Antibacterial Efficacy of Electrochemically Activated Solution for Poultry Spraying and Chilling

Z. Yang, Y. Li, and M. F. Slavik

ABSTRACT

An electrochemically activated solution (EAS) was evaluated for its antibacterial efficacy against *Salmonella typhimurium* on chicken carcasses during inside/outside (I/O) birdwasher spraying at 20°C and 413 kPa for 17s and chilling at 4°C for 45 min in a pilot plant. The EAS with 50 ppm of oxidants in terms of free chlorine (*Cl*) in the I/O spray reduced *Salmonella* on carcasses by 1.39 $\log_{10}$ CFU/carcass, whereas tap water and a 50 ppm (*Cl*) hypochlorite solution reduced *Salmonella* by 0.86 and 0.87 $\log_{10}$ CFU/carcass, respectively. Further chilling using iced EAS (50 ppm of *Cl*) did not reduce *Salmonella* on carcasses but eliminated *Salmonella* in the chiller water.

Key Words: antibacterial, electrochemically activated, spraying, chilling, chicken carcasses.

INTRODUCTION

CHLORINATION OF WATER IN SPRAY WASHERS OR CHILLERS HAS been used to reduce microbial contamination and cross-contamination of chicken carcasses in poultry processing plants (Sanders and Blackshear, 1971). Most studies have shown that water with a chlorine concentration from 5 to 200 ppm did not reduce *Salmonella* on chicken carcasses by more than 1 log CFU/carcass but prevented cross-contamination (Thomson et al., 1976; Lillard, 1980; Tsai et al., 1991; James et al., 1992). Greater reduction did not occur probably because of the abundance of organic material and nitrogenous compounds associated with chicken carcasses and consequent reaction to inactive forms of chlorine (Gelinas and Goulet, 1983).

To improve the bactericidal effectiveness of chlorinated water, another chlorinated compound, chlorine dioxide (ClO_2), has been used. Several studies have been reported on the use of ClO_2 to reduce bacterial populations in poultry chiller water and on chicken carcasses (Lillard, 1980; Thiessen et al., 1984; Villarreal et al., 1990). The mechanism of bactericidal action of ClO_2 involves loss of cell membrane permeability control with nonspecific oxidative damage to the outer membrane and consequent destruction of the transmembrane ionic gradient (Berg et al., 1986). Chlorine dioxide has more effective bactericidal activity than chlorine in the presence of organic materials (Lillard, 1980).

As an alternative to chlorine forms and other oxidants, electrochemically activated solutions (EAS) containing a mixture of chlorine, peroxide and chlorine oxides have been effective in destroying a wide variety of microorganisms (Sato et. al., 1989; Patermarakis and Fountoukidis, 1990). The EAS may have potential as disinfectants in medicine, agriculture and industry (Vineyard, 1997). However, EAS has not been evaluated for reducing bacterial contamination on poultry and meat products. The objective of this study was to determine the effect of an EAS generated by a specific electrochemical reactor (Bakhir and Zadorozhny, 1997; Bakhir et al., 1997) on reducing *Salmonella* on chicken carcasses during inside/outside birdwasher spraying and during chilling processes in a poultry processing pilot plant.

Authors Yang and Li are affiliated with the Dept. of Biological & Agricultural Engineering and author Slavik is affiliated with the Dept. of Poultry Science, Univ. of Arkansas, Fayetteville, AR 72701. Address inquiries to Dr. Yanbin Li.

MATERIALS & METHODS

Electrochemically activated solution (EAS)

Electrochemically activated solution (EAS) was generated by an electrochemical reactor (Bakhir et al., 1997) provided by RSCE-CAT, USA, Inc. (Las Vegas, NV). The electrochemical reactor (Fig. 1) consisted of 8 electrochemical cells in parallel (Bakhir and Zadorozhny, 1997). Each cell consisted of a coaxial external cylindrical electrode, an internal rod electrode and a ceramic diaphragm placed between them. The external electrode was made of titanium and connected to the negative pole of the power supply (cathode). The internal electrode was made of titanium, coated with ruthenium oxide and connected to the positive pole of the power supply (anode). Tap water flowed into the system at 100 L/h. The tap water was

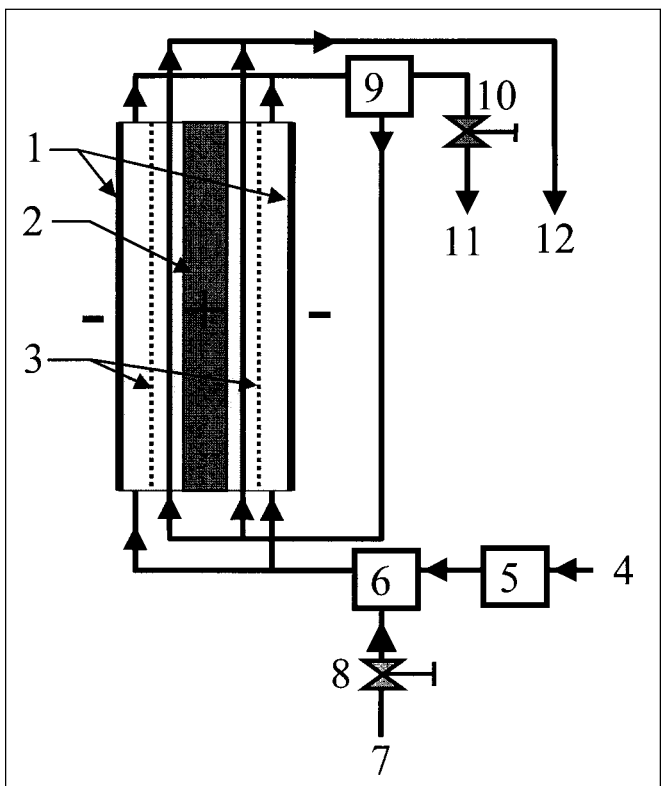

Fig. 1—Schematic of electrochemical reactor: (1) cathode; (2) anode; (3) ceramic diaphragm; (4) tap water; (5) flowmeter; (6) injection pump; (7) 15% NaCl; (8) Injection valve; (9) catalyst chamber; (10) pH adjusting value; (11) catholyte; and (12) original EAS.

mineralized by introducing 15% NaCl (Fisher, Pittsburgh, PA) online before entering the electrochemical cell. The mineralized tap water containing 3 g/L NaCl flowed into the cathode chamber, flowed through a catalyst chamber that contained a mixture of carbon and manganese dioxide particles, and then flowed into the anode chamber. The electrochemical reactor was operated by applying a cell voltage of 30V of direct current. The total current in the electrochemical reactor was 9A. The total oxidants concentration in the original EAS from the electrochemical reactor was 300 ppm in terms of free chlorine (*Cl*) with a pH of 6.5 which was controlled by drawing catholyte through the bypass. The original EAS was further diluted to 50 ppm of *Cl* with tap water for spraying and chilling, based on conventional chlorine treatments in poultry processing (Thomson et al., 1976). The total oxidants concentration (*Cl*) in the EAS was determined by an iodometric method (Greenberg, 1995) with 0.01M of sodium thiosulfate standard solution (Fisher, Pittsburgh, PA).

For comparison, a sodium hypochlorite solution (HCS, 50 ppm of *Cl*) was prepared from 8.4% Ecolab® sodium hypochlorite (Quantum E, Garland, TX). This preparation has been used mainly for disinfecting equipment but not for treating chicken carcasses. All of HCS used in the tests was titrated and contained the total oxidants concentration (*Cl*) needed for the experiments. In addition, this formulation contained no additional bactericidal agents other than sodium hypochlorite (manufacturer analysis data).

Chicken carcass samples

Chicken carcasses were taken from the end of an eviscerating line at a poultry processing plant, placed in plastic bags, and transported to the poultry processing pilot plant (Fig. 2). Since the number of *Salmonella* naturally attached to a pre-chilled chicken carcass was very low (0-100 CFU/carcass), the chicken carcasses had to be inoculated with *Salmonella* to get a higher and standard initial number for the studies. The chicken carcasses were hung on an independent shackle line in an isolated area. Each carcass was inoculated with *S. typhimurium* ATCC 14028 at 10^6 CFU/mL by spraying 1 mL of the inoculum on both the breast and back sides, and 1 mL on the inside of the cavity. *Salmonella* attachment to the carcasses was allowed to proceed for 30 min at 20°C. The inoculated carcasses were then manually rinsed on the shackle with 0.5 L/carcass of tap water at 20°C for 5s to wash off loosely attached *Salmonella*.

Inside/outside spraying treatment

An inside/outside (I/O) spraying system has been described (Yang et al., 1998). Briefly, the I/O spraying system consisted of a modified Sani-Kleen I/O birdwasher (Johnson™ Food Equipment, Kansas City, KS), a spraying solution storage tank, a pressure regulator, and a shackle line in the pilot plant. Each group of 10 inoculated chicken carcasses was hung on the shackle line connected to the I/O birdwasher. The shackle line operated at a speed of 57 birds/min. The chicken carcasses on the shackle line moved into the I/O birdwasher, were sprayed with a designated solution at 20°C and 413 kPa for 17 s, and then exited the I/O birdwasher.

After being sprayed, the chicken carcasses were left on the shackle line for a setting time of 60 s to increase exposure to the sprayed solution. They were then rinsed with tap water at 20°C and 413 kPa for 17 s to remove chemical residue.

Chilling treatment

Three plastic rectangular containers (80 cm × 50 cm × 24 cm) were used as chillers for treatments of carcasses. Each of the containers was filled with 78L of a designated iced solution. The center of the bottom of each chiller was connected to the designated iced solution storage tank through a NK-2 utility pump (Little Giant Pump Co., Oklahoma City, OK) for overflow control. The overflow rate was set at ≈1.9 L/carcass during chilling treatments. The chilling treatment was set for 45 min at 4°C.

Microbial enumeration

All carcasses from the treatment and control groups were tested for *Salmonella* using a whole carcass rinse. Each carcass was placed in a plastic bag (Bel-Art products, Pequannock, NJ) with 100 mL of 0.1% buffered peptone water at pH 7.3 (Difco laboratories, Detroit, MI) and mechanically shaken for 1 min. This wash water (2 mL) from each carcass was diluted with 18 mL of sterile phosphate-buffered solution (pH 7.3), then serially diluted to 10^{-4} with the phosphate-buffered water. For each treatment, 2 mL of samples were also taken from the chiller water after treatment and serially diluted to 10^{-4} with the phosphate-buffered water.

The 3 tube most probable number (MPN) method (Peeler et al., 1992) was used for *Salmonella* enumeration. A 5 mL portion of each dilution was transferred to 3 test tubes, each containing 5 mL of double-strength buffered peptone water (BPW, Difco). After incubation for 20h at 37°C, 1 mL of each culture was diluted with 9 mL of tetrathionate-hajna broth (Difco) and incubated for 20h at 43°C. Cultures were streaked on brilliant green sulfa (BGS) agar (Difco), modified lysine iron agar (MLIA) and xylose lysine tergitol 4 (XLT4) agar and then incubated for 20 to 24h at 37°C. Suspect colonies were confirmed by serological tests using poly-O antiserum (Difco). The *Salmonella* count was calculated using the MPN table and converted to $\log_{10}$ CUF/carcass.

Experimental design

I/O spraying. This trial was designed to evaluate antibacterial efficacy of the EAS applied only in the I/O spraying treatment. Each group of 10 inoculated carcasses was sprayed with either tap water (TW), HCS or EAS, then further processed using iced TW in the chilling treatment. A control group of 5 inoculated carcasses received neither the spraying nor chilling treatments. Therefore, a total of 35 inoculated chicken carcasses were used. This trial was repeated 3 times.

Combined I/O spraying-chilling. In order to enhance the antibacterial efficacy of the EAS, a combined I/O treatment was used to apply the EAS in both the spraying and chilling treatments in this trial. Each group of 10 inoculated carcasses received either TW spraying-TW chilling, ($TW_s - TW_c$), HCS spraying-HCS chilling ($HCS_s - HCS_c$) or EAS spraying-EAS chilling (EAS_s-EAS_c) treatments. A control group of 5 inoculated carcasses received neither spraying nor chilling treatment. A total of 35 inoculated carcasses were used for this trial. This trial was repeated 3 times.

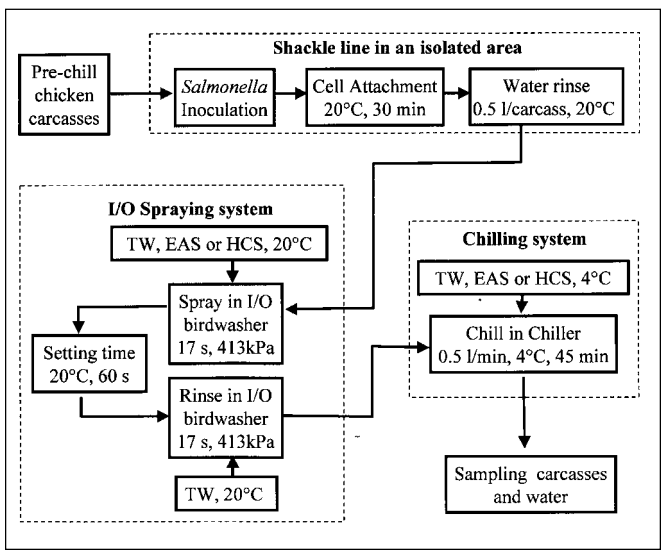

Fig. 2—Flow chart of poultry spraying and chilling processes. TW=Tap water, HCS=Hypochlorite solution, EAS=Electrochemically activated solution.

Statistical analysis

The *Salmonella* reduction attributable to the treatments was calculated by subtracting individual populations of *Salmonella* on the treated carcasses $(\log_{10} \text{CFU/carcass})_{treated}$ from an average population of *Salmonella* of the control group $(\log_{10} \text{CFU/carcass})_{control}$ in each trial. *Salmonella* population and means of *Salmonella* reduction on chicken carcasses for all trials were analyzed using the general linear model (GLM) and Duncan's multiple range test procedures of SAS (SAS Institute, Inc., 1993) with the level of significance defined at $P<0.05$.

RESULTS & DISCUSSION

Effect of I/O spraying treatments

Average *Salmonella* populations found on the carcasses were computed after treatment by I/O spraying with either TW, HCS (50 ppm of *Cl*) or EAS (50 ppm of *Cl*), followed by the same TW chilling treatment (Table 1). In order to determine the antibacterial efficacy of the EAS, means of *Salmonella* reduction on carcasses were computed (Fig. 3). The average reduction of *Salmonella* on carcasses (n= 30) sprayed with EAS was 1.39 $\log_{10}$ CFU/carcass, whereas the *Salmonella* reductions on carcasses sprayed with TW or HCS were 0.86 and 0.87 $\log_{10}$ CFU/carcass, respectively, as compared to unprocessed carcasses (controls). Results indicate that EAS had a stronger bactericidal activity on chicken carcasses during the I/O spraying processes than did HCS, although their total oxidant concentrations were the same.

Effect of combined I/O spraying-chilling treatments

The I/O spraying treatment of the EAS was further combined with a chilling treatment using iced EAS solution to determine if greater reduction of *Salmonella* could be achieved. Average *Salmonella* populations found on the carcasses were computed after the combined I/O spraying-chilling treatments with either TW, HCS (50 ppm of *Cl*) or EAS (50 ppm of *Cl*) (Table 2). The combined I/O spraying-chilling treatment with TW reduced *Salmonella* by 0.92 $\log_{10}$ CFU/carcass as compared to unprocessed carcasses (Fig. 4). The combined spraying-chilling treatments with HCS and EAS gave nearly identical reductions of *Salmonella* (1.69 and 1.67 $\log_{10}$ CFU/carcass, respectively), as compared to untreated carcasses (Fig. 4).

Compared with the corresponding I/O spraying treatments alone, the combined I/O spraying-chilling treatment with HCS increased ($P<0.05$) *Salmonella* reduction from 0.87 to 1.69 $\log_{10}$ CFU/carcass. Additional bactericidal effect using HCS was found in the HCS chilling treatment. The combined I/O spraying-chilling treatments with EAS did not further reduce *Salmonella* on chicken carcasses as compared to EAS spraying and TW chilling. *Salmonella* reduction from the combined I/O spraying-chilling with EAS was 1.67 $\log_{10}$ CFU/carcass, and the I/O spraying with EAS alone reduced *Salmonella* by 1.39 $\log_{10}$ CFU/carcass. This indicated that reduction of *Salmonella* on chicken carcasses by the combined I/O spraying-chilling with EAS was mainly contributed by the short period I/O spraying treatment using the EAS at 20°C, rather than by the chilling treatment using the EAS at 4°C for 45 min. The color of chicken carcasses was not changed by the I/O spraying or chilling with EAS.

Table 1—*Salmonella* counts on chicken carcasses after I/O spraying treatments (means and standard deviations)

Treatment[d]	Spraying	Chilling	*Salmonella* count (log CFU/carcass)		
			Trial 1	Trial 2	Trial 3
Control	None	None	5.90±0.41[a]	6.12±0.40[a]	6.91±0.51[a]
TW_s-TW_c	Tap water	Tap water	4.92±0.50[c]	5.41±0.60[b]	6.03±0.50[b]
HCS_s-TW_c	HCS	Tap Water	5.40±0.60[b]	5.50±0.60[b]	5.41±0.45[c]
EAS_s-TW_c	EAS	Tap water	5.01±0.50[c]	4.68±0.50[c]	5.07±0.37[c]

[a-c]Values in each column followed by same letter not significantly different ($P > 0.05$).
[d] n= 10 for each treatment. TW=Tap water, HCS=Hypochlorite solution, EAS=Electrochemically activated solution, s=spraying, c=chilling.

Table 2—*Salmonella* counts on chicken carcasses after combined I/O spraying-chilling treatments (means and standard deviations)

Treatment[d]	Spraying	Chilling	*Salmonella* count (log CFU/carcass)		
			Trial 1	Trial 2	Trial 3
Control	None	None	6.63±0.32[a]	7.19±0.31[a]	6.70±0.18[a]
TW_s-TW_c	Tap water	Tap water	5.88±0.36[b]	6.00±0.38[b]	5.88±0.29[b]
HCS_s-HCS_c	HCS	HCS	5.22±0.72[c]	5.14±0.52[c]	5.10±0.40[c]
EAS_s-EAS_c	EAS	EAS	5.27±0.45[c]	5.27±0.46[c]	4.96±0.27[c]

[a-c]Values in each column followed by the same letter not significantly different ($P > 0.05$).
[d]n=10 for each treatment. Abbreviations same as Table 1.

Effect of chilling treatments on Salmonella in chiller water

Salmonella populations were compared (Fig. 5) in iced TW, HCS (50 ppm of *Cl*) and iced EAS (50 ppm of *Cl*) after removing the treated carcasses from the chillers. *Salmonella* populations were higher than 10^3 CFU/L in iced TW, but no *Salmonella* survived in iced HCS or EAS. Although iced EAS did not reduce *Salmonella* on chicken carcasses ($P>0.05$), the activated components in iced EAS eliminated *Salmonella*, preventing cross-contamination of carcasses during chilling.

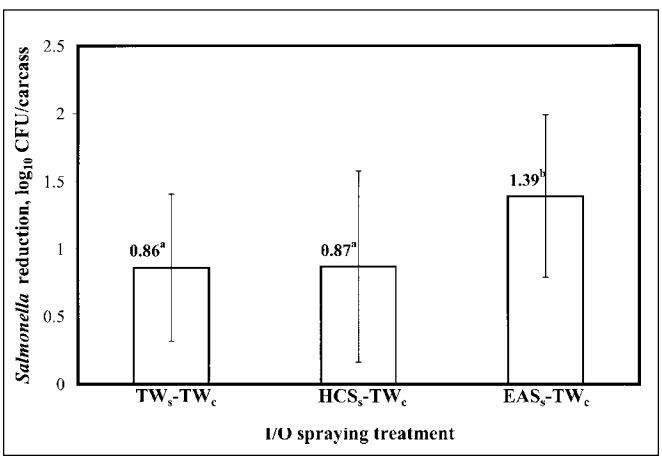

Fig. 3—*Salmonella* reduction on chicken carcasses after spraying treatments with TW, HCS (50 ppm of *Cl*) or EAS (50 ppm of *Cl*) at 20°C and 413 kPa for 17s, and iced TW chilling treatment at 4°C for 45 min. Values in each column followed by same letter are not significantly different (P>0.05). Abbreviations same as Fig. 1.

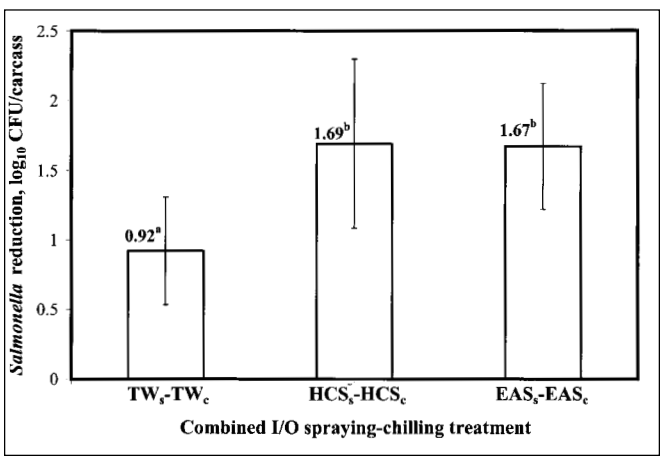

Figure 4—*Salmonella* reduction on chicken carcasses after combined spraying (20°C, 413 kPa, 17 s)-chilling (4°C, 45 min) treatments with iced TW, HCS (50 ppm of *Cl*) or EAS (50 ppm of *Cl*). Values in each column followed by same letter not significantly different (P>0.05). Abbreviations same as Fig. 1.

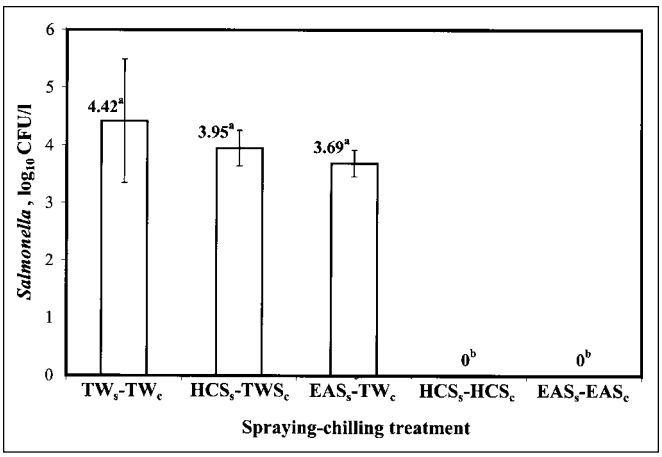

Fig. 5—*Salmonella* counts in chiller water, either iced TW, HCS (50 ppm of *Cl*) or EAS (50 ppm of *Cl*) after I/O spraying or combined I/O spraying-chilling treatments. Values in each column followed by same letter not significantly different (P>0.05). Abbreviations same as Fig. 1.

CONCLUSIONS

RESULTS HAVE SHOWN ANTIBACTERIAL EFFICACY OF EAS (50 PPM of *Cl*) in I/O spraying and chilling treatments of chicken carcasses. The I/O spraying with EAS reduced *Salmonella* by 1.39 $\log_{10}$ CFU/carcass, whereas the I/O spraying with TW and HCS (50 ppm of *Cl)* reduced *Salmonella* by 0.86 and 0.87 $\log_{10}$ CFU/carcass, respectively. Additional chilling using EAS did not reduce *Salmonella* on carcasses but eliminated *Salmonella* in the chiller water. Further study is needed to investigate the effects of the EAS method on quality of chicken products, such as texture and sensory.

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Ms received 7/28/98; revised 11/9/98; accepted 12/26/98.

This research was supported in part by Johnson Food Equipment, Inc. (Kansas City, KS). The authors thank H. Wang, A. O'Leary, C. Brownmillen, M. Ji and D. Fos for their assistance in the microbial and chemical tests.