Effect of Aqueous Chlorine Dioxide Treatment on the Microbial Growth and Quality of Chicken Legs during Storage

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Abstract

The effect of aqueous chlorine dioxide (ClO₂) treatment on microbial growth and quality of chicken leg during storage was examined. Chicken leg samples were treated with 0, 50, and 100 ppm of ClO₂ solution and stored at 4°C. Aqueous ClO₂ treatment significantly decreased the populations of total aerobic bacteria, yeast and mold, and coliforms in chicken leg. One hundred ppm ClO₂ treatment reduced the initial populations of total aerobic bacteria, yeast and mold, and coliforms by 0.93, 1.15, and 0.94 log CFU/g, respectively. The pH and volatile basic nitrogen values in the chicken leg decreased with increasing aqueous ClO₂ concentration, while concentrations thiobarbituric acid reactive substances (TBARS) increased during storage regardless of aqueous ClO₂ concentration. Sensory evaluation results revealed that the quality of the chicken leg treated with aqueous ClO₂ during storage was better than that of the control. These results indicate that aqueous ClO₂ treatment can be useful for improving the microbial safety of chicken leg during storage.

Key words: chicken leg, aqueous chlorine dioxide, microbial growth, storage

INTRODUCTION

Although the consumption of chicken products is increasing, the microbial safety of chicken during storage and marketing remains a concern (1-4). Chicken products are highly perishable, and food poisoning can occur as a result of careless processing and storage (1). Major bacterial contaminants in chicken include Salmonella, Listeria, Campylobacter, and Escherichia coli, present in the intestinal microflora of chicken (5). Therefore, to improve the microbial safety of chicken during processing and storage, various processing techniques have been used for reduction of bacterial contaminants to extend shelf life (6-9).

As a food preservation method, aqueous chlorine dioxide (ClO₂) has been used, since it has a broad biocidal effectiveness (9-11). Aqueous ClO₂ has been approved by the FDA for use in chiller water during poultry processing, and can be used to chill poultry carcasses for about 1 hr in chilling solution containing sodium chlorite between 50 and 150 ppm (12). Thus, there have been several reports on the use of aqueous ClO₂ for the sanitization of food products (13-15), but few studies are available for application of aqueous ClO₂ on chicken products.

Therefore, this study was conducted to examine the effect of aqueous ClO₂ treatment on microbial growth, pH, volatile basic nitrogen (VBN), lipid oxidation, and sensory evaluation of chicken leg during storage.

MATERIALS AND METHODS

Materials

Chicken leg samples (refrigerated, leg part only) were purchased from a local market in Daejeon, Korea.

Chlorine dioxide preparation and treatment

Aqueous ClO₂ was prepared using a chlorine dioxide generating system (CH₂O Inc., Olympia, WA, USA) as described previously (16). Samples were treated by dipping in 0, 50, or 100 ppm aqueous ClO₂ solution for 10 min. Chlorine dioxide concentration was determined according to the method of the American Public Health Association (17). Samples were then individually packaged in polyethylene terephthalate containers and stored at 4 ± 1°C.

Microbiological analysis

After ClO₂ treatment, samples (5 g) were removed using a sterile scalpel and 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. Total bacterial counts were determined
by plating appropriately diluted samples onto plate count agar (PCA, Difco Co., Detroit, MI, USA). Yeast and mold were plated onto potato dextrose agar (PDA, Difco Co.), and coliforms were plated onto Chromogenic E. coli (Coliform Medium (EC, Oxoid Ltd., Basingstoke, Hants, England). PCA, PDA, and coliforms plates were then incubated at 37°C for 72, 96, and 24 hr, respectively. Each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU/g.

**pH measurement**

Samples (5 g) were homogenized in 45 mL of distilled water using a grinder for 1 min. Sample solutions were centrifuged for 15 min at 2000 × g, and the pH was measured using a pH meter (Corning Inc., Corning, NY, USA).

**Measurement of volatile basic nitrogen (VBN)**

VBN was determined according to the micro-diffusion method (18). Samples (5 g) were homogenized with 45 mL of distilled water using a grinder for 30 sec, and after centrifugation for 20 min, the supernatant was filtered using Whatman No 1. One mL of the filtrate was put in the left outside of Conway dish, and 1 mL of 0.01 N H3BO3 50 μL of Conway reagent (0.066% methyl red, 0.066% bromocresol green) was added to the dish. In the right outside of the dish, 1 mL of saturated K2CO3 was added and the lid was closed. After reaction with the sample in the left outside at 37°C, samples were allowed to stand for 2 hr and then titrated with 0.02 N H2SO4.

**Measurement of lipid oxidation**

The degree of lipid oxidation of the chicken leg was determined using the method of Ahn et al. (19). Samples (5 g) were homogenized in 15 mL of distilled water using a blender for 1 min. Sample solution (1 mL) was then transferred into a disposable test tube, and 2 mL of 20 mM 2-thiobarbituric acid/15% trichloroacetic acid (TBA/TCA) solution was added. The mixture was vortexed and boiled in a water bath for 15 min. The sample was cooled at room temperature for 10 min and centrifuged for 15 min at 2,000 × g. The absorbance of the resulting supernatant solution was determined at 531 nm. Thiobarbituric acid reactive substances (TBARS) values were calculated from a standard curve and expressed as mg malondialdehyde per kg sample (MDA/kg).

**Sensory evaluations**

Samples were analyzed for their freshness, texture, odor, spoilage, and overall acceptability by 8 trained panelists. Sensory qualities of the samples were evaluated using a five point scoring method. Sensory scores were 5, very good; 4, good; 3, fair; 2, poor; and 1, very poor. In particular, for spoilage of samples, panelists assessed the degree of spoilage by appearance.

**Statistical analysis**

Experimental data were analyzed by ANOVA followed by Duncan’s multiple range test using a SAS program (1999, SAS Institute, Inc., Cary, NC, USA).

**RESULTS AND DISCUSSION**

**Microbiological changes**

Aqueous ClO2 treatment significantly decreased the populations of microorganisms in chicken, compared to the control (Fig. 1). It has been known that ClO2 causes the death of the microorganisms by damaging cell membranes and inactivating mRNA. After ClO2 treatment, populations of total aerobic bacteria in the chicken leg were 5.13, 4.81, and 4.20 log CFU/g for 0, 50, and 100 ppm of ClO2 treatment, respectively (Fig. 1A). In particular, 100 ppm ClO2 treatment reduced total aerobic bacteria by 0.93 log CFU/g, compared to the control. In addition, after 10 days of storage, the control reached 8.84 log CFU/g, while the populations of total aerobic bacteria for the samples treated with 50 and 100 ppm of ClO2 had 8.21 and 7.99 log CFU/g, respectively, demonstrating that the initial decrease in the populations of total aerobic bacteria affects the bacterial growth during storage.

Yeast and mold exhibited a similar pattern to that of total aerobic bacteria (Fig. 1B). Populations of yeast and mold in the chicken leg were 3.71, 3.41, and 2.56 log CFU/g for 0, 50, and 100 ppm ClO2 treatment, respectively. One hundred ppm ClO2 treatment reduced yeast and mold by 1.15 log CFU/g, compared to the control. However, after 10 days of storage, the difference in terms of the populations of yeast and mold was not as great as right after treatment. While the control reached 6.48 log CFU/g, the populations of yeast and mold for the samples treated with 50 and 100 ppm of ClO2 had 6.36 and 6.11 log CFU/g, respectively.

Populations of coliforms in the chicken leg were 4.24, 3.87, and 3.30 log CFU/g for 0, 50, and 100 ppm of ClO2 treatment, respectively (Fig. 1C). In particular, 100 ppm ClO2 treatment reduced coliforms by 0.94 log CFU/g, compared to the control. After 10 days of storage, the control reached 7.29 log CFU/g, while the populations of coliforms for the samples treated with 50 and 100 ppm of ClO2 had 7.04 and 6.61 log CFU/g, respectively. Overall, based on the microbial growth data during storage, it was unambiguous that aqueous ClO2 treatment significantly decreased the populations of pre-existing microorganisms in chicken.
Chouliara et al. (3) reported that treatment with oregano essential oil along with modified atmosphere packaging extended the shelf life of chicken breast meat during storage. However, the processing is not practical considering the scale-up as well as cost. Therefore, aqueous chlorine dioxide treatment in our study is better in terms of microbial decontamination. There have been several reports on the efficacy of aqueous ClO₂ on other food products. Unda et al. (20) reported that aerobic mesophilic bacteria on fresh beef steaks treated with 100 ppm ClO₂ decreased by 1 log cycle. Wu and Kim (15) also reported that treatment of blueberries with 15 ppm ClO₂ decreased yeast and mold by 2.86 log cycle.

Our results suggest that aqueous ClO₂ treatment can inhibit the growth of total aerobic bacteria, yeast and mold, and coliforms in chicken leg during storage, and 100 ppm ClO₂ treatment can extend the shelf life.

**Change in pH and VBN**

The pH of the chicken leg decreased a little with increasing ClO₂ concentration (Fig. 2). These results are in good agreement with those of Jimenez-Villarreal et al. (13,21), in which the pH of beef decreased after treatment with 200 ppm ClO₂ solution. Initial pH values for the chicken leg were 6.92, 6.88, and 6.83 after...
treatment with 0, 50, and 100 ppm of ClO₂ solution, respectively. After 2 days of storage, chicken leg showed rapid increases in pH value. In general, the pH value of meat products increase during storage, and our results are in good agreement with other studies (22,23).

VBN value is one of the indicators for deterioration of food products, which is determined by amine and ammonia content in foods (24). VBN values of the chicken legs during storage increased, due to decomposition of proteins by microorganism (25). These results are in good agreement with those of Kim and Park (26). Initial VBN values after treatment with 0, 50, and 100 ppm of ClO₂ solution were 1.82, 1.54, and 1.96 mg%, respectively (Fig. 3). After 2 days of storage, all samples showed rapid increases in VBN values. In particular, after 7 days, the samples treated with 0, 50, and 100 ppm reached 9.10, 8.12, and 5.42 mg%, respectively, demonstrating that ClO₂ treatment reduced VBN values during storage. However, there were no significant differences among treatments after 10 days of storage. This can be attributed to the poor quality of all chicken leg samples after 10 days of storage.

**Lipid oxidation and sensory evaluation**

Thiobarbituric acid reactive substance (TBARS) value represents the degree of lipid oxidation of foods. Lipid oxidation is an important factor of oxidative deterioration of poultry meat (27,28). TBARS values of the chicken leg increased during storage, regardless of ClO₂ concentration (Fig. 4). These results are in good agreement with those of Kim et al. (29,30), where salmon

![Fig. 3. Changes in VBN of chicken legs treated with aqueous ClO₂ during storage. ●: control, △: 50 ppm, ■: 100 ppm.](image)

![Fig. 4. Changes in TBARS of chicken legs treated with aqueous ClO₂ during storage. ●: control, △: 50 ppm, ■: 100 ppm.](image)

**Table 1.** Sensory evaluation of chicken leg treated with aqueous ClO₂ during storage

<table>
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<th>ClO₂ treatment (ppm)</th>
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<th>2</th>
<th>4</th>
<th>7</th>
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<td>3.75±0.46b</td>
<td>2.13±0.35b</td>
<td>1.00±0.00d</td>
<td>1.00±0.00d</td>
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<td>4.25±0.71b</td>
<td>2.50±0.54b</td>
<td>1.38±0.52b</td>
<td>1.00±0.00d</td>
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<tr>
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<td>4.88±0.35a</td>
<td>3.00±0.00a</td>
<td>2.00±0.00a</td>
<td>1.00±0.00d</td>
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<tr>
<td><strong>Texture</strong></td>
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<td>3.88±0.35b</td>
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<td>1.50±0.54a</td>
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<tr>
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<td>3.13±0.64ba</td>
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<tr>
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<td>2.00±0.00a</td>
<td>1.00±0.00e</td>
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</table>

Any means with different letters within a column are significantly different (p<0.05).
and red grouper samples treated with aqueous ClO₂ treatment had increases in TBARS values during storage. However, it should be mentioned that there was no significant change in TBARS values among treatments in this study.

Sensory evaluations of the chicken legs during storage are shown in Table 1. Sensory qualities such as freshness, texture, decay, and odor were evaluated during storage. After 7 days of storage, the ClO₂ treated chicken leg had better sensory scores than the control. These results indicate that ClO₂ treatment can improve sensory qualities and extend shelf life of the chicken leg during storage at 4°C.

In conclusion, this study clearly demonstrated that aqueous ClO₂ treatment significantly decreases the populations of preexisting microorganisms in chicken legs, and helps maintain quality during storage.

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