



Pulsed Electric Field Effects to Reduce the Level of *Campylobacter* spp. in Scalding and Chilling Water during Broiler Chicken Processing

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ABSTRACT : To evaluate the effects of pulsed electric field (PEF) application on scalding and chilling water on *Campylobacter* contamination, four different treatments under three different water conditions including hard scalding water (55°C), soft scalding water (45°C) and chilling water, were applied as follows: i) a control treatment with no salt and no electric treatment, ii) a PEF only treatment, iii) a PEF treatment with 0.5% salt water, and iv) a PEF treatment with 1% salt water treatment. The use of PEF in hard scalding water showed an effect of reducing *Campylobacter* when compared to the control during the 200 s timeframe. With the addition of salt, the intervention caused at least 5.81 log CFU/ml reduction of *Campylobacter* counts after 200 s of PEF exposure. Similar effects were observed under soft scalding conditions. *Campylobacter* reductions were evident under chilling conditions with up to 2.00 log for PEF only, 5.77 log for PEF+0.5% salt and 2.69 log for PEF+1% salt treatment in water. Therefore, the current PEF setting for the scalding and chilling water can be successfully used to reduce pathogenic loads of *Campylobacter* on broiler chicken carcasses, and further research may be necessary to apply it in the poultry processing industry. (**Key Words :** *Campylobacter*, Pulsed Electric Field, Scalding, Chilling)

INTRODUCTION

Campylobacter is the most common bacterial cause of human gastro-intestinal infections, and it has been estimated that approximately 2.5 million cases of foodborne campylobacteriosis occur annually, leading to approximate 124 deaths (Smith et al., 1999). Generally, campylobacters have been known to cause diseases in humans since the early 1900's and the chicken has long been associated with the presence of *Campylobacter* spp. that can cause human enteric illnesses (Deming et al., 1987; Altekruse et al., 1999). The most common vehicles of *Campylobacter* transmission in chicken products are upon the feathers of chickens arriving at the processing plants or upon the carcasses that become cross-contaminated with intestinal

contents during processing (Oosterom et al., 1983; Lillard, 1989; USDA-FSIS, 2004). Cross-contamination can occur at many points in the field or during processing where fecal matter or digestive tract can be contacted to chickens or its carcasses.

In poultry processing, broiler chicken carcasses and parts are frequently contaminated; this contamination is easily spread to other carcasses from the intestinal tract or from fecal material on feet and feathers (Deming et al., 1987). Therefore, cross-contamination is a particular problem at critical steps during processing including defeathering, evisceration and chilling. Research has focused on effective methods to substantially decrease contamination during the final stages of processing (Thompson et al., 1979; James et al., 1992) while minimal efforts have been implemented at the initial stages, where most contamination occurs. Several methods for chemical and mechanical decontamination of carcasses have been tested and reported in the literature (Cox et al., 1978; Bautista et al., 1997), but the demand for better safety and quality, less energy consumption, and lower costs have compelled poultry processors and researchers to devise better technologies and approaches to address the matter.

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Recently, a great amount of attention has been devoted to bacterial inactivation by electrical treatment in several food processing applications (Toepfl et al., 2007). These efforts are aimed at minimizing the use of thermal energy and chemicals as antimicrobial interventions. In the last few years, electrical treatments that rely on pulsed electrical fields (PEF) have received the greatest emphasis (Korolczuk et al., 2006; Toepfl et al., 2007). Schoenbach et al. (1997, 2000) demonstrated that bacteria inactivation by PEF can be achieved using the appropriate pulse width (60 ns to 1 ms), amplitude (100 V/cm-100 kV/cm), and single-shot or repetitive operation approach. Ravishankar et al. (2002) also found that temperature and pH level play a role in PEF treatments which can reduce bacteria count by 3 log. Another research based on PEF and its effects on eggs had been completed by Wesierska and Trziszka (2007), and they reported that PEF reduced bacteria counts by as much as 5 log during egg processing. Therefore, the objective of this study was to validate the use of PEF to inactivate *Campylobacter* spp. in scalding and chill water.

MATERIALS AND METHODS

The electrical pulses generated by an electric stunner device (Model SF-700, Simmons. Eng. Co., Dallas, GA) equipped with copper jacketed graphite electrodes was applied to scalding and chiller waters to minimize the number of *Campylobacter* spp. Therefore, to increase the lethality of *Campylobacter* spp., the electrical pulses were combined with a salt (0, 0.5 or 1%) and a temperature to treat poultry scalding (45 or 55°C) and chiller (4°C) submersion water. For scalding water simulation, water was collected from a local commercial poultry processor by selecting the water overflow of the final section of the scalding tank, and water contained a lot of organic matters as usual. Local tap water was supplied as a chiller water, and the addition of 0 or 25 ppm of sodium hypochlorite (NaClO) was performed to simulate poultry processing plant conditions.

Campylobacter strains were inoculated on Bolton broth tubes supplemented with lysed horse blood plates and placed in air tight plastic bag (Ziploc, Johnson & Son Inc., Racine, WI, USA). All air was removed from the bag, and a mixture of 5% O₂, 10% CO₂ and 85% N₂ gas was added to inflate the bag and produce a microaerobic environment. Bags were then incubated for 48 h at 42°C and stored under refrigeration temperatures until needed. Forty eight hours before each experiment, each *Campylobacter* strain was enriched in Bolton broth and incubated at 42°C under microaerophilic conditions. Immediately before conducting the PEF experiments the individual strains of *Campylobacter* were combined in a 50 ml test tube to produce a cocktail inoculum.

One milliliter of *Campylobacter* cocktail was added to 99 ml of either scalding or chiller water, and the copper electrodes were deeply inserted to a 200 ml beaker containing either scalding or chiller water. A stir bar was now activated and then PEF generator was operated at 40 v, yielding ~0.54 amps. The pulses were produced with an interval of 10 s on and 5 s off. One milliliter of *Campylobacter* inoculated sample water was collected at 0, 40, 80, 120, 160 and 200 s of PEF apply, and each water sample was stored with a 9 ml of aseptic 0.1% buffered peptone water (BPW). A serial dilution was conducted using a 0.1% BPW and 0.1 ml of diluted sample was placed onto Campy-Cefex plates for *Campylobacter* enumeration. Campy-Cefex plates were placed in air tight plastic bags. All air of the plastic bags was removed, and a mixture of 5% O₂, 10% CO₂ and 85% N₂ gas was added to produce a microaerobic environment. Bags were then incubated for 48 h at 42°C, and enumeration of *Campylobacter* was conducted. Similar processing steps were conducted for treatments applying two different combinations of salt and PEF to either scalding or chiller water; i) a PEF treatment with 0.5% salt water, and ii) a PEF treatment with 1% salt water treatment.

Count data obtained were transformed logarithmically and reported as log₁₀ CFU/ml. Each experiment was independently conducted on three different processing days, and two plates per treatment were sampled (replication×treatment×plate = 3×4×2). Data were analyzed by one-way ANOVA using SAS statistical analysis software program (1998), version 6.12 (SAS Institute Inc., Cary, NC, USA). p-values <0.05 were considered statistically significant.

RESULTS

Two scalding temperatures, 55°C and 45°C, were evaluated with *Campylobacter* spp. cocktails (Table 1 and 2). Significant differences (p<0.05) were noticed at 200 s with both temperatures. At 55°C *Campylobacter* was reduced below detection levels on all treatments, which was greater than 5.00 log. The greatest reduction (6.11 log) was achieved with 0.5% NaCl. With the lower temperature of 45°C, a 5.77 and 5.52 log reduction was achieved with PEF only and PEF combined with 0.5% NaCl, respectively. These results were below the detection limits of dilution 1. A reduction of 4.53 log was reached with 1% NaCl in the medium.

Treatments with *Campylobacter* spp. cocktails in chilling conditions seem that the colder medium reduced PEF's performance. However, with the use of 0.5% NaCl increased the inactivation performance (Table 3). *Campylobacter* was significantly reduced on all treatments when compared to the control (p<0.05). The PEF showed to reduce *Campylobacter* the best with 0.5% NaCl. A

Table 1. Effect of pulsed electric fields (PEF) to reduce *Campylobacter* spp. at 55°C of scalding water (log CFU/ml)¹

	0 s ²	40 s	80 s	120 s	160 s	200 s
Control	6.33±0.04 ^a	6.35±0.03 ^a	6.31±0.03 ^a	6.31±0.03 ^a	6.19±0.03 ^a	6.10±0.03 ^a
PEF only	6.03±0.06 ^{bw}	5.13±0.05 ^{abx}	2.68±0.06 ^{cy}	0.70±0.00 ^{cz}	0.70±0.00 ^{bz}	0.70±0.00 ^{bz}
0.5 NaCl ³	6.11±0.12 ^{bx}	4.27±1.59 ^{by}	0.70±0.71 ^{dz}	0.00±0.00 ^{dz}	0.00±0.00 ^{cz}	0.00±0.00 ^{cz}
1 NaCl ⁴	6.51±0.06 ^{av}	6.14±0.06 ^{aw}	5.35±0.17 ^{bx}	2.58±0.19 ^{by}	0.70±0.00 ^{bz}	0.70±0.00 ^{bz}

¹ Each data entry represents the mean±standard deviation. ² Second. ³ PEF and 0.5% NaCl. ⁴ PEF and 1% NaCl.^{a-d} Means followed by different letter within the column are significantly different (p<0.05).^{v-z} Means followed by different letter within the row are significantly different (p<0.05).**Table 2.** Effect of pulsed electric fields (PEF) to reduce *Campylobacter* spp. at 45°C of scalding water (log CFU/ml)¹

	0 s ²	40 s	80 s	120 s	160 s	200 s
Control	6.40±0.05 ^a	6.50±0.05 ^a	6.41±0.07 ^a	6.42±0.07 ^a	6.40±0.09 ^a	6.49±0.09 ^a
PEF only	6.47±0.03 ^{av}	6.29±0.08 ^{avw}	6.16±0.04 ^{bw}	5.09±0.23 ^{bx}	1.36±0.10 ^{cy}	0.70±0.00 ^{cz}
0.5 NaCl ³	6.22±0.03 ^{bw}	5.90±0.31 ^{bx}	5.05±0.08 ^{cy}	0.70±0.00 ^{dz}	0.70±0.00 ^{dz}	0.70±0.00 ^{cz}
1 NaCl ⁴	6.45±0.11 ^{aw}	6.34±0.13 ^{aw}	6.11±0.08 ^{bw}	4.22±0.03 ^{cx}	3.64±0.12 ^{by}	1.92±0.58 ^{bz}

¹ Each data entry represents the mean±standard deviation. ² Second. ³ PEF and 0.5% NaCl. ⁴ PEF and 1% NaCl.^{a-d} Means followed by different letter within the column are significantly different (p<0.05).^{v-z} Means followed by different letter within the row are significantly different (p<0.05).

reduction of 5.77 log was detected with 0.5% NaCl. The PEF only and 1% NaCl with PEF did not show as good of results as 0.5% NaCl did. A decrease of 2.00 log for PEF only and a decrease of 2.69 log with the 1% NaCl and PEF combination were recorded. *Campylobacter* was eliminated below detectable limits on all dilutions with the combination of 0.5% NaCl and 25 ppm NaClO. Results were not statistically reported due to the lack of detectable colony forming units at all six time intervals.

DISCUSSION

PEF's effects on *Campylobacter* spp. in scalders were magnified at both temperatures tested. PEF was efficiently influenced on the number of *Campylobacter* spp. load in scalding water when high temperature scalding water (55°C) was provided to a scalding tank. *Campylobacter* spp. was reduced below detection levels when PEF was applied to scalding water for 200 s and it was a far below as compared to control samples. However, significant variation was experienced between replications. The variation can partially be attributed to the temperature, being so close to

the lethal temperature for *Campylobacter* (Murphy et al., 2006) and to its lack of survivability in environments that are less than ideal for its survival. This being said; PEF affected *Campylobacter* greatest with 0.5% NaCl, reducing its presence by 5.52 log within 200 s compared to controls containing only scalding water. Scalding water set to run at lower temperatures (45°C) had lower *Campylobacter* counts after PEF treatment, but the reduction was less than those seen with the higher temperature scalders, but still significant. *Campylobacter* spp. was reduced by 6.11 log when 0.5% NaCl+PEF was used. These results were obtained without the use of 1% NaCl, both 0.5 and 0% NaCl with PEF killed the bacteria better than with the use of 1% NaCl.

Poultry processing companies are at higher risk to *Campylobacter* spp. contamination during chilling of the eviscerated carcasses than at any other steps during processing, and in need of effective interventions to control it (Stern et al., 2001). PEF results indicated that it too could be a possible alternative. *Campylobacter* was reduced by 5.77 log with the use of 0.5% NaCl in the chiller water and no NaClO was used to obtain these numbers. A widely

Table 3. Effect of pulsed electric fields (PEF) to reduce *Campylobacter* spp. in chilling water (log CFU/ml)¹

	0 s ²	40 s	80 s	120 s	160 s	200 s
Control	6.61±0.05 ^a	6.46±0.05 ^a	6.45±0.07 ^a	6.56±0.07 ^a	6.64±0.09 ^a	6.49±0.12 ^a
PEF only	6.12±0.05 ^{cx}	6.06±0.08 ^{abx}	5.89±0.18 ^{bx}	5.40±0.12 ^{by}	5.36±0.04 ^{by}	4.12±0.19 ^{bz}
0.5 NaCl ³	6.47±0.06 ^{bv}	5.59±0.57 ^{bw}	2.37±0.14 ^{cx}	1.46±0.66 ^{dy}	0.70±0.00 ^{dz}	0.70±0.00 ^{dz}
1 NaCl ⁴	6.41±0.13 ^{bw}	6.27±0.04 ^{aw}	5.73±0.14 ^{bx}	3.92±0.03 ^{cy}	3.78±0.10 ^{cyz}	3.72±0.04 ^{cz}

¹ Each data entry represents the mean±standard deviation. ² Second. ³ PEF and 0.5% NaCl. ⁴ PEF and 1% NaCl.^{a-d} Means followed by different letter within the column are significantly different (p<0.05).^{v-z} Means followed by different letter within the row are significantly different (p<0.05).

accepted intervention in western poultry processing is in the use of NaClO in the chiller water at varying concentrations (Lee et al., 2004). PEF effectiveness with 25 ppm of NaClO and 0.5% NaCl was tested. When compared to controls only containing NaClO, counts were reduced by 4.51 log; which was below detectable limits under the experimental conditions tested. *Campylobacter* spp. counts were drastically reduced with this combination; however, as compared to controls results were unable to report a significant difference due to the reduction beyond detectable limits.

Our research demonstrated the ability of PEF to be a very powerful and efficient tool in reducing *Campylobacter* spp. loads in scalding and chiller water. However, the use of a properly sized PEF generator is essential for PEF to be effective as a lethal alternative to other microbial interventions. With further validation, PEF can be used to meet the USDA organic certification criteria needed to allow broiler chicken products to be labeled as "Organic", since the interventions does not require the use of chemicals which may not be approved. Once certification is achieved, organic poultry processing plants can make use of PEF to assist them on reducing bacterial loads and control pathogens, while still meeting the many demanding federal and state regulations concerning safe poultry processing methods.

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