Pathogen Reduction and Quality of Lettuce Treated with Electrolyzed Oxidizing and Acidified Chlorinated Water

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ABSTRACT: The efficacy of electrolyzed oxidizing (EO) and acidified chlorinated water (45 ppm residual chlorine) was evaluated in killing *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce. After surface inoculation, each leaf was immersed in 1.5 L of EO or acidified chlorinated water for 1 or 3 min at 22 °C. Compared to a water wash only, the EO water washes significantly decreased mean populations of *E. coli* O157:H7 and *L. monocytogenes* by 2.41 and 2.65 $\log_{10}$ CFU per lettuce leaf for 3 min treatments, respectively ($p \leq 0.05$). However, the difference between the bactericidal activity of EO and acidified chlorinated waters was not significant ($p \geq 0.05$). Change in the quality of lettuce subjected to the different wash treatments was not significant at the end of 2 wk of storage.

Keywords: lettuce, electrolyzed oxidizing water, chlorinated water, *Escherichia coli* O157:H7, *Listeria monocytogenes*

Introduction

MANY OUTBREAKS OF FOODBORNE ILLNESS HAVE BEEN associated with consumption of fresh vegetables. The presence of pathogens on fresh produce is not uncommon (Nguyen-the and Caroline 1994; Beuchat 1996; Brackett 1999). Pathogens can contaminate raw vegetables through agricultural practices and survive during processing and distribution (Beuchat 1996; Brackett 1999). Furthermore, pathogens can grow on fresh vegetables during storage. *Listeria monocytogenes* grew on whole and shredded lettuce held at 5 °C (Beuchat and Brackett 1990), on whole tomatoes at 21 °C (Beuchat and Brackett 1991) and on asparagus, broccoli, and cauliflower at 15 °C (Berrang and others 1989a). Abdual-Raouf and others (1993) also reported that *E. coli* O157:H7 can grow on shredded lettuce at 12 and 21 °C. Similarly, *Salmonella* Hadar grew on fresh-cut cabbage at 12 and 20 °C (Piagentini and others 1997). *Aeromonas hydrophila* growth occurred on asparagus, broccoli, and cauliflower at 4 and 15 °C (Berrang and others 1989b). Hence, the prevalence of pathogens on many types of raw vegetables and growth potential of many foodborne pathogens at normal storage temperature suggest that certain types of fresh vegetables are potentially high risk foods.

A variety of chemical disinfectants have been used to reduce the populations of pathogens on fresh produce. Chlorine is among the most frequently used chemical disinfectants in fresh produce washing (Adams and others 1989; Beuchat and others 1998); however, a variety of other chemicals, including chlorine dioxide (Reina and others 1995; Zhang and Faber, 1996), hydrogen peroxide (Sapers, 1996), organic acids (Karapinar and Gonul 1992; Nguyen-the and others 1996), trisodium phosphate (Zhang and Faber 1996; Zhuang and Beuchat 1996), and ozone (Burrows and others 1999) have been evaluated and in some instances are being used. However, many of the chemicals evaluated have minimal effect on inactivating pathogens on fresh produce. Hence, more efficacious treatments to inactivate pathogens on fresh produce are needed (Beuchat 1996, 1999).

Recently, a new concept for application as a sanitizer, electrolyzed oxidizing (EO) water, has been introduced. EO water has been determined to be an effective treatment for sanitizing kitchen cutting boards (Venkitanarayanan and others 1999a) and killing food pathogens *in vitro* conditions (Kim and others 2000; Venkitanarayanan and others 1999b). EO water from the anode stream possesses at least 3 antimicrobial properties that include low pH (ca. 2.5), high oxidation-reduction potential (ORP; ca. > 1,100mV), and chlorine-based reactants (10 to 90 ppm) (Anonymous 1997). The concentration of chlorine reactants in EO water is influenced by the amperage of the EO water generator.

Izumi (1999) showed that EO water containing 15 to 50 ppm of available chlorine was effective in reducing microbial flora on several fresh-cut vegetables. However, neither direct comparison of the antimicrobial activity of EO water with equivalent concentrations of chlorine has been made nor have the specific quality attributes (except color) of treated foods been determined. The objectives of this study were to compare the efficacy of EO and acidified chlorinated waters in killing *E. coli* O157:H7 and *L. monocytogenes* on lettuce and to examine the effect of EO and acidified chlorinated waters on the quality of treated lettuce during 2 wk of storage at 4 °C.

Materials and Methods

Bacterial strains used

A 5-strain mixture of *L. monocytogenes* (ATCC 19117, sheep isolate; 109, pepperoni isolate; 201, milk isolate; 315, salami isolate; and 116, cheese isolate) or nalidixic acid-resistant *E. coli* O157:H7 (932, human isolate; 994, salami isolate; E0018, calf fecal isolate; H1730, human isolate; and F4546, human isolate) were used as inoculum. Each bacterial strain was cultured individually in 10 ml of tryptic soy broth (Difco, Detroit, Mich.) at 37 °C for 24 h. Each strain was cultured successively at least 3 times before use as inoculum on lettuce. Each bacterial culture was centrifuged (2,000 × g, 22 °C,

15 min) and its pellet was suspended in 2 ml of 0.1% sterile peptone water (pH 7.1). For each pathogen, equal portion (2 ml each) from each of the 5 strains was combined to make the inoculum. The bacterial population of each inoculum was also determined by surface plating 0.1-ml portion of appropriate dilutions (0.1% peptone water) of the suspension on duplicate tryptic soy agar (TSA; Difco, Detroit, Mich.) plates and incubating the plates at 37 °C for 24 h.

Electrolyzed oxidizing and chlorinated water

Electrolyzed oxidizing (EO) water was generated using a Hoshizaki EO water generator (model ROX 20TA, Hoshizaki Electric Co. Ltd., Toyoake, Aichi, Japan). For chlorine germicidal concentration test, the current passing through the EO water generator was set at 8.0 ± 0.2, 14.0 ± 0.2, or 19.0 ± 0.2 Amperes (A). Only EO water generated at 14 A was used for lettuce study. A 12% solution of sodium chloride and deionized water from a laboratory supply line was simultaneously pumped into the generator and the final concentration of sodium chloride solution passing through electrodes in the EO water generator chamber was about 0.1%. When the Amperage of the generator was stabilized, 1.5-L portions of acidic EO water (45 ppm of residual chlorine) were collected from the anode outlet in sterile 2-L beakers for treatment of whole lettuce leaves. Properties of EO water, including pH, ORP, and residual chlorine concentration, were determined. Residual chlorine concentration was measured by an iodometric method using a digital titrator (model 16900; Hach Co., Loveland, Colo., U.S.A.). The pH and ORP of the EO water were measured in duplicate using pH and ORP electrodes (ACCUMET®, model 50, Denver Instrument Co., Denver, Colo., U.S.A.). Chlorinated water (45 ppm of residual chlorine) was prepared using appropriate amount of sodium hypochlorite (Aldrich, Milwaukee, Wis., U.S.A.) and pH of the chlorinated water was adjusted to 2.5 by adding hydrochloric acid into 1.5 L of chlorinated water. The treatment solutions (22 °C) were prepared in 2-L beakers immediately before each experiment then covered with aluminum foil and used within 1 h.

Chlorine germicidal equivalent concentration

AOAC Official Method 955.16 (AOAC 1998) was used to determine chlorine germicidal equivalent concentration of EO water. Triplicate determinations were performed for each EO water sample. *Salmonella typhi* F3514 used for this assay was provided by the Centers for Disease Control and Prevention, Atlanta, GA.

Preparation of whole lettuce leaf

Iceberg lettuce heads were purchased from a local grocery store. For the bacterial challenge study, the outer 2 leaves of the lettuce head were discarded. Whole leaves, with weights ranging between 52 to 62 g, were used. Uninoculated lettuce leaves were used for the quality evaluation. The 2 outermost leaves also were discarded in testing the quality of lettuce leaves. Only the 3rd, 4th, and 5th leaf of each head of lettuce was used for the quality evaluation study. Sixty-three lettuce leaves were prepared with 9 used in each of the 7 treatment groups [unwashed control, washed with tap water (1 or 3 min), washed with EO water (1 or 3 min), and washed with chlorinated water (1 or 3 min)].

Procedure for inoculating lettuce leaves

Fifty microliters of each bacterial mixture was inoculated onto the outer-side of each lettuce leaf by placing along the stem, 15 to 20 drops of inoculum with a micropipettor. Inoculated leaves were air-dried under a laminar flow hood for 1 h at 22 °C before receiving a washing treatment.

Procedure for treating lettuce leaves

Treatment of inoculated lettuce leaves was performed by immersing an individual whole lettuce leaf in 1.5 L of the appropriate treatment solution (22 °C) in a 2-L glass beaker while shaking at 100 rpm for 1 or 3 min. On termination of the treatment, the lettuce leaf was transferred into a sterile stomacher bag using a sterile tong. The leaf was added to 200 ml of Bacto® neutralizing buffer (Difco, Detroit, Mich.) water and then macerated for 1 min at medium speed in a stomacher (model 80, Seward, London, U.K.). The lettuce homogenate was then subjected to bacterial assay. For quality determinations, treated lettuce leaves were drained on 2 layers of paper towels for 10 min at 22 °C and put into a 1-gallon Ziploc® vegetable bag (Dow, Indianapolis, Indiana). Each individually bagged lettuce leaf was then placed in a partitioned box (49 × 49 × 33 cm; 7 bags per box) to prevent contact of each bag during storage. Nine boxes of lettuce leaves in total were stored in a walk-in refrigerator (4 °C) for the 14-d experimental period.

Bacterial analysis

The populations of *E. coli* O157:H7 and *L. monocytogenes* in EO, acidified chlorinated, and deionized water after use for washing lettuce and in lettuce homogenate were determined. Undiluted lettuce homogenate was surface-plated in quadruplicate (0.25 ml) and serially (1:10) diluted in 0.1% peptone water and plated in duplicate (0.1 ml) on appropriate enumeration media. Each wash solution (25 ml) was combined with 225 ml of Bacto® neutralizing buffer water and 0.25 ml was surface-plated in quadruplicate and serially diluted and plated on appropriate enumeration media according to the procedure described above. *E. coli* O157:H7 was enumerated on sorbitol MacConkey agar (Oxoid, Basingstoke, Hampshire, U.K.) with 50 mg of nalidixic acid/ml (SMAC) which were incubated at 37 °C for 20-24 h. Selected colonies on SMAC were confirmed by *E. coli* O157 latex agglutination test (Unipath-Oxoid, Columbia, Md., U.S.A.) and API 20E diagnostic kits (bio Mérieux Vitek, Inc., Hazelwood, Mo., U.S.A.). *L. monocytogenes* was enumerated on Oxford Listeria selective agar containing selective supplements (Gene-Trak, Framingham, Mass., U.S.A.) which were incubated at 37 °C for 48 h. Selected presumptive colonies of *L. monocytogenes* on the selective agar were further confirmed by appropriate biochemical tests (Golden and others 1988). At least triplicate trials were performed for each pathogen studied.

Quality of lettuce leaves

Three expert evaluators assessed the quality of lettuce during 2 wk of storage period. The weight of each lettuce sample was determined on each sampling day. Lettuce quality was evaluated visually or by touching based on turgor, visual quality, decay, stem discoloration, wilting, and other defects (like spotted or torn not counted in other quality evaluations) using a 9-point hedonic scale for each quality attribute (Table 1). Color change of lettuce leaves was determined objectively during storage by color measurements at 2 locations on each leaf using a Minolta colorimeter (model CR200, Minolta Co., Japan). The 1st color measurement location was at the middle of the leaf stem at 1 cm from the end of the leaf and the 2nd location was at the middle of the leaf about 2 cm from the edge of the leaf. Standard C.I.E. 1976

Table 1—Nine-point hedonic scale for each quality factor

Turgor	Visual quality*	Decay	Stem discoloration	Wilting	Other defects**
9 very stiff	9 excellent	9 none	9 none	9 none	9 none
7 stiff	7 good	7 slight	7 slight	7 slight	7 slight
5 turgid	5 fair	5 moderate	5 moderate	5 moderate	5 moderate
3 fairy stiff	3 poor	3 severe	3 severe	3 severe	3 severe
1 limp	1 extremely poor	1 extreme	1 extreme	1 extreme	1 extreme

*Visual quality: general overall visual impression.
**Other defects: minor defects like spotted or torn not counted in Tables 4 to 6.

Table 2—Inactivation of *E. coli* O157:H7 and *L. monocytogenes*[a] on lettuce at 22°C by EO and acidified chlorinated waters[b]

		Mean population[c] (log_{10} CFU/ml of solution or leaf)			
		E. coli O157:H7		*L. monocytogenes*	
Treatment	Time (min)	In solution	On lettuce	In solution	On lettuce
Unwashed	—	—	7.11a	—	7.07a
Deionized water	1	3.76	5.73b	3.00	5.51b
	3	3.63	5.35b	3.68	5.32b
Chlorinated water	1	nd[d]	3.54c	nd	3.45c
	3	nd	2.77d	nd	3.54c
EO water	1	nd	2.95cd	nd	3.13c
	3	nd	2.94cd	nd	2.67c

[a]Initial inoculum (log_{10} CFU/leaf) per lettuce: 7.91 for *E. coli* O157:H7 and 8.22 for *L. monocytogenes*.
[b]Properties of acidified chlorinated and EO waters were pH 2.5, 45 ppm of residual chlorine, and 1,130 mV of ORP.
[c]Values in the same column that are not followed by the same letter are significantly different ($p \leq 0.05$).
[d]Not detected by direct plating. Minimum level of detection was 10 CFU/ml of solution.

Table 3—Determination of chlorine germicidal equivalent concentration

		S. typhi subculture series[c]									
Ger.[a]	Conc. ppm[b]	1	2	3	4	5	6	7	8	9	10
Cl	200	–	–	–	–	+	+	+	+	+	+
	100	–	–/+/+	+	+	+	+	+	+	+	+
	50	–	+	+	+	+	+	+	+	+	+
19 A	80	–	–	–	–	–	–	–	–	–/–/+	+/–/+
14 A	45	–	–	–	–/+/+	+	+	+	+	+	+
8 A	18	–	+	+	+	+	+	+	+	+	+

[a]Germicides were 3 levels of Cl (chlorinated) and EO waters generated at 8, 14, and 19 Amp.
[b]Residual chlorine cocentration.
[c]+ indicates growth; – indicates no growth; a single – indicates -/-/- for all 3 reps; and a single + indicates +/+/+ for all 3 reps.

chromatic coordinates (L*, a*, and b*) were determined. A white tile (L* = + 97.47, a* = − 0.67, b* = + 2.56) and a green tile (L* = + 71.69, a* = − 22.96, b* = + 25.35) were used to calibrate the colorimeter. Other chromatic attributes determined included chroma (C*), hue angle (H*) and total color difference (ΔE) using the following equations:

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{0.5}$$
$$\text{Hue angle } (H^*) = \tan^{-1}(b^*/a^*)$$

$$\text{Total color difference } (\Delta E) = [(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2]^{0.5}$$

where L_o^*, a_o^*, and b_o^* represent respective initial chromatic parameters of the green tile. Color measurements were determined at 0, 7, and 14 d of storage. On the last day of storage after the color measurement, the shear force of each lettuce leaf was determined with an Instron Universal Testing Machine (50-kg load cell; 100 mm/min cross-head speed).

Statistical analysis

Results of bacterial challenge and lettuce quality studies were analyzed by analysis of variance and Duncan's multiple range test (SAS 1995).

Results and Discussion

Bacterial challenge study

Results of the effect of EO and acidified chlorinated waters having the same pH (2.5), ORP (1,130 mV), and residual chlorine concentration (45 ppm) in killing *E. coli* O157:H7 and *L. monocytogenes* on lettuce are shown in Table 2. The populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated on lettuce were 7.91 and 8.22 log_{10} CFU per leaf, respectively. Drying the inoculated lettuce reduced both bacterial populations by ca. 1 log_{10} CFU per leaf. Rinsing lettuce with deionized water reduced pathogen populations by approximately 1.5 log_{10} CFU per leaf, regardless of rinsing time. Generally, a water wash only decreased initial microbial counts by 1 log_{10} CFU on fresh produce (Brackett 1987; Adams and others 1989; Izumi 1999). Immersing lettuce in 45 ppm EO water for 1 min was effective in further significantly reducing the mean populations of *E. coli* O157:H7 and *L. monocytogenes* (2.78 and 2.38 log_{10} CFU/leaf, respectively) compared to a deionized water rinse. However, there was no significant difference in efficacy between chlorinated and EO water in killing *E. coli* O157:H7 or *L. monocytogenes* on lettuce ($p \geq 0.05$). Also, there was no significant difference in efficacy of the 2 treatments based on treatment time except that there was a greater reduction of *E. coli* O157:H7 after treatment with acidified chlorinated water for 3 min than 1 min. No *E. coli* O157:H7 or *L. monocytogenes* was detected in the acidified chlorinated or EO water used to treat the lettuce.

Many studies have revealed that the effect of chemical disinfectants for decontaminating pathogens or natural microflora on fresh produce was minimal. A wash treatment with tap water decreased the microbial population on chopped lettuce by 92.4%, whereas treatment with 100 ppm active chlorine reduced the microbial load by 97.8% (Adams and others 1989). Zhang and Faber (1996) determined that of the disinfectants they used to treat shredded cabbage or lettuce, there was only ca. 1 log_{10} reduction in *L. monocytogenes* populations. Immersing Brussels sprouts in a 200 ppm active chlorine solution reduced *L. monocytogenes* populations by 1 log_{10} compared to a water wash (Brackett 1987).

To further evaluate the efficacy of EO water, a chlorine germicidal equivalent concentration test (AOAC Official Method 955.16, 1998) of 3 different batches of EO waters were performed (Table 3). EO waters generated at 8 A (18 ppm of residual chlorine), 14 A (45 ppm), and 19 A (80

ppm) were equivalent or more effective in killing *S. typhi* than 50, 100, and 200 ppm chlorine solutions, respectively. For example, EO water generated at 19 A had only 80 ppm residual chlorine but could completely inactivate 8 *S. typhi* subculture series whereas 200 ppm chlorine solution could only inactivate up to 4 subculture series. In the present study, the bactericidal activity of acidified chlorinated water was similar to that of EO water. EO water would predictably have greater bactericidal activity on lettuce than unacidified chlorinated water because of the acidic pH of EO water and the presence of other oxidants including hydrogen peroxide and hydroxyl radical in EO water in addition to hypochlorous acid (Shimizu and Hurusawa 1992). Acidification of chlorinated water (from pH 9 to 4.5 – 5.0) enhanced its microbial activity by 1.5 to 4.0 fold on shredded lettuce compared to an unacidified chlorine wash (Adams and others 1989). Results obtained from the current study revealed that EO water has potential of reducing pathogens on lettuce by at least 2 $\log_{10}$ CFU per leaf.

Lettuce quality characteristics

The effects of acidified chlorinated and EO water treatments on the quality of lettuce held for 2 wk at 4 °C are shown in Tables 4 to 6. The range of weight loss at the end of storage was between 9.5 g (unwashed) and 12.5 g (tap water wash for 1 min), with an average weight loss of 10.6 ± 0.6 g. The weight loss of lettuce leaf during storage was statistically significant; however, it was not significantly different among the different treatments at each sampling time. Turgor changes of treated lettuce leaves during storage are shown in Table 5. Washed lettuce leaves immediately after treatment (day 0) had higher turgor than the unwashed leaves. Turgor gradually decreased with time from stiff (score of 7) at day 0 to turgid (score of 5) at day 14. There was no significant difference in turgor (ranged from 4.3 to 5.2) at the end of storage, among all of the treatments ($p \geq 0.05$). These indicate that different treatment had no significant effect on turgor of lettuce leaf when compared with control (unwashed) leaf. Reduction on lettuce leaf turgor was primary due to the length of storage not the treatment.

Initial good (average score of 8) visual quality of treated lettuce leaves generally decreased to fair (average score of 5) by the end of storage. Decay of treated lettuce leaves occurred slightly by the end of storage (reduced from an average initial score of 8.7 to 7.5). However, there was no substantial difference in visual quality and degree of decay among all treatments at the end of storage. Other quality parameters such as stem discoloration, wilting, and other defects of treated lettuce leaves followed the same trend as decay, with scores ranging from 7 to 8 (very slight) at the beginning of storage to 5 to 6 (moderate) at the end of storage. There was also no substantial quality difference at the end of storage, in the quality attributes of lettuce receiving any of the different treatments.

Shear force is a destructive measurement of firmness. The lettuce leaves treated in chlorinated water for 1 min had the highest shear force of 20.7 Newton (N), whereas EO water treatment for 3 min had the lowest at 16.4 N. However, there was no substantial difference among any of the treated lettuce leaves, except between those treated with EO water for 3 min and acidified chlorinated water for 1 min. The effect of treatments on color change of lettuce leaves during the 2 wk of storage is shown in Table 6. Three chromatic parameters including chroma, hue angle, and total color difference were used to measure color change on lettuce leaf. There was no significant color difference in the 3 chromatic parameters among all 3 treatments ($p \geq 0.05$) at both locations on the leaves examined at the end of storage. Our results agree with those of Izumi (1999) that treatment of shredded lettuce with EO water (50 ppm of active chlorine) did not significantly affect quality characteristics such as color and general appearance compared with a water only treatment.

Table 4—Effect of storage time at 4°C and rinse treatment on weight loss of lettuce

	Treatment*						
Day	1	2	3	4	5	6	7
0	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
2	2.0b	2.3b	2.2b	2.3b	2.2b	2.2b	2.2b
4	3.3c	3.9c	3.6c	3.8c	3.6c	3.6c	3.7c
7	5.3d	6.2d	5.6d	6.1d	5.7d	5.9d	5.9d
9	6.6e	7.7e	7.0e	7.5e	7.2e	7.3e	7.4e
11	7.7f	9.1f	8.3f	8.9f	8.6f	8.7f	8.8f
14	9.5g	11.3g	10.3g	11.0g	10.8g	10.7g	10.9g

*Treatments are as follows: 1, unwashed; 2, tap water wash for 1 min; 3, tap water wash for 3 min; 4, EO water wash for 1 min; 5, EO water wash for 3 min; 6, Chlorinated water wash for 1 min; 7, Chlorinated water wash for 3 min. Values not followed by the same letter within the same treatment are significantly different ($p \leq 0.05$).

Table 5—The effect of storage time at 4 °C and rinse treatment on turgor of lettuce

	Treatment*						
Day	1	2	3	4	5	6	7
0	6.1a	6.9a	7.1a	7.4a	7.4a	7.3a	6.8a
2	5.1b	6.7a	6.7ab	6.8a	7.0ab	6.9a	6.6ab
4	4.8b	6.6a	6.2b	6.7a	6.6b	6.9a	6.4ab
7	4.9b	5.9b	5.4c	5.7b	5.8c	6.1b	5.8bc
9	4.8b	5.2c	5.3cd	5.4b	5.7c	5.7bc	5.1cd
11	4.7b	4.7cd	4.9cd	5.3b	5.3c	5.1cd	4.7d
14	4.5b	4.3d	4.6d	5.2b	4.6d	5.0d	4.7d

*Treatments are as follows: 1, unwashed; 2, tap water wash for 1 min; 3, tap water wash for 3 min; 4, EO water wash for 1 min; 5, EO water wash for 3 min; 6, chlorinated water wash for 1 min; 7, chlorinated water wash for 3 min. Values not followed by the same letter within the same treatment are significantly different ($p \leq 0.05$).

Conclusions

THE RESULTS OF THIS STUDY REVEALED THAT IMMERSING INoculated whole leaves in EO water was equally effective as acidified chlorinated water of equivalent pH, ORP and residual chlorine concentration in killing *L. monocytogenes* or *E. coli* O157:H7. Treatment time (1 or 3 min) did not significantly affect the antimicrobial activity of the EO water and the acidified chlorinated water treatments, except that acidified chlorinated water rinse for 3 min was more effective in killing *E. coli* O157:H7 than acidified chlorinated water rinse for 1 min. Overall, the quality characteristics of whole lettuce leaves subjected to the different rinse treatments were not significantly different among the treatments at each sampling time. This suggested that EO water could be an effective disinfectant for killing *E. coli* O157:H7 and *L. monocytogenes* on lettuce without affecting the quality of lettuce. However, all quality attributes of the treated lettuce gradually decreased during storage.

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Table 6—Color coordinates of treated lettuce leaf stored for 2 wk at 4°C

			Treatment[1]						
Loc.[2]	Color para.[3]	Day	1	2	3	4	5	6	7
A	C	0	25.1 a	21.7 a	26.7 a	24.6 a	24.1 a	24.9 a	26.0 a
		7	24.5 a	23.9 a	22.2 b	25.4 a	24.3 a	24.0 a	28.1 a
		14	24.9 a	24.4 a	27.4 a	25.4 a	24.6 a	26.4 a	26.9 a
	Hue	0	116.1 a	114.0 a	116.8 a	118.8 a	115.0 a	116.5 a	117.6 a
		7	116.9 a	115.6 a	116.8 a	118.2 a	115.0 a	116.3 a	117.1 a
		14	116.2 a	113.3 a	116.3 a	117.2 a	113.8 a	116.0 a	115.6 a
	ΔE	0	14.7 a	17.4 a	14.6 a	15.6 a	15.5 a	15.2 a	14.8 a
		7	14.9 a	15.4 a	16.7 a	15.8 a	15.5 a	15.3 a	12.3 a
		14	14.7 a	15.2 a	14.5 a	15.6 a	14.5 a	13.7 a	13.7 a
B	C	0	20.8 a	19.0 a	20.4 a	20.4 a	19.1 a	19.8 a	19.3 a
		7	21.8 a	18.0 a	19.8 a	20.1 a	20.8 a	20.1 a	21.1 a
		14	21.9 a	19.3 a	22.7 a	23.7 a	22.9 a	21.7 a	21.7 a
	Hue	0	115.2 a	113.1 a	115.8 a	117.5 a	115.5 a	115.4 a	116.2 a
		7	116.1 a	114.3 a	116.8 a	117.1 a	113.7 a	115.6 a	116.3 a
		14	115.0 a	111.7 a	116.2 a	117.8 a	114.1 a	113.9 a	115.4 a
	ΔE	0	17.9 a	18.9 a	18.9 a	18.7 a	20.8 a	18.8 a	19.7 a
		7	18.0 a	19.0 a	19.9 a	19.2 a	18.6 ab	19.8 a	17.1 a
		14	17.2 a	20.4 a	16.0 a	16.9 a	16.2 b	17.6 a	16.8 a

[1] Treatments are as follows: 1, unwashed; 2, tap water wash for 1 min; 3, tap water wash for 3 min; 4, EO water wash for 1 min; 5, EO water wash for 3 min; 6, chlorinated water wash for 1 min; 7, chlorinated water wash for 3 min. Values not followed by the same letter within same treatment location and chromatic coordinate are significantly different ($p \leq 0.05$).
[2] Location A was at the middle of the leaf stem at 1 cm from the end of the leaf and location B was 2 cm from the edge of the leaf.
[3] Color parameters are C. (Chroma), Hue angle, and ΔE (Total color difference).

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