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Research Report

Sensory and Microbiological Qualities of Romaine Lettuce and Kale Affected by a Combined Treatment of Aqueous Chlorine Dioxide and Ultraviolet-C

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Abstract. The effects of a combined treatment of aqueous chlorine dioxide (ClO_2) and ultraviolet-C (UV-C) on the sensory and microbiological qualities of Romaine lettuce and kale were examined. Samples of Romaine lettuce and kale were artificially inoculated with *Escherichia coli* O157:H7 and *Salmonella enterica* serotype Typhimurium. The inoculated Romaine lettuce and kale, as well as fresh samples, were treated with 50 $\mu L \cdot L^{-1}$ aqueous ClO_2, 10 $kJ \cdot m^{-2}$ UV-C, and a combination of aqueous ClO_2 and UV-C, and stored at 4℃ for 7 days. The combined treatment of aqueous ClO_2 and UV-C reduced the initial populations of total aerobic bacteria in the Romaine lettuce and kale by 2.07 and 2.49 log CFU/g, compared to 6.36 and 6.07 log CFU/g for the control, respectively. The combined treatment also reduced the populations of yeast and mold in the samples by 1.85 and 4.25 log CFU/g, respectively. In particular, *E. coli* O157:H7 and *S.* Typhimurium inoculated in the Romaine lettuce and kale were eliminated by the combined treatment. The Hunter '*L*', '*a*', and '*b*' values of Romaine lettuce and kale were not significantly different among the treatments. Sensory evaluation results indicated that the combined treatment provided better scores than did the control. Our results suggest that the combined treatment of 50 $\mu L \cdot L^{-1}$ aqueous ClO_2 with 10 $kJ \cdot m^{-2}$ UV-C can be useful for maintaining the microbial safety and sensory qualities of Romaine lettuce and kale.

***Additional key words*:** foodborne pathogens, microbial safety, non-thermal treatment, shelf-life, storage

Introduction

The antioxidant and anticarcinogenic properties of fresh vegetables containing vitamin C, vitamin E, carotenoids, phenolic compounds, and flavonoids reduce the risk of cardiovascular disease and some types of cancers (Murcia et al., 2009). Recently, the demand for minimally processed vegetables produced by washing, trimming, cutting, and peeling has increased as consumers become more concerned with health, nutrition, and convenience (Sagong et al., 2011). Romaine lettuce being used as minimally processed vegetables has a crisp texture, good aroma, and fresh appearance as well as plentiful phytochemicals (Martinez-Sanchez et al., 2011). Kale also is a leafy green vegetable having a good source of vitamin C, ferulic acid, and polyphenols (Korus and Lisiewska, 2011; Podsedek, 2007).

Minimally processed vegetables can be a vehicle for the transmission of bacterial, parasitic, and viral pathogens capable of causing human illnesses (Oliveira et al., 2010). In addition, their cut surfaces greatly increase the probability for the attachment and growth of bacteria (Akbas and Olmez, 2007). Among the foodborne pathogens, *Escherichia coli* O157:H7 and *Salmonella enterica* serotype Typhimurium are the typical pathogens associated with minimally processed vegetables (Kroupitski, 2011). *E. coli* O157:H7 is capable of producing large quantities of toxins that cause hemorrhagic diarrhea (Koohmaraie et al., 2007). The US Center for Disease Control and Prevention attributed 20 outbreaks and 634 cases of illness to the consumption of lettuce contaminated with *E. coli* O157:H7 between 1998 and 2005 (Carey et al., 2009). Food poisoning due to *S.* Typhimurium can cause gastroenteritis in humans, and the symptoms of salmonellosis usually appear about 12 to 36 h after eating contaminated food (El-Gazzar and Marth, 1992; Hur et al., 2011). Therefore, an appropriate decontamination treatment is required to control foodborne pathogens such as *E. coli* O157:H7 and *S.* Typhimurium in

the processing of minimally processed vegetables.

To improve the microbial safety of minimally processed vegetables, various processing methods, such as washing with chlorine or electrolyzed water and electron beam irradiation have been used to the reduce bacterial counts and to extend shelf-life (Akbas and Olmez, 2007; Han et al., 2004; Keskinen et al., 2009; Olmez and Kretzschmar, 2009). Chlorine was an effective treatment for reducing microbial contamination in fresh produce and the treatment at 50-200 $\mu L \cdot L^{-1}$ is the most commonly used sanitizing method in the United States (Akbas and Olmez, 2007; Keskinen et al., 2009). However, chlorine treatment has health risks associated with trihalomethanes and haloacetic acids (Olmez and Kretzschmar, 2009). In contrast, electron beam irradiation decreases the firmness of Romaine lettuce as the irradiation dosage increases (Han et al., 2004). It has been reported that inactivation of *E. coli* O157:H7 by 50 ppm acidic electrolyzed water treatment on lettuce leaves was less effective than 20 ppm aqueous ClO_2 (Keskinen et al., 2009).

Microbial inactivation on the surfaces of various vegetables by aqueous ClO_2 treatment has been extensively studied (Chen et al., 2010). Aqueous ClO_2 has been used as a powerful oxidant to reduce microbial pathogens with minimal effect on color and sensory qualities (Stivarius et al., 2002). Aqueous ClO_2 treatment is FDA-approved for washing fruits and vegetables (FDA, 1998).

UV-C irradiation is widely used as an aid to chemical sterilization for obtaining synergistic effects in the microbial reduction in food products (Jiang et al., 2010). UV-C treatment does not require chemicals or heat, and it causes fewer changes in the nutritional and sensory quality of foods (Chun et al., 2009). In addition, UV-C has been approved by the FDA as a disinfectant method for the surface sterilization of foods (Song et al., 2011).

Hurdle technology, which is the combination of chemical and physical disinfection treatments, has been introduced to improve microbial safety and to extend the shelf-life of vegetables and fruits. Bang et al. (2011) reported that the combined treatment of chlorine dioxide and dry heat was effective in inactivating microorganism on radish seeds. Additionally, Singla et al. (2011) reported that the combined treatment of malic acid and ozone with the addition of polyphenol and flavonoids had synergistic effects in reducing *Shigella flexneri* in radish and mung bean sprouts. However, few studies have been conducted on the application of the combined treatment of aqueous chlorine dioxide and UV-C treatment for fresh vegetables. Therefore the objective of this study was to determine the effect of a combined treatment of aqueous ClO_2 and UV-C for improving microbiological quality of Romaine lettuce and kale maintaining sensory qualities during storage.

Materials and Methods

Materials

Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) and kale (*Brassica oleracea* L. var. *acephala*) were purchased from a local market in Daejeon, Korea, and immediately kept at 4 ± 1℃. Damaged outer leaves were removed from each head of lettuce and kale and cut into approximately 2 × 2 cm pieces with a sharp stainless steel knife. Midribs were excised and discarded.

Bacterial Strains and Culture Preparation

Strains of *E. coli* O157:H7 (NCTC 12079) and *S.* Typhimurium (ATCC 14028, KCTC 2057, KCTC 2514) were used in this study. Each strain was maintained at -70℃ in tryptic soy broth (Difco, Detroit, MI, USA), containing 10% glycerol. *E. coli* O157:H7 and three strains of *S.* Typhimurium were streaked on tryptic soy agar and then incubated at 37℃ for 24 h. Following incubation, a single colony from *E. coli* O157:H7 and three strains of *S.* Typhimurium was chosen for further use and then added to 25 mL of selective medium and incubated overnight at 37℃ with shaking at 150 rpm. Cultures were subjected to centrifuge at 2,000 × *g* for 15 min, and the cells were then washed twice in 25 mL of 0.1% peptone water (Difco). The concentration of the inoculums was approximately 7 log CFU/mL, as determined by serially diluting the inoculums in 0.1% peptone water and plating on TSA. These inoculums were used in subsequent experiments.

Inoculation of Pathogenic Bacteria onto Romaine Lettuce and Kale

Prior to inoculation, the Romaine lettuce and kale samples were exposed to UV irradiation for 30 min in a laminar flow hood at room temperature to remove preexisting microorganisms. The samples were then placed on a plastic tray where *E. coli* O157:H7 and cocktails of *S.* Typhimurium (1 mL) were spot-inoculated on the surface of the Romaine lettuce and kale. To allow attachment of the pathogens, inoculated Romaine lettuce and kale were air-dried for 1 h in the laminar flow hood.

Aqueous ClO_2 Treatment

For single treatment, samples were treated by dipping into either of the solution, distilled water or 50 $\mu L \cdot L^{-1}$ aqueous ClO_2 for 5 min and then air-dried in a laminar flow hood for 30 min at room temperature. The concentration and contact time of aqueous ClO_2 were chosen based on the previous study (Kim et al., 2009) and a preliminary study using Romaine lettuce and kale samples. Aqueous ClO_2 was prepared using an aqueous ClO_2 generating system (CH_2O Inc., Olympia, WA, USA) according to the manufacturer's

instruction without detectable free and total chlorine. Aqueous ClO_2 concentration was determined using an iodometric method (APHP, 1995). After treatment, the samples were packed hermetically using polyethylene terephthalate containers (143 × 143 × 25 mm) and stored at 4 ± 1℃ for 7 days, respectively.

UV-C Irradiation

For single treatment, samples were placed on a stainless steel tray and irradiated with eight germicidal UV-C emitting lamps (15W, Sylvania, G15T8, Phillips, Netherlands) placed on both the upper and lower sides, inside a custom made metal cabinet (80 × 55 × 47 cm) that was described in previous studies (Chun et al., 2010; Kim et al., 2010). The UV-C cabinet was designed according to the description given by Bolton and Linden (2003), and the UV lamps were warmed up for 30 min before UV-C irradiation in order to ensure reproducible results. UV-C radiation intensity was measured at 254 nm using a UV radiometer (UV-340, Lutron Electronic Co., Ltd., Taipei, Taiwan). UV-C irradiation dosage was changed by altering exposure time (dose rate; 12 $W \cdot m^{-2}$). The Romaine lettuce and kale samples were laid in the UV-C cabinet without overlapping and exposed at 10 $kJ \cdot m^{-2}$ for 13 min 30 s at room temperature. After irradiation, the samples were hermetically packed using polyethylene terephthalate containers and stored at 4 ± 1℃ for 7 days.

Combined Treatment of Aqueous ClO_2 and UV-C

For the combined treatment, samples were first treated with 50 $\mu L \cdot L^{-1}$ aqueous ClO_2 and then with 10 $kJ \cdot m^{-2}$ UV-C irradiation.

Microbiological Analysis

After treatment, each of the Romaine lettuce and kale samples (20 g) was placed in a sterile stomacher bag containing 180 mL of 0.1% peptone water. Samples were homogenized using a stomacher (MIX2, AES Laboratoire, Combourg, France) for 3 min, and then filtered through a sterile cheese cloth. After homogenization, samples were diluted with 0.1% peptone water for microbial counts. Serial dilutions were performed in triplicate. Total aerobic bacteria counts were determined by plating the diluted samples onto plate count agar (PCA, Difco, Detroit, MI, USA) and incubating the plates at 37℃ for 48 h. Yeasts and mold were plated on potato dextrose agar (PDA, Difco) and the plates were incubated at 37℃ for 72 h. *E. coli* O157:H7 counts were determined by spread-plating appropriately diluted samples onto Sorbitol MacConkey agar (SMAC, Difco Co.). *S.* Typhimurium was plated onto Xylose Lysine Deoxychliate agar (XLD, Difco Co.). Plates were incubated at 37℃ for 24 h, and colonies were subsequently enumerated. The mean of three microbial counts was recorded and expressed as the log CFU (Colony Forming Unit)/g.

Color Measurement

The color on the surface of the samples was analyzed using a colorimeter (CR-400 Minolta Chroma Meter, Konica Minolta Sensing Inc., Tokyo, Japan). Samples were placed on a white standard plate, and Hunter values '*L*', '*a*', and '*b*' were measured. Hunter values for the standard plate were $L = 98.35$, $a = -0.04$, and $b = 1.65$. Five measurements were taken at different locations for each sample.

Sensory Evaluation

During storage, samples were analyzed for their appearance, odor and overall acceptability by eight trained panelists (3 male, 5 female, ages 23-31). Sensory qualities of samples were evaluated using a nine point scoring method. Sensory scores of 8-9 were given for very good; 6-7 for good; 4-5 for fair; 2-3 for poor; and 1 for very poor.

Statistical Analysis

Analyses of variance and Duncan's multiple range tests were performed to analyze the data using the SAS program (SAS Institute, Cary, NC, USA). Differences of $p < 0.05$ were considered significant. All results were expressed as the mean ± standard deviation.

Results and Discussion

This study was performed to compare the effect of single treatment (distilled water, aqueous chlorine dioxide, and UV-C) and a combined treatment of aqueous chlorine dioxide and UV-C on reducing the populations of microorganisms on Romaine lettuce and kale. The initial populations of total aerobic bacteria in the lettuce and kale were 6.36 log CFU/g and 6.07 log CFU/g, respectively (Table 1). Water washing of the samples resulted in 5.12 and 5.44 log CFU/g, which is a difference of 1.24 and 0.63 log CFU/g, respectively, compared to the control (Table 1). In contrast, the populations of total aerobic bacteria on the lettuce and kale were reduced to 3.49 and 3.00 log CFU/g after treatment with 50 $\mu L \cdot L^{-1}$ aqueous chlorine dioxide. Irradiation with 10 $kJ \cdot m^{-2}$ UV-C resulted in 4.25 and 4.05 log CFU/g, respectively. Therefore, aqueous ClO_2 and UV-C treatment were more effective in reducing the total aerobic bacteria than water washing. Similar results were reported in that 100 $\mu L \cdot L^{-1}$ aqueous ClO_2 treatment of asparagus lettuce caused a reduction of approximately 3 log CFU/g in the population of total aerobic bacteria (Chen et al., 2010).

The greatest reduction was achieved with the combined treatment of aqueous ClO_2 and UV-C, where the popu-

Table 1. Change in the populations of total aerobic bacteria of non-thermal treated Romaine lettuce and kale during storage at 4°C. (log CFU/g)

Samples	Treatment	Storage time (d)				
		0	1	3	5	7
Romaine lettuce	Control	6.36 ± 0.04Ac[z]	6.51 ± 0.26Ac	6.71 ± 0.04Ab	6.73 ± 0.04Ab	6.92 ± 0.11Aa
	Water	5.12 ± 0.12Bb	5.51 ± 0.21Ba	5.53 ± 0.09Ba	5.58 ± 0.17Ba	5.60 ± 0.06Ba
	ClO_2[y]	3.49 ± 0.15De	3.65 ± 0.05Dd	3.91 ± 0.07Dc	4.22 ± 0.21Db	4.75 ± 0.03Da
	UV-C[x]	4.25 ± 0.21Cc	4.31 ± 0.15Cc	4.58 ± 0.16Cb	4.81 ± 0.41Cab	4.96 ± 0.03Ca
	ClO_2 + UV-C[w]	2.07 ± 0.12Ed	2.53 ± 0.12Ec	2.64 ± 0.04Ebc	2.72 ± 0.13Eb	3.30 ± 0.02Ea
Kale	Control	6.07 ± 0.09Ae	6.16 ± 0.01Ad	6.50 ± 0.07Ac	6.58 ± 0.01Ab	6.72 ± 0.05Aa
	Water	5.44 ± 0.01Bd	5.90 ± 0.07Bc	5.98 ± 0.08Bc	6.12 ± 0.16Bb	6.25 ± 0.13Ba
	ClO2	3.00 ± 0.05De	3.19 ± 0.09Dd	3.65 ± 0.10Dc	4.06 ± 0.04Db	4.58 ± 0.18Da
	UV-C	4.05 ± 0.11Cc	4.20 ± 0.06Cb	4.29 ± 0.17Cb	4.32 ± 0.06Cb	4.99 ± 0.15Ca
	ClO_2 + UV-C	2.49 ± 0.13Ed	2.85 ± 0.19Ec	2.92 ± 0.09Ebc	3.04 ± 0.13Eab	3.14 ± 0.13Ea

[z]Any means in the same column (A-E) or row (a-e) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[y]50 ppm ClO_2.
[x]10 kJ·m^{-2} UV-C.
[w]Combination of 50 ppm ClO_2 and 10 kJ·m^{-2} UV-C.

Table 2. Change in the populations of yeast and mold of non-thermal treated Romaine lettuce and kale during storage at 4°C. (log CFU/g)

Samples	Treatment	Storage time (d)				
		0	1	3	5	7
Romaine lettuce	Control	3.09 ± 0.06Ac[z]	3.47 ± 0.08Ab	4.63 ± 0.08Aa	4.66 ± 0.11Aa	4.70 ± 0.15Aa
	Water	2.88 ± 0.07Bc	2.95 ± 0.03Bc	3.70 ± 0.08Bb	3.75 ± 0.12Bb	4.00 ± 0.10Ba
	ClO_2[y]	1.80 ± 0.03Cd	2.10 ± 0.06Cc	2.32 ± 0.13Dbc	2.42 ± 0.08Db	2.96 ± 0.11Da
	UV-C[x]	2.71 ± 0.03Bb	2.82 ± 0.03Bb	3.34 ± 0.03Ca	3.35 ± 0.19Ca	3.37 ± 0.09Ca
	ClO_2 + UV-C[w]	1.24 ± 0.06Dc	1.36 ± 0.09Dc	2.22 ± 0.09Db	2.21 ± 0.14Db	2.55 ± 0.08Ea
Kale	Control	5.45 ± 0.12Ac	5.53 ± 0.12Ac	5.90 ± 0.10Ab	5.92 ± 0.11Ab	6.12 ± 0.10Aa
	Water	4.38 ± 0.15Bc	4.49 ± 0.08Bbc	4.60 ± 0.20Bab	4.72 ± 0.16Ba	4.77 ± 0.16Ba
	ClO_2	2.36 ± 0.23Cd	2.67 ± 0.11Dc	2.84 ± 0.16Dc	3.15 ± 0.12Db	3.48 ± 0.13Da
	UV-C	2.52 ± 0.11Cd	2.80 ± 0.10Cc	3.41 ± 0.24Cb	3.99 ± 0.12Ca	4.10 ± 0.05Ca
	ClO_2 + UV-C	1.20 ± 0.16De	1.36 ± 0.09Ed	1.69 ± 0.08Ec	1.84 ± 0.04Eb	2.26 ± 0.05Ea

[z]Any means in the same column (A-E) or row (a-e) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[y]50 ppm ClO_2.
[x]10 kJ·m^{-2} UV-C.
[w]Combination of 50 ppm ClO_2 and 10 kJ·m^{-2} UV-C.

lations of the total aerobic bacteria in the lettuce and kale were 2.07 and 2.49 log CFU/g, respectively, resulting in a difference of 4.29 and 3.58 log CFU/g, respectively, compared to the control (Table 1). Youm et al. (2005) reported that the total population of aerobic bacteria in salad experienced a 3.75 log CFU/g difference after the combined treatment with 50 μL·L^{-1} aqueous ClO_2 and 1% citric acid, compared to the control. After 7 days of storage, the total aerobic bacteria populations in the samples that had undergone the combined treatment were 3.30 and 3.14 log CFU/g, compared to 6.92 and 6.72 log CFU/g for the control lettuce and kale samples. During storage, the combined treatment maintained a difference of 3.31-4.07 log CFU/g in the total aerobic bacteria populations in lettuce and kale, while single treatments had 1.73-2.97 log CFU/g difference (Table 1). Accordingly, the combined treatment of aqueous ClO_2 and UV-C provided a better result as a hurdle technique.

Yeast and mold populations had a similar pattern to that of total aerobic bacteria (Table 2). Initial populations of yeast and mold in the lettuce and kale were 3.09 and 5.45 log CFU/g, respectively, while water washing reduced them to 2.88 and 4.38 log CFU/g. The results of this study in-

dicate that water washing reduces the populations of yeast and mold in the lettuce and kale by less than 1.07 log CFU/g. In contrast, after aqueous ClO_2 treatment, the yeast and mold populations were 1.80 and 2.36 log CFU/g, indicating a difference of 1.29 and 3.09 log CFU/g, respectively. The populations of yeast and mold in the lettuce and kale irradiated with 10 kJ·m^{-2} UV-C were reduced to 2.71 and 2.52 log CFU/g. Similar to the present study, Kim et al. (2010) reported that single treatment of strawberries in 50 μL·L^{-1} aqueous ClO_2 or 10 kJ·m^{-2} UV-C resulted in about 1.0-1.5 log CFU/g reduction in the population of yeast and mold, while water washing caused a difference of 0.38 log CFU/g. The combined treatment with aqueous ClO_2 and UV-C resulted in 1.24 and 1.20 log CFU/g of yeast and mold population, showing differences of 1.85 and 4.25 log CFU/g, compared to the control, respectively (Table 2). Kim et al. (2011) reported that the population of yeast and mold in red chicory was reduced from 5.68 to 3.27 log CFU/g after the combined treatment of 50 μL·L^{-1} aqueous ClO_2 and 10 kJ·m^{-2} UV-C, while single treatment reduced the yeast and mold counts by 1.38 log CFU/g. Therefore, it is suggested that appropriate combination of sterilization methods is needed to obtain synergistic effects.

During storage, the yeast and mold populations for all treatments increased with storage time. The population of yeast and mold in the control lettuce and kale samples reached 4.70 and 6.12 log CFU/g, respectively, while the population of yeast and mold for the lettuce and kale treated with the combination of aqueous ClO_2 and UV-C were 2.55 and 2.26 log CFU/g after 7 days of storage, respectively (Table 2).

For the inoculated lettuce and kale, the initial populations of *E. coli* O157:H7 on the Romaine lettuce and kale were 6.22 and 6.32 log CFU/g, respectively (Table 3). Water washing resulted in 5.17 and 5.29 log CFU/g, which were differences of 1.05 and 1.03 log CFU/g, respectively, compared to the control. Keskinen et al. (2009) reported that water washing only reduced the population of *E.* coli O157:H7 by 0.55 log CFU/g in inoculated Romaine lettuce. According to these results, water washing is not effective in reducing bacterial populations, suggesting that other treatment is needed, such as chlorine, aqueous ClO_2, or acidic electrolyzed water.

In contrast, after treatment with 50 μL·L^{-1} aqueous ClO_2, the populations of *E. coli* O157:H7 in the lettuce and kale were reduced to 4.19 log CFU/g and 3.17 log CFU/g, indicating a difference of 2.03 and 3.15 log CFU/g, compared to the control (Table 3). Bang et al. (2011) reported that treatment with 500 μL·L^{-1} aqueous chlorine dioxide for 5 min in radish seeds resulted in 1.20 log CFU/g reduction in the population of *E. coli* O157:H7, indicating that aqueous ClO_2 treatment is more effective than water washing. In UV-C-irradiated samples, the populations of *E. coli* O157:H7 were reduced to 3.36 and 2.10 log CFU/g, showing differences of 2.86 and 4.22 log CFU/g, respectively. These results suggest that UV-C irradiation treatment is more effective treatment for reducing *E. coli* O157:H7 populations than aqueous ClO_2 treatment. Guan et al. (2012) reported that the population of *E. coli* O157:H7 on the mushroom was reduced from 7.28 to 6.15 log CFU/g after 2.7 kJ·m^{-2} UV-C irradiation. Meanwhile, after the combined treatment

Table 3. Effect of non-thermal treatment on the survival of *E. coli* O157:H7 inoculated on the Romaine lettuce and kale during storage at 4℃. (log CFU/g)

Samples	Treatment	Storage time (d)				
		0	1	3	5	7
Romaine lettuce	Control	6.22 ± 0.10Ab[z]	6.35 ± 0.16Ab	6.74 ± 0.08Aa	6.75 ± 0.06Aa	6.91 ± 0.05Aa
	Water	5.17 ± 0.09Bc	5.82 ± 0.11Bb	5.81 ± 0.06Bb	5.83 ± 0.09Bb	6.10 ± 0.08Ba
	ClO_2[y]	4.19 ± 0.07Cc	4.98 ± 0.08Cb	5.00 ± 0.07Cb	5.01 ± 0.07Dab	5.13 ± 0.04Da
	UV-C[x]	3.36 ± 0.06Dd	4.95 ± 0.14Cc	5.03 ± 0.04Cc	5.24 ± 0.13Cb	5.57 ± 0.09Ca
	ClO_2 + UV-C[w]	ND[v]	ND	ND	ND	ND
Kale	Control	6.32 ± 0.08Ad	6.52 ± 0.02Ac	6.94 ± 0.22Ab	7.32 ± 0.07Aa	7.45 ± 0.10Aa
	Water	5.29 ± 0.09Bd	5.67 ± 0.05Bc	5.78 ± 0.07Bc	5.92 ± 0.10Bb	6.07 ± 0.15Ba
	ClO_2	3.17 ± 0.08Ce	3.33 ± 0.10Cd	3.72 ± 0.09Cc	3.85 ± 0.06Cb	4.10 ± 0.10Ca
	UV-C	2.10 ± 0.16Dd	2.52 ± 0.06Dc	2.66 ± 0.18Dc	2.92 ± 0.09Db	3.10 ± 0.08Da
	ClO_2 + UV-C	ND	ND	2.03 ± 0.08Eb	2.14 ± 0.15Eab	2.25 ± 0.20Ea

[z]Any means in the same column (A-E) or row (a-e) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[y]50 ppm ClO_2.
[x]10 kJ·m^{-2} UV-C.
[w]Combination of 50 ppm ClO_2 and 10 kJ·m^{-2} UV-C.
[v]ND: not detected.

of aqueous ClO_2 and UV-C, *E. coli* O157:H7 in the inoculated lettuce was eliminated during storage (Table 3). The combined treatment also eliminated *E. coli* O157:H7 counts at the beginning of storage in the kale, although the bacteria appeared again during storage. Song et al. (2011) reported similar result that the combined treatment of 10 $\mu L \cdot L^{-1}$ aqueous ClO_2 and 5 $kJ \cdot m^{-2}$ UV-C eliminated the inoculated *E. coli* O157:H7 in cherry tomatoes during storage. However, it should be noted that the total aerobic bacteria and yeast and mold populations decreased by about 1.85 and 4.29 log CFU/g after the combined treatment in the lettuce and kale, unlike the inoculated lettuce and kale samples. The difference between the inoculated and natural lettuce and kale samples may be attributed to the formation of biofilm on the surface of vegetables, since biofilm formation protects the attached bacteria cells during UV-C radiation for the natural lettuce and kale (Bernbom et al., 2011). Poulsen (1999) also reported that biofilm has a resistance to antibiotic, various chemicals, heat, light, and drying. Therefore, it appears that total aerobic bacteria and yeast and mold can form a strong biofilm, differently from the inoculated *E. coli* O157:H7 and *S.* Typhimurium.

During storage, the population of *E. coli* O157:H7 increased slightly in the Romaine lettuce and kale. The control samples reached 6.91 and 7.45 log CFU/g after 7 d, respectively, while the population of *E. coli* O157:H7 for aqueous ClO_2-treated samples had 5.13 and 4.10 log CFU/g and UV-C-irradiated had 5.57 and 3.10 log CFU/g (Table 3). For the combined treatment of aqueous ClO_2 and UV-C, the lettuce did not have the bacteria, and kale had 2.25 log CFU/g. Therefore, compared to the results obtained for the single treatments, the combined treatment distinctly enhanced the reduction of *E. coli* O157:H7 during storage.

The populations of *S.* Typhimurium inoculated in the samples exhibited a pattern similar to those of *E. coli* O157:H7. Initial populations of *S.* Typhimurium in the lettuce and kale were 6.26 and 6.06 log CFU/g, respectively. Water washing resulted in 4.24 and 4.30 log CFU/g, a difference of 2.02 and 1.76 log CFU/g, respectively (Table 4). Similar results were reported in the case of the Romaine lettuce treated by aerosolized distilled water, where it had a difference in the population of *S.* Typhimurium by 1.19 log CFU/g (Choi et al., 2012). In contrast, after aqueous ClO_2 treatment, the populations of *S.* Typhimurium in the lettuce and kale were 3.81 and 2.20 log CFU/g, resulting in differences of 2.45 and 3.86 log CFU/g (Table 4). In addition, the populations of *S.* Typhimurium in the lettuce and kale irradiated with 10 $kJ \cdot m^{-2}$ UV-C were reduced to 3.15 and 2.00 log CFU/g, respectively (Table 4). These results are comparable with similar studies that 7.2 $kJ \cdot m^{-2}$ UV-C irradiation reduced the population of *Salmonella enterica* on baby spinach by 3.0 log CFU/g, compared to the control (Escalona et al., 2010). In particular, the combined treatment of aqueous ClO_2 and UV-C exhibited elimination in the population of *S.* Typhimurium during storage (Table 4).

After 7 d of storage, the control groups of lettuce and kale exhibited *S.* Typhimurium populations of 6.86 and 6.97 log CFU/g, while the water-washed groups showed 4.93 and 5.07 log CFU/g, respectively (Table 4). In contrast, the populations of *S.* Typhimurium in the lettuce and kale reached

Table 4. Effect of non-thermal treatment on the survival of *S. Typhimurium* inoculated on the Romaine lettuce and kale during storage at 4°C. (log CFU/g)

Samples	Treatment	Storage time (d)				
		0	1	3	5	7
Romaine lettuce	Control	6.26 ± 0.05Ab[z]	6.27 ± 0.06Ab	6.32 ± 0.06Ab	6.64 ± 0.03Aa	6.86 ± 0.10Aa
	Water	4.24 ± 0.08Bc	4.57 ± 0.10Bb	4.64 ± 0.11Bb	4.87 ± 0.10Ba	4.93 ± 0.13Ba
	ClO_2[y]	3.81 ± 0.03Cc	3.99 ± 0.20Cb	4.06 ± 0.05Cab	4.08 ± 0.05Cab	4.16 ± 0.07Ca
	UV-C[x]	3.15 ± 0.03Db	3.22 ± 0.02Db	3.99 ± 0.11Ca	4.07 ± 0.13Ca	4.08 ± 0.14Ca
	ClO_2 + UV-C[w]	ND[v]	ND	ND	ND	ND
Kale	Control	6.06 ± 0.12Ad	6.27 ± 0.06Ac	6.35 ± 0.17Ac	6.76 ± 0.02Ab	6.97 ± 0.04Aa
	Water	4.30 ± 0.10Bc	4.66 ± 0.30Bb	4.79 ± 0.16Bb	4.88 ± 0.13Bab	5.07 ± 0.15Ba
	ClO_2	2.20 ± 0.13Ce	2.53 ± 0.09Cd	2.98 ± 0.10Cc	3.14 ± 0.20Cb	3.30 ± 0.11Ca
	UV-C	2.00 ± 0.11Dd	2.10 ± 0.16Dd	2.63 ± 0.23Dc	2.85 ± 0.09Db	3.15 ± 0.10Da
	ClO_2 + UV-C	ND	ND	ND	ND	ND

[z]Any means in the same column (A-E) or row (a-e) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[y]50 ppm ClO_2.
[x]10 $kJ \cdot m^{-2}$ UV-C.
[w]Combination of 50 ppm ClO_2 and 10 $kJ \cdot m^{-2}$ UV-C.
[v]ND: not detected.

4.16 and 3.30 log CFU/g after treatment of 50 ppm aqueous chlorine dioxide treatment. For the 10 kJ·m^{-2} UV-C irradiation, they reached 4.08 and 3.15 log CFU/g after 7 days of storage. Populations of *S.* Typhimurium under the combined treatment were not detected in either lettuce and kale samples (Table 4). It was reported that the combined treatment of aqueous ClO_2 or fumaric acid with UV-C was more effective in reducing the population of *S.* Typhimurium than any single treatment (Kim et al., 2009). Singh et al. (2002) also reported that the combined treatment with aqueous ClO_2, ozonated water, and thyme oil was more effective than any single treatment. The combined treatment of 0.38 kJ·m^{-2} UV and 1.5 % H_2O_2, also reduced the population of *Salmonella* Montevideo in Romaine lettuce to 3.75 log CFU/g, compared to 5.23 log CFU/g for the control (Hadjok et al., 2008). In addition, Park et al. (2008) reported that the treatment with 200 ppm aqueous ClO_2 and 2% citric acid in radish seeds resulted in a 2.89 log CFU/g reduction of *S.* Typhimurium,

Table 5. Change in Hunter color values of non-thermal treated Romaine lettuce and kale during storage at 4℃.

Samples	Color paraeter	Treatment	Storage Time (d)				
			0	1	3	5	7
Romaine lettuce	*L*[z]	Control	38.71 ± 0.63Ab[y]	39.29 ± 1.18Aab	39.03 ± 0.56Ab	39.09 ± 0.84Ab	40.31 ± 0.51Aa
		Water	38.57 ± 0.20Ab	40.39 ± 0.36Aa	39.17 ± 0.72Aab	39.55 ± 0.33Aab	39.88 ± 0.59Aab
		ClO_2[x]	38.12 ± 0.34Ab	39.38 ± 1.31Aa	39.87 ± 0.63Aa	39.94 ± 1.05Aa	39.95 ± 0.60Aa
		UV-C[w]	38.59 ± 0.35Ab	39.05 ± 0.28Aab	39.47 ± 0.62Aab	38.72 ± 1.23Ab	40.02 ± 1.10Aa
		ClO_2 + UV-C[v]	38.23 ± 0.70Ab	39.16 ± 1.13Aab	39.51 ± 0.81Aa	39.24 ± 0.42Aab	39.67 ± 0.80Aa
	a	Control	-10.62 ± 0.25Aa	-10.88 ± 0.45Aab	-11.06 ± 0.62Aab	-11.49 ± 0.47Ab	-11.56 ± 0.81Ab
		Water	-10.34 ± 0.82Aa	-10.50 ± 0.42Aa	-10.80 ± 0.66Aa	-11.48 ± 1.33Aab	-12.33 ± 0.56Ab
		ClO_2	-10.88 ± 0.21Aab	-10.89 ± 0.61Aab	-10.59 ± 0.76Aa	-11.56 ± 1.24Aab	-11.89 ± 0.52Ab
		UV-C	-10.53 ± 0.17Aa	-10.67 ± 0.82Aa	-10.61 ± 0.54Aa	-11.10 ± 0.92Aab	-11.58 ± 0.35Ab
		ClO_2 + UV-C	-10.38 ± 0.19Aa	-10.65 ± 0.79Aa	-10.86 ± 0.66Aa	-11.11 ± 0.49Aab	-11.72 ± 0.30Ab
	b	Control	13.50 ± 0.71Aab	12.71 ± 0.59Ac	13.30 ± 0.45Abc	13.81 ± 0.38Aab	14.05 ± 0.37Aa
		Water	13.44 ± 1.09Aab	12.48 ± 0.22Ab	14.08 ± 0.77Aa	13.77 ± 0.66Aa	14.15 ± 0.78Aa
		ClO_2	13.64 ± 0.41Ab	12.06 ± 0.51Ab	13.87 ± 0.79Ab	14.32 ± 0.55Aab	14.19 ± 0.51Aa
		UV-C	13.30 ± 1.09Aab	12.43 ± 0.25Ab	13.98 ± 0.69Aa	13.99 ± 0.50Aa	13.51 ± 0.48Aa
		ClO_2 + UV-C	12.99 ± 0.71Ab	12.37 ± 0.51Ab	14.26 ± 0.71Aa	14.30 ± 0.27Aa	14.07 ± 0.38Aa
Kale	*L*	Control	40.72 ± 0.65Aa	40.58 ± 0.36Aa	40.61 ± 0.59Aa	40.65 ± 0.40Aa	40.33 ± 0.26Aa
		Water	40.51 ± 0.28Aa	40.60 ± 0.26Aa	41.09 ± 0.64Aa	40.77 ± 0.38Aa	40.74 ± 0.34Aa
		ClO_2	40.60 ± 0.57Aa	40.71 ± 0.22Aa	41.08 ± 0.63Aa	40.73 ± 0.40Aa	40.46 ± 0.37Aa
		UV-C	40.77 ± 1.46Aa	40.57 ± 0.34Aa	41.16 ± 0.68Aa	40.31 ± 0.35Aa	40.89 ± 1.01Aa
		ClO_2 + UV-C	41.73 ± 0.85Aa	41.01 ± 0.56Aab	41.00 ± 0.49Aab	40.66 ± 0.33Ab	40.42 ± 0.28Ab
	a	Control	-11.53 ± 0.26Aa	-11.38 ± 0.53Aa	-11.73 ± 0.30Aa	-11.27 ± 0.37Aa	-11.22 ± 1.02Aa
		Water	-11.50 ± 0.66Aa	-10.85 ± 0.46Aa	-11.37 ± 0.59Aa	-11.59 ± 0.75Aa	-11.26 ± 1.20Aa
		ClO_2	-11.44 ± 0.72Aa	-10.96 ± 0.40Aa	-11.58 ± 0.76Aa	-11.60 ± 1.01Aa	-10.81 ± 0.13Aa
		UV-C	-11.12 ± 0.16Aa	-10.77 ± 0.44Aa	-10.98 ± 0.46Aa	-10.80 ± 0.99Aa	-11.09 ± 0.37Aa
		ClO_2 + UV-C	-11.28 ± 0.49Aab	-11.16 ± 0.44Aab	-11.26 ± 0.43Aab	-10.62 ± 0.05Aa	-11.35 ± 0.73Ab
	b	Control	13.81 ± 0.69Aa	13.71 ± 1.07Aa	13.95 ± 0.70Aa	13.18 ± 0.34Aa	13.79 ± 0.75Aa
		Water	13.53 ± 0.72Aab	13.03 ± 0.64Aab	13.92 ± 0.83Aa	12.94 ± 0.66Ab	13.48 ± 0.19Aab
		ClO_2	14.12 ± 0.89Aa	13.93 ± 0.23Aab	13.71 ± 0.57Aab	13.16 ± 0.83Ab	13.77 ± 0.38Aab
		UV-C	13.19 ± 0.91Aa	13.14 ± 0.73Aa	13.60 ± 0.59Aa	13.20 ± 1.15Aa	13.15 ± 0.57Aa
		ClO_2 + UV-C	13.60 ± 0.68Aa	13.85 ± 0.69Aa	13.05 ± 0.76Aa	13.04 ± 0.15Aa	13.18 ± 0.58Aa

[z]*L*; degree of whiteness (0 black ~ 100 White), *a*; degree of redness (-80 greenness ~ 100 redness), *b*; degree of yellowness (-80 blue ~ 70 yellowness).
[y]Any means in the same column (A) are not significantly ($p < 0.05$) different and any means in the same row (a-b) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[x]50 ppm ClO_2.
[w]10 kJ·m^{-2} UV-C.
[v]Combination of 50 ppm ClO_2 and 10 kJ·m^{-2} UV-C.

and a 200 ppm aqueous ClO_2 and 0.5% glycerol resulted in a 2.14 log CFU/g difference compared to the control. Along with these reports, the results of this study suggest that the combined treatment of aqueous ClO_2 and UV-C may be the most suitable hurdle technology for inactivating pathogenic bacteria in minimally processed vegetables such as Romaine lettuce and kale.

The Hunter '*L*', '*a*', and '*b*' values of the Romaine lettuce and kale are shown in Table 5. Color is considered to be an important factor in minimally processed vegetables because consumers judge quality on the basis of color. Regarding the Hunter '*L*', '*a*', and '*b*' values of the samples, there were no significant differences among treatments during storage. Kim et al. (2011) reported that the combined treatment of 50 μL·L^{-1} aqueous ClO_2 and 10 J·m^2 UV-C did not affect the color of red chicory and pak choi, which are comparable to the results presented here. On the other hand, Han et al. (2004) reported that 3.5 kGy of electron beam irradiation accelerated the discoloration of the Romaine lettuce, resulting in a lower '*L*' value compared to the control. However, the results of

Table 6. Sensory evaluation of non-thermal treated Romaine lettuce and kale during storage at 4°C.

Samples	Organoleptic parameter	Treatment	Storage Time (d)				
			0	1	3	5	7
Romaine lettuce	Appearance	Control	9.00 ± 0.00Aa[z]	8.75 ± 0.46Aab	8.13 ± 0.64Bb	7.13 ± 0.83Cc	6.25 ± 0.89Cd
		Water	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.00 ± 0.53Bb	7.38 ± 0.52Bc	6.63 ± 0.92Bd
		ClO_2[y]	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.75 ± 0.46Aa	8.00 ± 0.53ABb	7.38 ± 0.92ABc
		UV-C[x]	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.25 ± 0.71Ab	7.75 ± 0.71Ac
		ClO_2 + UV-C[w]	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.63 ± 0.52Aa	7.88 ± 0.83Ab
	Odor	Control	9.00 ± 0.00Aa	8.75 ± 0.46Aab	8.38 ± 0.74Aab	8.13 ± 1.13Aab	7.75 ± 1.58Ab
		Water	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.50 ± 0.76Aa	8.38 ± 0.92Aa	8.13 ± 1.25Aa
		ClO_2	9.00 ± 0.00Aa	8.88 ± 0.35Aab	8.88 ± 0.35Aab	8.63 ± 0.52Aab	8.38 ± 0.92Ab
		UV-C	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.63 ± 0.52Aab	8.38 ± 0.74Aab	8.13 ± 0.83Ab
		ClO_2 + UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.63 ± 0.52Aab	8.25 ± 0.71Ab
	Overall	Control	9.00 ± 0.00Aa	8.75 ± 0.46Aab	8.13 ± 0.64BCb	7.13 ± 0.83Cc	6.38 ± 0.92Bd
		Water	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.00 ± 0.53Cb	7.38 ± 0.52Cc	6.50 ± 0.93Bd
		ClO_2	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.63 ± 0.52ABa	7.75 ± 0.71BCb	7.13 ± 1.13ABb
		UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.25 ± 0.71ABb	7.63 ± 0.74Ac
		ClO_2 + UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.88 ± 0.35Aa	8.63 ± 0.52Aa	7.88 ± 0.83Ab
Kale	Appearance	Control	9.00 ± 0.00Aa	8.63 ± 0.52Ba	6.88 ± 0.83Cb	6.88 ± 0.83Bb	6.00 ± 1.07Ac
		Water	9.00 ± 0.00Aa	8.88 ± 0.35ABa	7.50 ± 0.53BCb	7.00 ± 0.53Bb	6.00 ± 1.07Ac
		ClO_2	9.00 ± 0.00Aa	8.88 ± 0.35ABa	7.50 ± 0.93BCb	7.00 ± 0.76Bb	6.00 ± 0.93Ac
		UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.25 ± 0.71ABb	7.38 ± 0.74ABc	6.63 ± 0.92Ad
		ClO_2 + UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.38 ± 0.52Aab	7.88 ± 0.64Ab	7.00 ± 1.07Ac
	Odor	Control	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.00 ± 0.76Ab	7.25 ± 1.39Ac
		Water	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aab	8.00 ± 0.76Ab	7.13 ± 1.64Ac
		ClO_2	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aab	8.00 ± 0.76Ab	7.13 ± 1.64Ac
		UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.00 ± 0.76Ab	7.25 ± 1.39Ac
		ClO_2 + UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.75 ± 0.46Aa	8.00 ± 0.76Ab	7.25 ± 1.39Ac
	Overall	Control	9.00 ± 0.00Aa	8.63 ± 0.52Ba	7.13 ± 0.99Bb	6.88 ± 0.83Bb	6.00 ± 1.07Ac
		Water	9.00 ± 0.00Aa	8.88 ± 0.35ABa	7.63 ± 0.74ABb	7.00 ± 0.53Bb	6.00 ± 1.07Ac
		ClO_2	9.00 ± 0.00Aa	8.88 ± 0.35ABa	7.63 ± 0.74ABb	7.00 ± 0.76Bb	6.00 ± 0.93Ac
		UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.13 ± 0.64Ab	7.38 ± 0.74ABc	6.63 ± 0.92Ad
		ClO_2 + UV-C	9.00 ± 0.00Aa	9.00 ± 0.00Aa	8.25 ± 0.46Ab	7.88 ± 0.64Ab	6.88 ± 0.99Ac

[z]Any means in the same column (A-C) or row (a-c) followed by different letters are significantly ($p < 0.05$) different by Duncan's multiple range test.
[y]50 ppm aqueous ClO_2.
[x]10 kJ·m^{-2} UV-C.
[w]Combination of 50 ppm aqueous ClO_2 and 10 kJ·m^{-2} UV-C.

this study indicate that the combined treatment maintains the color of the Romaine lettuce and kale, unlike electron beam irradiation.

Sensory evaluation of the Romaine lettuce and kale during storage is shown in Table 6. Sensory qualities of minimally processed vegetables exposed to disinfection treatment are important for food industry and consumers. Sensory qualities such as appearance, odor, and overall aspect were evaluated among treatments during storage. The sensory scores of the lettuce and kale during storage decreased with increasing storage time for all treatments. However, the appearance and overall qualities of the lettuce treated with the combination of aqueous ClO_2 and UV-C had higher scores than other treatments after 7 days of storage, reflecting the inhibition of browning in cut-surface of the samples. As suggested by Du et al. (2009), aqueous ClO_2 treatment not only provided better inhibitory effect on the browning of fresh-cut lotus root, but also maintained higher overall visual quality than the control during storage. Therefore, it is suggested that the combined treatment used in the present investigation is most favorable for maintaining the quality of the lettuce and kale during storage. For kale samples, the combined treatment also had higher scores that any other treatments during storage. For the odor of the lettuce and kale, there were no significant differences among treatments during storage, indicating that aqueous ClO_2 and UV-C did not significantly impair the odor of the lettuce and kale. Chun et al. (2007) reported that 50 and 100 $\mu L \cdot L^{-1}$ aqueous ClO_2 treatment of fresh ginseng had better sensory scores such as freshness, texture, decay, and odor than the control during 8 weeks of storage. Kim et al. (2010) also concluded that the combined postharvest treatments (aqueous ClO_2, fumaric acid, and UV-C) of strawberry fruit showed higher scores than did the either control or any single treatment after 12 days of storage. The results of this study are comparable with those results.

In summary, microbiological data indicated that the populations of total aerobic bacteria, yeast and mold, *E. coli* O157:H7 and *S.* Typhimurium were significantly decreased with combined treatment of aqueous 50 $\mu L \cdot L^{-1}$ ClO_2 and 10 $kJ \cdot m^{-2}$ UV-C resulting in improved microbial safety without altering the color of Romaine lettuce and kale during storage. The sensory qualities of the Romaine lettuce and kale treated with combination of aqueous 50 $\mu L \cdot L^{-1}$ ClO_2 and 10 $kJ \cdot m^{-2}$ UV-C were better than those of the control. Therefore, the use of the combined treatment using aqueous ClO_2 and UV-C can be an effective decontamination method.

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