

## Inactivation of *Listeria monocytogenes* and *Campylobacter jejuni* in Chicken by Aqueous Chlorine Dioxide Treatment

– Research Note –

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### Abstract

Aqueous chlorine dioxide (ClO<sub>2</sub>) treatment was used for the inactivation of *Listeria monocytogenes* and *Campylobacter jejuni* in chicken. Chicken breasts and legs were inoculated with 8~9 log CFU/g of *Listeria monocytogenes* and *Campylobacter jejuni*, respectively, and then treated with 0, 50, and 100 ppm of ClO<sub>2</sub> solution. Aqueous ClO<sub>2</sub> treatment decreased the populations of the pathogenic bacteria on the chicken samples. One hundred ppm ClO<sub>2</sub> treatment on the chicken breast and leg reduced the populations of *Listeria monocytogenes* and *Campylobacter jejuni* by 0.61~1.93 and 0.99~1.21 log CFU/g, respectively. Aqueous ClO<sub>2</sub> treatment affected the microbial growth during storage at 4°C by decreasing the initial microbial populations. These results clearly suggest that aqueous ClO<sub>2</sub> treatment should be useful in improving the microbial safety of chicken during storage and extending the shelf life.

**Key words:** chicken, aqueous chlorine dioxide, *Listeria monocytogenes*, *Campylobacter jejuni*

### INTRODUCTION

Consumption of chicken products has been increasing due to high nutritional value and relatively low cost, but the microbial safety of chicken remains a concern (1-3). Chicken products are highly perishable, and food poisoning by pathogens can be caused by careless processing and storage. Major bacterial contaminants in chicken include *Salmonella spp.*, *Listeria monocytogenes*, and *Campylobacter*. *Campylobacter jejuni* is present in the intestinal microflora of chicken, and it contaminates the surface of chicken during processing, causing foodborne illness in humans (4). Another food pathogen, *Listeria monocytogenes*, also causes food poisoning, and it can grow at refrigerated temperature and on dry surfaces. In particular, during slaughtering process of chicken, there is a high probability of cross-contamination by intestinal microorganism. Therefore, to enhance the microbial safety of chicken during processing and storage, various processing techniques such as microwave exposure (5), trisodium phosphate and sodium hydroxide (3), potassium sorbate (6), lactic acid and sodium benzoate (7), and lactic acid and lauricidin (8) have been used for reduction of bacterial counts as well as extension of shelf life.

As a food preservation method, aqueous chlorine dioxide has been used. Chlorine dioxide is a strong oxidizing agent and has a broad biocidal effectiveness (9). In addition, it does not produce hazardous trihalomethanes and

is considered as safe (9,10). Chlorine dioxide is more effective than chlorine for reducing the number of bacteria present in poultry-processing water (10,11). Aqueous chlorine dioxide has been approved by the FDA as an antimicrobial agent in chiller water during poultry processing, and could be used to chill poultry carcasses for about 1 hr in chilling solutions containing sodium chlorite between 50 and 150 ppm (12). There have also been several reports on the effect of aqueous chlorine dioxide on beef (13,14), seafood (15), and fruit and vegetable products (10,16,17).

Therefore, the objectives of this study were to examine the effect of aqueous chlorine dioxide treatment to inactivate the major pathogens, *Listeria monocytogenes* and *Campylobacter jejuni*, on chicken breast and leg, and to provide a suitable processing method for microbial safety of chicken products.

### MATERIALS AND METHODS

#### Preparation of chicken

Chicken samples were purchased from a local market in Daejeon, Korea.

#### Bacterial strains and culture preparation

*Listeria monocytogenes* culture was grown at 37°C or 48 hr in 50 mL tubes containing 25 mL of *Listeria* enrichment broth base (Oxoid, Basingstoke, UK). *Campylobacter jejuni* culture was grown at 42°C or 24 hr in

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50 mL tubes containing 25 mL of Brucella broth (Difco Laboratoires, Detroit, MI, USA) with 10% sheep blood under microaerophilic atmosphere.

### Inoculation

Chicken breasts and legs were washed using distilled water and treated with UV light for 30 min to reduce preexisting microorganisms. The decontaminated chicken breast and leg were then immersed in *Listeria monocytogenes* (ATCC 19111) and *Campylobacter jejuni* (ATCC 33291) inoculum solutions for 10 min, respectively, and allowed to drain for 30 min. Initial levels of *Listeria monocytogenes* and *Campylobacter jejuni* were 8~9 log CFU/g.

### Chlorine dioxide preparation and treatment

Aqueous ClO<sub>2</sub> was prepared using a chlorine dioxide generating system (CH<sub>2</sub>O Inc., Olympia, WA, USA) as described previously (18). Samples were treated by dipping in 0, 50, or 100 ppm aqueous chlorine dioxide solution for 10 min. Chlorine dioxide concentration was determined according to the method of APHA (19). Samples were then individually packaged in polyethylene terephthalate containers and stored at 41°C.

### Microbiological analysis

After chlorine dioxide treatment, samples (5 g) were removed using a sterile scalpel. Samples were then homogenized using a Stomacher (MIX 2, AES Laboratoire, France) for 3 min, filtered through a sterile cheese cloth, and diluted with peptone water (0.1% sterile peptone, w/v) for microbial count. Serial dilutions were performed in triplicate on each selective agar plate. *Listeria monocytogenes* counts were determined by plating appropriately diluted samples onto *Listeria* Selective Agar Base (Oxoid, Basingstoke, UK). Samples were evenly spread

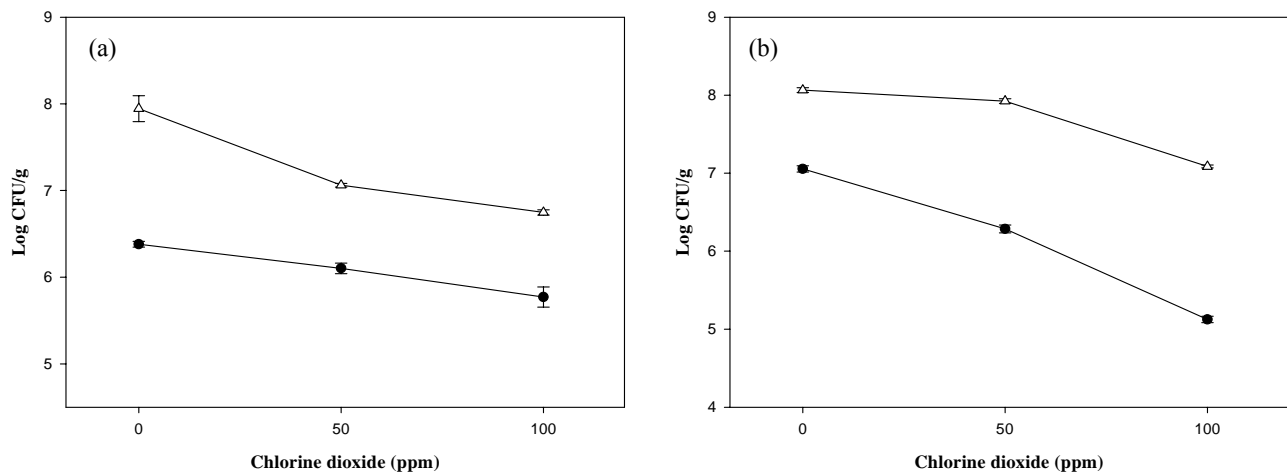
on the surface of the plates with a sterile glass rod. Plates were incubated at 37°C for 48 hr. For *Campylobacter jejuni*, samples were plated onto Brucella Agar (Difco Laboratories, Detroit, MI, USA) having 10% sheep blood, and plates were incubated at 42°C under microaerophilic atmospheric conditions for 24 hr. Each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU/g.

## RESULTS AND DISCUSSION

### Microbiological changes

Initial populations of *Listeria monocytogenes* on the inoculated chicken breast and leg were 6.38 and 7.05 log CFU/g, respectively, and for *Campylobacter jejuni*, they were 7.94 and 8.08 log CFU/g, respectively (Fig. 1).

Aqueous chlorine dioxide treatment significantly decreased the populations of *Listeria monocytogenes* and *Campylobacter jejuni* on the chicken breast and leg, compared to the control (Fig. 1). In addition, increased chlorine dioxide concentrations decreased the microbial populations of the pathogenic bacteria. After chlorine dioxide treatment, the populations of *Listeria monocytogenes* on chicken breast were 6.10 and 5.77 log CFU/g for 50 and 100 ppm of chlorine dioxide, respectively (Fig. 1-a), and for chicken leg, they were 6.29 and 5.12 log CFU/g for 50 and 100 ppm of chlorine dioxide, respectively (Fig. 1-b). In particular, one hundred ppm of chlorine dioxide reduced the populations of *Listeria monocytogenes* by 0.61~1.93 log cycle in comparison to the chicken treated at 0 ppm of chlorine dioxide. González-Fandos and Dominguez (6) reported that dipping of poultry in 2.5%~5% of potassium sorbate for 5 min decreased the populations of *Listeria mon-*



**Fig. 1.** Effect of aqueous ClO<sub>2</sub> treatment on the survival of the pathogenic bacteria inoculated on chicken. Bars represent standard error. (a) chicken breast (b) chicken leg. ●: *Listeria monocytogenes* △: *Campylobacter jejuni*.

*ocytogenes* by 0.3~1.3 log CFU/g, suggesting that aqueous chlorine dioxide treatment is more effective than potassium sorbate.

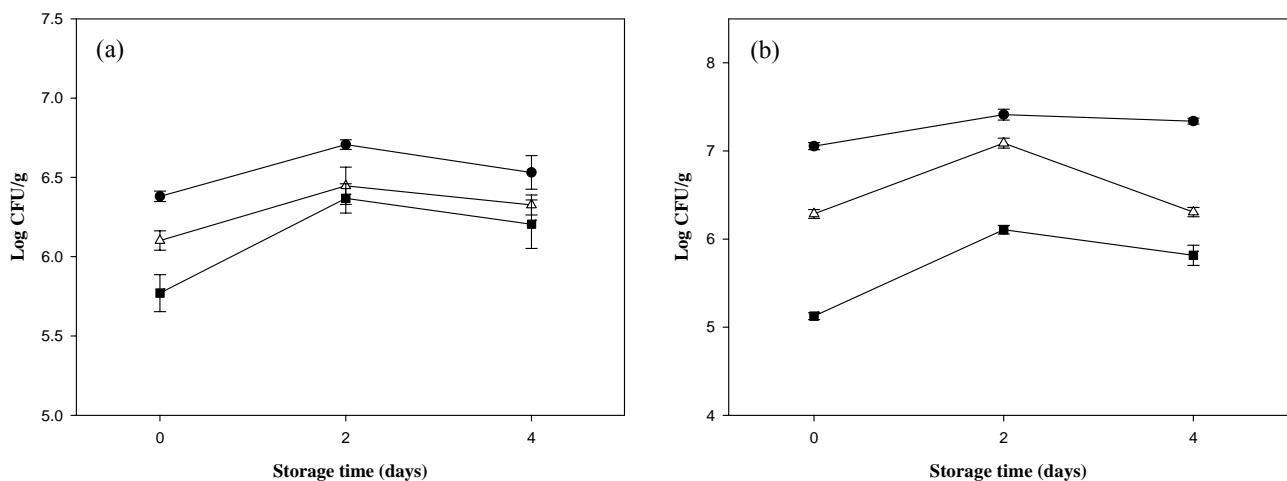
After 4 days of storage, populations of *Listeria monocytogenes* in the chicken breasts treated with 50 and 100 ppm of chlorine dioxide had 6.33 and 6.20 log CFU/g, respectively, while the control had 6.53 log CFU/g (Fig. 2-a). During storage of the chicken at 4°C the growth of *Listeria monocytogenes* was not vigorous, and the bacterial populations increased only slightly after day 2. Our results are comparable with the report of Hwang and Beuchat (7), where the populations of *Listeria monocytogenes* on chicken wings increased during storage at 4°C. However, after day 4, the populations of the bacteria decreased. This change can be explained by the availability of essential nutrients for the microbial growth during storage. It should also be noted that the effect of chlorine dioxide treatment was not greater than right after treatment.

Chicken leg controls had 7.34 log CFU/g of *Listeria monocytogenes* however, the chicken legs treated with 50 and 100 ppm of chlorine dioxide were 6.31 and 5.82 log CFU/g, respectively (Fig. 2-b). These results indicate that the decrease in the initial populations following chlorine dioxide treatment affects the microbial growth during storage, and that the difference in the degree of decrease between the chicken breast and leg can be attributed to the different tissue characteristics, resulting in a different growth pattern as well as degree of penetration by aqueous chlorine dioxide.

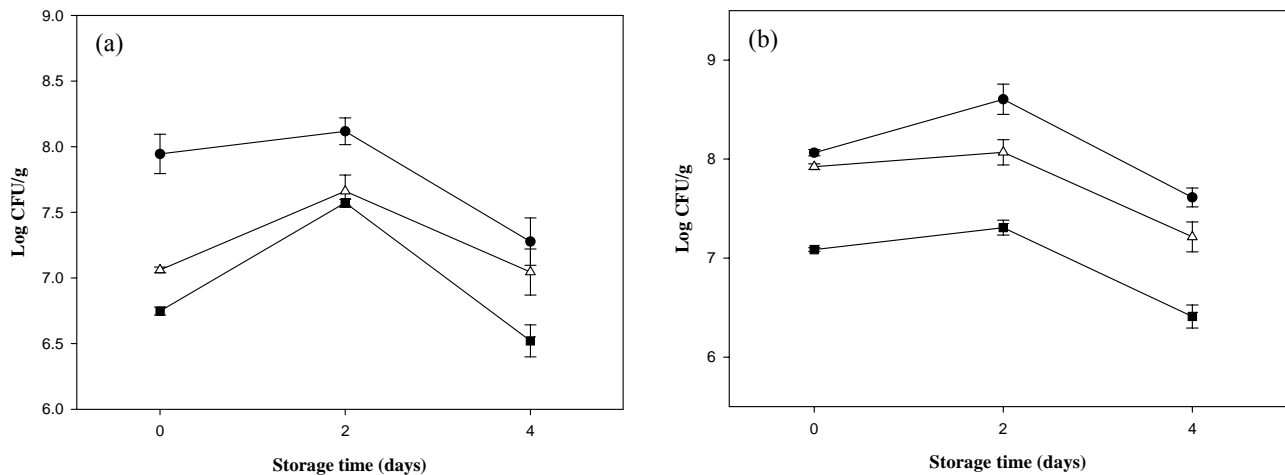
*Campylobacter jejuni* was similarly affected by aqueous chlorine dioxide treatment. After treatment, the control chicken breast had 7.94 log CFU/g, whereas the populations of *Campylobacter jejuni* for the samples treated

with 50 and 100 ppm of chlorine dioxide were 7.06 and 6.73 log CFU/g, respectively (Fig. 1-a). The control chicken leg had 8.08 log CFU/g, while the populations of *Campylobacter jejuni* in the samples treated with 50 and 100 ppm of chlorine dioxide had 7.92 and 7.09 log CFU/g, respectively (Fig. 1-b). In particular, one hundred ppm of chlorine dioxide treatment reduced the populations of *Campylobacter jejuni* by 0.99~1.21 log CFU/g. In addition, after 4 days of storage, the control chicken breast had 7.28 log CFU/g, whereas the populations of *Campylobacter jejuni* in the samples treated with 50 and 100 ppm of chlorine dioxide had 7.05 and 6.52 log CFU/g, respectively (Fig. 3-a). The control chicken leg had 7.61 log CFU/g, while the populations of *Campylobacter jejuni* for the samples treated with 50 and 100 ppm of chlorine dioxide had 7.21 and 6.41 log CFU/g, respectively (Fig. 3-b). Therefore, our results clearly indicate that aqueous chlorine dioxide treatment is effective for the inactivation of the major pathogens in the chicken during processing and storage. Thus, aqueous chlorine dioxide treatment can be used for microbial decontamination of raw chicken during processing such as in chilled water treatment.

Chouliara et al. (2) reported that the use of oregano essential oil and modified atmosphere packaging extended the shelf life of the chicken breast for 3~4 days. However, the processing is not practical considering the scale-up and cost. Hwang and Beuchat (7) also reported that treatment with 0.5% of lactic acid and 0.05% of benzoic acid decreased *Listeria monocytogenes* and *Campylobacter jejuni* by 1.0 log CFU/mL. Compared to this report, aqueous chlorine dioxide treatment of 100 ppm (equivalent to 0.01%) in our study was better than 0.5% of lactic acid and 0.05% benzoic acid.



**Fig. 2.** Effect of aqueous  $\text{ClO}_2$  treatment on the growth of *Listeria monocytogenes* in chicken during storage. Bars represent standard error. (a) chicken breast (b) chicken leg. ●: 0 ppm △: 50 ppm ■: 100 ppm.



**Fig. 3.** Effect of aqueous ClO<sub>2</sub> treatment on the growth of *Campylobacter jejuni* in chicken during storage. Bars represent standard error. (a) chicken breast (b) chicken leg. ●: 0 ppm △: 50 ppm ■: 100 ppm.

There have been several studies on the effect of aqueous chlorine dioxide on food products (14,17). Unda et al. (14) reported that aerobic mesophilic bacteria on fresh beef steaks treated with 100 ppm chlorine dioxide were decreased by 1 log cycle. Wu and Kim (17) also reported that aqueous chlorine dioxide treatment of blueberries reduced the populations of five pathogens, and yeast and molds. Our results in this study showed that aqueous chlorine dioxide treatment decreased the populations of *Listeria monocytogenes* and *Campylobacter jejuni* in chicken during storage up to 4 days.

Overall, aqueous chlorine dioxide treatment appears to achieve microbial decontamination. Therefore, our results clearly suggest that aqueous chlorine dioxide treatment should decrease the growth of pathogenic bacteria such as *Listeria monocytogenes* and *Campylobacter jejuni* in chicken.

### ACKNOWLEDGEMENT

This work was supported by a grant from the Rural Development Administration, Korea.

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(Received October 22, 2007; Accepted November 28, 2007)