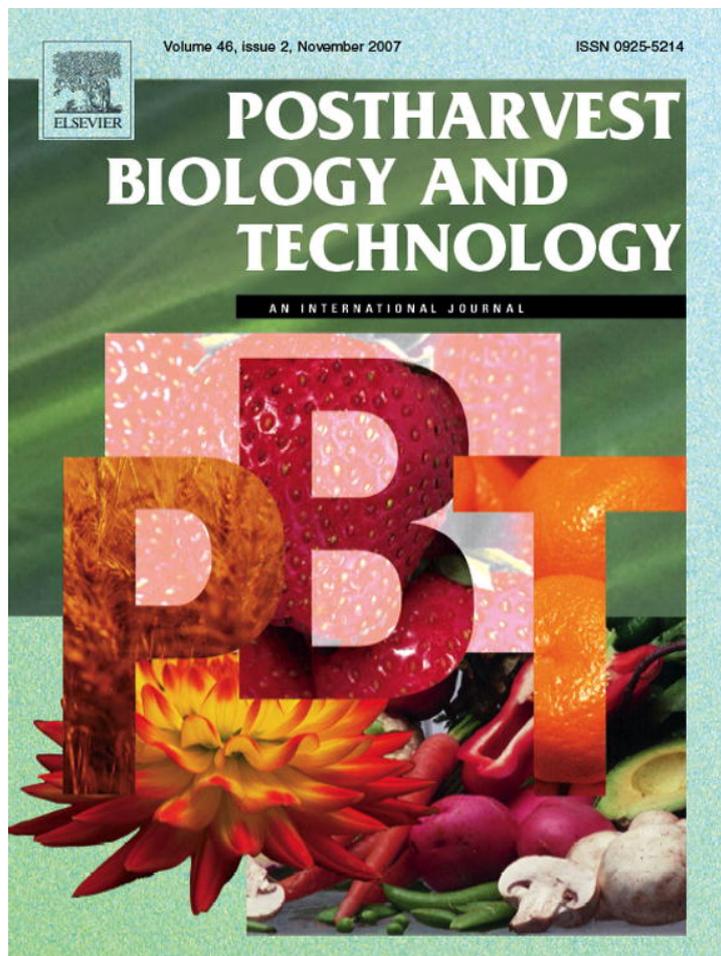


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Effect of the sequential treatment of 1-methylcyclopropene and acidified sodium chlorite on microbial growth and quality of fresh-cut cilantro

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Abstract

This study investigated the effects of 1-methylcyclopropene and sanitizer (acidified sodium chlorite or sodium hypochlorite), treated alone or in combination, on microbial growth and quality of packaged fresh-cut cilantro (*Coriandrum sativum* L.). Cilantro bunches were treated with 1.5 mg L⁻¹ 1-methylcyclopropene or air for 18 h at 10 °C. The samples were then cut and washed in tap water, 100 mg L⁻¹ sodium hypochlorite, or 100 mg L⁻¹ acidified sodium chlorite solution for 1 min. The washed cilantro leaves were centrifugally dried, packaged with 29.2 pmol s⁻¹ m⁻² Pa⁻¹ oxygen transmission rate film, and stored at 5 °C for 14 d. Results indicated that 1-methylcyclopropene significantly ($P < 0.0001$) delayed the decrease in O₂ and accumulation of CO₂ partial pressures in the headspace of sample packages. Acidified sodium chlorite application significantly reduced initial coliform/*Escherichia coli* counts ($P < 0.001$), and reduced decay rate at the end of storage ($P < 0.05$). A combination treatment of 1-methylcyclopropene and acidified sodium chlorite, followed by acidified sodium chlorite treatment alone, maintained the lowest decay rates and the highest overall quality scores at the end of storage.

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Keywords: Postharvest physiology; Decay; MA packaging; Overall quality

1. Introduction

Cilantro, the leaves and stems of the Coriander plant (*Coriandrum sativum* L.), has a unique aroma and flavor (Potter and Fagerson, 1990) and is an important culinary herb in the United States, widely used in Mexican, Asian, and Caribbean cuisine. There is an increased demand for packaged fresh-cut culinary herbs in both retail and food service sectors. However, the preparation of fresh-cut cilantro with an acceptable quality and shelf-life remains a challenge due to its high perishability (Loaiza and Cantwell, 1997). The quality of fresh cilantro is best maintained by low temperature and high-humidity storage (Cantwell and Reid, 1993; Loaiza and Cantwell, 1997). Modified atmosphere (MA) packaging has been used to delay senescence and decay of cilantro (Aharoni et al., 1989). Our earlier work demonstrated that MA packages prepared with 16.5 and 29.2 pmol s⁻¹ m⁻² Pa⁻¹ oxygen transmission rate films was

beneficial in maintaining quality of fresh-cut cilantro leaves (Luo et al., 2004); however, microbial populations remained at a high level.

Washing fresh-cut produce after cutting and prior to packaging is an important step in reducing microbial populations. Chlorine (sodium hypochlorite) has been widely used in produce washes in order to inactivate microorganisms and ensure quality and safety (Li et al., 2001; Wei et al., 1985). However, recent studies have shown that chlorine lacks efficacy on pathogen reduction (Zhang and Farber, 1996; Beuchat et al., 1998); the formation of chlorine by-products is also deleterious to human health (Richardson et al., 1998). Thus, there is much interest in developing a safer and more effective antimicrobial alternative to chlorine.

Acidified sodium chlorite (ASC, Alcide Corp., Redmond, Wash.) has recently been approved by the FDA for spray or dip application on various food products, including fresh and fresh-cut produce (FDA, 2001). Studies have shown that ASC has strong antimicrobial efficacy against various human pathogens inoculated onto cantaloupes and asparagus (Park and Beuchat, 1999). Our earlier studies demonstrated ASC at 1.20 g L⁻¹ had a

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strong efficacy on pathogen inactivation with up to 5 log reduction of *E. coli* O157:H7 on shredded carrots (González et al., 2004). Fresh-cut carrots treated with ASC demonstrated the lowest growth rate of aerobic bacteria, compared to those treated with other sanitizers, throughout the storage period (Ruiz-Cruz et al., 2006). However, tissue damage and loss of shelf life of shredded carrots were noticed at the application rate of 1.20 g L⁻¹ (Ruiz-Cruz et al., 2006), whereas a concentration of 100–250 mg L⁻¹ ASC reduced microbial populations without causing quality deterioration of carrots (Ruiz-Cruz et al., 2006). Additional studies on fresh-cut cilantro also showed that application of ASC at 100–250 mg L⁻¹ for 1 min caused a significant reduction of *E. coli* O157:H7 (Allende et al., 2007). However, no study on the quality maintenance of fresh-cut cilantro treated with ASC was reported.

Literature indicates that 1-methylcyclopropene (1-MCP) delays ripening and extends storage-life of apples by blocking ethylene action (Ekman et al., 2004; Sisler and Blankenship, 1996; Blankenship and Dole, 2003). Effective application concentrations of 1-MCP are very low with no detectable residues left on the produce. Various studies have shown that 1-MCP delays ethylene-induced senescence (Blankenship and Dole, 2003; Tay and Perera, 2004), inhibiting tissue-softening, and delaying color change in many fruits and vegetables (Blankenship and Dole, 2003). 1-MCP inhibited ethylene-induced effects during fresh-cut processing of leafy Asian vegetables, such as Chinese mustard and choy sum (Able et al., 2003), and reduced the development of russet spotting, leaf yellowing and delayed senescence in shredded lettuce (Tay and Perera, 2004). Specifically, 1-MCP has been shown to delay chlorophyll degradation and senescence of cilantro leaves (Jiang et al., 2002). However, 1-MCP has no antimicrobial effect and the reports on the growth of spoilage microorganisms on 1-MCP treated produce have been inconsistent (Blankenship and Dole, 2003).

The main objective of this study was to evaluate the effects of 1-MCP treatment and sanitizer wash, alone and in combination on microbial growth, physiology, and quality of packaged fresh-cut cilantro.

2. Materials and methods

2.1. Plant material, processing and packaging

Fresh cilantro (*Coriandrum sativum* L.) was obtained from a local wholesale market and placed in sealed 208 L stainless steel chambers equipped with fans to facilitate air circulation. Cilantro bunches were then exposed to 1.5 mg L⁻¹ 1-methylcyclopropene gas (1-MCP) for 18 h at 10 °C with the concentration of 1-MCP monitored periodically following a modified procedure from Janisiewicz et al. (2003). The control samples were also placed in an identical chamber and exposed to ambient air (Air). Following 1-MCP treatment and before cutting, cilantro bunches were placed on trays and air equilibrated for 2 h to allow for 1-MCP out-gassing. Damaged and yellowed leaves were removed from both 1-MCP treated and untreated bunches, and stems were cut off below the low-

est leaves. Cilantro leaves were cut into ~3 cm segments and washed with either 100 mg L⁻¹ acidified sodium chlorite (ASC, pH 2.5, Alcide Corp., Redmond, Wash.) or 100 mg L⁻¹ sodium hypochlorite (SH) solution (NaOCl, pH 6.5) at a product mass (kg) to wash solution volume (L) ratio of 1:20 for 1 min with gentle agitation (shaken manually). A water wash (WA) at the same product to water ratio was also included as a control. All wash solutions, including tap water, were maintained at 5 °C. Washed samples were centrifuged with a commercial salad centrifugal dryer (Model T-304, Meyer Machine Co., San Antonio, Texas) at 10.8 s⁻¹ (~110 × g) for 2.5 min to remove excess water. Fresh-cut cilantro samples (0.05 kg each) were packaged in 29.2 pmol s⁻¹ m⁻² Pa⁻¹ OTR film (140 mm × 220 mm) and stored at 5 °C for 14 d for subsequent evaluation.

2.2. Respiration rate, ethylene production, and gas composition within packages

Fresh-cut cilantro samples (0.20 kg each) were placed in sealed containers at 5 °C with humidified air at a flow rate of 0.33 mL s⁻¹. The CO₂ and ethylene concentrations of the outlet streams from sample containers were monitored every 1 h for the first 2 d and every 6 h for the next 7 d. Quantification of CO₂ was done using a gas chromatograph (GC; HP 5890a, Hewlett Packard Co., Rockville, MD) fitted with a Hayesep Q column (2.4 m × 3 mm) and a thermal conductivity detector. The ethylene content of the outlet streams was also measured using a GC (HP 5890a) equipped with a GS-Q column (3.0 m × 0.53 mm; J&W Scientific, Folsom, CA) and a flame ionization detector. The partial pressures of O₂ and CO₂ within packages of fresh-cut cilantro was measured, on days 1, 4, 8, 11 and 14, using a gas analyzer system (Combi Check 9800-1, PBI Dansensor Co., Ringsted, Denmark).

2.3. Quality assessment of packaged fresh-cut cilantro

Color was measured using a Minolta Chroma Meter (Model CR-300, Minolta Corp., Osaka, Japan). A total of 10 sampling points were taken on each side of each bag. The color values of *a*^{*} and *b*^{*} were further converted into hue angle [hue = tan⁻¹(*b/a*)] according to Nunes and Emond (1998).

Ethanol partial pressures in the headspace of packages were determined using a gas chromatographic procedure (Kim et al., 2005). Gas samples (250 μL) were collected from the headspace of fresh-cut cilantro packages using a gas-tight syringe and injected into a glass-lined splitless injection port of a gas chromatograph (Model 6890N, Agilent Technologies, Rockville, MD) equipped with a flame ionization detector. Volatiles were separated using a capillary column (DB-WAX, 30 m × 0.32 mm i.d.; 1.0 μm coating thickness; J&W Scientific, Folsom, Calif.) following the procedure of Kim et al. (2005).

Decay rate was calculated by the weight of all decayed pieces in each package, divided by the total fresh weight of the sample and multiplied by 100 to obtain percentage (Lopez-Galvez et al., 1997; Loaiza and Cantwell, 1997). A panel of three trained personnel evaluated sensory quality. The samples were coded with three-digit numbers to mask the treatment identity in an effort

to minimize the test subjectivity and to ensure test accuracy. Off-odor was evaluated immediately after opening the packages and scored on a five-point scale where 0 = none; 1 = slight; 2 = moderate; 3 = strong; and 4 = severe (Lopez-Galvez et al., 1997; Luo et al., 2004); a score of 3 or above was considered unacceptable. Overall quality was evaluated after 14 d storage using a 9-point hedonic scale where: 9 = like extremely; 7 = like moderately; 5 = neither like nor dislike; 3 = dislike moderately; and 1 = dislike extremely (Meilgaard et al., 1991; Luo et al., 2004); a score of 6 was considered the limit of marketability (Loaiza and Cantwell, 1997; Lopez-Galvez et al., 1997). In order to minimize the effect of temperature variation during testing, all quality evaluations were performed in a temperature-controlled room at 5 °C.

2.4. Microbial enumeration

Cilantro samples of 0.03 kg each were macerated in sterile peptone water with a Stomacher blender (model 400, Seward Limited, London, U.K.) for 2 min at 3.83 s^{-1} and filtered with sterile glass wool. For the aerobic plate counts (APC), the homogenate and its serial dilutions were logarithmically spread onto Tryptic Soy Agar (Difco Lab, Sparks, MD) with an automatic spiral plater (Autospiral™ DW Scientific Ltd., West Yorkshire, U.K.). Plates were incubated at 28 °C for 2 d with the microbial colonies counted using a Protos Colony Counter (Model 50000; Synoptics Ltd., Cambridge, U.K.). For coliform/*E. coli* counts, the homogenate and its serial dilutions were directly plated on 3 M coliform/*E. coli* petrifilms (3 M Petrifilm, St. Paul, Minn.) and incubated at 37 °C for 24–48 h, using an official AOAC method (AOAC International, 2000). Only colonies showing typical coliform/*E. coli* morphology were counted.

2.5. Experimental design and statistical analyses

The experiment was conducted according to a factorial design with three replications. The main factors evaluated include gas treatment (Air, 1-MCP), sanitizer (WA, SH, ASC) and storage time. The experiment was conducted twice with similar trends from both trials. The results from the second trial with precisely controlled experimental conditions were reported here. Data were analyzed using the Proc Mixed procedure of SAS (SAS Inst., Cary, N.C.).

3. Results and discussion

3.1. Carbon dioxide and ethylene production rates as well as package atmospheres

Respiration rates of fresh-cut cilantro, measured as evolved CO_2 , increased rapidly upon processing, peaked at ~12 h, and then declined (Fig. 1A). Although samples treated with 1-MCP had lower respiration rates, no significant difference was observed between 1-MCP-treated and untreated fresh-cut cilantro. Similar findings were reported by Jiang et al. (2002).

Samples treated with 1-MCP had a sharp increase in ethylene production compared to control samples (Fig. 1B). Similar

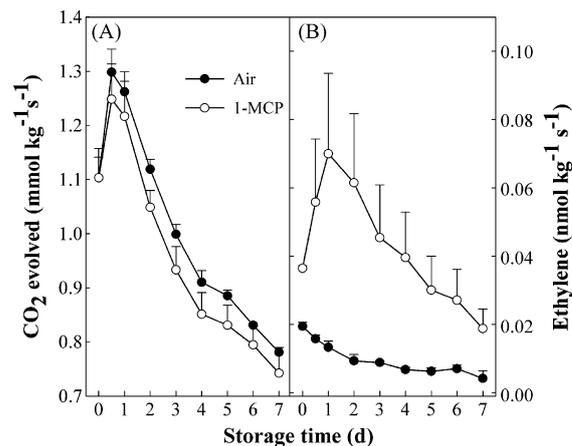


Fig. 1. Effect of 1-MCP on respiration (A) and ethylene production (B) in fresh-cut cilantro stored at 5 °C for 7 d. Each symbol is the mean of three replications. Vertical lines represent S.E.

results were reported on several non-climacteric fruits including pineapple (Mullins et al., 2000) and grapefruit (Selvarajah et al., 2001). However, most studies on a broad range of climacteric fruits and vegetables found that 1-MCP treatment inhibited ethylene production (Blankenship and Dole, 2003). Although the exact mechanism for ethylene promotion by 1-MCP treatment in fresh-cut cilantro has not been determined, the trend observed in this study suggests that a feedback control system of ethylene synthesis in fresh-cut cilantro may be induced, rather than inhibited, by 1-MCP (Jiang et al., 2002).

The O_2 partial pressure in packages decreased rapidly and appeared to reach a steady state around day 8 (Fig. 2A). Samples treated with 1-MCP displayed a significantly ($P < 0.01$) higher O_2 partial pressure than the other treatments from day 4 and maintained a significantly ($P < 0.01$) higher O_2 partial pressure throughout the rest of the storage period. The treatment that combined 1-MCP and ASC also equilibrated at a slightly higher O_2

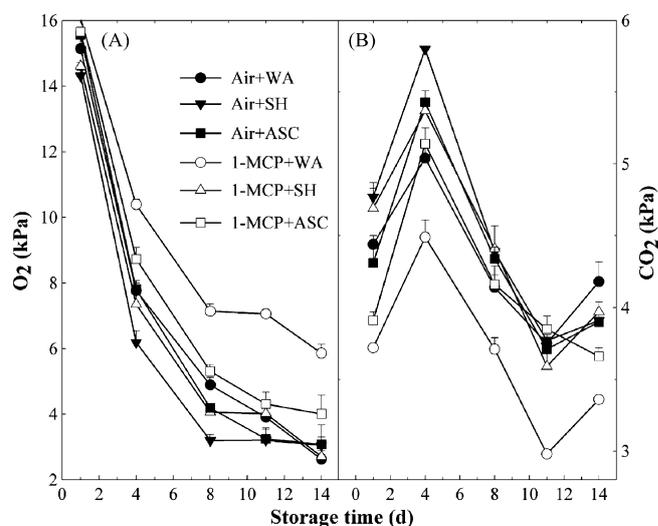


Fig. 2. Changes of O_2 (A) and CO_2 (B) partial pressures in the headspace of packaged fresh-cut cilantro stored at 5 °C for up to 14 d. Each symbol is the mean of three replications. Vertical lines represent S.E.; S.E. bars were not shown when masked by the symbol.

partial pressure than the other treatments; however, the treatment difference was not consistently significant.

Carbon dioxide partial pressures generally increased rapidly initially, peaked on day 4, decreased gradually until day 11, and finally increased slightly at the end of storage (Fig. 2B). There was a significant difference in CO₂ partial pressures over time ($P < 0.0001$) and among all treatments ($P < 0.0001$). Packages with the 1-MCP treatment had the lowest CO₂ accumulation, in agreement with the highest O₂ partial pressures in the packages. All other treatments had a similar trend in CO₂ accumulation. The gas composition of water washed packaged fresh-cut cilantro at equilibrium observed in this experiment was similar to that reported by Luo et al. (2004) under the same package configuration and film O₂ transmission rate.

3.2. Changes in microbial populations

The initial aerobic plate count (APC) was relatively high and ranged from 5.0 to 6.25 log cfu g⁻¹ (Fig. 3A). This is similar to the findings from Luo et al. (2004) and Allende et al. (2007) on fresh-cut cilantro and higher than most other fresh-cut vegetables (Babic and Watada, 1996; Garg et al., 1990; Magnuson et al., 1990). Sanitizer treatment had a significant ($P < 0.0001$) effect on APC while 1-MCP had no significant ($P > 0.05$) effect on APC. All treatments containing either SH or ASC significantly ($P < 0.001$) reduced initial APC on fresh-cut cilantro compared to those washed with tap water alone. There was no significant ($P > 0.05$) difference in APC between cilantro samples treated with ASC and SH on day 0. During storage, there was a significant ($P < 0.0001$) increase in APC among all treatments after 4 days in storage. Treatments containing ASC had significantly ($P < 0.05$) lower APC than the water alone treatment throughout most of the storage period, except on day 8.

Treatments containing ASC had significantly ($P < 0.01$) higher reductions on coliform/*E. coli* counts on fresh-cut cilantro on day 0 than all other treatments including SH (Fig. 3B; approximately a reduction of 1.6 log cfu g⁻¹ compared to the water control, and 0.8 log cfu g⁻¹ compared to all other treatments). Similar to APC, the counts of coliform/*E. coli* increased signifi-

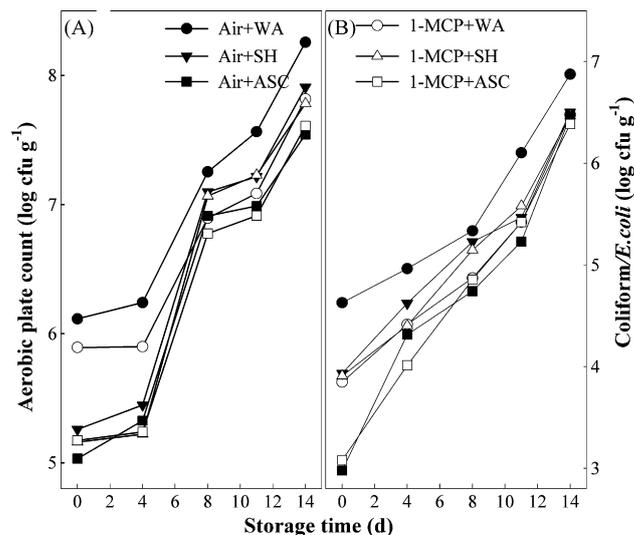


Fig. 3. Changes in aerobic plate counts (A) and coliform/*E. coli* counts (B) on fresh-cut cilantro during storage at 5 °C. Each symbol is the mean of three replications. Vertical lines represent S.E.; S.E. bars were not shown when masked by the symbol.

cantly ($P < 0.0001$) on cilantro leaves in all of the treatments during storage. However, all treatments containing ASC maintained smaller coliform/*E. coli* counts than the water washed control samples throughout storage. Samples containing SH also had significantly lower coliform/*E. coli* counts than the water control through most of the storage period, except on day 8. The application of 1-MCP had no significant ($P > 0.05$) effect on coliform/*E. coli* growth.

3.3. Changes in product quality

Chlorophyll degradation and the resultant loss of green color during storage is a major quality defect for most leafy green vegetables, such as cilantro and broccoli (Jiang et al., 2002; Ku and Wills, 1999). Studies have shown that 1-MCP delayed the yellowing of broccoli, Chinese mustard (Able et al., 2003) and choysum (Fan and Mattheis, 2000), and inhibited chloro-

Table 1
Changes in color values b^* and hue angle of fresh-cut cilantro during storage at 5 °C

	Treatment	Storage time (d)				
		0	4	8	11	14
b^*	Control		13.6 ± 0.7 ^a	13.8 ± 0.5	13.7 ± 0.6	15.2 ± 0.9
	1-MCP		12.7 ± 0.4	13.2 ± 0.4	12.9 ± 0.4	15.0 ± 0.7
	Chlorine	12.8	13.3 ± 0.4	12.9 ± 0.4	12.7 ± 0.4	14.5 ± 0.5
	1-MCP + Chlorine	± 0.4	13.2 ± 0.5	12.9 ± 0.4	13.6 ± 0.4	13.7 ± 0.4
	ASC		13.2 ± 0.4	13.4 ± 0.5	13.3 ± 0.4	13.6 ± 0.5
	1-MCP + ASC		12.6 ± 0.4	13.5 ± 0.4	13.4 ± 0.5	13.9 ± 0.4
Hue	Control		126.5 ± 0.6	125.8 ± 0.7	125.1 ± 0.8	123.7 ± 0.9
	1-MCP		126.8 ± 0.7	126.6 ± 0.5	126.6 ± 0.6	124.2 ± 0.7
	Chlorine	126.1	126.2 ± 0.5	125.7 ± 0.7	126.1 ± 0.6	124.7 ± 0.5
	1-MCP + Chlorine	± 0.4	126.9 ± 0.6	126.0 ± 0.6	125.8 ± 0.7	125.0 ± 0.5
	ASC		126.6 ± 0.6	126.6 ± 0.6	125.3 ± 0.6	125.1 ± 0.7
	1-MCP + ASC		126.9 ± 0.4	126.3 ± 0.4	126.9 ± 0.8	125.5 ± 0.4

^a Values are the means of three-replicated samples ± S.E.

phyll degradation in a number of vegetables (Jiang et al., 2002; Blankenship and Dole, 2003). In this study, the b^* values (yellow coloration) of cilantro leaves ranged from 12.6 to 15.2 (Table 1). Although there was a slight increase in b^* during storage, neither 1-MCP nor sanitizer treatment had any significant effect ($P > 0.05$) on the changes of b^* value. The hue angle of cilantro ranged from 123.1 to 126.9 (Table 1). There were more pronounced changes in hue angle over time and among treatment than the changes in b^* . The values of hue angle were significantly affected by storage time ($P < 0.0001$) and treatment ($P < 0.05$). Although all treatments maintained a relatively stable hue angle from day 0 to day 11, there was a significant ($P < 0.001$) decrease in hue on day 14 for cilantro leaves that received WA, SH, and 1-MCP treatments. On the contrary, no significant difference in hue angle was found on samples that received the combination treatment of 1-MCP with ASC or SH, and ASC alone during the entire storage period.

There was a significant difference in decay rate (%) during storage ($P < 0.0001$) and among treatments ($P < 0.0001$) with a significant interaction between storage time and treatment ($P = 0.0132$). The water washed fresh-cut cilantro was the first treatment to show decay symptoms on all of the replications, starting on day 4 (Fig. 4). Samples treated with ASC or SH had no decay at this stage, and those treated with 1MCP+ASC, 1-MCP+SH, and 1-MCP+WA had sporadic slight decay development on only some of the replications. At the end of storage, the sequential treatment of 1-MCP and ASC, followed by ASC alone, had a significantly ($P < 0.05$) lower decay rate than any other treatment. This finding was in agreement with microbial assays which indicated that all treatments containing ASC had lower microbial growth than control and other treatments.

Ethanol production is associated with anaerobic respiration of fresh produce under low O_2 and high CO_2 atmospheric con-

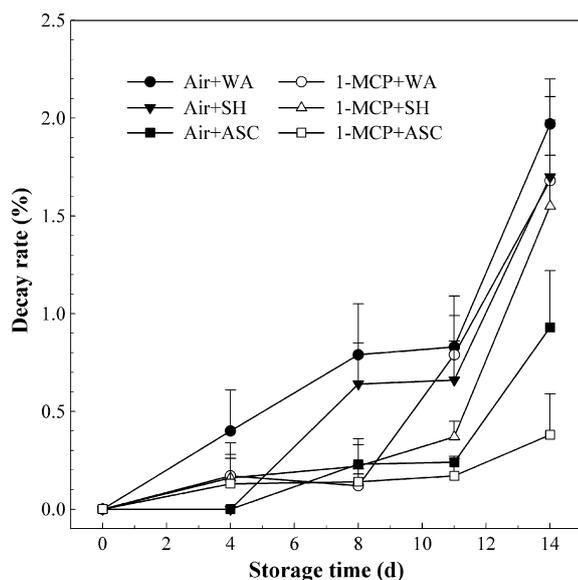


Fig. 4. Changes in decay rate of fresh-cut cilantro stored at 5 °C for up to 14 d. Each symbol is the mean of three replications. Vertical lines represent S.E. Decay rate was calculated as percentage weight of affected tissues vs. total tissues in each package.

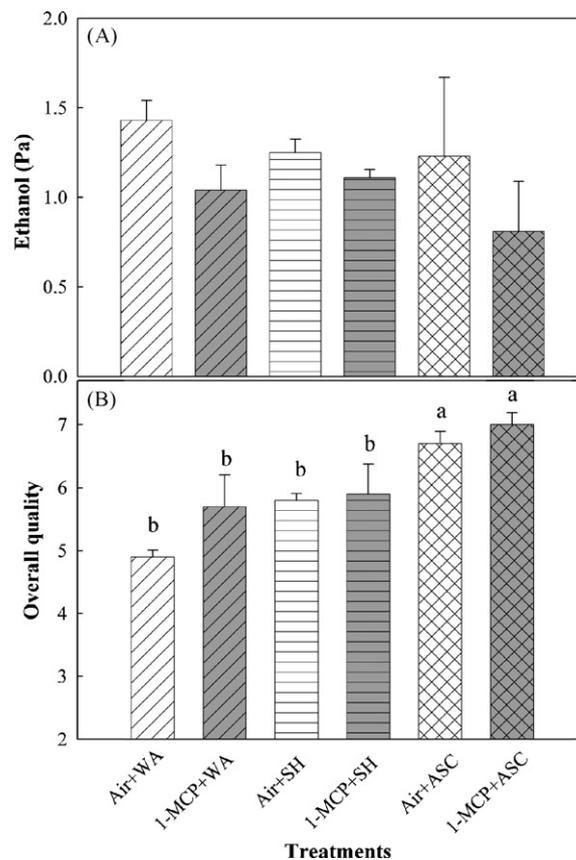


Fig. 5. Ethanol partial pressure in the headspace of packaged fresh-cut cilantro (A), and overall quality (B) of fresh-cut cilantro stored at 5 °C for 14 d. Each histogram bar is the mean of three replications; vertical lines represent S.E. Overall quality was scored by three trained panelists using a 1–9 hedonic scale where 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; and 9 = like extremely. Bars labeled with different letters are significantly different at $P < 0.05$.

ditions. At the end of storage, small amounts of ethanol were detected in the package headspace of all samples, ranging from 0.82 to 1.43 Pa (Fig. 5A). Although no significant ($P > 0.05$) difference was found among treatments, all treatments containing 1-MCP had lower ethanol vapor pressures than their corresponding treatments without 1-MCP.

A slight off-odor was detected on control samples at the end of storage (data not shown), corresponding to the higher ethanol partial pressure in this group. No off-odor was detected from any samples treated with either 1-MCP, SH, ASC or their combinations. Lack of off-odor is in accordance with the smaller ethanol partial pressures measured in these packages and the odor detection threshold of 1 Pa (Kim et al., 2005; Sigma–Aldrich, 2003).

The overall visual quality of fresh-cut cilantro was assessed at the end of storage (Fig. 5B). There were significant differences among treatments in overall quality (Fig. 5B). All treatments containing ASC displayed significantly ($P < 0.0001$) higher overall visual quality scores than other treatments. Among all treatments, samples that received the sequential treatment of 1-MCP and ASC had the highest score (a score of 7.0 on a 1–9.0 point scale) and cilantro leaves appeared to be near their initial

condition with green, fresh appearance and no yellowing, dehydration or off-odor. Cilantro leaves treated with ASC alone also had higher scores than other treatments, mainly due to the lower decay rate. The control samples with water wash had the lowest overall quality score, with relatively high levels of yellowing and decay. Although SH alone or in combination with 1-MCP resulted in better quality scores than the control, SH was less effective in maintaining quality than ASC.

4. Conclusion

Washing fresh-cut cilantro leaves with 100 mg L⁻¹ acidified sodium chlorite solution significantly reduced the initial aerobic bacterial populations and coliform/*E. coli* counts and decreased the decay rate. Packaged fresh-cut cilantro leaves that received a sequential treatment of 1-methylcyclopropene and acidified sodium chlorite wash maintained the lowest decay rate and highest overall quality at the end of storage.

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