J. Microbiol. Biotechnol. (2008), **18**(4), 742–745

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* Inoculated on Chicken by Aqueous Chlorine Dioxide Treatment

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Received: October 4, 2007 / Accepted: November 19, 2007

Inactivation of *Escherichia coli* O157:H7 and *Salmonella typhimurium* was evaluated on inoculated chicken by aqueous chlorine dioxide (ClO_2) treatment. Chicken samples were inoculated with 6–7 log CFU/g of *Escherichia coli* O157:H7 and *Salmonella typhimurium*, respectively. The chicken samples were then treated with 0, 50, and 100 ppm of ClO_2 solution and stored at 4±1°C. Aqueous ClO_2 treatment decreased the populations of the pathogenic bacteria on the chicken breast and drumstick. In particular, 100 ppm ClO_2 treatment on the chicken breast and drumstick reduced *Escherichia coli* O157:H7 and *Salmonella typhimurium* by 1.00–1.27 and 1.37–1.44 log CFU/g, respectively. Aqueous ClO_2 treatment on the growth of the bacteria was continuously in effect during storage, resulting in the decrease of the populations of *Escherichia coli* O157:H7 and *Salmonella typhimurium*. These results suggest that aqueous ClO_2 treatment should be useful in improving the microbial safety of chicken during storage.

Keywords: Chicken, aqueous chlorine dioxide, microbial growth, storage

Recently, consumption of poultry meat has increased worldwide [5]. Poultry products are highly perishable, and depending on the processing condition, their spoilage varies significantly even under refrigeration [15]. The major bacterial contamination on chicken includes pathogens such as *Salmonella* spp. and *Escherichia coli* [9, 11]. In particular, *Salmonella* is one of the most common pathogens associated with food poisoning, and poultry is a good source of *Salmonella* [10]. In addition, *Escherichia coli* is present in the intestinal microflora of chicken, and can survive at refrigeration temperature [13]. Thus, *Salmonella* spp. and *Escherichia coli* are detected on raw chicken during processing after slaughter, especially in chilling processing [5].

Chicken is a very popular food commodity owing to its low fat content and high nutritional value [3]. However, there is always the possibility of contamination of the pathogens during the slaughtering process and storage of chicken. Therefore, to enhance the safety of chicken, various processing techniques such as potassium sorbate [6], trisodium phosphate and sodium hydroxide [4], lactic acid and sodium benzoate [8], lactic acid and lauricidin [1], and microwave [7] treatments, and modified atmosphere packaging [5, 15] have been used for the reduction of bacterial counts and the extension of the shelf life.

As a food preservation method, aqueous chlorine dioxide has been used. Chlorine dioxide (ClO_2) is a strong oxidizing agent and has a broad biocidal effectiveness [11]. In addition, it does not produce hazardous trihalomethanes. Regarding the effectiveness of chlorine and ClO_2 in reducing the number of bacteria present in poultry-processing water, ClO_2 is more effective than chlorine [12, 16]. However, there has been no report available on the inactivation by aqueous ClO_2 of the pathogenic bacteria inoculated on chicken products. Therefore, the objective of this study was to examine the feasibility of using aqueous ClO_2 treatment to inactivate *Escherichia coli* O157:H7 and *Salmonella typhimurium* on chicken breast and drumstick samples.

Chicken samples were purchased from a local market in Daejeon, Korea, and they were dipped in distilled water and treated with UV light for 30 min. The decontaminated chicken breasts and drumsticks were then immersed in *Escherichia coli* O157:H7 (NCTC 12079) and *Salmonella typhimurium* (ATCC 14028) for 10 min and allowed to drain for 30 min. The initial level of *Escherichia coli* O157:H7 and *Salmonella typhimurium* was 6–7 log CFU/g. Before inoculation, *Escherichia coli* O157:H7 and *Salmonella typhimurium* cultures were grown at 37°C for 24 h in 50-ml tubes containing 25 ml of Luria-Bertani broth (LB; Difco Laboratoires, Detroit, MI, U.S.A.) and

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Tryptic soy broth (TSB; Difco Laboratoires, Detroit, MI, U.S.A.). After inoculation, the chicken samples were treated by dipping in 0, 50, and 100 ppm aqueous chlorine dioxide (ClO_2) solution for 10 min, respectively. The samples were then individually packaged in polyethylene terephthalate containers and stored at 4±1°C. Aqueous ClO_2 was prepared using a chlorine dioxide generating system (CH_2O Inc., Olympia, WA, U.S.A.) as described previously [18]. After ClO_2 treatment, the samples (5 g) were removed using a sterile scalpel. The 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. *Escherichia coli* O157:H7 counts were determined by plating appropriately diluted samples onto Chromogenic *E. coli*/coliform medium (EC; Oxoid, Basingstoke, U.K.). The samples were evenly spread on the surface of the plates with a sterile glass rod. *Salmonella typhimurium* was plated onto *Salmonella* Chromogenic agar base (Oxoid, Basingstoke, U.K.). All plates were incubated at 37°C for 24 h. Each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU/g.

The initial populations of *Escherichia coli* O157:H7 on the inoculated chicken breast and drumstick were 5.92 and 5.69 log CFU/g, respectively (Fig. 1A). The initial populations of *Salmonella typhimurium* were 5.33 and 5.28 log CFU/g, respectively (Fig. 1B). Aqueous ClO_2 treatment significantly decreased the populations of *Escherichia coli* O157:H7 and *Salmonella typhimurium* on the chicken breasts and drumsticks, compared with the control (Fig. 1).

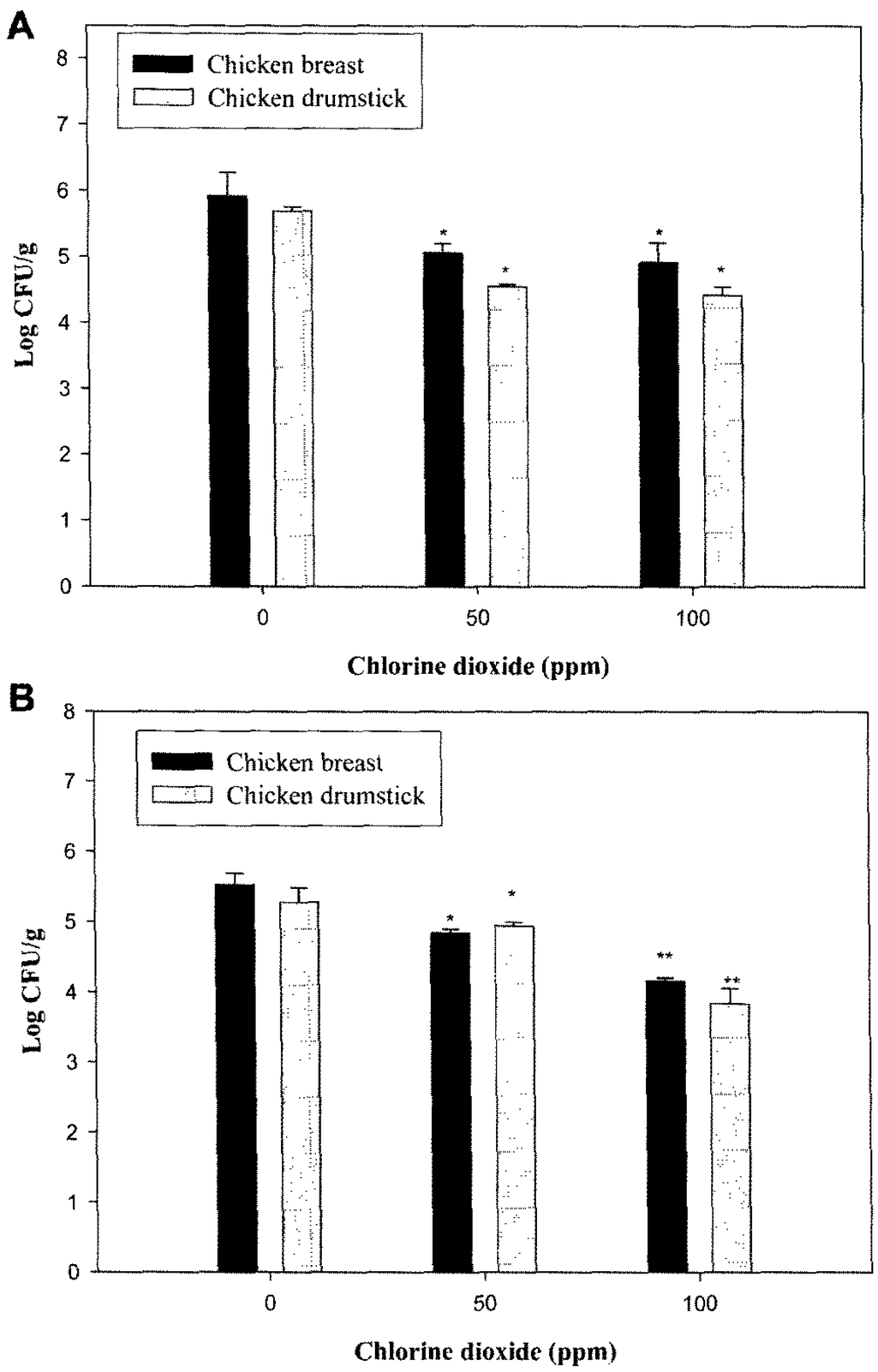

Fig. 1. Effect of aqueous ClO_2 treatment on the survival of the pathogenic bacteria inoculated on chicken. Bars represent standard error.
*, Significantly different at $p<0.05$ compared with the control (0 ppm). **, Significantly different at $p<0.01$ compared with the control (0 ppm). **A**. *Escherichia coli* O157:H7. **B**. *Samonella typhimurium*.

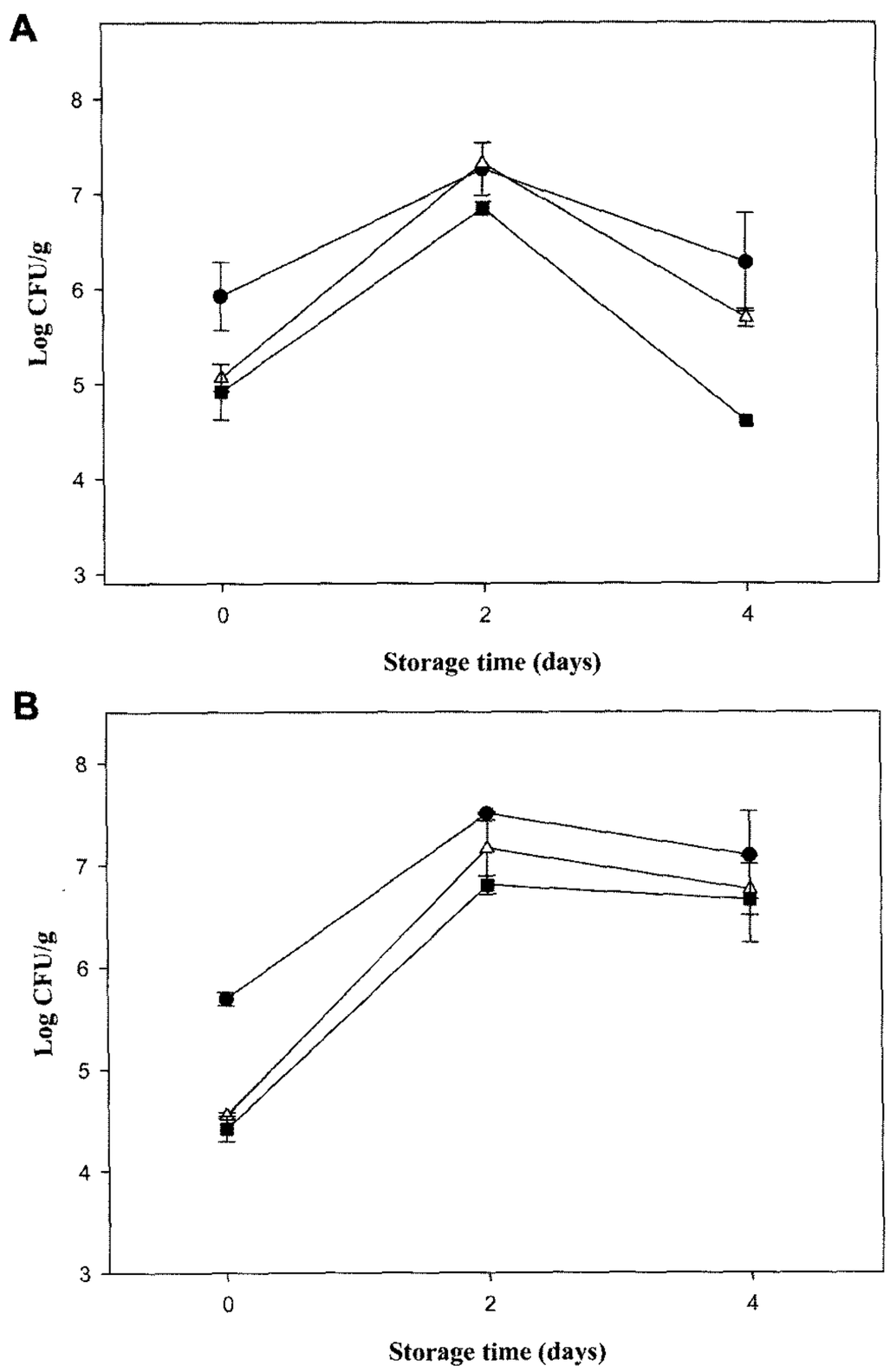

Fig. 2. Effect of aqueous ClO_2 treatment on the growth of *Escherichia coli* O157:H7 in chicken during storage. Bars represent standard error.
A. Chicken breast. **B**. Chicken drumstick. ●: Control; △: 50 ppm; ■: 100 ppm.

Increase of concentration of ClO_2 decreased the microbial populations of the bacteria. After ClO_2 treatment, the populations of *Escherichia coli* O157:H7 on the chicken breasts were 5.92, 5.06, and 4.92 log CFU/g for 0, 50, and 100 ppm ClO_2, respectively. The populations of *Escherichia coli* O157:H7 on the drumsticks were 5.69, 4.55, and 4.42 log CFU/g for 0, 50, and 100 ppm ClO_2, respectively (Fig. 1A). In particular, 100 ppm ClO_2 reduced *Escherichia coli* O157:H7 by 1.0–1.27 log cycle reduction. After 4 days of storage, the control of the chicken breast had 6.27 log CFU/g, whereas the populations of *Escherichia coli* O157:H7 for the samples treated with 50 and 100 ppm ClO_2 had 5.69 and 4.60 log CFU/g, respectively (Fig. 2A). The control of the drumstick had 7.09 log CFU/g, whereas the populations of *Escherichia coli* O157:H7 for the samples treated with 50 and 100 ppm ClO_2 had 6.76 and 6.66 log CFU/g, respectively (Fig. 2B). During storage of the chicken at 4°C, the populations of the bacteria increased slightly after day 2, and decreased after day 4. Our results are comparable with the report of Hwang and Beuchat [8], where the populations of *Escherichia coli* O157:H7 on chicken wings steadily decreased during storage at 4°C for 8 days. The change after day 4 in this study can be explained by the growth condition of the bacteria with regards the availability of nutrients for the growth during storage. In addition, it should also be noted that the effect of ClO_2 treatment was not greater than immediately after treatment.

Salmonella typhimurium also had a similar effect as *Escherichia coli* O157:H7 by aqueous chlorine dioxide treatment. The control of the chicken breast had 5.53 log CFU/g, whereas the populations of *Salmonella typhimurium* for the samples treated with 50 and 100 ppm ClO_2 had 4.84 and 4.16 log CFU/g, respectively. The control of the drumstick had 5.28 log CFU/g, whereas the populations of *Salmonella typhimurium* for the samples treated with 50 and 100 ppm ClO_2 had 4.95 and 3.84 log CFU/g, respectively (Fig. 1B). Specifically, 100 ppm ClO_2 reduced *Salmonella typhimurium* by 1.37–1.44 log CFU/g. In addition, after 4 days of storage, the control of the chicken breast had 5.65 log CFU/g, whereas the populations of *Salmonella typhimurium* for the samples treated with 50 and 100 ppm ClO_2 had 5.29 and 4.95 log CFU/g, respectively (Fig. 3A). The control of the drumstick had 5.47 log CFU/g, whereas the populations of *Salmonella typhimurium* for the samples treated with 50 and 100 ppm ClO_2 had 5.09 and 4.51 log CFU/g, respectively (Fig. 3B). Therefore, our results clearly indicate that ClO_2 treatment is an efficient way of inactivating foodborne pathogens in the chicken breast and drumstick during processing and storage. In addition, it should be noted that the efficiency of aqueous ClO_2 treatment was different depending on the chicken parts. The difference between the chicken breast and drumstick can be attributed to a difference in texture and

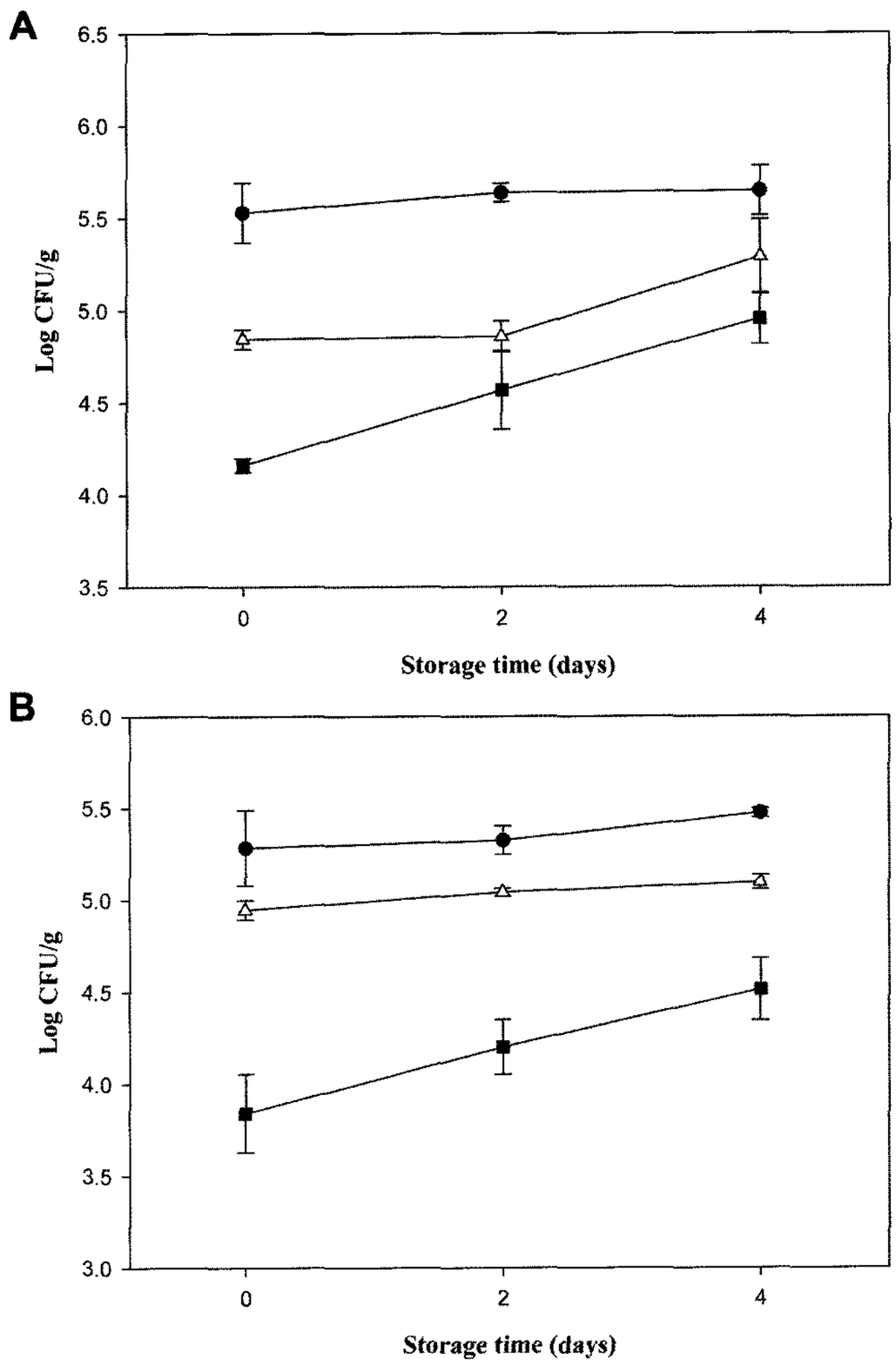

Fig. 3. Effect of aqueous ClO_2 treatment on the growth of *Samonella typhimurium* in chicken during storage. Bars represent standard error.
A. Chicken breast. **B**. Chicken drumstick. ●: Control; △: 50 ppm; ■: 100 ppm.

lipid composition of the chicken parts. Park *et al.* [14] reported that microbial growth on the surface of chicken is different for the chicken breast and drumstick. Overall, aqueous ClO_2 treatment can be used to obtain the microbial decontamination of raw chicken during processing such as chilling water treatment.

Chouliara *et al.* [5] have reported the combined effect of oregano essential oil and modified atmosphere packaging on the shelf-life extension of chicken breast meat. They reported the extension of shelf life, but the processing was not practical considering the scale-up as well as cost. Anang *et al.* [1] also reported the effects of 0.5–2% of lactic acid and lauricidin on the survival of *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli* O157:H7 in the chicken breast. Dipping of 2% of lactic acid or lauricidin for 10 min decreased the populations of *Salmonella enteritidis* and *Escherichia coli*

O157:H7 by 0.91–1.23 and 0.87–1.79 log CFU/g, respectively [1]. Compared with these reports, the aqueous ClO_2 treatment of 100 ppm (equivalent to 0.01%) in our study is more effective and has successfully provided better efficacy than other agents.

There have been several reports on the effect of aqueous ClO_2 on food products [16, 17]. Singh *et al.* [16] have reported that *Escherichia coli* O157:H7 on lettuce and baby carrots treated with 20 mg/l of aqueous ClO_2 decreased by 1.72 and 2.54 log CFU/g, respectively. Wu and Kim [17] have also reported that 15 ppm of ClO_2 treatment on blueberries for 20 min reduced the populations of *Salmonella typhimurium* by 3.32 log CFU/g. Our results in this study showed that the aqueous ClO_2 treatments at 50 and 100 ppm significantly decreased the populations of *Escherichia coli* O157:H7 and *Salmonella typhimurium* inoculated on the chicken breast and drumstick during storage up to 4 days.

Overall, these results suggest that ClO_2 treatment should decrease the growth of pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella typhimurium* on chicken products.

Acknowledgment

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

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