International Food Research Journal 21(2): 533-539 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

The effects of immersion methods and concentration of ozonated water on the microbial counts and the quality and sensory attributes of fresh-cut broccoli

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Article history

Received: 1 November 2013
Received in revised form: 28 November 2013
Accepted: 29 November 2013

Keywords

Aqueous ozone
Brassica oleracea L.
Food safety
Immersion
Minimally processed
Microbial contamination

Abstract

The effects of immersion methods and concentrations of ozonated water on natural microbial contamination as well as the quality and sensory attributes of fresh-cut broccoli were studied. The first group of fresh-cut broccoli was immersed in air-ozonated water (containing 0.56 ppm ozone [O_3]) for 5 min and remained immersed in this water for 5 min (i.e., low O_3 concentration and long contact time; LL). The second group of fresh-cut broccoli was immersed in O_2 gas-ozonated water (containing 1.60 ppm O_3) for 5 min and remained immersed in this water for 3 min (i.e., high O_3 concentration and short-contact time; HS). Fresh-cut broccoli treated with tap water served as the control. All samples were stored at 4°C for 6 days. The results revealed that washing fresh-cut broccoli with ozonated water (LL and HS) and tap water (control) reduced the amount of microbes compared with the initial microbial loads of unwashed fresh-cut broccoli. HS was the most effective treatment with regard to reducing aerobic bacteria, coliforms, and yeasts and molds throughout storage to ranges of 1.41-2.61, 0.55-1.75, and 1.46-1.71 log CFU.g^{-1}FW, respectively. Furthermore, this treatment did not have negative effects on color (lightness, a^*, b^*, and hue angle values), chlorophyll content, or sensory attributes (overall visual quality, visible color, and odor). The LL treatment reduced the microbial counts in fresh-cut broccoli on the first day after the treatment; however, the quality and sensory attributes of the LL-treated broccoli were significantly decreased compared with the control. The results indicate that treating fresh-cut broccoli with water ozonated with a high concentration of O_3 (1.60 ppm) for 5 min followed by immersion in the same water for 3 min reduces natural microbial contamination without any negative effects on the quality or sensory attributes of the broccoli.

Introduction

Fresh-cut or minimally processed broccoli is a popular vegetable. Consumers demand that these types of products maintain a high level of quality with regard to appearance, taste, flavor, nutrition, and convenience. These products should also be safe for direct consumption (Garcia and Barrett, 2002). The shelf life of fresh-cut broccoli florets is limited by the yellowing of florets due to chlorophyll degradation and contamination with pathogenic or spoilage microorganisms (Yamauchi and Watada, 1998; Skog and Chu, 2001; Stringer *et al.*, 2007). Treatments that delay chlorophyll degradation and inhibit microbial growth, thereby maintaining a fresh-like quality, are required to prolong the shelf life of fresh-cut broccoli.

The United States Food and Drug Administration (US FDA) has classified the antimicrobial agent ozone (O_3) as generally recognized as safe (GRAS); thus, it can be directly applied to food products (FDA, 2012). O_3 is a strong oxidizer, and there are numerous applications for gaseous and dissolved O_3 (ozonated water) in disinfection and food processing. Because O_3 is highly unstable and decomposes rapidly, it does not leave a potentially harmful residue on fresh produce (Skog and Chu, 2001; Rice, 1999). O_3 gas and ozonated water have been used on cilantro (Wang *et al.*, 2004), celery (Zhang *et al.*, 2005), lettuce (Beltrán *et al.*, 2005; Selma *et al.*, 2007; Ölmez and Akbas, 2009), and cantaloupe (Selma *et al.*, 2008). In fresh-cut broccoli, Forney *et al.* (2003) reported that the application of O_3 gas at 0.7 ppm combined with or without 1-MCP at 1.0 µL.L^{-1} delayed the yellowing process. Kumar and Kim (2010) compared the antimicrobial effects of sanitizers (chlorinated water, electrolyzed water, and ozonated water) and contact times (90 and 180 sec) for washing fresh-cut broccoli. Continuous washing with ozonated water for a longer contact time (180 sec) caused a greater

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decrease in total aerobic bacteria and coliform plate counts when compared with other sanitizers, and the color of the broccoli florets was not altered. Renumarn *et al.* (2013) found that exposure to water with a high O_3 concentration (1.50 ppm) for 15 min reduced the coliform count, total bacteria, yeasts, and molds in fresh-cut broccoli florets only one day after treatment. Conversely, exposure to ozonated water induced yellowing and reduced visual quality during storage. The effectiveness of O_3 as a disinfectant depends on the application method, O_3 concentration, exposure or contact time, types of pathogenic or spoilage microorganisms, levels of initial inoculums, and sensitivity of fresh produce to O_3 (Liew and Prange, 1994). Most O_3 studies have focused on antimicrobial efficacy; however, little information exists regarding the effect of O_3 on the nutritional constituents and sensory quality of fresh and fresh-cut produce (Rico *et al*., 2007; Ölmez and Kretzschmar, 2009). Additionally, the effects of immersion method and concentration of ozonated water on microbial loads and sensory quality of fresh-cut broccoli have not been investigated. Therefore, in the current study, we examined the effects of immersion methods and concentration of ozonated water on the decontamination of microbes as well as the quality and sensory attributes of fresh-cut broccoli.

Materials and Methods

The preparation of fresh-cut broccoli florets

Fresh broccoli (*Brassica oleracea* L.) heads were purchased from the Royal Project Foundation in Chiang-Mai province, Thailand. Sixty heads of broccoli were cut into small florets (approximately 2.5 cm in diameter and 4 cm in length) with a sharp, stainless steel knife. The mixed broccoli florets were prewashed with cold tap water (8-10°C) for 2 min, spin-drained, and randomly sampled for ozonated water treatments at room temperature (25±2°C).

Ozonated water preparation

O_3 gas was generated using an O_3 generator (Ozonizer Model B6APT, Thailand; O_3 production rate of 2,500 mg/h) using two types of inlet gas: 1) ambient air containing approximately 21% O_2 (used to generate water with a low concentration of ozone, referred to herein as "air-ozonated water") with a flow rate of 8.0 $L.min^{-1}$ and 2) 99% O_2 gas from an O_2 cylinder (used to generate water with a high concentration of ozone, referred to herein as "O_2 gas-ozonated water") with a flow rate of 2.0 $L.min^{-1}$. When the O_3 generator was turned on, O_3 gas was produced and transferred via a rubber outlet tube connected to four blueball airstones to facilitate the dissolution of gaseous O_3 into 6 L of tap water at room temperature (25±2°C). Fresh-cut broccoli florets (600 g) were immersed in air-ozonated water (0.56 ppm O_3) or O_2 gas-ozonated water (1.60 ppm O_3) for 5 min. Thereafter, the O_3 generator was turned off, and the fresh-cut broccoli remained immersed in the air-ozonated water for 5 min (i.e., low O_3 concentration and long contact time; LL) or in the O_2 gas-ozonated water for 3 min (i.e., high O_3 concentration and short contact time; HS). Fresh-cut broccoli immersed in tap water for 5 min was used as a control. After immersion, the excess water was removed from the fresh-cut broccoli florets using a manual salad spinner. Fresh-cut broccoli florets (approximately 120 g) from each treatment were packed into polyvinylchloride boxes and stored at 4°C for 6 days. Each treatment was performed with 18 boxes. Six boxes of treated broccoli samples were randomly sampled to determine microbial populations, visual quality (i.e., floret color and chlorophyll content), and sensory quality on Days 0 (first day), 3, and 6 of storage compared with the control.

Microbiological analysis

Samples of fresh-cut broccoli florets (25 g) were homogenized with 225 ml of 1% sterile peptone water using a stomacher (IUL Instruments Masticator, Barcelona, Spain) for 1 min. A tenfold dilution series was prepared in sterile peptone water for plating. The following culture media and conditions were used to enumerate the microbial cells: (1) plate count agar (PCA, HiMedia) incubated at 37°C for 24 h to determine aerobic bacteria counts; (2) eosin methylene blue agar (EMB, HiMedia) incubated at 37°C for 24-36 h to determine total coliform counts; and (3) potato dextrose agar (PDA, HiMedia) incubated at 28°C for 7 days to determine yeast and mold counts. Microbial counts were expressed as log colony-forming units per gram fresh weight (log $CFU.g^{-1}$ FW).

Measuring color change and chlorophyll content

The floret color was measured at the center of 15 florets from each of the treatments as lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) values as well as hue angle (h°) with a Konica Minolta Chroma Meter (model CR-400; Konica Minolta, Tokyo, Japan). The total chlorophyll, chlorophyll *a*, and chlorophyll *b* contents were analyzed using *N,N*-dimethylformamide extracts of fresh-cut broccoli floret samples according to the method of Moran (1982).

Sensory evaluation

An untrained panel of five people performed the sensory evaluation. Sensory attributes, such as overall visual quality, color, and odor, were evaluated during storage using a 9-point scale, where 1 denoted complete deterioration and 9 denoted excellent quality and freshness. The horizontal line at 5 represents the lowest acceptable shelf life score because customers would likely deem lower scores as “unacceptable”.

Determination of O_3 concentration in water

The concentrations of O_3 dissolved in water were measured immediately using the indigo colorimetric method according to Eaton *et al.* (2005).

Statistical analyses

Statistical analyses were performed using SPSS 12.0 (SPSS, SPSS Inc., Chicago. IL, USA). The data obtained from three replicates were subjected to analysis of variance (ANOVA), and mean differences were determined using Duncan’s multiple range tests. The least significant difference (LSD) test was used to analyze significant differences ($P \leq 0.05$) between the means of different treatments. All data are presented as means and standard deviations.

Results and Discussion

The effect of air- or O_2 gas-ozonated water treatments on microbial reduction

The effects of air-ozonated water (LL) or O_2 gas-ozonated water (HS) treatments on the microbial counts of fresh-cut broccoli florets stored under refrigerated conditions (4°C) are shown in Figure 1 and Table 1. The initial counts of total aerobic bacteria, coliforms, and yeasts and molds in the original untreated broccoli (without washing with tap or ozonated water) were 6.93, 5.60, and 6.55 log CFU.g^{-1} FW, respectively. The microbial counts significantly decreased by 0.91-0.94, 1.24-2.38, and 1.71-2.61 log CFU.g^{-1} FW when the fresh-cut broccoli was treated with tap water, LL, and HS, respectively. This result indicates that washing with tap water removes a small amount of microbes from fresh-cut broccoli, which has been demonstrated previously for shredded carrots (1.0 log reduction of total aerobic bacteria; Gonzalez *et al*., 2004) and fresh-cut carrots (0.5 log reduction of *E. coli* and *Salmonella*; Ruiz-Cruz *et al*., 2007). Ozonated water effectively decreased the microbial contamination in fresh-cut broccoli due to its strong oxidative power against a wide spectrum of microorganisms (i.e., bacteria, viruses, fungal spores, and fungal vegetative cells; Guzel-Seydim *et al*., 2004).

Ozonated water (both the LL and HS treatments)

Table 1. The effects of ozonated water treatments on aerobic bacteria, coliforms, and yeasts and molds in fresh-cut broccoli

Ozonated water treatments*	Time after treatment at 4°C		
	0 d	3 d	6 d
Aerobic bacteria (log CFU g^{-1} FW)			
Untreated	6.93 ± 0.08	-	-
Tap water	6.02 ± 0.69 $^{a, A}$	6.15 ± 0.18 $^{a, A}$	6.34 ± 0.78 $^{a, A}$
LL	5.69 ± 0.01 $^{ab, C}$	6.52 ± 0.04 $^{b, B}$	7.39 ± 0.10 $^{a, A}$
HS	4.77 ± 0.56 $^{b, B}$	5.12 ± 0.23 $^{c, AB}$	5.52 ± 0.30 $^{b, A}$
Total coliforms (log CFU g^{-1} FW)			
Untreated	5.60 ± 0.03	-	-
Tap water	4.66 ± 0.03 $^{a, B}$	5.61 ± 0.38 $^{a, A}$	5.87 ± 0.28 $^{a, A}$
LL	3.35 ± 0.06 $^{c, C}$	5.36 ± 0.32 $^{a, B}$	6.55 ± 0.04 $^{b, A}$
HS	3.85 ± 0.10 $^{b, B}$	4.55 ± 0.29 $^{b, A}$	5.05 ± 0.48 $^{c, A}$
Yeasts and molds (log CFU g^{-1} FW)			
Untreated	6.55 ± 0.03	-	-
Tap water	5.62 ± 0.21 $^{a, A}$	5.44 ± 0.07 $^{a, A}$	5.71 ± 0.25 $^{a, A}$
LL	4.17 ± 0.03 $^{c, C}$	5.04 ± 0.04 $^{ab, B}$	6.04 ± 0.07 $^{a, A}$
HS	4.84 ± 0.06 $^{b, A}$	4.87 ± 0.36 $^{c, A}$	5.09 ± 0.27 $^{b, A}$

Each value represents the mean ± standard deviation of three replicates within each experiment.
[a] Within the columns, different lowercase letters for each storage period indicate significant differences between treatments($P \leq 0.05$).
[A] Within the rows, different capital letters for each treatment indicate significant differences between the storage periods ($P \leq 0.05$).
[*] Exposure to O_3 treatment for 5 min at the initial concentration with subsequent exposure to O_3 residues in water for the previously stated times.

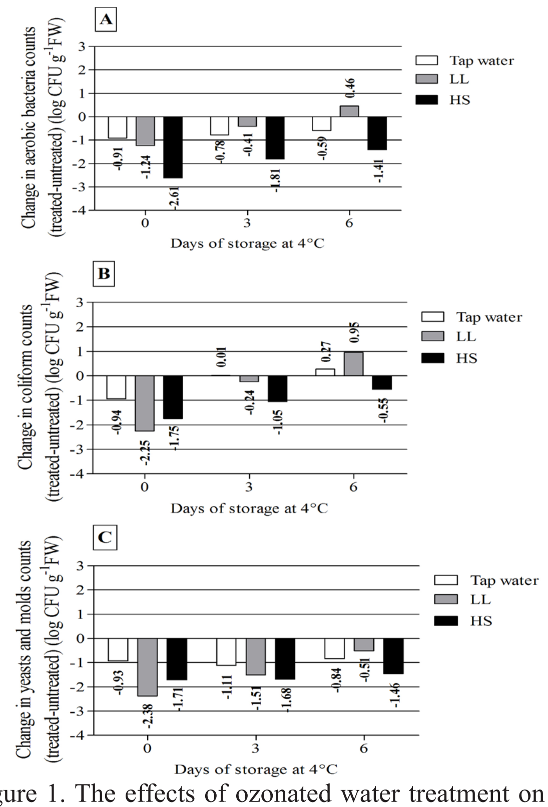

Figure 1. The effects of ozonated water treatment on the changes in aerobic bacteria (A), coliforms (B), and yeasts and molds (C) of fresh-cut broccoli. Fresh-cut broccoli samples were treated with a low O_3 concentration and a long contact time (LL; ▩); a high ozone concentration and short contact time (HS; ■); or tap water (control; □) compared with untreated samples prior to storage at 4°C for 6 days

reduced the microbial contamination in fresh-cut broccoli compared with tap water. The HS treatment was most effective at reducing the microbial populations throughout the storage period

(6 days); however, the LL treatment reduced the microbial populations only on the first day (Day 0), and its antimicrobial effect decreased after the fresh-cut broccoli was stored for 4 and 6 days. In general, O_3 is highly unstable in water and rapidly decomposes into oxygen (Khadre *et al*., 2001). Thus, the decomposition of O_3 at low concentrations (0.56 ppm) should occur faster than it does at high concentrations (1.60 ppm). The organic matter released from the cut surface of fresh-cut broccoli exposed to the LL treatment might interfere with the antimicrobial action of O_3. Suslow (2004) stated that the half-life of O_3 in fresh-cut processing water containing organic matter or suspended soil might be less than 1 min. Moreover, the effectiveness of aqueous O_3 depends on microorganism type, temperature, pH, water turbidity, and the presence of O_3-oxidizable substances (Alexandre *et al*., 2011).

The data regarding the antimicrobial effects of O_3 on the different types of microorganisms showed that aerobic bacteria were more sensitive to the O_3 treatment than coliforms and yeasts and molds (Table 1). In a review, Pascual *et al.* (2007) found that bacteria are more sensitive to O_3 than yeasts and molds, gram-positive bacteria are more sensitive than gram-negative bacteria, and fungal vegetative cells (mycelia) are more sensitive than spores. O_3 kills bacterial cells by attacking the glycoproteins and/or glycolipids in the bacterial membrane (Guzel-Seydim *et al*., 2004), resulting in cell envelope disruption, cell leakage, and cell death (Pascual *et al*., 2007).

Although the O_3 treatment reduced the microbial population in fresh-cut broccoli, the population gradually increased when the storage life was extended. Compared with their population sizes on Day 0, on the last day of storage (Day 6), the population of aerobic bacteria and coliforms increased by approximately 1.0-1.5 log CFU.g^{-1} FW, and the population of yeasts and molds increased by 0.5-1.0 log CFU.g^{-1} FW (Table 1). Yuk *et al.* (2007) reported similar results, showing that an aqueous O_3 treatment at 5 ppm for 5 min reduced *E. coli* and *Listeria* sp. in fresh-cut lettuce by 1.09 and 0.94 log CFU.g^{-1} FW on Day 1, whereas after storage at 5°C for 10 days, the microbial population increased to 9 log CFU.g^{-1} FW. This result indicates that the antimicrobial activity of O_3 residue in fresh-cut produce is nonexistent. During the storage of fresh-cut produce, increases in the microbial population damage plant tissue. When plant cells are damaged, sap cells release nutrients that supplement microbial growth (Moreira *et al*., 2008).

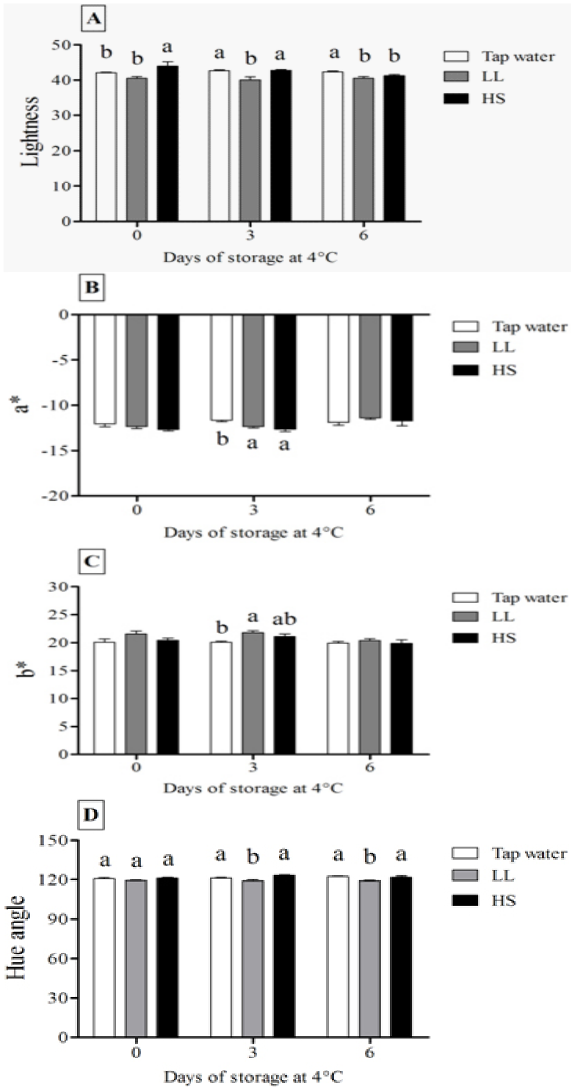

Figure 2. The effects of ozonated water treatment on the L* (A), a* (B), and b* (C) values as well as the hue angle (D) of fresh-cut broccoli during storage at 4°C for 6 days. Fresh-cut broccoli samples were treated with a low O_3 concentration and a long contact time (LL; ▇), a high O_3 concentration and short contact time (HS; ■), or tap water (control; □). The bars represent the mean ± standard error. Identical lower case letters within each storage period are not significantly different (P > 0.05).

The effect of the air- or O_2 gas-ozonated water treatments on floret color

Broccoli is a highly perishable vegetable that senesces rapidly; its storage life is reduced due to chlorophyll loss, floret yellowing, water loss, decay, and off-odors (Forney *et al.*, 2003; Lucera *et al.*, 2011). Changes in the color of fresh-cut broccoli, including L*, a*, and b* values as well as hue angle (h°), are shown in Figure 2. The L* values of fresh-cut broccoli changed slightly (within the range of 40.54-44.03) during storage across all treatments (Figure 2A). On the last day, the L* value of the control

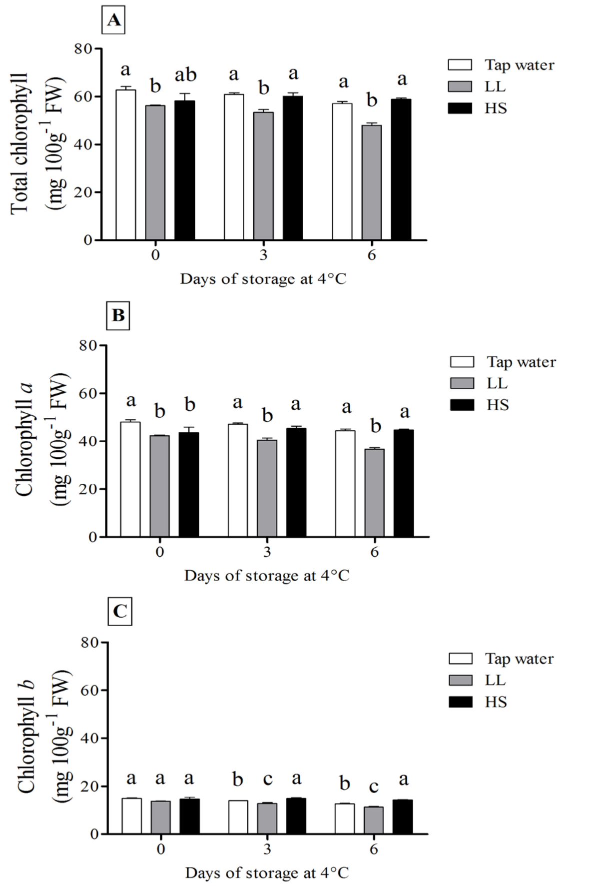

Figure 3. The effects of ozonated water treatment on the contents of total chlorophyll (A), chlorophyll *a* (B), and chlorophyll *b* (C) in fresh-cut broccoli during storage at 4°C for 6 days. Fresh-cut broccoli samples were treated with a low ozone concentration and long contact time (LL; ▩), a high ozone concentration and short contact time (HS; ■), and control conditions (tap water wash; □). The bars represent the mean ± standard error. Identical lowercase letters within each storage period are not significantly different (P > 0.05)

was significantly higher than that of the broccoli subjected to ozonated water treatments (LL and HS). In addition, the a* and b* values did not significantly differ between the O_3 treatments and the control (tap water; Figure 2B and 2C). During storage for 6 days, the hue angle of the HS-treated broccoli was similar to that of the control; the HS treated samples ranged from 121.16-123.24° (green), whereas the control ranged from 120.80-122.35° (green). The hue angle of the LL-treated sample (118.94-119.26°) was significantly lower than those of the HS-treated and control samples (Figure 2D). This result indicates that the LL treatment might not be suitable for maintaining the color of fresh-cut broccoli. Visible browning on the cut surface of broccoli stalks was not observed over 6 days of storage (data not shown).

The effect of the air- or O_2 gas-ozonated water treatments on chlorophyll content

During the 6-day storage period, the chlorophyll contents (total chlorophyll, chlorophyll *a*, and chlorophyll *b*) of the O_3-treated and untreated fresh-cut broccoli decreased (Figure 3). The LL-treated fresh-cut broccoli exhibited the lowest chlorophyll content compared with the other treatments. This decrease in the chlorophyll content of LL-treated fresh-cut broccoli may have been caused by the long post-ozonation immersion time (5 min) in the air-ozonated water compared to the short post-ozonation immersion time (3 min) in the O_2 gas-ozonated water under the HS treatment. This result is similar to that of Meyer *et al.* (1997), who found that the decreased chlorophyll content of spring wheat leaves treated with O_3 depended on O_3 exposure time and the application methods. Moreover, the continuous application of a high O_3 concentration (1.50 ppm) with a long contact time (15 min) was toxic to

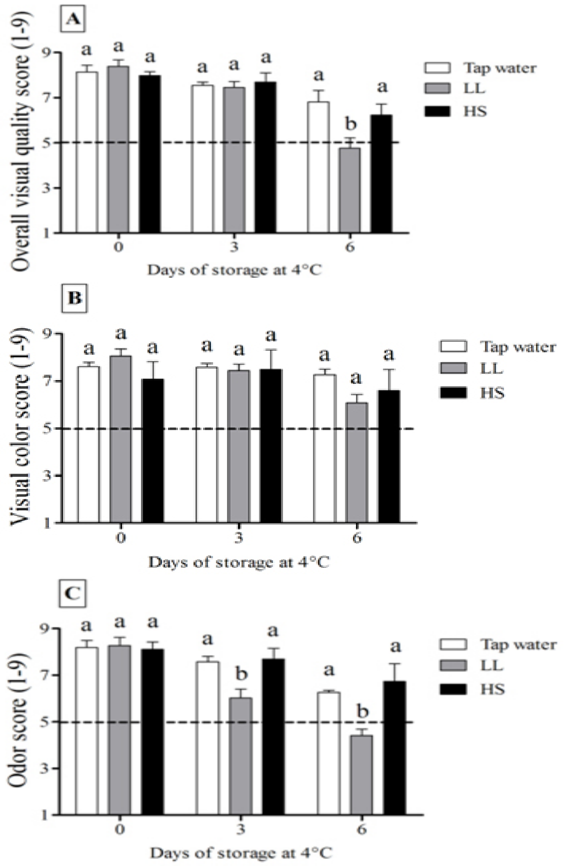

Figure 4. The effects of ozonated water treatment on the sensory attributes of fresh-cut broccoli during storage at 4°C for 6 days: overall visual quality (A), color (B), and odor/off-odor (C). Fresh-cut broccoli samples were treated with a low ozone concentration and long contact time (LL; ▩), a high ozone concentration and short contact time (HS; ■), and control conditions (tap water wash; □). The bars represent the mean ± standard error. Identical lowercase letters within each storage period are not significantly different (P > 0.05). Overall visual quality score: 9 = fresh appearance, 1 = unusable. Color score: 9 = dark green, 1 = 100% yellow. Odor score: 9 = no off-odor, 1 = extreme off-odor. The horizontal line marks the unacceptable quality limit for customers.

fresh-cut broccoli tissue and induced chlorophyll degradation after 3 days of storage (Renumarn *et al*., 2013). However, in the current study, significant differences were not observed with regard to chlorophyll content between the HS-treated fresh-cut broccoli and the control (Figure 3). Chan *et al.* (2007) reported that the chlorophyll contents of O_3-treated blade-leaf vegetables did not significantly differ from the control; this negative result might have occurred because chlorophyll was not sensitive to O_3 under the conditions of that particular study. Our results might indicate that the HS treatment does not affect chlorophyll degradation, whereas the LL treatment may induce chlorophyllase activity and cause chlorophyll degradation.

Sensory analysis of fresh-cut broccoli

On the first day of storage, the mean sensory attribute scores provided by an untrained panel with regard to the overall visual quality, color, and odor of the fresh-cut broccoli were similar, and no significant ($P > 0.05$) differences were observed between the untreated and O_3-treated samples (Figure 4). The sensory attributes of all fresh-cut broccoli treatments decreased during storage at 4°C. On the last day of storage, the mean sensory attribute score of the LL-treated broccoli was lower than that of the control and the HS-treated fresh-cut broccoli. The overall visual quality and odor scores were significantly lower than the unacceptable scores provided by customers (i.e., scores of 5 or less; Figure 4A and 4C). Other researchers have not found significant effects regarding the organoleptic changes in fresh-cut lettuce, rocket leaves, and carrot shreds after treatment with a lower concentration of ozonated water (Beltrán *et al.*, 2005; Martínez-Sánchez *et al.*, 2006; Kim, 2012).

Conclusions

Ozonated water is an alternative sanitizing treatment that might improve microbial safety without negatively affecting color, total chlorophyll content, or the sensory attributes of fresh-cut produce when suitable treatments are applied. In the current study, immersing the fresh-cut broccoli in ozonated water with a high O_3 concentration (1.60 ppm) for 5 min and allowing the broccoli to remain immersed in the ozonated water for 3 min reduced the natural microbial contamination (i.e., aerobic bacteria, coliforms, and yeasts and molds) without any negative effects on quality (color and chlorophyll content) or sensory attributes. Therefore, this finding indicates that the presence of O_3 in washing water at a suitable concentration has the potential to reduce the microbial population and maintain the quality of fresh-cut broccoli.

Acknowledgements

This research was financially supported by a grant from the Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree from the Commission on Higher Education, Thailand. The authors would like to thank Prof. Dr. Julian Hey from Massey University, New Zealand, for proofreading the manuscript.

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