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Article

Effect of Aqueous Chlorine Dioxide Treatment on the Microbial Growth and Qualities of Iceberg Lettuce during Storage

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Effects of aqueous chlorine dioxide (ClO_2) treatment on the microbial growth and the quality of iceberg lettuce during storage were examined. Lettuce samples were treated with 0, 5, 10, and 50 ppm of ClO_2 solution and stored at 4°C. Aqueous ClO_2 treatment significantly decreased the populations of total aerobic bacteria, yeasts and molds, and coliforms on the shredded lettuce. Fifty ppm ClO_2 treatment reduced the initial populations of total aerobic bacteria, yeasts and molds, and coliforms by 1.77, 1.34, 1.10 log CFU/g, respectively. Aqueous ClO_2 treatment caused negligible changes in the Hunter color L, a, and b values during storage. Sensory evaluations exhibited that there were no significant changes among treatments. These results indicate that the aqueous ClO_2 treatment can be useful in improving the microbial safety of the iceberg lettuce during storage and extending the shelf life.

Key words: *aqueous chlorine dioxide, iceberg lettuce, microbial growth, storage*

Minimally processed vegetables are prepared for use as ready-to-eat or ready-to-use products. They are usually trimmed, peeled, cut, washed, and sometimes disinfected. Fresh-cut produce market has undergone a rapid growth due to thenew trend in life-style, short processing time, and low cost of shipping and storage [Ortega *et al.*, 1997].

However, the use of fresh-cut vegetables such as shredded lettuce is limited due to the microbial contamination during storage. Sources of microbial contamination for minimally processed vegetables include raw materials, plant workers, processing environment, and handling equipments. In particular, the surface of the food products is exposed to the microbial contamination during the cutting process [Watada *et al.*, 1996]. Therefore, appropriate preservation techniques are needed for extending the shelf-life of fresh-cut vegetables.

Many raw fruits and vegetables are washed with chlorinated water to reduce the population of the microorganisms [Cherry, 1999]. However, the use of chlorine has brought on health concerns due to the presence of trihalomethanes generated in the presence of organic materials [Beuchat *et al.*, 1998; Kim *et al.*, 1999]. Recent studies showed aqueous chlorine dioxide (ClO_2) could be an effective alternative to chlorine [Kim *et al.*, 1999; Owusu-Yaw *et al.*, 1990]. Chlorine dioxide has much higher oxidation capacity than chlorine [Bennarde *et al.*, 1965] and does not generate hazardous chemicals. Food and Drug Administration has allowed the use of aqueous chlorine dioxide in washing fruits and vegetables.

Therefore, this study was conducted to examine the effects of aqueous chlorine dioxide treatment on the microbial growth, color, and sensory qualities of shredded lettuce during storage, and to improve the microbial safety and qualities of iceberg lettuce.

Materials and Methods

Preparation of lettuce. Fresh iceberg lettuce samples were purchased from a local market in Daejeon, Korea. Samples were cut into 3 cm × 3 cm pieces, and rinsed with cold tap water for 1 min.

Chlorine dioxide preparation and treatment. Chlorine dioxide (ClO_2) was prepared using a chlorine dioxide-generating system (CH_2O Inc., Olympia, WA, USA) as described previously [Youm *et al.*, 2004]. Shredded lettuce samples were treated by dipping in 0, 5, 10, and 50 ppm ClO_2 solutions for 10 min. After ClO_2 treatment, the samples (80 g each) were individually packaged and stored at 4 ± 1°C.

Microbiological analysis. After the ClO_2 treatment, 10 g of the shredded lettuce was placed with 90 mL of 0.85% sterile saline (w/v) in a sterile stomacher bag. The

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samples were then homogenized using a Stomacher (MIX 2, AES Laboratoire, France) for 3 min, filtered through a sterile cheese cloth, and diluted with 0.85% sterile saline for the microbial count. Serial dilutions were performed in triplicate on each selective agar plate. Total bacterial counts were determined by plating appropriately diluted samples onto the plate count agar (PCA, Difco, Detroit, MI, USA). The samples were evenly spread on the surfaces of the plates using a sterile glass rod. Yeasts and molds were plated on the potato dextrose agar (PDA, Difco, Detroit, MI, USA), and coliforms were plated on the *E. coli*/coliform medium (Oxoid, Basingstoke, Hampshire, U.K). *E. coli*/coliform, PCA, and PDA plates were then incubated at 37°C for 24, 48, and 72 h. Each microbial count was the mean of three determinations. The microbial counts were expressed as log CFU/g.

Color measurement. Colors of the samples were analyzed using a colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan). The samples were placed on a white standard plate, and Hunter's color values (L, a, b) were measured. Hunter's L, a, and b values for the standard plate were L = 97.47, a = –0.02, and b = 1.67, respectively. Five measurements were taken at different locations of each sample.

Sensory evaluation. The samples were analyzed for their freshness, color, texture, flavor, and overall acceptability by eight trained panels. The sensory qualities were evaluated using the five-point scoring method: 5, very good; 4, good; 3, fair; 2, poor; and 1, very poor.

Statistical analysis. Analysis of variance and Duncan's multiple range tests were performed to analyze the results using the SAS program (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

Aqueous chlorine dioxide treatment significantly decreased the populations of total aerobic bacteria as compared to the control. After the ClO_2 treatment, the populations of total aerobic bacteria in shredded lettuce were 5.44, 4.98, 4.64, and 3.67 log CFU/g after 0, 5, 10, and 50 ppm of the ClO_2 treatments, respectively (Fig. 1). Fifty ppm treatment reduced total aerobic bacteria population by 1.77 log CFU/g, compared to the control. These results indicate that the ClO_2 treatment at 50 ppm may be sufficient to extend the shelf life of the iceberg lettuce. In addition, initial decrease in the populations of total aerobic bacteria by the ClO_2 treatment affected the microbial growth during the storage of the iceberg lettuce. After 8 days of storage, the control reached 6.56 log CFU/g, whereas the population of total aerobic bacteria in the samples treated with 50 ppm of ClO_2 was 5.66 log CFU/g.

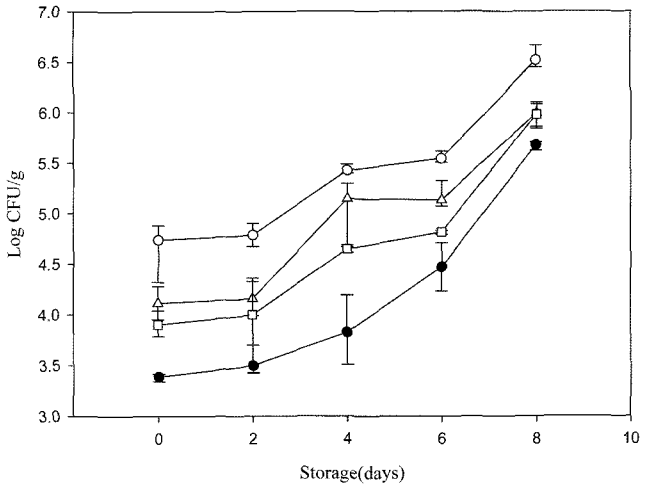

Fig. 1. Changes in total aerobic bacteria population of ClO_2-treated iceberg lettuce during storage. ○: control, △: 5 ppm, □: 10 ppm, ●: 50 ppm.

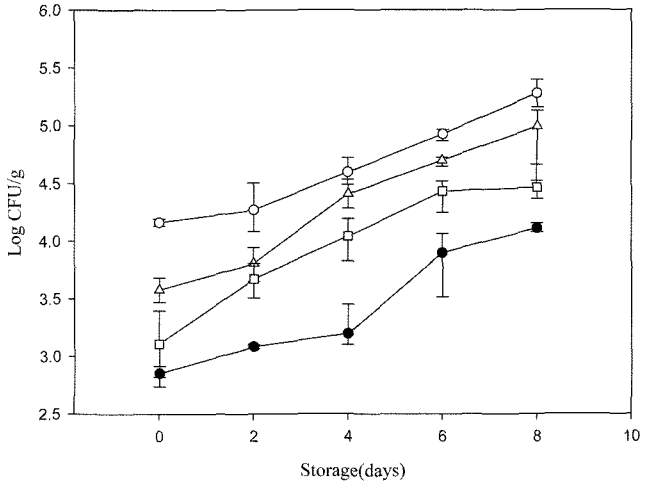

Fig. 2. Changes in yeast and mold populations of ClO_2-treated iceberg lettuce during storage. ○: control, △: 5 ppm, □: 10 ppm, ●: 50 ppm.

Yeasts and molds showed a similar pattern as that of the total aerobic bacteria (Fig. 2). Populations of yeasts and molds in the shredded lettuce were 4.16, 3.58 3.11, and 2.82 log CFU/g after 0, 5, 10, and 50 ppm of the ClO_2 treatment, respectively. Fifty ppm treatment reduced the populations of yeasts and molds by 1.34 log CFU/g, compared to the control. This difference in the populations was attributed to the duration of storage. After 8 days of storage, the control reached 5.28 log CFU/g, whereas the populations of yeasts and molds in the samples treated with 50 ppm of ClO_2 showed 4.12 log CFU/g.

Populations of the coliforms in the shredded lettuce were 2.70, 2.54 2.51, and 1.60 log CFU/g after 0, 5, 10,

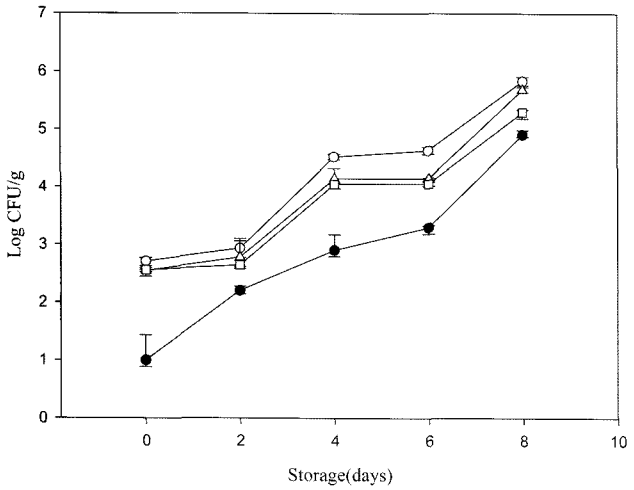

Fig. 3. Changes in coliform populations of ClO_2-treated iceberg lettuce during storage. ○: control, △: 5 ppm, □: 10 ppm, ●: 50 ppm.

and 50 ppm of the ClO_2 treatment, respectively (Fig. 3). In particular, 50 ppm treatment reduced the coliform population by 1.10 log CFU/g. In addition, after 8 days of storage, the population of the control reached 5.83 log CFU/g, while that of the coliforms in the samples treated with 50 ppm ClO_2 was 4.93 log CFU/g. These results were similar with the bactericidal effect of ClO_2 treatment in lettuces and baby carrots inoculated with *Escherichia coli* 0157:H7 [Singh *et al.*, 2002]. A number of studies have reported on the effects of the ClO_2 treatment on the microbial decontamination of the food products. Lee *et al.* [2004] reported that the treatment of 40 ppm ClO_2 on apples significantly decreased the number of *Alicyclobaillus acidoterrestris* spores. Wu and Kim [2007] also reported that the treatment of 15 ppm ClO_2 on the blueberries decreased the populations of yeasts and molds by 2.86 log cycle. The results of the present study suggest that the aqueous ClO_2 treatment decreases the populations of total aerobic bacteria, yeast and molds, and coliforms in the shredded lettuce, and 50 ppm of the ClO_2 treatment can extend the shelf life by inhibiting the growth of the microorganisms.

Color of the shredded lettuce was determined using a colorimeter (Table 1). Regarding Hunter's L, a, and b values of samples, no significant differences were observed among treatments. However, it should be noted that Hunter's b values of the samples increased significantly during storage, and this trend was the same for all treatments. Therefore, our results clearly indicate that the ClO_2 treatment does not cause a change in color, in good agreement with the results of other studies [Ihl *et al.*, 2003; Baur *et al.*, 2004]. In addition, sensory qualities such as freshness, color, texture, flavor, and overall acceptance were examined among the samples during storage. Table 2 clearly indicates that the sensory qualities

Table 1. Color measurement of shredded lettuce treated with aqueous ClO_2 during storage

Color parameter	Storage time (day)	Concentration (ppm) 0	5 ppm	10 ppm	50 ppm
L	0	66.95 ± 2.01^{Ab}	67.27 ± 0.97^{Ab}	67.13 ± 5.69^{Aa}	67.03 ± 3.24^{Ab}
	2	68.06 ± 1.80^{Aba}	69.17 ± 3.14^{Aba}	71.12 ± 0.74^{Aa}	70.32 ± 2.11^{Aba}
	4	69.52 ± 1.59^{Aa}	70.45 ± 0.16^{Aa}	70.27 ± 2.02^{Aa}	70.60 ± 0.84^{Aba}
	6	70.26 ± 2.54^{Aa}	70.81 ± 1.71^{Aa}	72.49 ± 1.76^{Aa}	73.00 ± 0.48^{Aa}
	8	71.00 ± 0.91^{Aa}	71.63 ± 0.46^{Aa}	72.15 ± 1.63^{Aa}	71.57 ± 1.95^{Aa}
a	0	-18.61 ± 2.22^{Aa}	-18.40 ± 0.53^{Aa}	-18.84 ± 1.83^{Aa}	-20.04 ± 0.57^{Aa}
	2	-20.33 ± 0.46^{Aa}	-20.17 ± 1.90^{Aba}	-20.41 ± 0.50^{Aa}	-21.34 ± 0.70^{Aa}
	4	-20.20 ± 0.16^{Aa}	-21.08 ± 0.75^{Ab}	-21.10 ± 0.92^{Aba}	-21.37 ± 1.35^{Aa}
	6	-20.69 ± 3.44^{Aa}	-21.04 ± 0.46^{Ab}	-21.17 ± 2.50^{Aba}	-21.64 ± 1.92^{Aa}
	8	-20.96 ± 4.11^{Aa}	-21.13 ± 1.24^{Ab}	-22.03 ± 0.76^{Ab}	-21.50 ± 1.07^{Aa}
b	0	29.07 ± 2.06^{Ab}	29.51 ± 1.27^{Ac}	28.78 ± 2.23^{Ac}	29.05 ± 1.67^{Ad}
	2	32.37 ± 1.44^{Aba}	33.12 ± 0.47^{Ab}	32.61 ± 1.85^{Ab}	33.29 ± 1.13^{Ac}
	4	33.39 ± 1.19^{Aba}	33.99 ± 1.79^{Aba}	34.11 ± 1.09^{Aba}	34.91 ± 1.57^{Abc}
	6	34.73 ± 2.79^{Aa}	35.37 ± 2.35^{Aa}	36.30 ± 0.72^{Aa}	36.59 ± 0.37^{Aba}
	8	35.29 ± 1.53^{Aa}	35.56 ± 0.55^{Aa}	35.97 ± 2.09^{Aa}	37.61 ± 0.14^{Aa}

$^{a-d}$Any means in the same column followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.

^{A}Any means in the same row followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.

Table 2. Sensory evaluation of shredded lettuce treated with aqueous ClO_2 during storage

Organoleptic parameter	Concentration (ppm)	Storage time (day)				
		0	2	4	6	8
Freshness	0	5.00 ± 0.00^{Aa}	3.63 ± 0.52^{Ab}	2.38 ± 0.52^{Bc}	1.63 ± 0.52^{Ad}	1.00 ± 0.00^{Ae}
	5	5.00 ± 0.00^{Aa}	3.50 ± 0.54^{Ab}	2.75 ± 0.46^{BAc}	1.38 ± 0.52^{Ad}	1.13 ± 0.35^{Ad}
	10	5.00 ± 0.00^{Aa}	3.88 ± 0.35^{Ab}	2.88 ± 0.64^{BAc}	1.63 ± 0.74^{Ad}	1.00 ± 0.00^{Ae}
	50	5.00 ± 0.00^{Aa}	4.00 ± 0.54^{Ab}	3.00 ± 0.54^{Ac}	1.88 ± 0.35^{Ad}	1.13 ± 0.35^{Ae}
Color	0	5.00 ± 0.00^{Aa}	3.13 ± 0.35^{Cb}	2.13 ± 0.36^{Ac}	1.38 ± 0.52^{Ad}	1.00 ± 0.00^{Ae}
	5	5.00 ± 0.00^{Aa}	3.25 ± 0.46^{BCb}	2.50 ± 0.54^{Ac}	1.38 ± 0.52^{Ad}	1.13 ± 0.35^{Ad}
	10	5.00 ± 0.00^{Aa}	3.88 ± 0.36^{Ab}	2.63 ± 0.74^{Ac}	1.50 ± 0.54^{Ad}	1.00 ± 0.00^{Ae}
	50	5.00 ± 0.00^{Aa}	3.63 ± 0.52^{BAb}	2.63 ± 0.92^{Ac}	1.50 ± 0.54^{Ad}	1.00 ± 0.00^{Ad}
Texture	0	5.00 ± 0.00^{Aa}	3.75 ± 0.46^{BAb}	2.63 ± 0.74^{Ac}	1.38 ± 0.52^{Ad}	1.00 ± 0.00^{Ad}
	5	5.00 ± 0.00^{Aa}	3.63 ± 0.52^{Bb}	3.25 ± 0.71^{Ab}	1.63 ± 0.52^{Ac}	1.00 ± 0.00^{Ad}
	10	5.00 ± 0.00^{Aa}	4.00 ± 0.54^{BAb}	3.13 ± 0.99^{Ab}	1.88 ± 0.35^{Ac}	1.13 ± 0.35^{Ad}
	50	5.00 ± 0.00^{Aa}	4.25 ± 0.71^{Ab}	3.215 ± 0.71^{Ac}	1.88 ± 0.64^{Ad}	1.00 ± 0.00^{Ae}
Flavor	0	5.00 ± 0.00^{Aa}	3.38 ± 0.52^{Bb}	2.38 ± 0.74^{Ac}	1.25 ± 0.46^{Ad}	1.00 ± 0.00^{Ae}
	5	5.00 ± 0.00^{Aa}	3.75 ± 0.46^{BAb}	2.63 ± 0.74^{Ac}	1.38 ± 0.52^{Ad}	1.00 ± 0.00^{Ad}
	10	5.00 ± 0.00^{Aa}	3.63 ± 0.52^{BAb}	2.75 ± 0.71^{Ac}	1.63 ± 0.52^{Ad}	1.13 ± 0.35^{Ae}
	50	5.00 ± 0.00^{Aa}	4.00 ± 0.00^{Ab}	2.88 ± 0.64^{Ac}	1.63 ± 0.52^{Ad}	1.25 ± 0.46^{Ad}
Overall	0	5.00 ± 0.00^{Aa}	3.63 ± 0.52^{Ab}	2.25 ± 0.71^{Bc}	1.25 ± 0.46^{Bd}	1.00 ± 0.00^{Ad}
	5	5.00 ± 0.00^{Aa}	3.88 ± 0.35^{Ab}	2.63 ± 0.52^{BAc}	1.63 ± 0.52^{Bad}	1.00 ± 0.00^{Ae}
	10	5.00 ± 0.00^{Aa}	3.88 ± 0.35^{Ab}	2.88 ± 0.35^{Ac}	1.50 ± 0.54^{Bd}	1.00 ± 0.00^{Ae}
	50	5.00 ± 0.00^{Aa}	4.00 ± 0.00^{Ab}	2.75 ± 0.46^{BAc}	2.00 ± 0.00^{Ad}	1.00 ± 0.00^{Ae}

[A-B]Any means in the same column followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.

[a-e]Any means in the same row followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.

of the shredded lettuce were the same among treatments during storage.

In summary, these results indicate that the aqueous ClO_2 treatment can be useful in improving the microbial safety of the iceberg lettuce during storage and extending the shelf life.

References

Baur S, Klaiber R, Hammes WP, and Carle R (2004) Sensory and microbiological quality of shredded, packaged iceberg lettuce as affected by pre-washing procedures with chlorinated and ozonated water. *Innovat Food Sci Emerg Technol* **5**, 45-55.

Bennarde MA, Israel BM, Oliveri VP, and Granstrom ML (1965) Efficiency of chlorine dioxide as a bactericide. *Appl Microbiol* **13**, 776.

Beuchat LR, Nail BV, Adler BB, and Clavero MRS (1998) Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J Food Prot* **61**, 1305-1311.

Cherry JP (1999) Improving the safety of fresh produce with antimicrobials. *Food Technol* **53**, 54-59.

Ihl M, Aravena L, Scheuermann E, Uquiche E, and Bifani V (2003) Effect of immersion solution on shelf-life of minimally processed lettuce. *Lebensmitt-wiss Technol* **36**, 591-599.

Kim JM, Maurice R, Marshall MR, Du WX, Otwell WS, and Wei CI (1999) Determination of chlorate and chlorite and mutagenicity of seafood treated with aqueous chlorine dioxide. *J Agric Food Chem* **47**, 3586-3591.

Lee SY, Gray PM, Dougherty RH, and Kang DH (2004) The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. *Food Microbiol.* **92**, 121-127.

Ortega RM, Requejo AM, Sanchez-Muniz FJ, Quintas ME, Sanches-Quiles B, Andres P, Redondo MR, and Lopez-Sobaler AM (1997) Concern about nutrition and its relation to the food habits of a group of young university students from Madrid. *Z Ernahrungswiss* **36**, 16-22.

Owusu-Yaw J, Toth JP, Wheeler WB, and Wei CI (1990) Mutagenicity and identification of the reaction products of aqueous chlorine dioxide with L-tryptophan. *J Food Sci* **55**, 1714-1719.

Singh N, Singh PK, Bhunia AK, and Stroshine RL (2002)

Efficacy of chlorine dioxide, Ozone, and thyme essential oil or a sequential washing in killing Escherichia coli 0157:H7 on lettuce and baby carrots. *Lebensmitt-wiss Technol* **35**, 720-729.

Watada AE, Ko NP, and Minott DA (1996) Factors affecting quality of fresh-cut horticultural products. *Postharvest Biol Technol* **9**, 115-125.

Wu VCH and Kim B (2007) Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiol* **24**, 794-800.

Youm HJ, Ko JK, Kim MR, and Song KB (2004) Inhibitory effect of aqueous chlorine dioxide on survival of *Escherichia* coli 0157:H7, *Salmonella Typhimurium, and Listeria monocytogenes* in pure cell culture. *Korean J Food Sci Technol* **36**, 514-517.