populations of *S.* Typhimurium in trout, chicken and beef muscle after treatment for 0 (control), 1, 3, 5, and 10 min with either AC-EW or DW. A time-dependent inhibitory pattern was observed for the AC-EW against the pathogen. One minute treatment with AC-EW resulted in about 1 log reduction in *S.* Typhimurium inoculated onto fish compared to the control and the number of survivors decreased by about 1.5 logs after 10 min treatment time. In comparison, treatment with DW for the corresponding time resulted in about 1 log reduction in *S.* Typhimurium count. Likewise, *S.* Typhimurium was significantly ($P < 0.05$) decreased by approximately 1.5 logs in chicken after 10 min treatment with AC-EW compared to only 0.7 logs reduction with DW at same treatment time. In beef, the AC-EW diminished *S.* Typhimurium by about 1.4 logs compared to only 0.6 logs reduction in the DW after 10 min of treatment.

Treating trout muscle with AC-EW resulted in a gradual reduction in the *L. monocytogenes* count. Approximately 1.2 logs reduction was observed after 10 min treatment with AC-EW compared to the control and the reduction was significantly different ($P < 0.05$). Meanwhile, treating trout with DW resulted in an initial reduction in *L. monocytogenes* count, but longer treatment time did not result in further reduction in the count (Fig. 1). The decontaminative effect of AC-EW and sterile DW against *L. monocytogenes* in chicken is shown in Fig. 2. The AC-EW resulted in approximately 1.1 log reduction in *L. monocytogenes* count; however, DW elicited only 0.15 log reduction after 10 min treatment time. For beef, AC-EW led to about 1.3 logs reduction in *L. monocytogenes* count, while DW brought about 0.5 logs reduction after 10 min treatment time.

Figs. 1–3 present the total mesophilic counts in trout fish, chicken legs and beef meat after treatment with either AC-EW or DW. Treating of the food items for 10 min with AC-EW resulted in reductions that reached to more than 2 logs reduction in the total

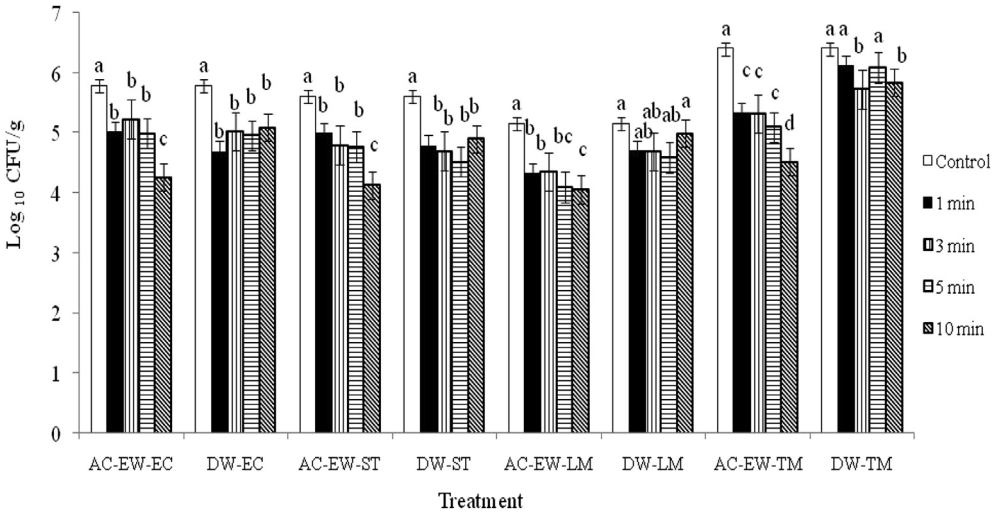

Fig. 2. Survivors of *Escherichia coli* O157:H7 (EC), *Salmonella* Typhimurium (ST), *Listeria moncocytogenes* (LM) and total mesophiles (TM) inoculated on chicken surface after treatment with either acidic electrolyzed water (AC-EW) or sterile distilled water (DW) for different periods of times. The error bars indicate 95% confidence intervals. Control is inoculated but untreated samples. Bars with different lowercase letters are significantly different ($P < 0.05$).

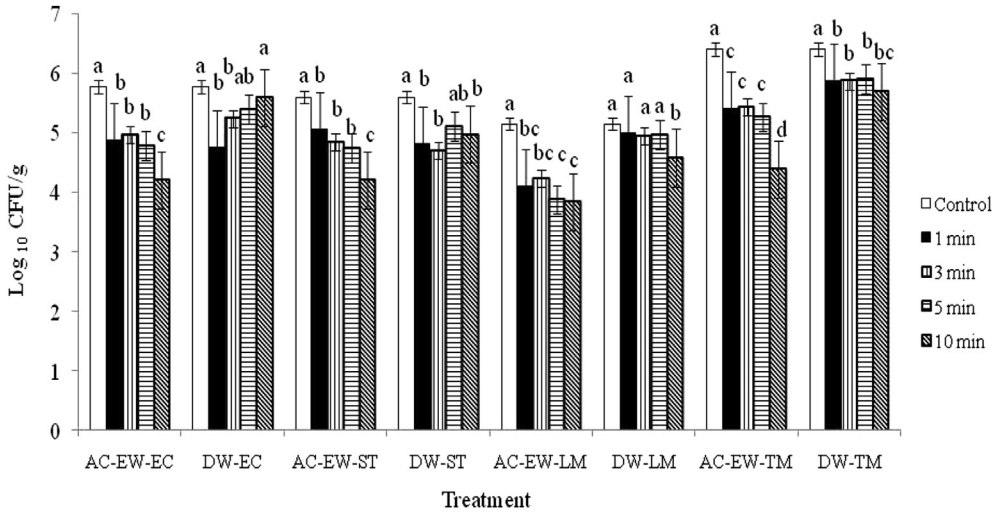

Fig. 3. Survivors of *Escherichia coli* O157:H7 (EC), *Salmonella* Typhimurium (ST), *Listeria moncocytogenes* (LM) and total mesophiles (TM) inoculated on beef surface after treatment with either acidic electrolyzed water (AC-EW) or sterile distilled water (DW) for different periods of times. The error bars indicate 95% confidence intervals. Control is inoculated but untreated samples. Bars with different lowercase letters are significantly different ($P < 0.05$).

mesophiles compared to not more than 0.7 logs reduction in the DW treatment for the same corresponding time period and the effect was significantly different in the three food items ($P < 0.05$).

AC-EW presented significant antibacterial activity against *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* and total mesophilic bacteria in trout, chicken, and beef muscles. Generally, the bactericidal capacity of the AC-EW increased with increasing treatment time. Generally, Gram-negative bacteria are more sensitive than Gram-positives to AC-EW disinfection (Jay, Loessner, & Golden, 2005) and this study confirms this. The efficacy of AC-EW was more pronounced against *E. coli* O157:H7 and *S.* Typhimurium compared to *L. monocytogenes*. Treatment of the inoculated food items used in the current study with DW resulted in reducing microbial count; however, this reduction is due to the physical removal of the pathogens cells off the food surfaces.

The presence of organic matter with AC-EW negatively impacts the effectiveness of AC-EW. For example, the addition of bovine serum to AC-EW solution resulted in increasing organic load and hence reduced the efficacy of AC-EW against *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* inoculated on the surfaces of green onions and tomatoes (Park, Alexander, Taylor, Costa, & Kang, 2009). Additionally, the disinfection of AC-EW was remarkably compromised by the presence of chicken serum with AC-EW solution and the number of the surviving cells of *L. monocytogenes* considerably increased (Ayebah, Hung, & Frank, 2006). Organic matter, especially protein, which is highly available in the products used in the current study; can react quickly with free available chlorine and reduces AC-EW bactericidal properties. One more factor that restricts the antimicrobial activity of AC-EW is that sanitizers can inflict stronger bactericidal activities on smoother surfaces rather than rough surfaces. Thereby, the irregular surfaces existing in fish, chicken and beef tested here, provides a safer haven for pathogens.

The oxidation reduction potential (ORP) of the AC-EW is relatively high. The ORP of a solution is an indicator of its ability to oxidize or reduce, with positive and higher ORP values correlated to greater oxidizing strength. An ORP of +200 to +800 mV is optimal for growth of aerobic microorganisms, whereas an optimum range of −30 to −550 mV is favored for growth of anaerobic microorganisms. For most facultative anaerobes the ORP in the range between +200 and −250 mV is needed for members of this category to grow normally (Jay et al., 2005). Since the ORP of AC-EW is greater than 1100 mV, the ORP is likely to play an influential role, in combination with low pH and free chlorine, in killing microorganisms. Several studies have indicated that available chlorine, including HOCl, hypochlorite ion (OCl-) and chlorine gas (Cl2), were the primary factors that accounted for the AC-EW bactericidal activity (Abadias, Usall, Oliveira, Alegre, & Vinas, 2008; Len, Hung, Erickson, & Kim, 2000; Xiong, Liu, Liu, & Li, 2010). In addition to free chlorine, reactive oxygen species (ROS) such as $\bullet OH^{-}$ and O_3 have been identified as bactericidal agents in AC-EW (Jeong, Kim, & Yoon, 2006). AC-EW inactivates bacteria by increasing membrane permeability and leakage of intracellular content as well as by decreasing dehydrogenase and nitrate reductase activities (Kiura et al., 2002; Zeng et al., 2010). However, the hydroxyl radicals play a minor role in the disinfection capability of AC-EW and the major role was due to the existing chlorine compounds (Hao et al., 2012).

The efficacy of AC-EW against *E. coli* O157:H7, *S.* Enteritidis and *L. monocytogenes* was explored in a model broth system. Regardless of the nature of the cell membrane (Gram positive or Gram negative), AC-EW was effective in reducing the microbial load in all the tested microbes, especially at higher temperatures (35 and 45 °C) and longer holding times (15 min) (Venkitanarayanan, Ezeike, Hung, & Doyle,1999a). In another study, the impact of using AC-EW to inactivate a cocktail mixture of five strains of *Salmonella* in alfalfa seeds and sprouts was investigated. The combination of AC-EW with sonication resulted in 3.3 logs reduction of the inoculated *Salmonella* population compared to the control (sterile deionized water) (Kim, Hung, Brackett, & Lin, 2003). AC-EW was distinctively effective in reducing *S.* Tyhimurium microbial load on the chicken surface and the effect persisted during the storage period of 7 days, where no *Salmonella* cells were detected at the end of the storage period. In comparison, other antimicrobial treatments such as trisodium phosphate, acetic acid and ozonated water were less effective. Yet, acetic acid resulted in undesirable changes in the visual quality of chicken (Fabrizio, Sharma, Demirci, & Cutter, 2002). In comparison, AC-EW did not influence the visual quality of the product.

Listeria is considered a common problem in seafood products (Al-Holy, Lin, & Rasco, 2005; Vongkamjan, Fuangpaiboon, Jirachotrapee, & Turner, 2015). Treatment of salmon fillet with

AC-EW resulted in approximately 0.50–1 log reduction in the numbers of *L. monocytogenes* Scot A inoculated on the surface of salmon fillet at 35 °C (Ozer & Demirci, 2006). In addition, AC-EW demonstrated an effective disinfection capability of food contact surfaces and reducing the potential of cross-contamination. Treatment of plastic kitchen cutting boards that have been inoculated with high counts of *L. monocytogenes* or *E. coli* O157:H7 with AC-EW reduced populations of the pathogens to undetectable levels on the surfaces or in the AC-EW dipping solution after soaking (Venkitanarayanan, Ezeike, Hung, & Doyle, 1999b). AC-EW reduced *L. monocytogenes* contamination on gloves and thereby minimized the possibility of transferring *L. monocytogenes* from gloves to RTE seafoods in seafood processing plants (Liu & Su, 2006).

4. Conclusions

The use of AC-EW to decontaminate trout, chicken and beef muscles was effective in reducing pathogenic microorganisms such as *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes.* While chlorine dips are usually used during processing of meat and poultry to reduce pathogens risk, AC-EW could serve as potential alternative to eliminate or substantially decrease bacterial population on fish, chicken and beef. In the current study, no complete elimination of the inoculated pathogens was obtained after treatment with AC-EW and this is possibly due to the presence of organic matter and blood residue in the dipping solution that decreased free available chlorine. Hence, to have a more effective utilization of the AC-EW by the food processing industry, it is suggested that a pre-treatment of the different meat products with clean water to remove blood and any residual organic matter debris prior to AC-EW application. This may result in more efficient bactericidal effect of AC-EW. It is also recommended to use AC-EW for long time intervals and to change the AC-EW dipping solutions frequently to impart more effective decontamination.

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