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Effect of Chlorine Dioxide Treatment on Microbial Growth and Qualities of Chicken Breast

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Abstract

Chlorine dioxide (ClO_2) treatment was evaluated for microbial growth inhibition and its effects on the quality of vacuum-packaged chicken breasts. Chicken breast samples were treated with 3, 50, and 100 ppm of ClO_2 solution, respectively. After ClO_2 treatment, chicken breast samples were individually vacuum-packaged and stored at 4°C, a typical storage temperature for meat and meat product, for 7 days. The vacuum-packaged chicken breasts treated with ClO_2 had significantly lower total bacteria, yeast and mold, total coliform, and *Salmonella* spp. were significantly reduced by ClO_2 treatment. D_{10}-values of total bacteria count, yeast and mold, total coliform, and *Salmonella* spp. in vacuum-packaged chicken breasts was 93, 83, 85, and 50 ppm, respectively. The pH of vacuum-packaged chicken breasts decreased with increasing ClO_2 concentration. Thiobarbituric acid reacted substance (TBARS) values of vacuum-packaged chicken breasts increased during storage, regardless of ClO_2 concentration. ClO_2 treatment caused negligible changes in Hunter L, a, and b values in the vacuum-packaged chicken breasts. Sensory evaluation of the vacuum-packaged chicken breasts showed that there were no significant changes among the samples treated with various ClO_2 concentration. These results indicate that ClO_2 treatment could be useful in improving the microbial safety and quality of meat products.

Key words: chlorine dioxide, chicken, microbial growth, storage, quality

INTRODUCTION

Foodborne diseases continue to present public health hazards, demonstrating the need for improvement in food safety (1). In general, deterioration of foods is caused by physical factors such as temperature, pH, moisture content, and condition of packaging, but a major factor is microbial contamination (2,3). Therefore, safety of meat products is associated with controlling the growth of microorganisms during processing procedures, storage, and transportation (2-5).

Consumption of poultry is increasing, partly due to its nutritional value and presumed beneficial health effects (6). Poultry products are prone to deterioration after slaughtering, even under low temperature storage (6-8) and then to have higher pathogenic and spoilage bacterial counts than any other food (9). Although poultry is usually washed in chilled water before packaging, removal of microorganisms by this treatment has been limited (1, 10). Therefore, to enhance the safety of poultry products, various processing techniques such as irradiation (11), washing with organic acids (lactic, acetic, propionic acids) or sanitizers (2,5,12,13), hot water and steam pasteurization (3), chlorine (12-15), phosphates (12,13), and ozone (16) treatments have been used for the reduction of bacterial counts and extension of shelf life (17).

Regarding the use of chlorine, there have been some health concerns due to the discovery of potentially mutagenic/carcinogenic reaction byproducts like trihalomethanes (THMs), and chlorophenols generated during chlorination in the presence of organic materials (18-20). Therefore, there have been many studies on chlorine dioxide (ClO_2) as an effective alternative to chlorine (20-23), because it can extend the shelf life of meat and meat products (24-27), seafood (28-31), and vegetables and fruits (32-37), resulting in reductions in microbial counts due to its strong sterilizing power and the absence of health concern (24-39). The U.S. Food and Drug Administration (FDA) amended the food additive regulations in 1995 to allow 3 ppm residual of ClO_2 for controlling microbial populations in poultry.

Therefore, this study was conducted to determine the effectiveness of chlorine dioxide for controlling microbial growth and changes of D-value, pH, lipid oxidation,

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color and sensory evaluations of chicken breasts during storage at 4°C, a commonly used storage temperature for meat and meat product and to provide optimal processing parameters for vacuum packaged chicken breasts.

MATERIALS AND METHODS

Materials

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

Chlorine dioxide preparation and treatment

Chlorine dioxide (ClO_2) was prepared by using a chlorine dioxide generating system (CH_2O Inc., Olympia, WA, USA). Samples were treated by dipping in a solution of 0, 3, 50 and 100 ppm ClO_2 solutions concentration, which was determined according to the method of APHA (40). After ClO_2 treatment, samples were individually vacuum-packaged in PE/PP/nylon bags using a vacuum packer (M-7EG, leepack, Korea Electronic Inc., Pucheon, Korea) and stored at 4°C for 7 days.

Microbiological analysis and D-value

After ClO_2 treatment, samples (10 g) were removed from vacuum packaged chicken breasts using a sterile scalpel. Samples were placed in 90 mL of 0.1% peptone water in a sterile stomacher bag and then homogenized using a Stomacher (MIX 2, AES Laboratoire, France) for 3 min, filtered through a sterile cheese cloth, and diluted with peptone water 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). Samples were evenly spread on the surface of the plates with a sterile glass rod. Yeast and mold were plated on potato dextrose agar (PDA, Difco Co., Detroit, MI, USA). Both plates were incubated at 37°C for 48 hr. For total coliform counts, Chromogenic *E. coli*/Coliform Medium (EC, Oxoid Ltd., Basingstoke, Hants., England) was used, and plates were incubated at 37°C for 24 hr. *Salmonella* spp. counts were plated on salmonella chromogenic agar base (Oxoid Ltd., Basingstoke, Hants., England). Plates were incubated at 37°C for 48 hr. During storage at 4°C, changes of residual total mesophilic bacteria, yeast and mold, total coliform counts, and *Salmonella* spp. counts were determined. Each microbial count was the mean of three determinations and microbial counts were expressed as log CFU/g.

D_{10}-values were determined from the slope of the regression line obtained from the survival plots by plotting the log survival (N/N_0) vs concentration of ClO_2 solution (41).

pH measurement

Samples (5 g) were homogenized using a grinder (Model MCH600SI, Tong Magic Co., Seoul, Korea) for 1 min. Sample solutions were centrifuged for 15 min at 2,000 × g, and the pH was measured using a pH meter (Corning Inc., Corning, NY, USA).

Measurement of lipid oxidation

Lipid oxidation was determined according to the method of Ahn et al. (11). Each sample (5 g) was homogenized using a grinder for 1 min. One mL of each sample solution was transferred to a disposable test tube, and 2 mL of 2-thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution was added. The mixture was then vortexed and boiled in a water bath for 15 min. The samples were cooled at room temperature for 10 min and then centrifuged for 15 min at 2,000 × g. The absorbance of the resulting supernatant solution was determined at 531 nm. TBARS were expressed as mg malondialdehyde (MDA)/kg of muscle.

Color measurement

Color of samples were analyzed using a colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan). Samples were placed on a white standard plate and Hunter values (L, a, b) were measured and total color difference values were expressed as ΔE value. Hunter L, a, and b values for the standard plate were L=98.34, a=-0.03, b=1.62, respectively. Five measurements were taken at different locations of each sample.

Sensory evaluations

Samples were analyzed for their freshness, texture, odor, spoilage, and overall acceptability by 10 trained panelists. Sensory qualities of samples were evaluated using five point scoring method.

Statistical analysis

Differences were analyzed by Duncan's multiple range tests and analysis of variance using a SAS program (1999, SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Microbiological changes during storage at 4°C

Initial populations of total bacteria, yeast and mold, total coliform, and *Salmonella* spp. of chicken breasts were 5.6, 4.6, 5.0 and 3.5 log CFU/g, respectively. These results confirmed a need for sterilization to assure the microbial safety of chicken breasts.

Fig. 1 shows that populations of total bacteria, yeast and mold, total coliform, and *Salmonella* spp. in chicken

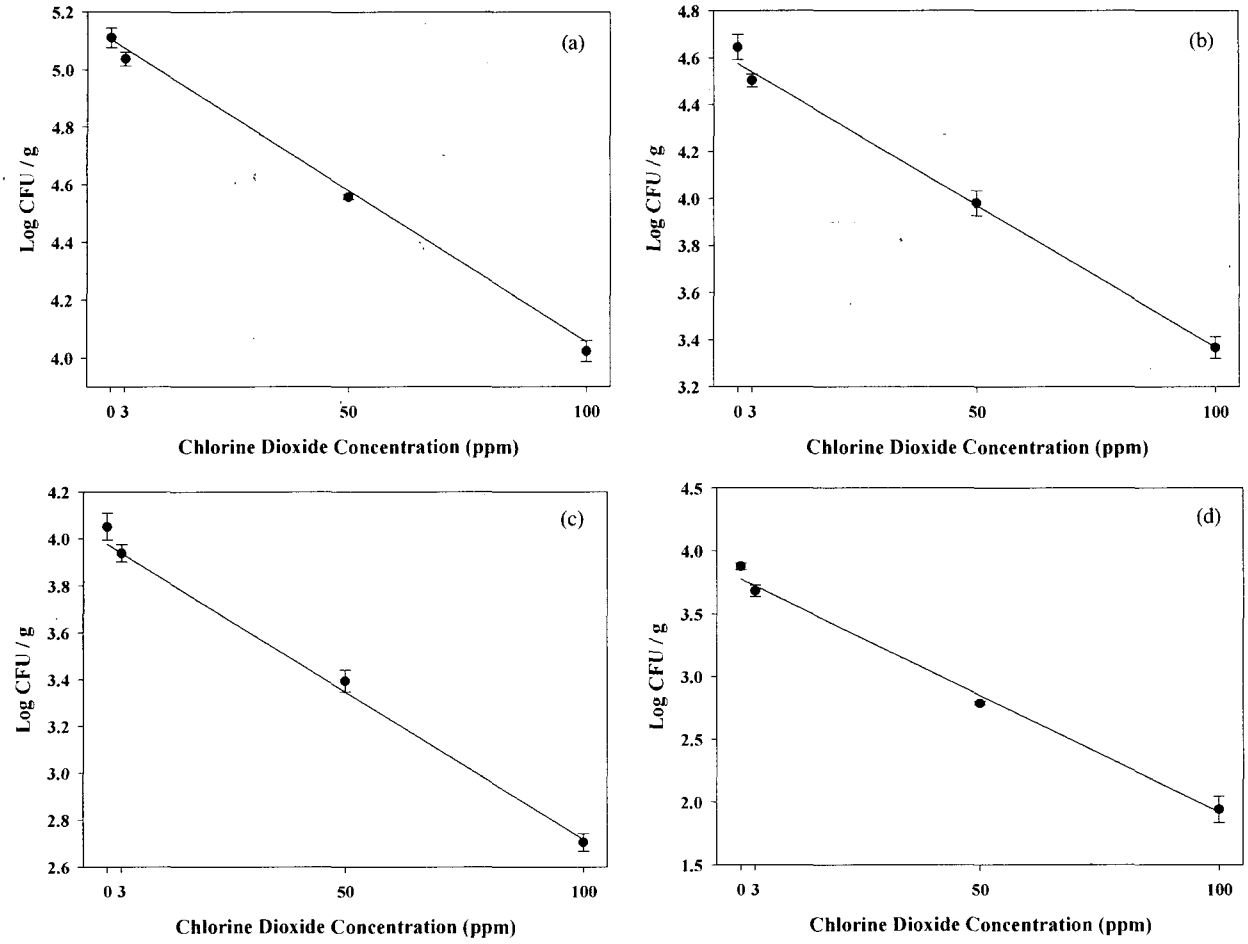

Fig. 1. Effect of chlorine dioxide treatment on the survival of microorganisms in vacuum-packaged chicken breast at 4°C. Bars represent standard error. (a) Total aerobic bacteria, (b) Yeast and mold, (c) Coliforms, (d) *Salmonella* spp.

breasts were significantly reduced by ClO_2 treatment. Populations of microorganism on samples treated with ClO_2 solution at 3~100 ppm were reduced by 0.3~2 log CFU/g. ClO_2 is known to cause protein denaturation resulting in the death of microorganism by damage to cell membrane and inactivation of mRNA (15,18,20). Kim et al. (29,30) have reported that 40 ppm ClO_2 treatment caused reduction of 0.3 and 0.6 log CFU/g of microorganism in salmon fillet and grouper fillet, respectively. Singh et al. (32) also have reported that *Escherichia coli* O157:H7 on shredded lettuce and baby carrots were significantly reduced by increasing ClO_2 concentrations. The effect of ClO_2 treatment in this study was in good agreement with the results reported by Kim et al. (29,30) and Singh et al. (32,37).

Chicken breast samples showed a rapid increase in total bacteria on vacuum-packaged chicken breasts during storage at 4°C for 7 days, reaching populations in excess of 7 log CFU/g after 3 days of storage (Fig. 2a). Considering 7 log CFU/g as the maximum allowable level of microbial population, shelf life of vacuum-packaged chicken breasts was below 3 days under normal storage conditions. After 3 days, the populations of total bacterial counts on samples treated with 3, 50, and 100 ppm of ClO_2 were 6.7, 6, and 5.3 log CFU/g, respectively. Treatments of 50 and 100 ppm of ClO_2 delayed the time required for total bacterial counts to reach 7 log CFU/g to 7 days.

These results show that ClO_2 treatment is an efficient method for preventing microbial spoilage during storage of vacuum-packaged chicken breasts at 4°C. Yeast and mold increased in a similar pattern as did total bacteria (Fig. 2b). After 3 days, the control reached 6.4 log CFU/g, while populations of yeast and mold for samples treated with 3, 50 and 100 ppm of ClO_2 had 6.1, 5.8, and 4.5 log CFU/g, respectively. During storage, the control reached populations in excess of 7 log CFU/g, while samples treated with 3, 50 and 100 ppm of ClO_2 had 6.6, 6.2, and 5.1 log CFU/g, respectively. Coliforms also showed a similar pattern as total bacterial count during storage (Fig. 2c). After 3 days, the control reached 6.4 log CFU/g, while samples treated with 3, 50 and

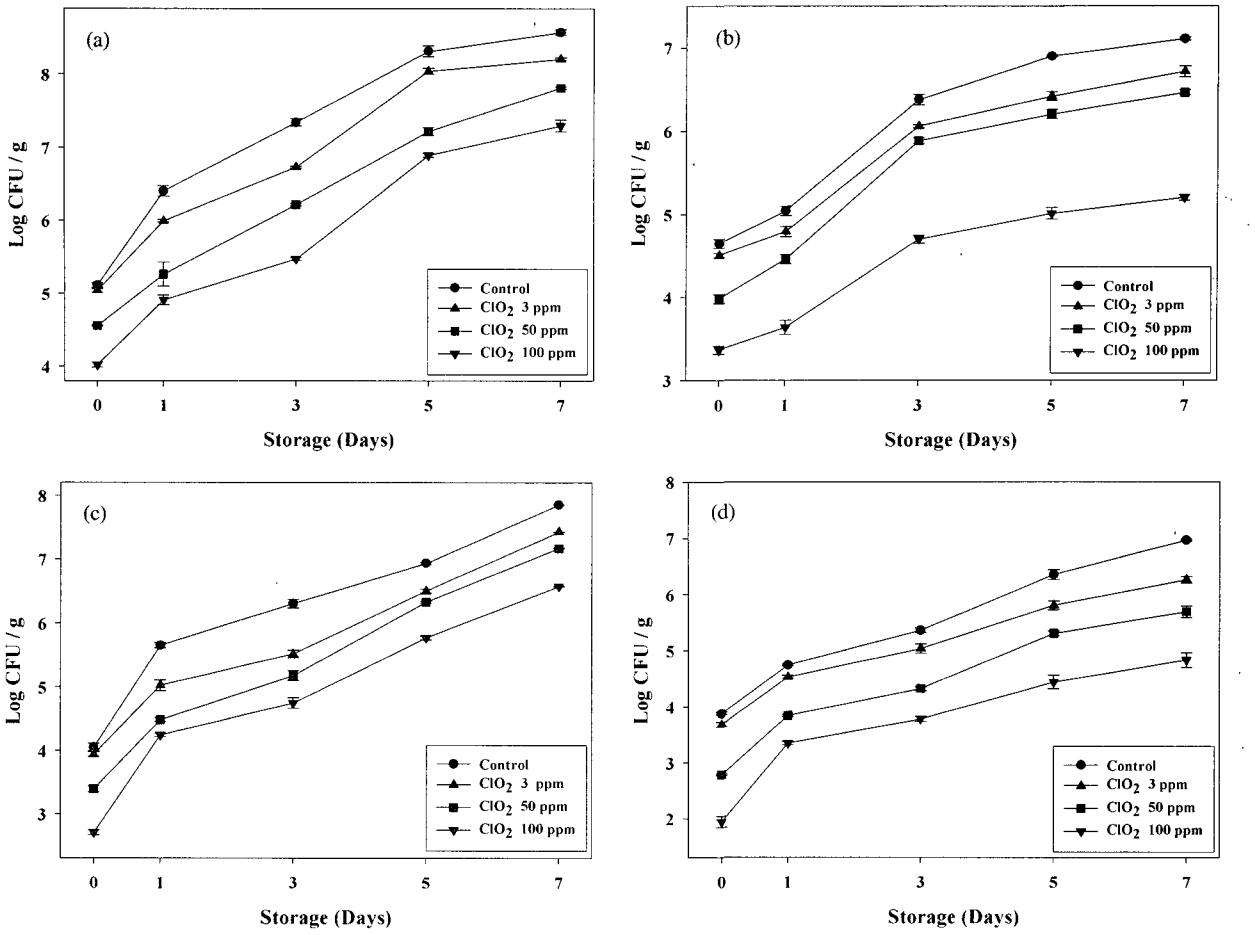

Fig. 2. Changes in microbial populations of vacuum-packaged chicken breast treated with ClO_2 during storage at 4°C. Bars represent standard error. (a) Total aerobic bacteria, (b) yeast and mold, (c) Coliforms, (d) *Salmonella* spp.

100 ppm of ClO_2 had 5.6, 5.2, and 4.8 log CFU/g, respectively. After 7 days, the control reached 8 log CFU/g, while samples treated with 3, 50, and 100 ppm of ClO_2 had 7.2, 6.9, and 6.2 log CFU/g, respectively. These results indicate that ClO_2 treatment effectively reduced initial populations of coliform in vacuum-packaged chicken breasts, but had less effect on the growth of coliforms during growth as previously reported in the literature (32-34,36-39).

Salmonella spp., which is one of the major pathogenic bacteria causing food poisoning, is the main concern regarding the microbial safety of poultry and poultry products (7,8,26,27,39). *Salmonella* spp. showed a similar pattern as other microorganisms during storage (Fig. 2d). After 3 days, the control reached 5.3 log CFU/g, while samples treated with 3, 50, and 100 ppm of ClO_2 had 5.1, 4.3, and 3.8 log CFU/g, respectively. After 7 days, populations were below 6.5 log CFU/g for vacuum-packaged chicken breasts. These results showed that ClO_2 treatment delayed the increase in population of *Salmonella* spp. in vacuum-packaged chicken breasts.

D_{10} value

D_{10}-values of total bacteria, yeast and mold, total coliforms, and *salmonella* spp. for vacuum-packaged chicken breasts are shown in Table 1. *Salmonella* spp. treated with ClO_2 had a lower D_{10}-value than other microorganisms. D_{10}-values of microorganisms are usually affected by the method of inoculation, type of microorganism, initial microbial population, type of samples, physicochemical condition of samples, and many other environmental conditions such as treatment temperature or medium, and storage conditions (7-9). Therefore, these results could be due to the difference in sensitivity of

Table 1. D_{10} value[1)] of microorganisms in vacuum-packaged chicken breast

Microorganisms	Initial populations of microorganism (log CFU/g)	D_{10} value (ppm)
Total aerobic bacteria	5.6	93.46
Yeast and mold	4.6	83.33
Escherichia coli	5.0	84.74
Salmonella spp.	3.5	50.25

[1)]Decimal reduction dose.

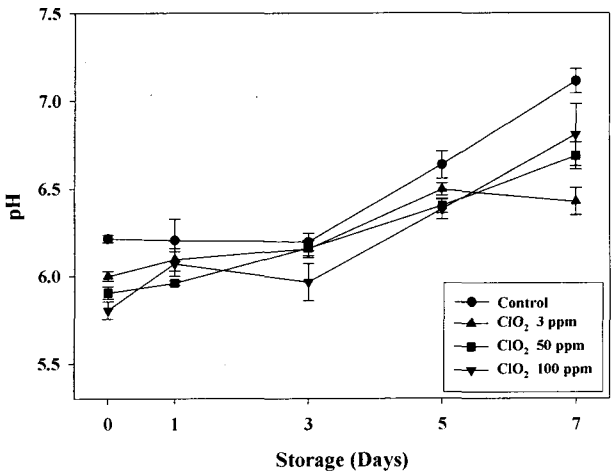

Fig. 3. Changes in pH of vacuum-packaged chicken breast treated with ClO_2 during storage at 4°C. Bars represent standard error.

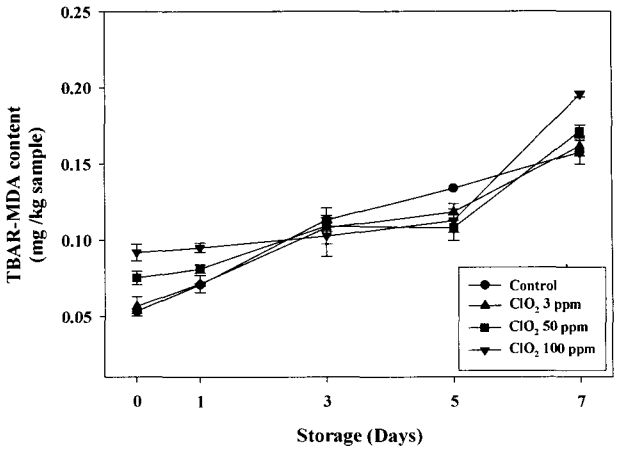

Fig. 4. Changes in TBARS of vacuum-packaged chicken breast treated with ClO_2 during storage at 4°C. Bars represent standard error.

bacteria cells to various ClO_2 treatment (15,30,38,39).

Change in pH and lipid oxidation during storage at 4°C

The pH of vacuum-packaged chicken breasts decreased with increasing ClO_2 concentration (Fig. 3). These results are in good agreement with those of Jimenez-Villarreal et al. (12,13), where the pH of beef decreased after treatment with 200 ppm of ClO_2 solution. Fig. 3 shows that the pH of vacuum-packaged chicken breasts treated with ClO_2 solution increased during storage at 4°C. Initial pH values after treatment of chicken breasts with 0, 3, 50, and 100 ppm of ClO_2 solution were 6.3, 6.0, 5.9, and 5.8, respectively. Chicken breasts treated with various ClO_2 solutions showed similar increases in pH value for 3 days. However, after 3 days, all chicken breast samples showed rapid increases in pH value. In general, pH value of meat and meat product increases with increasing storage time, because there is a decrease in electrolyte dissociation or an increase in the concentration of buffering protein and the formation of ammonia. Our results suggest that the pH of vacuum-packaged chicken breasts increases with formation of ammonia. These results are in good agreement with those of Holley et al. (42).

Lipid oxidation measurements are shown in Fig. 4. TBARS values of vacuum-packaged chicken breasts increased during storage at 4°C regardless of ClO_2 solution concentration. During 3 days of storage, samples treated with 3 ppm of ClO_2 increased in a similar pattern as the control, while those of 50 and 100 ppm ClO_2 treatments increased very slowly. However, after 5 days, all samples showed rapid increases in TBARS values. These results are in good agreement with those of Kim et al. (28,29), where salmon and red grouper samples with aqueous ClO_2 treatment had increases in TBARS values during storage at 4°C.

Color change and sensory evaluation

Hunter L, a, and b values of vacuum-packaged chicken breasts treated with ClO_2 solution are shown in Table 2. Hunter L values of vacuum-packaged chicken breasts treated with ClO_2 solution increased with increasing ClO_2 concentration immediately after treatment, and then gradually decreased during storage at 4°C. These results are similar to those of Jimenez-Villarreal et al. (12,13), where L values of ground beef with ClO_2 treatment decreased during storage at 4°C.

Hunter a values of vacuum-packaged chicken breasts treated with ClO_2 solution decreased with increasing ClO_2 concentration immediately after treatment, while Hunter b values increased with increasing ClO_2 concentration. During 3 days of storage, Hunter a values of vacuum-packaged chicken breasts gradually decreased. However, after 3 days of storage, Hunter a values increased. Hunter b values also decreased during 5 days of storage. After 5 days, Hunter b values increased. These results are similar to those of Pohlman et al. (24), where chlorine dioxide/trisodium phosphate (CT) treatment did not alter Hunter a and b values of ground beef during storage. These results indicate that ClO_2 treatment does not cause color change in vacuum-packaged chicken breasts.

Sensory evaluations of vacuum-packaged chicken breasts during storage are shown in Table 3. Sensory qualities such as freshness, texture, decay, and odor were examined to evaluate the samples during storage. After 7 days of storage, ClO_2 treated vacuum-packaged chicken breasts had better sensory scores than the control. These results indicated that ClO_2 treatment may improve sensory qualities and extend the shelf life of vacuum-packaged chicken breasts during storage at 4°C.

Table 2. Change in Hunter color values of vacuum-packaged chicken breast treated with ClO_2 solution during storage at 4°C

Color parameter[1)]	ClO_2 treatment (ppm)	Storage period (days)				
		0	1	3	5	7
L	0	55.00±1.31[b2)]	54.38±1.75[a]	56.15±1.81[b]	51.58±0.73[b]	49.29±1.46[b]
	3	55.16±1.70[a]	54.27±2.49[a]	56.16±1.71[b]	53.49±1.46[ab]	51.75±0.78[a]
	50	57.05±1.70[b]	55.94±3.26[a]	53.65±2.20[a]	51.69±1.22[b]	49.47±0.73[b]
	100	57.13±2.64[ab]	55.23±3.65[a]	54.25±0.81[ab]	54.04±2.07[a]	51.51±1.27[a]
a	0	1.70±0.22[a]	1.73±0.15[a]	1.48±0.96[b]	3.71±0.37[a]	3.74±0.75[a]
	3	1.21±0.36[ab]	1.19±0.54[b]	1.13±0.55[b]	2.32±0.54[b]	2.82±0.50[ab]
	50	1.16±0.62[ab]	1.99±0.44[a]	1.52±0.69[b]	2.13±0.62[b]	2.16±0.51[b]
	100	0.70±0.29[b]	1.17±0.88[b]	1.12±0.31[b]	2.14±0.58[b]	2.72±1.49[b]
b	0	4.10±0.49[b]	3.02±0.65[a]	3.38±0.94[b]	3.02±0.42[b]	4.79±0.83[b]
	3	4.30±1.29[b]	3.49±1.01[a]	3.72±0.78[b]	3.13±1.49[b]	3.15±0.48[a]
	50	4.80±0.63[b]	4.09±0.67[b]	3.61±1.11[b]	2.78±1.46[a]	4.12±1.49[b]
	100	5.59±1.13[a]	4.18±0.86[b]	3.78±0.40[b]	3.38±0.60[b]	4.23±2.21[b]

[1)]L: degree of whiteness (0 black~100 White), a: degree of redness (-80 greenness~100 redness), b: degree of yellowness (-80 blue~70 yellowness).
[2)]Means in the same row followed by the same letter are not significantly ($p<0.05$) different by Duncan's multiple range test.

Table 3. Sensory evaluation of vacuum-packaged chicken breast treated with ClO_2 solution during storage at 4°C

Items	ClO_2 treatment (ppm)	Storage period (days)				
		0	1	3	5	7
Freshness	0	5.00±0.00	4.80±0.45	2.80±0.84	1.60±0.55[b1)]	1.00±0.00
	3	5.00±0.00	4.80±0.45	3.00±1.22	2.00±0.71[ab]	1.20±0.45
	50	5.00±0.00	4.60±0.55	2.80±0.84	2.40±0.55[ab]	1.60±0.55
	100	5.00±0.00	4.60±0.55	3.60±1.14	2.60±0.55[a]	1.80±0.45
Texture	0	5.00±0.00	4.60±0.55	2.80±1.30	1.20±0.45[b]	1.20±0.45[b]
	3	5.00±0.00	4.40±0.55	3.20±1.10	1.60±0.55[ab]	1.40±0.55[b]
	50	5.00±0.00	4.00±1.00	3.20±1.10	2.60±0.55[a]	2.00±0.00[a]
	100	5.00±0.00	3.60±1.14	3.40±1.14	2.60±0.89[a]	2.20±0.45[a]
Decay	0	5.00±0.00	4.80±0.45	3.00±1.41	1.80±0.84	1.40±0.55
	3	5.00±0.00	4.80±0.45	3.40±1.52	2.00±1.00	1.80±0.45
	50	5.00±0.00	4.80±0.45	3.60±1.34	2.40±0.89	1.80±0.45
	100	5.00±0.00	4.80±0.45	4.00±1.00	3.00±0.71	2.00±0.00
Odor	0	5.00±0.00	5.00±0.00[a]	2.60±0.89	1.40±0.55	1.00±0.00[c]
	3	5.00±0.00	4.60±0.55[ab]	3.20±1.30	1.80±0.84	1.20±0.45[bc]
	50	5.00±0.00	4.40±0.55[ab]	3.20±1.30	2.40±0.55	1.60±0.55[ab]
	100	5.00±0.00	4.20±0.45[b]	3.40±1.14	2.40±0.55	1.80±0.45[a]
Overall acceptability	0	5.00±0.00	4.80±0.45	2.60±0.89	1.20±0.45[b]	1.00±0.00[c]
	3	5.00±0.00	4.40±0.55	3.00±1.41	1.60±0.55[ab]	1.20±0.45[bc]
	50	5.00±0.00	4.20±0.45	3.00±1.00	2.20±0.45[a]	1.60±0.55[ab]
	100	5.00±0.00	4.20±0.45	3.60±1.14	2.20±0.45[a]	2.00±0.00[a]

[1)]Means (±standard errors) with different superscripts within the same column are significantly different ($p<0.05$) by Duncan's multiple range test. Means of 3 replications.

In conclusion, this study clearly indicated that ClO_2 treatment of vacuum-packaged chicken breasts significantly decreases populations of microorganisms and reduces lipid oxidation during storage at 4°C. In addition, ClO_2 treatment was effective in maintaining the quality of vacuum-packaged chicken breasts. Therefore, ClO_2 treatment can extend the shelf life and improve the microbial safety of vacuum-packaged chicken breasts during storage at 4°C.

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