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Near Neutral Electrolyzed Water and Peroxyacetic Acid and Their Effect on the Survival of *Escherichia coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* Inoculated on Poultry Meat

ABSTRACT

This study was conducted to evaluate the antimicrobial efficacy of near-neutral electrolyzed water (NEW) and peroxyacetic acid (PAA) alone and in combination for reducing the foodborne pathogens *Salmonella* Typhimurium, *Escherichia coli*, and *Listeria monocytogenes* in pure culture and fresh chicken meat. The NEW treatments resulted in 100% inactivation of these organisms in pure culture at concentrations of 50, 100, and 200 µg/mL and 2 min of contact time at room temperature. The PAA treatments at concentrations of 100 and 200 µg/mL resulted in 100% inactivation of the tested pathogens. The combination of NEW and PAA had a greater bactericidal effect than did each individual treatment. The inoculated chicken meat samples were dipped for 10 min in each treatment solutions (100 and 200 µg/mL NEW, 200 and 400 µg/mL PAA, 100 µg/mL NEW + 200 µg/mL PAA) at room temperature. Samples dipped in water were used as a control. The greatest reduction was achieved with the combined treatment, which significantly ($P < 0.05$) reduced total cells and healthy cells of *Salmonella* Typhimurium, *E. coli*, and *L. monocytogenes* by 2.79 and 3.01, 2.63 and 2.75, and 1.47 and 1.99 log CFU/g, respectively. The findings of this study indicate that a combined treatment with NEW and PAA has potential as a novel antimicrobial agent to improve the microbial safety of fresh chicken meat.

INTRODUCTION

The consumption of poultry meat has been increasing over the last decades due to its high nutritional value, low fat content, and low cost of production *(10)*. Contamination of poultry meat with foodborne pathogens is an important human health issue, and food poisoning can result from mishandling of raw meat, careless processing, and storage *(22, 37)*. Consumption of contaminated poultry products is a major cause of foodborne disease outbreaks *(26)*. In some countries, decontamination procedures include chemical, physical, and biological treatments that are applied to reduce the prevalence of pathogens and microbial loads on poultry carcasses. Various chemical treatments have been developed in the meat and poultry industries to kill or inhibit microor-

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ganisms *(4, 11)*. The disruption of physiological processes, cellular membranes, or other cellular constituents are the main antimicrobial activities of chemical compounds *(34)*. However, most of these compounds have limitations, which include chemical residues, discoloration of meat, negative health effects on food handlers, corrosion of machinery, environmental pollution, and high costs *(40, 42)*. Red meat and poultry decontamination technologies have been applied for years in the United States *(6, 24)*. Electrolyzed water, a safe sanitizer, has been increasingly used in the food industry to reduce and control of microorganism on foods and food contact surfaces *(23)*. Near-neutral electrolyzed water (NEW), with a pH of 6.0 to 6.8, with an oxidation-reduction potential (ORP) of 800 to 900 mV, and containing 95% hypochlorous acid, 5% hypochlorite ion, and trace amounts of Cl_2, is generated by passing an aqueous salt solution (ca. 1% NaCl) through a nonmembrane electrochemical cell *(21, 33)*. Hypochlorous acid is the most active of the chlorine compounds and has high bactericidal activity *(36)*. In several studies, the efficacy of NEW has been evaluated for reduction of bacterial population in fresh meat *(12, 35, 42, 43)*, eggshells *(44)*, seafoods *(28)*, and vegetables *(1)*. If NEW were to be combined with other chemical disinfectants such as organic acids, its antimicrobial efficacy might increase. Peroxyacetic acid (PAA) is produced from the reaction of acetic acid with hydrogen peroxide *(31)*. Its disinfection efficiency is due to the strong oxidizing potential, which can result in disruption of the cell membrane and blockage of enzymatic and transport systems in microorganisms *(32, 46)*. Application of PAA for meat decontamination has been studied *(3, 30)*. The aim of the present study was to evaluate the efficacy of individual treatments with NEW and PAA and their combined effect on reduction of the foodborne pathogens *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* in vitro and in fresh chicken meat.

MATERIALS AND METHODS

Bacterial cultures

The bacterial strains used in this study were *L. monocytogenes* (PTCC 1297), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), and *E. coli* (ATCC 35218) (School of Veterinary Medicine, Shiraz University, Shiraz, Iran). To confirm *L. monocytogenes*, cultures were streaked on PALCAM (polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol) agar (Merck, Darmstadt, Germany) supplemented with nalidixic acid (50 µg/mL), amphotericin B (10 µg/mL), and acriflavin (30 µg/mL). For easy differentiation of *E. coli* and *Salmonella* Typhimurium from background bacteria of chicken breast meat, these pathogens were cultured to be resistant to nalidixic acid. Nonresistant cultures were inoculated into 100 mL of trypticase soy broth (TSB; Merck) and incubated at 37°C for 24 h, 100 mL of TSB containing 200 µg/mL nalidixic acid (1451000, Sigma-Aldrich, St. Louis, MO) was added, and this culture was subsequently incubated at 37°C for a further 24 h. A 0.1-mL aliquot of this culture was spread on the surface of a trypticase soy agar (TSA; Merck) plate containing 100 µg/mL nalidixic acid and incubated at 37°C for 24 h. A single colony was selected and restreaked onto another TSA plate to confirm resistance to nalidixic acid *(45)*. Each strain of pathogens was separately transferred into TSB with nalidixic acid (50 µg/mL) and incubated at 37°C for 24 h, and 10 mL of each culture was then centrifuged at 4,000 × g for 10 min. The supernatant was discarded, the cell pellet was washed twice with normal saline solution, and the final cell pellet was resuspended with the same solution. The density of the suspensions was compared to the 0.5 McFarland turbidity standard and adjusted to 1.5×10^8 CFU/mL.

Preparation of disinfectant solutions

NEW with a pH of 6.8, an ORP of 830 ± 5 mV, and available free chlorine (AFC) concentration of 800 µg/mL was obtained from Khosro Medisa Teb Co. (Envirolyte, Tehran, Iran). NEW was produced by electrolysis of a salt solution (ca. 1% NaCl in tap water) with an electrolysis device. Solutions with various AFC concentrations were prepared with sterile distilled water. PAA was obtained from Behban Shimi Co. (Percidine 15%, Golestan, Iran), and various concentrations were prepared with sterile distilled water (v/v).

MIC determination for NEW and PAA

The AFC concentration of NEW was measured with a chlorine test kit (Karizab Co., Tehran, Iran) immediately before use. The original NEW was then diluted with sterile distilled water to obtain AFC concentrations of 3.13, 6.25, 12.5, 25, 50, 100, and 200 µg/mL. A 0.1-mL aliquot of each bacterial suspension was combined with 9.9 mL of each concentration of NEW and vortexed for 5 s and then incubated at room temperature. The final level of each test pathogen per treatment was approximately 10^6 CFU/mL. Sterile distilled water was used as a control for each experiment. To evaluate the effect of treatment time on the reduction of bacterial cells, treatment tubes were sampled at 0, 1, 2, 3, and 4 min after inoculation. The results indicated that no additional reduction was observed after 2 min; therefore, 2 min of contact time at room temperature was chosen for the experiment. Following 2-min treatments, 0.1 mL of each sample was transferred to 9.9 mL of sterile neutralizing buffer (0.5% sodium thiosulphate + 0.03 M phosphate buffer solution), and the tubes were shaken. After neutralization, 0.1 mL of each treatment was plated on TSA with nalidixic acid and incubated at 37°C for 24 to 48 h. The colonies were enumerated with the plate count method. When necessary, 10-fold serial dilutions were made from samples before plating. Using this method, the presence of low numbers of surviving cells may not be detected. In parallel, a 100-µL aliquot of each treatment was added to 10

mL of TSB with nalidixic acid and incubated at 37°C for 24 to 48 h. Then, 100 µL of enriched broth was spread onto TSA plates with nalidixic acid and incubated at 37°C for 24 to 48 h. The plates were then checked for bacterial colonies *(19)*. The antibacterial effect of PAA at 3.13, 6.25, 12.5, 25, 50, 100, and 200 µg/mL was also determined using the method described above.

To evaluate the antimicrobial interaction and any synergism between NEW and PAA, 3.13, 6.25, 12.5, 25 and 50 µg/mL concentrations of each disinfectant were prepared, and the same concentrations were mixed in equal proportions and tested according to the previous method.

Sample preparation

Whole fresh chicken breasts with skin were obtained from a local poultry processing plant. The breasts were transported in ice, stored at 4°C, and used for treatments in < 2 h. The average weight of each chicken breast sample was 400 ± 30 g.

Inoculation of pathogens onto chicken meat samples

Chicken breasts were randomly divided into three batches, each containing 54 samples. Samples in each batch were dipped for 10 min into separate bacterial suspensions of *L. monocytogenes*, nalidixic acid-resistant *Salmonella* Typhimurium, and nalidixic acid-resistant *E. coli* at 10^7 CFU/mL in sterile normal saline. The ratio of chicken meat sample to bacterial suspension volume was 1:1 (w/v). For draining and bacterial attachment, the inoculated meat samples were placed on a grid plate inside a laminar flow hood at room temperature for 30 min *(2)*. Inoculated untreated meat samples were tested to determine the levels of the pathogenic bacteria.

Decontamination treatment

Inoculated chicken meat samples in each batch were randomly divided into six groups, each containing nine samples. Five treatments of NEW at 100 and 200 µg/mL, PAA at 200 and 400 µg/mL, and NEW 100 µg/mL + PAA 200 µg/mL were prepared in a sterile chilled water container (4°C). Sterilized chilled water was used to dip the control samples. Inoculated chicken meat samples with pathogens were dipped in treatment solutions for 10 min *(7, 16, 42)*. The ratio of chicken meat to treatment solution was 1:1 (w/v). Following treatments, samples were drained for 10 min at room temperature, packed individually into sterile reclosable zippered polyethylene bags, and stored at 4 ± 0.5°C.

Noninoculated untreated chicken meat samples were tested for background contamination with *L. monocytogenes*, nalidixic acid-resistant *Salmonella* Typhimurium, and nalidixic acid-resistant *E. coli*.

Microbiological analysis

Treated inoculated chicken meat samples were tested immediately after treatment and on days 2 and 4 of storage. Samples were transferred to sterile stomacher bags and weighed, and sterilized 0.1% (w/v) buffered peptone water (Merck) was added in at nine times the weight of each sample. To ensure that the samples were washed well, bags were hand massaged for 1 min. The rinsate was then collected, ten-fold serial dilutions were made in sterile 0.1% buffered peptone water. PALCAM agar, xylose lysine deoxycholate (XLD) agar, and MacConkey agar, each supplemented with nalidixic acid, were used to count healthy cells of *L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli*, respectively. A 100-µL aliquot of each sample or appropriate diluent was surface plated onto the appropriate medium and incubated at 37°C for 24 to 48 h.

After disinfectant treatment, some microorganisms may be only injured rather than killed *(48)*. For enumeration of injured bacterial cells, the overlay method was used *(27)*. For each sample or appropriate diluent, 100 µL was surface plated onto TSA and incubated at 37°C for 2 to 3 h to allow damaged bacterial cells to resuscitate, then agar plates overlaid with 7 to 12 mL of the appropriate selective medium. After the overlay solidified, the plates were incubated at 37°C for 24 to 48 h. The difference in counts between selective (PALCAM agar, XLD agar, and MacConkey agar) and nonselective (overlay method) media was considered the count of injured cells. The percentage of injured bacterial cells was calculated by using the formula *(20)*

$$\% \text{ injured cells} = \frac{\text{CFUnonselective} - \text{CFUselective}}{\text{CFUnonselective}} \times 100$$

Determination of residual chlorine

For the residue experiment, chicken breast meat samples were dipped in 100 µg/mL NEW, 200 µg/mL NEW, the combination treatment of 100 µg/mL NEW plus 200 µg/mL PAA, and sterile water (control) and then placed in a grid plate inside a laminar flow hood at room temperature for 4 h. After drying, the meat samples were rinsed in distilled water for 10 min at a 1:2 (w/v) ratio of samples:distilled water. The rinse water was used for determination of residual chlorine with a chlorine test kit (*N,N*-diethyl-*p*-phenylenediamine method; Karizab Co., Tehran, Iran).

Statistical analysis

The experiment was replicated three times. The data were analyzed with an analysis of variance in SPSS ver. 21 (SPSS Inc., IBM, Armonk, NY). Differences between treatments were assessed with Duncan's test and were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

MIC determination for NEW and PAA

The bactericidal activity of NEW against pure cultures of *E. coli*, *Salmonella* Typhimurium, and *L. monocytogenes* during the 2 min of contact time is shown in *Figure 1*. At time 0, the initial populations of *E. coli*, *Salmonella* Typhimurium,

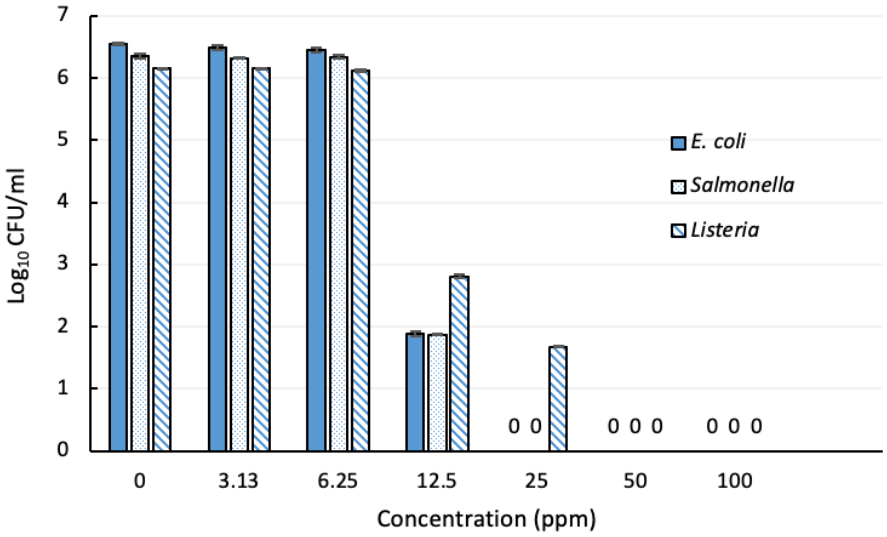

FIGURE 1. Effect of different concentrations of near-neutral electrolyzed water (NEW) on survival of *E. coli*, *S.* Typhimurium and *L. monocytogenes* after two minutes of exposure at 25°C. Zero values indicate no detectable survivors by a direct plating procedure and enrichment experiment.

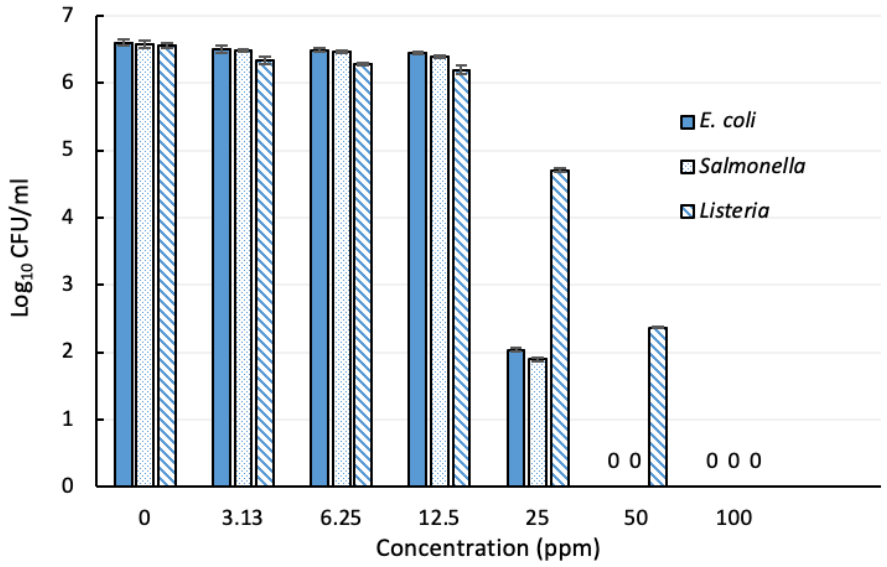

FIGURE 2. Effect of different concentrations of peroxyacetic acid (PAA) on survival of *E. coli*, *S.* Typhimurium and *L. monocytogenes* after two minutes of exposure at 25°C. Zero values indicate no detectable survivors by a direct plating procedure and enrichment experiment.

and *L. monocytogenes* were 6.45, 6.30, and 6.12 log CFU/mL, respectively. *Listeria* was more resistant than *E. coli* and *Salmonella*; the AFC concentration of 25 µg/mL completely eliminated *E. coli* and *Salmonella* Typhimurium but only reduced *L. monocytogenes* by 4.44 log CFU/mL. All microorganisms were completely eliminated when exposed to NEW at an AFC of 50 µg/mL. Survivors were not detected by direct plating or after enrichment. Guentzel et al. *(19)* treated pure cultures of *E. coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *L. monocytogenes*, and *Enterococcus faecalis* with NEW (pH 6.3 to 6.5; ORP 800 to 900 mV; 20, 50, 100, and 120 ppm of total residual chlorine) and obtained 100% elimination of these organisms after 10 min. However, in another study pure cultures of *Salmonella* Enteritidis were completely inactivated by NEW with an available chlorine concentration of >4 µg/mL, pH of 6.3 to 6.5, and ORP of 251.0 to 297.2 mV at 4, 20, and 45°C for 2 min *(5)*. Several researchers have also reported complete elimination of *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* after treatment with acidic electrolyzed water *(47)*.

PAA at 50 µg/mL effectively eliminated *E. coli* and *Salmonella* Typhimurium (survivors not detected by direct plating and culture enrichment), but the reduction of *L. monocytogenes* was only 3.75 log CFU/mL *(Fig. 2)*. At 25 and 12.5 µg/mL, NEW was more effective than PAA ($P < 0.05$).

The bactericidal effect of the combination of PAA and NEW against the three pathogens was higher than that of each disinfectant alone. The lowest concentration in the combined treatment required for complete inactivation of *E. coli* and *Salmonella* Typhimurium was 12.5 µg/mL, and the lowest for complete inactivation of *L. monocytogenes* was 25 µg/mL *(Fig. 3)*. In combination, lower concentrations of

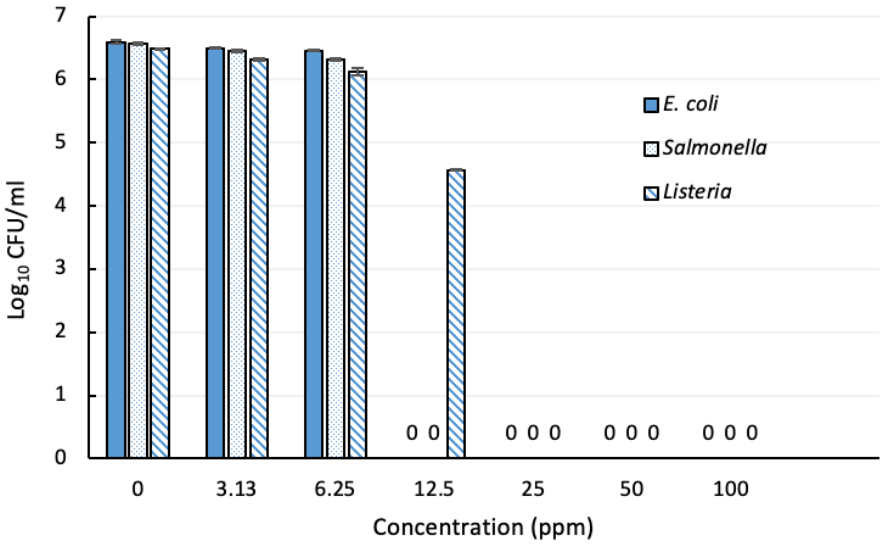

FIGURE 3. Effect of different concentrations of combination of NEW and PAA on survival of *E. coli*, *S.* Typhimurium and *L. monocytogenes* after two minutes of exposure at 25°C. Zero values indicate no detectable survivors by a direct plating procedure and enrichment experiment.

NEW and PAA were required to eliminate these pathogens, and significantly greater bactericidal activity was achieved by the two disinfectants combined than was achieved by each disinfectant alone.

Antimicrobial effect of treatments in fresh chicken meat

L. monocytogenes and nalidixic acid-resistant *Salmonella* Typhimurium and *E. coli* were not detected in noninoculated untreated chicken meat samples. The initial mean (±SD) populations of *E. coli*, *Salmonella* Typhimurium, and *L. monocytogenes* in chicken meat samples after inoculation were 6.92 ± 0.30, 6.78 ± 0.42, and 6.12 ± 0.32 log CFU/g, respectively.

NEW and PAA had destructive effects on these pathogens in chicken meat. However, reductions of the tested pathogens were lower in chicken meat than in the in vitro assay possibly because of the presence of organic materials and their protective effects on the pathogens.

Mean microbial reduction of healthy and total pathogen cells and the percentages of injured bacterial cells during 4 days of storage at 4°C are listed in *Tables 1 and 2*, respectively. A significant pathogen reduction was observed immediately after individual and combined treatments when compared with the water control, and further reduction of all tested pathogens was observed during 4 days of storage ($P < 0.05$). The reduction of pathogenic bacteria continued until day 4 of storage at 4°C probably because of the continued presence of strong antimicrobial compounds in NEW (hypochlorous acid and hypochlorite ion) and PAA (oxidizing compounds and undissociated acids) *(20, 32, 44)*.

L. monocytogenes was more resistant to all chemical solutions than were *E. coli* and *Salmonella* Typhimurium. NEW with AFC concentrations of 100 and 200 µg/mL reduced total cells of *E. coli*, *Salmonella* Typhimurium, and *L. monocytogenes* by 0.68 to 2.38 log CFU/g. The reduction achieved with 200 µg/mL NEW was significantly higher than that with 100 µg/mL for all three pathogens. Several researchers have assessed the effect of electrolyzed water on pork, chicken, and other meat samples for controlling *E. coli*, *L. monocytogenes, Campylobacter coli, Campylobacter jejuni,* and *Salmonella* Typhimurium and obtained reductions of 0.48 to 3.0 log CFU/g *(16, 17, 29, 41)*. Guentzel et al. *(19)* reported that a 10-min dip of spinach leaves inoculated with *E. coli*, *Salmonella* Typhimurium, *S. aureus, L. monocytogenes*, and *E. faecalis* in NEW at 100 and 120 ppm of total residual chlorine reduced all pathogens by 4.0 to 5.0 log CFU/mL. The efficacy of slightly acidic low concentration electrolyzed water (SlALcEW) and strong acidic electrolyzed water (StAEW) on inoculated fresh chicken breast meat against *E. coli* and *L. monocytogenes* was reported by Rahman et al. *(42)*. SlALcEW and StAEW had similar antimicrobial effects, but SlALcEW was preferred because of its semineutral pH and low chlorine concentration. The reductions found in our study are lower than those previously reported. The differences observed are related to differences in the type of sample, dipping time, bacterial strain, level of bacterial attachment, and application method *(7, 9, 18, 39)*.

Concentrations of 200 and 400 µg/mL PAA resulted in 0.99- to2.72-log reductions of total cells of the tested microorganisms. Similar to NEW, the reduction by 400 µg/mL PAA was significantly higher than that from 200 µg/mL ($P < 0.05$). The antimicrobial effect of PAA is a result of its high ORP, which disrupts cell membrane permeability and alters protein synthesis. The second antimicrobial mechanism is acidification of the meat surface and penetration of the bacterial cell by undissociated acids *(15)*.

Injured bacterial cells were detected in all treatment groups *(Table 2)*. Detection of injured cells is important because these cells may be able to repair themselves and become functionally normal under favorable conditions *(25)*. The production of injured cells and their reduction during 4 days of storage are indicative of the bacteriostatic and bactericidal effects of the

TABLE 1. Reduction of inoculated pathogens in fresh chicken meat treated with different concentrations of peroxyacetic acid (PAA) and near-neutral electrolyzed water (NEW) during 4 days of storage at 4°C

		Bacterial reduction (mean ± SD, log CFU/g chicken meat)					
		E. coli		*Salmonella* Typhimurium		*L. monocytogenes*	
Storage Time	Treatments	Total cells	Healthy cells	Total cells	Healthy cells	Total cells	Healthy cells
10 min	Control	1.04 ± 0.03^{Aa}	1.16 ± 0.04^{Aa}	1.00 ± 0.03^{Aa}	1.19 ± 0.09^{Aa}	0.62 ± 0.05^{Aa}	0.89 ± 0.03^{Ba}
	PAA200	1.72 ± 0.02^{Ac}	1.84 ± 0.02^{Bc}	1.65 ± 0.03^{Ac}	1.98 ± 0.02^{Bb}	0.99 ± 0.03^{Ac}	1.31 ± 0.01^{Bc}
	PAA400	1.89 ± 0.06^{Ae}	1.98 ± 0.02^{Ad}	2.10 ± 0.03^{Ae}	2.44 ± 0.08^{Ad}	1.30 ± 0.04^{Ae}	1.68 ± 0.02^{Be}
	NEW100	1.55 ± 0.02^{Ab}	1.77 ± 0.02^{Bb}	1.57 ± 0.04^{Ab}	1.92 ± 0.05^{Bb}	0.68 ± 0.05^{Ab}	1.10 ± 0.05^{Bb}
	NEW200	1.82 ± 0.01^{Ad}	2.00 ± 0.02^{Bd}	1.74 ± 0.05^{Ad}	2.30 ± 0.06^{Bc}	1.14 ± 0.02^{Ad}	1.45 ± 0.04^{Bd}
	NEW100+ PAA200	1.96 ± 0.06^{Af}	2.19 ± 0.05^{Af}	2.12 ± 0.02^{Ae}	2.58 ± 0.05^{Be}	1.32 ± 0.02^{Ae}	1.74 ± 0.03^{Bf}
2 days	Control	0.85 ± 0.05^{Aa}	1.02 ± 0.01^{Aa}	0.87 ± 0.01^{Aa}	1.01 ± 0.06^{Aa}	0.47 ± 0.05^{Aa}	0.8 ± 0.06^{Ba}
	PAA200	1.82 ± 0.02^{Ac}	2.14 ± 0.05^{Bc}	2.02 ± 0.02^{Ac}	2.44 ± 0.06^{Bc}	1.09 ± 0.05^{Ac}	1.39 ± 0.02^{Bc}
	PAA400	2.03 ± 0.02^{Ad}	2.37 ± 0.06^{Be}	2.37 ± 0.06^{Ad}	2.58 ± 0.05^{Ad}	1.34 ± 0.06^{Ae}	1.75 ± 0.02^{Ae}
	NEW100	1.72 ± 0.05^{Ab}	2.02 ± 0.01^{Bb}	1.66 ± 0.02^{Ab}	2.28 ± 0.13^{Bb}	0.81 ± 0.02^{Ab}	1.21 ± 0.06^{Bb}
	NEW200	2.17 ± 0.05^{Ae}	2.29 ± 0.02^{Ad}	2.05 ± 0.05^{Ac}	2.55 ± 0.03^{Bcd}	1.19 ± 0.02^{Ad}	1.69 ± 0.03^{Bd}
	NEW100+ PAA200	2.37 ± 0.11^{Af}	2.46 ± 0.01^{Af}	2.41 ± 0.02^{Ad}	2.73 ± 0.05^{Be}	1.41 ± 0.04^{Af}	1.87 ± 0.03^{Bf}
4 days	Control	0.71 ± 0.06^{Aa}	0.97 ± 0.03^{Ba}	0.68 ± 0.01^{Aa}	0.79 ± 0.02^{Aa}	0.41 ± 0.01^{Aa}	0.71 ± 0.03^{Ba}
	PAA200	2.07 ± 0.03^{Ac}	2.48 ± 0.01^{Bc}	2.16 ± 0.05^{Ac}	2.65 ± 0.03^{Bc}	1.15 ± 0.04^{Ac}	1.43 ± 0.01^{Bc}
	PAA400	2.27 ± 0.05^{Ad}	2.68 ± 0.04^{Be}	2.72 ± 0.05^{Ae}	2.96 ± 0.05^{Ae}	1.38 ± 0.02^{Ae}	1.80 ± 0.06^{Bd}
	NEW100	1.84 ± 0.05^{Ab}	2.05 ± 0.02^{Bb}	1.86 ± 0.06^{Ab}	2.53 ± 0.09^{Bb}	0.99 ± 0.02^{Ab}	1.31 ± 0.02^{Bb}
	NEW200	2.38 ± 0.04^{Ae}	2.59 ± 0.03^{Bd}	2.29 ± 0.01^{Ad}	2.75 ± 0.05^{Bd}	1.30 ± 0.05^{Ad}	1.87 ± 0.03^{Be}
	NEW100+ PAA200	2.63 ± 0.05^{Af}	2.75 ± 0.04^{Af}	2.79 ± 0.01^{Af}	3.01 ± 0.06^{Ae}	1.47 ± 0.03^{Af}	1.99 ± 0.02^{Bf}

PAA200 and PAA400, PAA at 200 and 400 µg/mL; NEW100 and NEW200, NEW at 100 and 200 µg/mL); NEW100 + PAA200, NEW at 100 µg/mL + PAA at 200 µg/mL. Different lowercase letters indicate a significant difference between treatment groups within each storage day ($P < 0.05$). Different uppercase letters indicate a significant difference between the values of total cells and healthy cells within each treatment ($P < 0.05$).

treatments. The reduction of healthy cells was greater than that of the total cells for each pathogen, but in the some the differences were not significant *(Table 2)*.

In our study, the greatest reduction was achieved with a combination of NEW and PAA compared with all individual treatments and the control ($P < 0.05$). With this combination treatment, maximum reductions during 4 days of storage for *Salmonella* Typhimurium, *E. coli*, and *L. monocytogenes* were 2.79 ± 0.01, 2.63 ± 0.05, and 1.47 ± 0.03 log CFU/g, respectively, for total cells and 3.01 ± 0.06, 2.75 ± 0.04, and 1.99 ± 0.02, respectively, for healthy cells. The second most effective compound was 200 µg/mL NEW for *E. coli* and 400 µg/mL PAA for *Salmonella* Typhimurium and *L. monocytogenes*. The enhanced antimicrobial effect of the combined NEW plus PAA treatment revealed the synergistic properties of these disinfectants. When PAA was added to NEW, the pH and AFC of the combined solution were reduced, but the ORP increased. These factors are responsible for the higher bactericidal activity of the combined treatment, even though the AFC was reduced *(35)*. McPherson *(38)* and Carlson *(8)* stated that the ORP value of the solution was more important than the concentration of free or total chlorine. Park et al. *(41)* reported that the high ORP of electrolyzed water could cause modification of metabolic fluxes and ATP production, probably due to the change in the electron flow in the cells. Electron microscopy and fluorescent measurements indicated that the ORP affects *E. coli* O157:H7 by damaging the outer and inner membranes *(33)*.

TABLE 2. Percentage of injured cells of three pathogenic bacteria in fresh chicken meat after treatment with different concentrations of peroxyacetic acid (PAA) and near-neutral electrolyzed water (NEW) during 4 days of storage at 4°C

		Injured cells (%)		
Storage Time	Treatment	*E. coli*	*Salmonella* Typhimurium	*L. monocytogenes*
10 min	Control	23.5[a]	34.66[a]	47.0[a]
	PAA200	23.0[a]	52.85[b]	52.0[ab]
	PAA400	18.8[a]	52.71[b]	57.6[bc]
	NEW100	38.3[b]	54.83[b]	59.4[d]
	NEW200	35.9[b]	72.54[c]	51.0[a]
	NEW100 + PAA200	41.0[b]	64.72[bc]	61.7[d]
2 days	Control	31.8[ab]	26.58[a]	54.8[ab]
	PAA200	52.4[c]	61.46[bc]	49.8[a]
	PAA400	53.9[c]	37.75[a]	62.2[cd]
	NEW100	50.3[bc]	75[d]	60.1[bc]
	NEW200	22.2[a]	68[cd]	68.8[d]
	NEW100 + PAA200	22.3[a]	51.73[b]	65.2[cd]
4 days	Control	44.0[bc]	22.2[a]	33.7[a]
	PAA200	60.7[c]	67.83[c]	47.6[b]
	PAA400	61.0[c]	42.14[b]	62.2[c]
	NEW100	37.8[ab]	80[d]	52.5[b]
	NEW200	38.8[ab]	65.24[c]	71.6[d]
	NEW100 + PAA200	25.3[a]	38.55[b]	64.5[cd]

PAA200 and PAA400, PAA at 200 and 400 µg/mL; NEW100 and NEW200, NEW at 100 and 200 µg/mL); NEW100 + PAA200, NEW at 100 µg/mL + PAA at 200 µg/mL. Different letters indicate a significant difference between treatment groups within each storage day ($P < 0.05$).

Chlorine and PAA residues

Measurement of the residual chlorine on the meat samples revealed that the 100 µg/mL NEW, combined treatment, and control groups had no residues, although NEW at 200 µg/mL left 0.3 µg/mL residual chlorine on meat samples, which was acceptable and considered safe for consumption. A maximum residue limit of 0.7 mg/L was stipulated by the European Food Safety Authority (EFSA) *(14)* in the European Union for all foodstuffs and drinking water.

In this study, the residual PAA was not evaluated because previous research has indicated that PAA is highly reactive and, when used in the presence of organic compounds, dissociates very rapidly and breaks down to acetic acid and water. According to the EFSA *(13)*, the residues of peroxyacids and hydrogen peroxide in chicken carcasses after dipping for 60 min in PAA solution (200 mg/L) were below the detection limit of 1 mg/L; thus, it is not necessary to subsequently remove the PAA solution from the poultry carcasses or poultry meat.

CONCLUSION

The results of the present study revealed that treating fresh chicken meat with NEW and PAA effectively reduced populations of *Salmonella* Typhimurium, *E. coli*, and *L. monocytogenes* immediately after the treatment and during storage at 4°C. Combined treatment with NEW and PAA significantly reduced populations of inoculated foodborne pathogens compared with NEW and PAA treatments alone. This combined treatment is a beneficial decontamination method and can improve the microbiological safety of chicken meat. The effectiveness of combined NEW plus PAA on the microbiological shelf life and some meat quality variables such as pH, lipid oxidation, and color is being evaluated. Further study is needed to evaluate the efficacy

of NEW plus PAA or other organic acids with different treatment methods and dipping times in commercial slaughter plants and in other meat products.

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