J. Microbiol. Biotechnol. (2010), **20**(2), 363–369
doi: 10.4014/jmb.0907.07009
First published online 10 December 2009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Microbial Quality and Safety of Fresh-Cut Broccoli with Different Sanitizers and Contact Times

Das, Basanta Kumar and Ji Gang Kim*

National Institute of Horticultural and Herbal Science, Rural Development Administration, Suwon 440-706, Korea

Received: July 9, 2009 / Revised: September 2, 2009 / Accepted: September 3, 2009

This study was conducted to investigate the effects of different sanitizers and contact times on storage quality and microbial growth in fresh-cut broccoli. Fresh broccoli samples were cut into small pieces, washed each for 90 s and 180 s in normal tap water (TW), 100 μl/l chlorinated water (CL, pH 7), electrolyzed water (EW, pH 7.2) containing 100 μl/l free chlorine, or 2 μl/l ozonated water (O_3). Then, samples were packaged in 30-μm polyethylene bags and stored at 5°C for 9 days. No significant differences were observed in gas composition and color parameters (L*, a*, b*, and hue angle) among different sanitizers with contact times. No off-odor was detected during the storage. A longer contact time was not effective in reducing microbial population, except with O_3 washing. O_3 with 90 s was not much effective in reducing microbial population compared with Cl or EW. However, samples washed with O_3 for 180 s observed the lowest numbers of total aerobic and coliform plate counts. The result suggested that, a longer contact time of ozone can be used as a potential sanitizer to maintain the microbial quality and safety of fresh-cut broccoli.

Keywords: Chlorinated water, contact time, electrolyzed water, ozonated water

The fresh-cut vegetable market has shown fast growth in recent years with demand for safer and better quality aspects. Broccoli is one of the popular fresh-cut commodities in Korea, owing to its high level of health-promoting compounds such as glucosinolates, flavonoids, and vitamins C, E, and A. However, fresh-cut broccoli is generally much more perishable than intact produce because it has been subjected to severe physical stress after harvest [8, 18, 24, 29]. The cutting of fruits and vegetables generally increases microbial spoilage of fresh-cut produce through transfer of microflora on the outer surface into the interior tissue. Careful attention is needed for the selection and application of antimicrobial agents and sanitizers during fresh-cut processing, which can improve or maintain the taste, quality, and shelf-life of the fresh-cut product [1, 17, 28, 33]. Washing with sanitizers can remove spoilage microorganisms partially or completely by decreasing the microbiological activity from raw fruit and vegetables. Bacteria cells can attach easily to the surface of fruit and vegetables and may escape from washing or sanitizing agents, so it is basically difficult to remove all cells by vigorous washing or treatment with sanitizers [1, 19, 20, 34].

Chlorine has been widely used to reduce microbial contamination in produce processing lines, mainly because of its antimicrobial activity and low cost. However, increasing public health concerns about the possible formation of chlorinated organic compounds (carcinogen), the emergence of new more-tolerant pathogens, and restrictions by regulatory agencies have raised doubts about the use of chlorine by the fresh-cut industry [7, 14, 27, 30, 31]. Similarly, electrolyzed water is strongly bactericidal and more effective than chlorine owing to its high oxidation reduction potential against pathogens and spoilage microorganisms [15, 21]. This is a relatively new concept based on a previously unknown law of anomalous changes of reaction and catalytic abilities of aqueous solutions subjected to electrochemical unipolar (either anoxic or cathodic) treatment. As associated with alternation of its chemical composition, acidity, and alkalinity within a wide range, it is used for technological processes to improve production quality and to reduce the labor-consuming practices. The advantage of the use of electrolyzed water at neutral pH shows good results for sanitizing fresh-cut vegetables [26]. However, the presence of chlorine and cost-effectiveness still remain a challenge to fresh-cut research and processing industries. Therefore, there is an increasing need to investigate the efficacy of new commercial sanitizers and other alternative technologies.

*Corresponding author
Phone: +82-043-871-5581; Fax: +82-043-871-5589;
E-mail: kjg3@korea.kr

Ozone is a strong antimicrobial agent with high reactivity, penetrability, and spontaneous decomposition to a non-toxic product [10, 11]. Research has shown that treatment with ozone appears to have a beneficial effect in extending the storage life of fresh produce such as cucumbers, apples, grapes, oranges, pears, raspberries, and strawberries, by reducing microbial populations and by oxidation of ethylene [11, 32]. The use of ozonated water has been applied to fresh-cut vegetables for sanitation purposes, reducing microbial populations and extending the shelf-life of some of these products [4, 5, 16, 30, 31, 35]. Application of ozone in food processing has been accepted as a potential sanitizer in many countries. When compared with chlorine, ozone has a greater antimicrobial effect against certain microorganisms and rapidly decomposes to oxygen, leaving no residues [25]. Inactivation by ozone is a complex process that attacks various cell membranes and wall constituents (*e.g.*, unsaturated fats) and cell content constituents (*e.g.*, enzymes and nucleic acids). The microorganism is killed by cell envelope disruption or disintegration, leading to leakage of the cell contents. Disruption or lysis is a faster inactivation mechanism than that of other disinfectants, which require the disinfectant agent to permeate through the cell membrane in order to be effective [11, 12]. Bacteria are more sensitive than yeasts and fungi. In the use of ozone, Gram-positive bacteria are more sensitive than Gram-negative organisms, and spores are more resistant than vegetative cells [22, 23]. However, little information exists on the effect of different sanitizers with contact times on the quality attributes and microbial numbers of fresh-cut broccoli. The objective of this study was to evaluate the effects of chlorine, electrolyzed water, and ozone washing, with different contact times, in reducing microbial population and maintaining the quality of fresh-cut broccoli during storage.

Materials and Methods

Preparation of Washing Solutions

Chlorine solution was prepared with food-grade bleach and the pH was adjusted to 7.0 with 1 N HCl. The available chlorine was determined with a portable chlorine colorimeter (Model-1200; LaMotte, U.S.A.). The solution was used within 30 min. Electrolyzed water (EW) was generated by using the Electrolyzed Water System (HBS-500; Han Bio, Republic of Korea). The pH was adjusted to 7.2 with a residual chlorine concentration of 100 µl/l and used within 1 h. Aqueous ozone solution was prepared by continuously circulating the water through an Ozone Water Sterilizing System (OS-800; Advanced Scientific Technology, Republic of Korea) and a stainless steel water tank. The ozone generator was equipped with a vortexer to facilitate dissolving of gaseous ozone in the water. The concentration of dissolved ozone was determined by a ozone measuring meter (DO3 Meter; DDK-TOA Corporation, Japan). Ozone solution of 2 µl/l was used immediately after the required ozone concentration reached.

Sample Preparation

Fresh broccoli (*Brassica oleracea,* Grace) harvested in Pyungchang was obtained from a local supermarket. Broccoli with defects in surface color, damage, and scratch were discarded, and the remaining parts were washed with tap water for 90 s and left to dry in room temperature for 10 min. Then, the broccoli were cut into small pieces (1.5 cm in diameter, 3 cm in length) using a sharp knife. These fresh-cut samples (1.8 kg in each net bag) were washed carefully with gentle agitation in separate buckets, each containing 20 l of solution from one of the following treatments: tap water (TW), chlorinated water (CL, 100 µl/l, pH 7), electrolyzed water (EW, pH 7.2), and ozonized water (O_3, 2 µl/l), for contact time of 90 s and 180 s individually and separately. The washed samples were centrifuged for 1 min with a dryer (WS 6501 T; Hanil, Republic of Korea) to remove excess water. Then, these dewatered samples of 100 g each were packaged in 30-µm polyethylene bags (20×25 cm), sealed, and stored at 5°C for 9 days. Sampling for quality evaluation was conducted at days 0, 3, 6, and 9.

Gas Composition

Changes in O_2 and CO_2 concentrations within the packages were measured by a gas analyzer, periodically monitored for up to 9 days of storage (Checkmate 9900; PBI Dansensor, Denmark). Gas measurement was performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bag, at a flow rate of 1.5 ml/min for 1 min, monitoring with a sensitivity of 0.001 (O_2) and 0.1 (CO_2). The accuracy is in percentage of reading: ±1% of the reading in the calibrated range and ±2% of the full range for CO_2. Three bags per treatment were evaluated for each experimental day.

Off-Odor, Electrolyte Leakage, and Color

Off-odor or sensorial quality was evaluated immediately after opening the bags by a three-member trained panel. Off-odor was scored on a 0 to 4 scale, where 0=no off-odor, 1=slight, 2=moderate, 3=strong, and 4=extremely strong. The electrolyte leakage of fresh-cut broccoli was measured immediately after treatment and during storage to determine possible tissue deterioration. Samples of 20 g of broccoli were submerged in 200 ml of deionized water for 30 min at room temperature. The conductivity (µs/cm) of the solution was determined with a conductivity meter (Orion 4 star portable pH/conductivity meter; Thermo Electron Corporation, U.S.A.) and used to characterize the electrolyte leakage of plant tissues. Color was measured on the surface of the broccoli florets as Lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) in the Hunter color system, with a Minolta Chroma Meter (model CR-400; Minolta Co., Japan) calibrated to a standard white tile (Y 93.5, x 0.3155, y 0.3320). A total of 9 samples were taken to ensure that the data obtained truly represented the color of the samples. The color values of a* and b* were further converted into hue angle [hue=$\tan^{-1}$ (b*/a*)].

Microbiological Assay

Samples of 20 g each were homogenized in 180 ml of 1% sterile buffered peptone water using filter stomacher bags (Stomachem 400, England) for 60 s at 230 rpm. One ml of the appropriate sample dilution was pour-plated on aerobic plate count (3 M Pertifilm; 3 M Microbiology, U.S.A.), incubated at 25°C for 48 h. Simultaneously, the appropriate sample dilution was pour-plated on coliform plate count (3 M Pertifilm; 3 M Microbiology, U.S.A.), incubated at 25°C for 24 h for coliform counts.

RESULTS

Gas Composition

In the packages of fresh-cut broccoli, the O_2 concentration decreased up to 0.2–0.5 kPa, and then increased slightly or maintained a steady level (Fig. 1). The CO_2 concentration in both 90 s and 180 s washing treatments increased up to 5.6–6.2 kPa during the first 3 days and then decreased slightly. No significantly difference in gas composition was observed irrespective of the different washing solutions and contact times experimented in this study.

Off-Odor, Electrolyte Leakage, and Color

Significantly, no off-odor was detected in the samples throughout the storage period (data not shown). The changes in electrolyte leakage of fresh-cut broccoli depended upon the type of washing solution and on the contact time (Fig. 2). O_3 washing for 180 s contact time (O_3-180s) initially showed the highest electrolyte leakage, but later on lowering and maintaining the leakage (11.36–3.72 µs/cm), in comparison with other washing solutions during the storage period. However, O_3 washing for 90 s (O_3-90s) showed the lowest, except for tap water initially (8.17 µs/cm). With regards the color, there was no marked difference in L* and a* values (data not shown) and the hue angle of the samples among different washing solutions and contact times during the storage periods. Tap water treatment maintained the b* value during storage, but sanitizers treatment showed a slightly increased b* value on the 9th day of observation (Table 1). However, visually, there was no color marked difference among the fresh-cut broccoli.

Microbiological Analysis

Among the sanitizers, O_3-180s maintained the lowest numbers of aerobic plate counts throughout the storage days in comparison with others (Fig. 3). The highest number of aerobic plate count was observed for tap water (TW-180S) washing with longer contact time on day 0 (2.93 log CFU/g) and on day 9 (4.28 log CFU/g) of the storage period. Absolutely no coliform was observed in EW-90s and O_3-180s on day 0. However, equal numbers of coliform counts (0.82 log CFU/g) were observed on O_3-180s and CL-180s

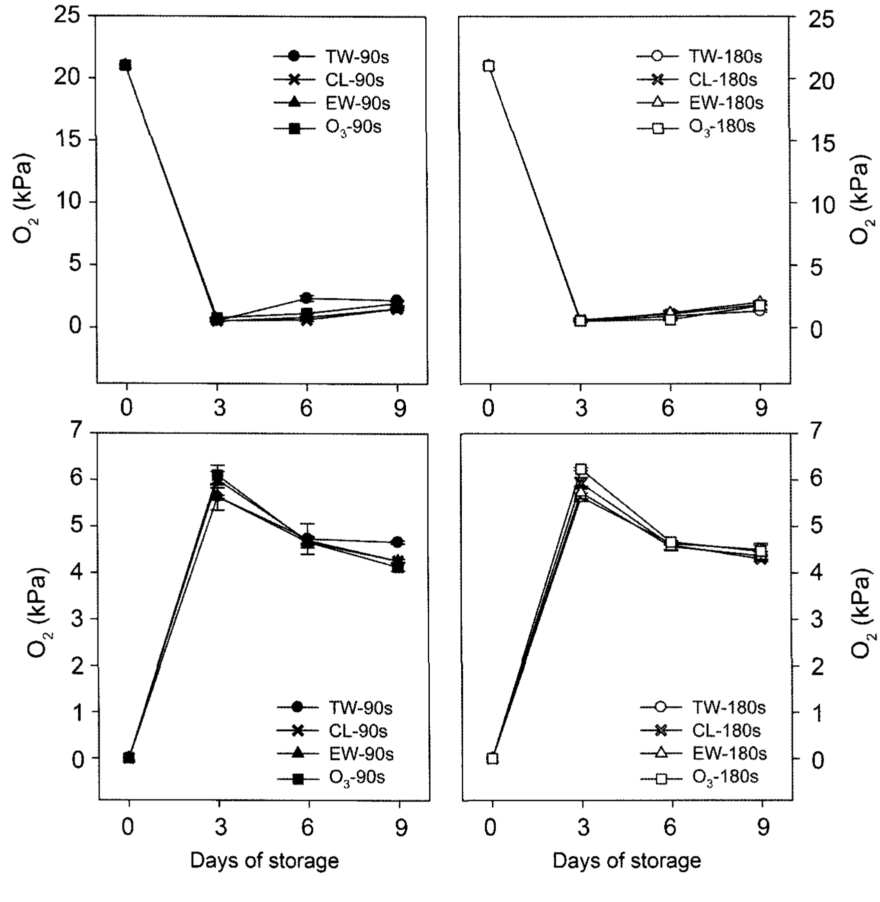

Fig. 1. O_2 (above) and CO_2 (below) compositions in fresh-cut broccoli during the storage period.
Samples were washed for 90 s and 180 s in tap water (TW), chlorinated water (CL), electrolyzed water (EW), or ozonated water (O_3). Data are the mean of three replications ± SE.

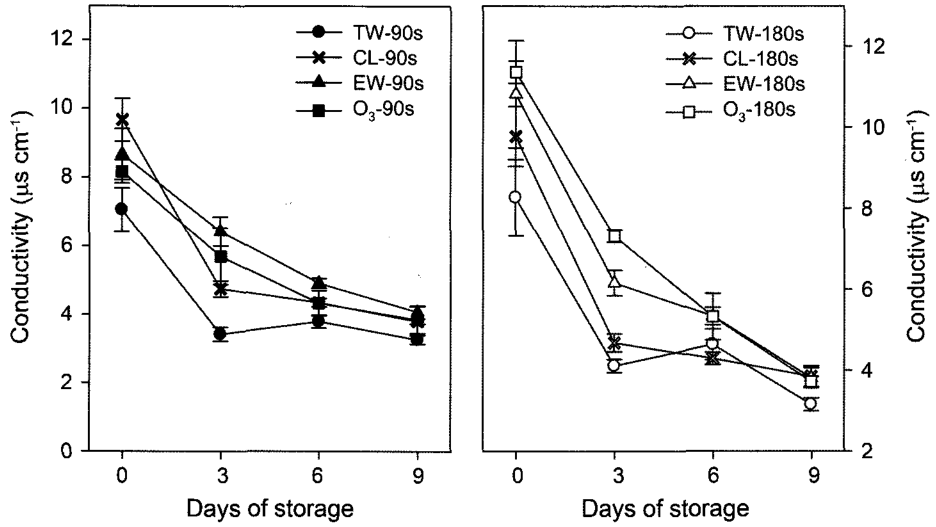

Fig. 2. Electrical conductivity of fresh-cut broccoli during the storage period.
Samples were washed for 90 s and 180 s in tap water (TW), chlorinated water (CL), electrolyzed water (EW), or ozonated water (O_3). Data are the mean of three replications ± SE.

washing at the end of storage. Highest number of coliform count was observed in tap water washing initially and on the final day of investigation as well (Fig. 4).

Discussion

The application of different sanitizers is necessary for the researchers and processing industries to maintain microbial quality and safety of fresh-cut produce during storage (*i.e.*, prior to reaching the consumers). Broccoli is a perishable commodity with a shorter postharvest shelf-life, and needs better and safer postharvest quality maintenance [4]. At the end of storage, all treatments reached similar O_2 levels (0.5–2 kPa) and CO_2 levels (4–5 kPa) with no significant differences among treatments (Fig. 1). The rate of respiration is often a good indication of the storage life of fresh-cut produce. In the current study, there was no detectable off-odor in the packaged fresh-cut broccoli during storage in all treatments, owing to neither further O_2 depletion nor increased CO_2 beyond the level. Generally, off-odor is always associated with the onset of aerobic respiration under lower O_2 and higher CO_2 levels; thus, the lack of off-odor indicates that no aerobic respiration had occurred. It has been reported that the off-flavor is mainly due to ethanol, aldehyde, ethyl acetate, volatile compounds, and ethylene associated compounds, which are basically responsible for off-odor during the storage period either by living cells or by microbes present in the packaging bags [9]. This might be due to the high oxygen transmission rate of the packaging film used in our investigation. Although the respiration rate of fresh-cut broccoli is high, the insignificant gas exchange rate data indicated that the packaging film used was appropriate [13].

Table 1. Hue angle and b* value calculated in fresh-cut broccoli on 0 and 9th day of the storage period.

Treatments	Hue angle		b* value	
	Day 0	Day 9	Day 0	Day 9
TW-90s	124.17±0.67	124.12±0.46	19.56±1.23	19.28±0.67
TW-180s	123.87±0.79	123.73±0.92	19.27±1.34	19.01±1.03
CL-90s	123.91±0.64	124.14±1.27	19.80±1.29	19.88±1.52
CL-180s	124.09±0.82	124.25±0.44	16.64±0.64	20.45±1.37
EW-90s	124.32±0.35	124.61±1.28	18.69±0.82	19.34±0.91
EW-180s	124.07±0.40	123.17±1.36	18.16±1.05	19.46±1.67
O_3-90s	124.43±0.57	123.82±1.70	18.59±0.86	19.73±1.17
O_3-180s	124.36±0.65	124.48±0.65	18.63±1.14	20.74±1.92

Samples were washed for 90 s and 180 s in tap water (TW), chlorinated water (CL), electrolyzed water (EW), or ozonated water (O_3). Data are the mean of nine replications ± standard error (SE).

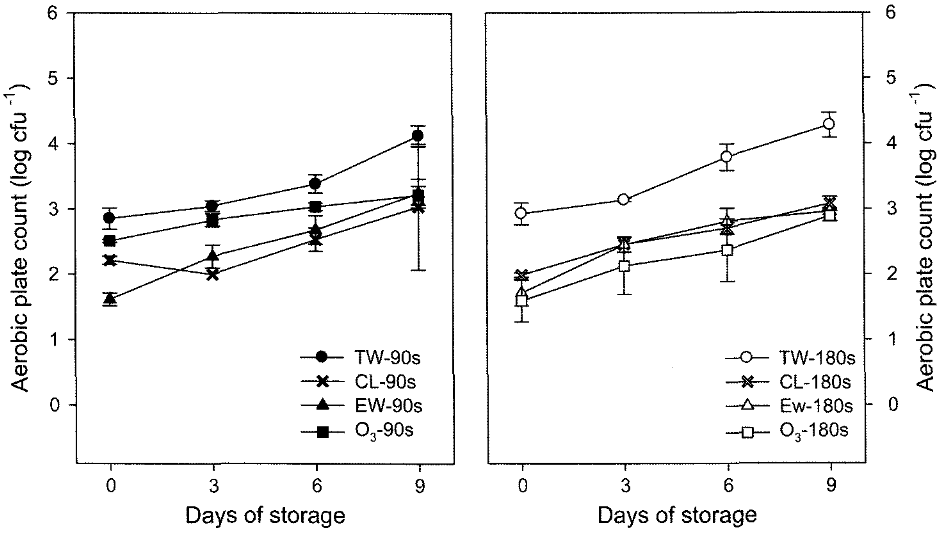

Fig. 3. Aerobic plate count of fresh-cut broccoli during the storage period.
Samples were washed for 90 s and 180 s in tap water (TW), chlorinated water (CL), electrolyzed water (EW), or ozonated water (O_3). Data are the mean of three replications ± SE.

Fresh-cut produce are attractive and eye-catching to a large degree because it contains, which is vital for quality maintenance. Chlorophyll degradation resulting in a loss of green color is a major quality defect for most leafy green vegetables, especially in broccoli. The insignificant change in L*, a*, hue angle, and visual color analysis showed that the washing solutions with different contact times had no adverse affect on the color of the fresh-cut broccoli. However, a slight increase of the b* value was marked on the 9th day, except tap water treatment (Table 1). This indicated that the application of sanitizers affected the color by slightly increasing the yellowness of the fresh-cut broccoli, as studied earlier by some researchers for different horticultural produce [6, 14, 34].

During processing or handling of the fresh-cut produce, common symptoms of the injury and physical damage lead to the unusual malfunctioning of membrane systems of the fresh-cut produce. Basically, stresses such as dehydration, temperature abuse, and toxic chemicals can induce functional impairments into the cellular membrane systems through alterations to membrane physicochemical properties such as activities of membrane-bound proteins, leading to the loss of normal physiological processes, membrane leakage, and tissue injury. The outer membrane is responsible for the

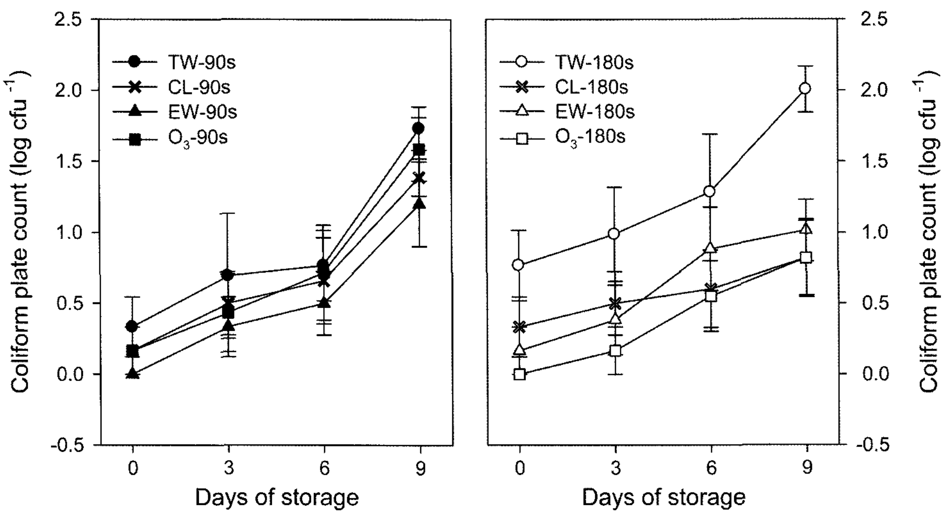

Fig. 4. Coliform count in fresh-cut broccoli during the storage period.
Samples were washed for 90 s and 180 s in tap water (TW), chlorinated water (CL), electrolyzed water (EW), or ozonated water (O_3). Data are the mean of three replications ± SE.

selective inflow and outflow of molecules, ions, water, *etc.* Simultaneously, it acts as a dynamic structure that performs a variety of functions. Moreover, the nutrients in leakage may play a key role in the growth and development of pathogens. Furthermore, electrical conductivity is generally considered as an indirect measure of plant cell membrane damage [3]. It was found that a longer contact time showed higher electrical conductivity than a short contact time, irrespective of sanitizers, on days 0 and 9 (Fig. 2). It might be due to the longer contact time, which washed off the cut ends of broccoli more prominently during the contact time [2]. In particular, in the current experiment, we observed that O_3-180s initially showed the highest electrical conductivity, owing to its highly oxidizing nature, than chlorine and electrolyzed washing, but at the end of the storage, the value was nearly equal to the above washings. It may be due to the continuous flow and highly oxidizing nature of ozone during the washing time initially and later check in the further damage after washing and during storage.

Ozone washing for a longer contact time showed decreased aerobic and coliform plate count numbers than other sanitizers throughout the storage period. At the same time, other treatments showed increasing aerobic and coliform plate count numbers likely because of the following causes: the surface wash-off might be attached to the samples again during the washing time; higher electrical conductivity observed during storage; and the reactivity against pathogen may be less effective in comparison with O_3 of 2 μl/l (Fig. 3 and 4). Application of aqueous ozone is well understood owing to its very high oxidizing nature, which washed off the primary pathogens more effectively than other sanitizers. Secondly, the lower electrical conductivity observed at the end of the storage is directly influenced by the microbial growth. However, there were no findings reported on the efficacy of ozone against foodborne pathogen in fresh-cut broccoli, but in lettuce it depends on the concentration and contact time of ozone [22, 35]. Ozone effectiveness against microorganisms depends not only on the amount applied, but also on the effectiveness of the ozone delivery method, type of material, the target microorganisms, and physiological state of the bacteria cells at the time of treatment. Furthermore, just after washing, the washing solutions may control the further proliferation of microorganisms during storage of fresh-cut produce [1, 19, 34].

From our observation, ozone (2 μl/l) washing for a longer contact time proved to be the best among washing solutions for maintaining the food safety and microbial quality of fresh-cut produce. Furthermore, the application of a higher concentration of ozone will work more prominently in decreasing microbial numbers during storage. In view of our findings, it is recommended that ozone can be used as an alternative to chlorine washing in fresh-cut broccoli.

References

1. Allende, A., M. V. Selma, F. López-Gálvez, R. Villaescusa, and M. I. Gil. 2008. Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biol. Technol.* **49:** 155–163.
2. Arvind, A. B., A. S. Robert, and A. A. Judith. 2004. Evaluation of wash treatments for survival of foodborne pathogens and maintenance of quality characteristics of fresh-cut apple slices. *Food Microbiol.* **21:** 319–326.
3. Bajji, M., J. Kinet, and S. Lutts. 2002. The use of the electrolyte method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul.* **36:** 61–70.
4. Beltran, D., M. V. Selma, A. Marin, and M. I. Gil. 2005. Ozonated water extends the shelf life of fresh-cut lettuce. *J. Agric. Food Chem.* **53:** 5654–5663.
5. Hassenberg, K., C. Idler, E. Molloy, M. Geyer, M. Plöchl, and J. Barnes. 2007. Use of ozone in a lettuce-washing process: An industrial trial. *J. Sci. Food Agric.* **87:** 914–919.
6. Hildebrand, P. D., C. F. Forney, J. Song, L. Fan, and K. B. McRae. 2008. Effect of a continuous low ozone exposure (50 nl l^{-1}) on decay and quality of stored carrots. *Postharvest Biol. Technol.* **49:** 397–402.
7. Jinhua, D., Y. Fu, and N. Wang. 2009. Effects of aqueous chlorine dioxide treatment on browning of fresh-cut lotus root. *LWT Food Sci. Technol.* **42:** 654–659.
8. Junhua, H., T. Weiyu, H. Huakun, Z. Bolin, J. Weibo, N. Tiangui, L. Quanhong, and C. Tongyi. 2006 Physiology and quality responses of fresh-cut broccoli florets pretreated with ethanol vapor. *J. Food Sci.* **71:** S385–S389.
9. Kader, A. A. (ed.). 2002. Postharvest Technology of Horticultural Crops. 3rd Ed. University of California, Division of Agriculture and Natural Resources, Oakland, U.S.A.
10. Khadre, M. A., A. E. Yousef, and J. G. Kim. 2001. Microbiological aspects of ozone applications in food: A review. *J. Food Sci.* **66:** 1242–1252.
11. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Applications of ozone for enhancing the microbiological safety and quality of foods: A review. *J. Food Protec.* **62:** 1071–1087.
12. Kim, J. G., A. E. Yousef, and M. A. Khadre. 2003. Ozone and its current and future application in the food industry. *Adv. Food Nutr. Res.* **45:** 167–218.
13. Kim, J. G., Y. Luo and K. C. Gross. 2004. Effect of package film on the quality of fresh-cut salad savoy. *Postharvest Biol. Technol.* **32:** 99–107.
14. Kim, J. G., Y. Luo, and Y. Tao. 2007. Effect of the sequential treatment of 1-methylcyclopropene and acidified sodium chlorite on microbial growth and quality of fresh-cut cilantro. *Postharvest Biol. Technol.* **46:** 144–149.
15. Kiura, H., K. Sano, S. Morimatsu, T. Nakano, C. Morita, M. Yamaguchi, T. Maeda, and Y. Katsuoka. 2002. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *J. Microbiol. Methods* **49:** 285–293.
16. Koseki, S. and I. Seichiro. 2006. Effect of ozonated water treatment on microbial control and on browning of iceberg lettuce (*Lactuca sativa* L.). *J. Food Protect.* **69:** 154–160.

17. Lukasik, J., M. L. Bradley, T. M. Scott, M. Dea, A. Koo, W. Y. Hsu, J. A. Bartz, and S. R. Farrah. 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. *J. Food Protect.* **66:** 188–193.
18. Mandelova, L. and J. Totusek. 2007. Broccoli juice treated by high pressure: Chemoprotective effects of sulforaphane and indole-3-carbinol. *Int. J. High Pressure Res.* **27:** 151–156.
19. Mandrell, R. E., L. Gorski, and M. T. Brandl. 2006. Attachment of microorganisms to fresh produce, pp. 33–73. *In* G. M. Sapers, J. R. Gorny, and A. E. Yousef (eds.). *Microbiology of Fruits and Vegetables*. CRC Press, Boca Raton, Florida, U.S.A.
20. Martínez-Sánchez, A., A. Allende, R. N. Bennett, F. Ferreres, and M. I. Gil. 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biol. Technol.* **42:** 86–97.
21. Muhammad, I. A., S. Junichi, and I. Seiichiro. 2005. Application of electrolyzed water in agriculture and food industries. *Food Sci. Technol. Res.* **11:** 135–150.
22. Ölmez, H. and M. Y. Akbas. 2009. Optimization of ozone treatment of fresh-cut green leaf lettuce. *J. Food Eng.* **90:** 487–494.
23. Pascual, A., I. Llorca, and A. Canut. 2007. Use of ozone in food industries for reducing the environmental impact of cleaning and disinfection activities. *Trends Food Sci. Technol.* **18:** S29–S35.
24. Pérez-Balibrea, S., D. A. Moreno, and C. García-Viguera. 2008. Influence of light on health-promoting phytochemicals of broccoli sprouts. *J. Sci. Food Agric.* **88:** 904–910.
25. Rico, D., A. B. Martín-Diana, J. M. Barat, and C. Barry-Ryan. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: A review. *Trends Food Sci. Technol.* **18:** 373–386.
26. Rico, D., A. B. Martín-Diana, C. Barry-Ryan, J. M. Frías, G. T. M. Henehan, and J. M. Barat. 2008. Use of neutral electrolyzed water for quality maintenance and shelf-life extension of minimally processed lettuce. *Innov. Food Sci. Emerging Technol.* **9:** 37–48.
27. Sapers, G. M. 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technol. Biotechnol.* **39:** 305–311.
28. Selma, M. V., A. M. Ibañez, A. Allende, M. Cantwell, and T. Suslow. 2008. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *E. coli* O157:H7 during cutting. *Food Microbiol.* **25:** 162–168.
29. Serrano, M., D. Martinez-Romero, F. Guillen, S. Castillo, and D. Valero. 2006. Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. *Postharvest Biol. Technol.* **39:** 61–68.
30. Singh, N., R. K. Singh, A. K. Bhunia, and R. L. Stroshine. 2002. Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Food Microbiol.* **19:** 183–193.
31. Singh, N., R. K. Singh, A. K. Bhunia, and R. L. Stroshine. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *LWT Food Sci. Technol.* **35:** 720–729.
32. Skog, L. J. and C. L. Chu. 2001. Effect of ozone on qualities of fruits and vegetables in cold storage. *Can. J. Plant Sci.* **81:** 773–778.
33. Ukuku, D. O., M. L. Bari, S. Kawamoto, and K. Isshiki. 2005. Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces. *Int. J. Food Microbiol.* **104:** 225–233.
34. Wang, H., H. Feng, and Y. Luo. 2004. Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Res. Int.* **37:** 949–956.
35. Yuk, H.-G., M.-Y. Yoo, J.-W. Yoon, K.-D. Moon, D. L. Marshall, and D.-H. Oh. 2006. Effect of combined ozone and organic acid treatment for control of *E. coli* O157:H7 and *L. monocytogenes* on lettuce. *J. Food Sci.* **71:** 83–87.