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Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce

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ABSTRACT

In this study, the efficacy of hydrogen peroxide vapor (HPV) for reducing *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce was investigated as well as its effect on lettuce quality. Lettuce was inoculated with a cocktail containing three strains of each pathogen then treated with vaporized hydrogen peroxide for 0, 2, 4, 6, 8 and 10 min. The concentrations of hydrogen peroxide used were 0, 1, 3, 5 and 10%. With increasing treatment time and hydrogen peroxide concentration, HPV treatment showed significant ($P < 0.05$) reduction compared to the control (0%, treated with vaporized distilled water). In particular, vaporized 10% hydrogen peroxide treatment for 10 min was the most effective combination for reducing the three pathogens on lettuce. The reduction levels of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on lettuce were 3.12, 3.15 and 2.95 $\log_{10}$ CFU/g, respectively. Furthermore, there were no significant ($P > 0.05$) quality changes (color and texture) of lettuce among all tested samples, and hydrogen peroxide residues were not detected after 36 h storage time in any of the treated samples. These results suggest that HPV treatment could be an alternative method for reducing *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on fresh produce.

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1. Introduction

Documented foodborne illness outbreaks associated with consumption of raw vegetables and fruits have increased in recent years (Cedric et al., 2010; Doyle & Erickson, 2008). Consequently, the concern about pathogens in fresh foods has also increased. Fresh produce can become contaminated with pathogenic microorganisms in the field through contact with improperly treated manure compost, contaminated irrigation water, the presence of wild and domestic animals, and unclean containers and tools used in harvesting (Beuchat, 2002; Lee, Costello, & Kang, 2004; Oliveira, Usall, Viñas, Solsona, & Abadias, 2011). In addition, contamination of produce with pathogenic microorganisms may occur during postharvest handling, processing, and distribution. Because of this, controlling pathogenic microorganisms on fresh produce plays a paramount role in maintaining product quality and microbiological safety (Han, Linton, Nielsen, & Nelson, 2000; Rahman, Ding, & Oh, 2010).

Pathogenic microorganisms such as *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* have all been implicated in outbreaks of foodborne illness associated with minimally processed fresh produce (Beuchat, 1996; Jablasone, Warriner, & Griffiths, 2005). *S.* Typhimurium is the most commonly isolated Salmonella serotype implicated in foodborne illness, and the symptoms of infection in humans are diarrhea, abdominal pain, mild fever and chills (Baird-Parker, 1990; Rhee, Lee, Dougherty, & Kang, 2003). *E. coli* O157:H7 has a low infective dose and causes hemorrhagic colitis, which is occasionally complicated by hemolytic uremic syndrome (Doyle, 1991; Griffin & Tauxe, 1991). *L. monocytogenes* causes meningitis, encephalitis or septicaemia in immuno-compromised patients, pregnant women, infants, and the elderly (Farber & Peterkin, 1991; Schuchat, Swaminathan, & Broome, 1991).

For sanitizing fresh fruits and vegetables, washing with water and chlorinated water (50–200 ppm chlorine) are widely used on a commercial scale (Parish et al., 2003; Weissinger, Chantarapanont, & Beuchat, 2000). In particular, chlorine based chemicals are the

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most widely used sanitizers for fresh produce disinfection in the food industry. However, this treatment results in a microbial reduction of less than 2 log CFU/g on fresh fruits and vegetables (Beuchat, 1999; Taormina & Beuchat, 1999; Zhang & Farber, 1996). For applying new decontamination technologies by industry, the required inactivation level of pathogens generally needs to exceed 99.9% (≥3 log reduction). Therefore, investigation of an alternative technology which can effectively reduce pathogenic microorganisms by at least 3 log on fresh produce while maintaining product quality is needed.

Microorganisms can become attached to injured or inaccessible surfaces of fresh produce and can thus show increased resistance to sanitization (Foschino, Nervegna, Motta, & Galli, 1998; Lindsay & Von Holy, 1999; Zottola, 1994). Microorganisms harbored in crevices under the surface can be a limiting factor of using aqueous sanitizers (Sapers, 2001). One potential method for overcoming this limiting factor is to apply them in gaseous form, as this method has greater penetration ability it may facilitate better control of pathogenic microorganisms on fresh produce. Currently, several studies have documented that gaseous chlorine dioxide has better penetration ability compared with its aqueous form and is therefore considered more effective in reducing microorganisms on fresh produce (Han, Linton, Nielsen, & Nelson, 2001; Lee et al., 2004).

To date, hydrogen peroxide (H_2O_2) has been widely tested as a sanitizing agent (Lin, Moon, Doyle, & McWatters, 2002; Moore, Patel, Jaroni, Friedman, & Ravishankar, 2011; Ukuku, 2004). H_2O_2 demonstrates activity against a wide range of organisms such as vegetative bacteria, bacterial spores, yeasts, fungi, and viruses (Block, 2001, 185–204; McDonnell & Russell, 1999). It is a potent oxidant, acting against bacteria and viruses by forming hydroxyl free radicals (•OH) which attack essential cell components, including lipids, proteins, and DNA (McDonnell & Russell, 1999). While aqueous H_2O_2 has a long history of use as a sterilant, the concept of H_2O_2 vapor sterilization has been utilized since the 1980s. Hydrogen Peroxide Vapor (HPV) is considered less toxic in terms of residual byproducts to humans than other fumigants such as formaldehyde, chlorine dioxide and ethylene oxide since hydrogen peroxide breaks down into water and oxygen (Daft, 1991; Klapes & Vesley, 1990). Therefore, HPV has been widely used for the decontamination of laboratory and medical equipment, health care institutions, pharmaceutical facilities and other applications (Charles, Roger, Ricardo, & Smilanick, 1991; French et al., 2004; Klapes & Vesley, 1990; Smilanick et al., 1994). However, to date, there has been a paucity of information regarding the effectiveness of HPV for controlling foodborne pathogens on foodstuffs (Simmons, Smilanick, John, & Margosan, 1997).

The objectives of this study were to evaluate the bactericidal effectiveness of HPV on lettuce, and investigate the quality changes following treatment with HPV. To be specific, we determined HPV concentration and exposure time required to reduce numbers of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on lettuce and its effect on color value and texture parameters. Additionally, levels of hydrogen peroxide residues on lettuce were measured after treatments.

2. Materials and methods

2.1. Bacterial strains

Three strains each of *Salmonella* Typhimurium (ATCC 19585, ATCC 43971, ATCC 700408), *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *L. monocytogenes* (ATCC 15315, ATCC 19114, ATCC 19115) were used in this experiment and were obtained from the Bacterial Culture Collection at Seoul National University (Seoul, Korea). All strains were stored at −80 °C in 0.7 ml of Tryptic Soy Broth (TSB; Difco Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol (vol/vol). Working cultures were maintained on TSA (Difco) slants at 4 °C and subcultured weekly.

2.2. Bacterial cultures and cell suspension preparation

Each strain of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* was cultured in 5 ml TSB for 24 h at 37 °C, harvested by centrifugation at 4000 × *g* for 20 min at 4 °C, and washed three times with Buffered Peptone Water (BPW; Difco). The final pellets were resuspended in BPW, corresponding to approximately $10^7 \sim 10^8$ CFU/ml. Subsequently, suspended pellets of each strain of the three pathogens were mixed to produce a culture cocktail.

2.3. Sample preparation and inoculation

Iceberg lettuce was purchased at a local grocery store (Seoul, South Korea) on the day before the experiment and kept at 4 °C prior to use. Two to three layers of outer leaves were removed and discarded. Intact inner leaves were removed and cut into 5 by 5 cm pieces. Samples were placed on sterile aluminum foil in a laminar flow hood with the fan running and 100 μl of previously described culture cocktail was inoculated onto the surface of each lettuce piece by distributing this volume between 15 droplets deposited at randomly selected locations with a micropipettor. The inoculated lettuce pieces were air-dried for 3 h in the hood with the fan running at room temperature (22 ± 2 °C). Spot inoculation is more consistent and produces more reproducible results for the inoculation of a known number of pathogen cells on lettuce surfaces than does the dipping inoculation method (Beuchat et al., 2001).

2.4. Preparation of treatments

Aqueous solutions containing 1%, 3%, 5% and 10% hydrogen peroxide (hydrogen peroxide, 30%, Junsei Chemical Co. Ltd., Tokyo, Japan) were prepared using sterile distilled water on a vol/vol basis. Freshly prepared solutions were used within 30 min at room temperature (22 ± 2 °C). Distilled water served as a control.

2.5. HPV treatment system

The experimental apparatus (Fig. 1) consisted of a HPV generation chamber (37 × 50 × 50 cm), a sample treatment chamber (37 × 50 × 50 cm), a solution tank, a volumetric pump (JWS600, Jeniewell, Seoul, Korea), and a modified bowl-shaped heater (300W, JY-22300, Exso, Busan, Korea). The HPV generation chamber and the sample treatment chamber were separated by a gap of ca. 5 cm to prevent temperature rise in the treatment chamber. For the HPV treatment, 1-L of solution of each previously described hydrogen peroxide concentration was placed in the 2-L solution tank. The solution was pumped to the heater by a volumetric pump, and the flow rate was maintained at 10 ml/min. When the solution dropped onto the bowl-shaped heater (ca. 600 °C of surface temperature), HPV was generated and transferred into the treatment chamber through a seamed pipe (with a fan) installed between the two chambers. 25 g of inoculated lettuce leaves (17–18 pieces, inoculum level of 10^7–10^8 CFU/sample) were placed on sterilized stainless rack and treated with HPV for 2, 4, 6, 8 and 10 min in the treatment chamber. When each treatment was terminated, the remaining HPV in treatment chamber was removed using an extractor fan. Temperature of the treatment chamber was monitored and recorded using a fiber optic temperature sensor (FOT-L; FISO Technologies Inc., Quebec, Canada), placed at the sample tray and a signal conditioner (TMI-4, FISO Technologies Inc., Quebec, Canada). The temperature of the sample tray was always less than

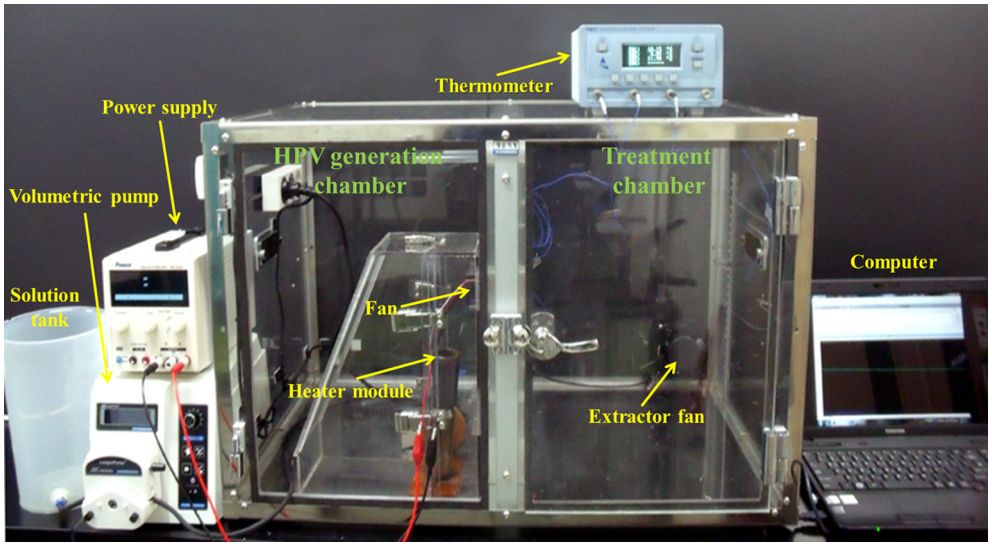

Fig. 1. Experimental hydrogen peroxide vapor (HPV) treatment system used in this study.

40 °C. This system was developed and constructed at Seoul National University (Seoul, South Korea). Before the chamber was fabricated, HPV treatment system simulations were performed with ANSYS (ANSYS Inc., Canonsburg, PA, USA), an engineering simulation software for optimization of HPV dispersion.

2.6. Microbial enumeration

After 2, 4, 6, 8 and 10 min of treatment, 25 g treated samples of lettuce leaves were immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of BPW and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of sample were serially 10-fold diluted with 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Xylose Lysine Desoxycholate Agar (XLD; Difco), Sorbitol MacConkey agar (Difco) and Oxford Agar Base (OAB; Difco) with antimicrobic supplement (Bacto Oxford Antimicrobic Supplement, Difco), were used as selective media to enumerate *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes*, respectively. All plates were incubated aerobically at 37 °C for 24–48 h, then typical colonies characteristic of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* were enumerated.

2.7. Enumeration of injured cells

Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to enumerate injured cells of *E. coli* O157:H7 (Rhee, Lee, Hillers, McCurdy, & Kang, 2003). After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 Latex Agglutination Test; Remel, Lenexa, KS, USA), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7. The overlay (OV) method was used to enumerate injured cells of *S.* Typhimurium and *L. monocytogenes* (Lee & Kang, 2001). TSA was used as a nonselective medium to repair and resuscitate injured cells. One hundred microliters of appropriate dilutions were spread onto TSA medium and plates were incubated at 37 °C for 2 h to allow injured microorganisms to repair and resuscitate. Plates were then overlaid with 7–8 ml of selective medium (XLD or OAB agar). After solidification, plates were further incubated for an additional 24–48 h at 37 °C. Following incubation, typical black colonies were enumerated.

2.8. Color and texture measurement

In order to identify the color and texture changes of lettuce leaves during storage following treatments, all treated samples were stored at 4 °C for 7 days in polyethylene zipper bags under aerobic conditions. Color change of lettuce leaves was measured with a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) at 3 locations on each leaf at 0, 1, 3, 5, and 7 days after treatment. Colors were expressed as L*, a*, and b* values. The parameter L* is a measure of lightness, a* is an indicator of redness, and the parameter b* is a measure of yellowness. Changes in texture of HPV-treated lettuce leaf were evaluated with a Brookfield texture analyzer (CT3-10k, Brookfield Engineering Laboratories, Inc., MA, USA) with a blade set probe. Three stacked samples (5 by 5 cm) were placed onto the press holder, and a blade was moved down at 2 mm/s. Maximum shear force required for cutting the sample was recorded using TexturePro CT software (version 1.2, Brookfield Engineering Laboratories, Inc.). The peak force required to shear the samples were referred as a measure of hardness. All experiments were performed three times, with independently-prepared samples.

2.9. Residual hydrogen peroxide

Peroxide residues on treated lettuce leaves were determined by hydrogen peroxide test strips (WaterWorksTM, Industrial Test Systems, Inc., SC, USA) with a minimum detection level of 0.05 ppm. Non-inoculated lettuce leaves were treated with various concentrations (1, 3, 5 and 10%) of HPV for 10 min as previously described. In order to investigate the changes of residual of hydrogen peroxide on lettuce leaves during storage, all treated samples were stored at 4 °C for 36 h. Twenty-five gram samples of treated lettuce leaves were transferred into sterile plastic bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 225 ml of distilled water and homogenized for 2 min with a stomacher at ambient laboratory temperatures. After homogenization, hydrogen peroxide test strips were submerged in sample leachate to measure the hydrogen peroxide concentration. All experiments were performed three times.

2.10. Statistical analysis

All microbial experiments were repeated three times with duplicate samples and averages of duplicate plate counts from three replications were converted to log CFU/g. Data were analyzed by the ANOVA procedure of SAS (Version 9.2. SAS Institute Inc., NC, USA). When the main effect was significant ($P < 0.05$), means were separated using the Duncan's multiple range test.

3. Results

3.1. Inactivation of pathogenic bacteria after HPV treatment and recovery of injured cells

Tables 1–3 show levels of surviving cells, including injured, of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes*, on the surfaces of lettuce leaves following distilled water or HPV treatment. When lettuce leaves were treated with vaporized distilled water (0% HPV) for 10 min, cell numbers of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* were not significantly reduced ($P > 0.05$). However, when inoculated lettuce was treated with HPV (1–10%), levels of the three pathogens were reduced significantly ($P < 0.05$) compared to untreated inoculated control samples (0 min). Survival of pathogens decreased with increasing treatment time and hydrogen peroxide concentration. After 10 min of 1, 3, and 5% HPV treatment, levels of *S.* Typhimurium were reduced by 1.48, 2.09, and 2.63 $\log_{10}$ CFU/g, respectively, on lettuce leaves. Also, there were 1.62, 2.14, and 2.94 $\log_{10}$ CFU/g reductions of *E. coli* O157:H7 after 10 min of 1%, 3%, and 5% HPV treatment, respectively. In the case of *L. monocytogenes*, 0.99, 1.63, and 2.24 $\log_{10}$ CFU/g of reductions were achieved after 10 min of 1%, 3%, and 5% HPV treatments, respectively. All pathogens were affected the most by treatment with vaporized 10% hydrogen peroxide, which reduced levels of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* by 3.12, 3.15 and 2.95 $\log_{10}$ CFU/g after 10 min, respectively.

When inoculated lettuce leaves were treated with HPV, slightly higher numbers of the three pathogens were observed by the agar OV method (SPRAB in the case of *E. coli* O157:H7) than on selective agar. However, statistically significant ($P > 0.05$) differences between the numbers of cells enumerated on selective agar (XLD, SMAC, and OAB) versus agar including the resuscitation step (OV-XLD, SPRAB, and OV-OAB) were not observed for most treatment times (Table 1–3).

3.2. The effect of HPV treatment on lettuce quality

Tables 4 and 5 show the color and texture parameters of lettuce stored at 4 °C for 7 days following treatments with 0% (vaporized distilled water) or 1%, 3%, 5%, and 10% HPV for 10 min, compared with non-treated samples. There were no significant differences ($P > 0.05$) in L*, a*, and b* color values and maximum load values of texture measurements among all tested samples, indicating treatment with up to 10% HPV did not significantly affect visual color and texture quality of lettuce following treatment and during 7 days storage.

3.3. Residual hydrogen peroxide

Hydrogen peroxide residues were detected immediately after exposure in all treated samples (Table 6). Following treatment with 1%, 3%, and 5% HPV for 10 min, the concentration of hydrogen peroxide on lettuce was 100, 250, and 500 mg/kg, respectively. At 10% HPV, the concentration of hydrogen peroxide exceeded the detection limit (1000 mg/kg). The residues declined over time. After 24 h, samples treated with 5% and 10% HPV had detectable

Table 1
Populations[a] ($\log_{10}$ CFU/g) of surviving cells and cells including injured *Salmonella* Typhimurium on lettuce surfaces exposed to hydrogen peroxide vapor (0, 1, 3, 5, and 10%).

Treatment time (min)	Population (log CFU/g)											
	0%		1%		3%		5%		10%			
	XLD[d]	OV-XLD	XLD	OV-XLD	XLD	OV-XLD	XLD	OV-XLD	XLD	OV-XLD
0	7.51 ± 0.13 A[b]a[c]	7.68 ± 0.12 Aa	7.87 ± 0.15 Aa	7.95 ± 0.06 Aa	7.73 ± 0.06 Aa	7.83 ± 0.03 Aa	7.93 ± 0.11 Aa	7.99 ± 0.14 Aa	7.89 ± 0.06 Aa	7.98 ± 0.02 Aa
2	7.51 ± 0.13 Aa	7.67 ± 0.11 Aa	7.26 ± 0.57 ABa	7.69 ± 0.15 ABa	7.01 ± 0.27 Ba	7.36 ± 0.17 Ba	6.71 ± 0.32 Ba	7.20 ± 0.34 Ba	6.57 ± 0.11 Ba	6.93 ± 0.19 Ba
4	7.54 ± 0.19 Aa	7.67 ± 0.11 Aa	6.93 ± 0.30 BCa	7.38 ± 0.11 BCa	6.47 ± 0.13 Ca	6.85 ± 0.23 Ca	6.21 ± 0.49 BCa	6.71 ± 0.34 BCa	6.16 ± 0.22 Ca	6.26 ± 0.50 Ca
6	7.55 ± 0.21 Aa	7.67 ± 0.11 Aa	6.65 ± 0.29 BCa	7.18 ± 0.22 CDa	6.24 ± 0.14 Ca	6.61 ± 0.21 CDa	6.03 ± 0.43 BCDa	6.58 ± 0.32 CDa	5.65 ± 0.40 Da	5.81 ± 0.39 CDa
8	7.51 ± 0.15 Aa	7.68 ± 0.12 Aa	6.44 ± 0.47 Ca	7.00 ± 0.22 DEa	5.78 ± 0.18 Da	6.33 ± 0.33 DEa	5.69 ± 0.50 CDa	6.16 ± 0.35 DEa	5.27 ± 0.24 Da	5.53 ± 0.16 DEa
10	7.49 ± 0.11 Aa	7.66 ± 0.10 Aa	6.40 ± 0.45 Ca	6.84 ± 0.22 Ea	5.64 ± 0.26 Da	5.93 ± 0.42 Ea	5.30 ± 0.32 Da	5.75 ± 0.21 Ea	4.77 ± 0.12 Eb	5.12 ± 0.13 Ea

[a] Data represent means ± standard deviations from three replications.
[b] Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$).
[c] Within each HPV concentration columns, means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[d] XLD, Xylose Lysine Desoxycholate; OV XLD, overlay XLD agar on TSA.

Table 2
Populations[a] (log_{10} CFU/g) of surviving cells and cells including injured *Escherichia coli* O157:H7 on lettuce surfaces exposed to hydrogen peroxide vapor (0, 1, 3, 5, and 10%).

Treatment time (min)	Population (log CFU/g)									
	0%		1%		3%		5%		10%	
	SMAC[d]	SPRAB	SMAC	SPRAB	SMAC	SPRAB	SMAC	SPRAB	SMAC	SPRAB
0	6.88 ± 0.18 A[b]a[c]	7.14 ± 0.12 Aa	7.66 ± 0.04 Aa	7.67 ± 0.06 Aa	7.24 ± 0.11 Aa	7.32 ± 0.15 Aa	7.67 ± 0.05 Aa	7.68 ± 0.05 Aa	7.79 ± 0.07 Aa	7.81 ± 0.11 Aa
2	6.89 ± 0.18 Aa	7.12 ± 0.14 Aa	7.04 ± 0.44 Ba	7.19 ± 0.38 ABa	6.45 ± 0.43 Ba	6.80 ± 0.19 Ba	6.16 ± 0.51 Ba	6.62 ± 0.25 Ba	6.42 ± 0.42 Ba	6.69 ± 0.45 Ba
4	6.86 ± 0.21 Aa	7.13 ± 0.13 Aa	6.72 ± 0.32 BCa	7.03 ± 0.28 ABCa	6.06 ± 0.27 BCa	6.37 ± 0.09 Ca	5.91 ± 0.45 BCa	6.29 ± 0.34 BCa	5.84 ± 0.44 BCa	6.10 ± 0.60 BCa
6	6.86 ± 0.20 Aa	7.17 ± 0.12 Aa	6.51 ± 0.22 BCDa	6.79 ± 0.43 BCa	5.78 ± 0.20 CDa	6.12 ± 0.10 CDa	5.47 ± 0.56 BCDa	6.02 ± 0.40 CDa	5.37 ± 0.43 CDa	5.75 ± 0.56 Ca
8	6.90 ± 0.17 Aa	7.17 ± 0.12 Aa	6.30 ± 0.29 CDa	6.63 ± 0.52 BCa	5.47 ± 0.26 DEa	5.80 ± 0.21 Da	5.16 ± 0.55 CDa	5.69 ± 0.34 Da	5.06 ± 0.32 DEa	5.37 ± 0.26 CDa
10	6.86 ± 0.20 Aa	7.09 ± 0.17 Aa	6.04 ± 0.47 Da	6.42 ± 0.47 Ca	5.10 ± 0.25 Ea	5.33 ± 0.45 Ea	4.73 ± 0.43 Da	5.16 ± 0.18 Ea	4.64 ± 0.07 Eb	4.94 ± 0.04 Da

[a] Data represent means ± standard deviations from three replications.
[b] Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$).
[c] Within each HPV concentration columns, means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[d] SMAC, Sorbitol MacConkey agar; SPRAB, Phenol red agar base with 1% sorbitol.

Table 3
Populations[a] (log_{10} CFU/g) of surviving cells and cells including injured *Listeria monocytogenes* on lettuce surfaces exposed to hydrogen peroxide vapor (0, 1, 3, 5, and 10%).

Treatment time (min)	Population (log CFU/g)									
	0%		1%		3%		5%		10%	
	OAB[d]	OV-OAB	OAB	OV-OAB	OAB	OV-OAB	OAB	OV-OAB	OAB	OV-OAB
0	6.76 ± 0.09 A[b]a[c]	6.86 ± 0.06 Aa	7.37 ± 0.21 Aa	7.49 ± 0.24 Aa	7.12 ± 0.07 Aa	7.22 ± 0.03 Aa	7.32 ± 0.12 Aa	7.38 ± 0.10 Aa	7.10 ± 0.10 Aa	7.19 ± 0.10 Aa
2	6.74 ± 0.07 Aa	6.85 ± 0.05 Aa	6.92 ± 0.31 ABa	7.26 ± 0.18 ABa	6.79 ± 0.19 Ba	7.06 ± 0.02 Aa	6.73 ± 0.21 Ba	7.10 ± 0.10 Aa	6.52 ± 0.37 Ba	6.83 ± 0.25 Aa
4	6.73 ± 0.06 Aa	6.88 ± 0.08 Aa	6.67 ± 0.32 Ba	7.14 ± 0.18 ABCa	6.40 ± 0.08 Cb	6.78 ± 0.05 Ba	6.49 ± 0.13 Ba	6.75 ± 0.13 Ba	5.79 ± 0.18 Ca	6.18 ± 0.31 Ba
6	6.76 ± 0.08 Aa	6.88 ± 0.08 Aa	6.60 ± 0.34 Ba	7.03 ± 0.19 BCa	6.22 ± 0.15 Ca	6.52 ± 0.10 Ca	6.02 ± 0.32 Ca	6.45 ± 0.11 Ca	5.42 ± 0.14 Ca	5.75 ± 0.22 Ca
8	6.76 ± 0.09 Aa	6.86 ± 0.06 Aa	6.50 ± 0.27 Ba	6.96 ± 0.19 BCa	5.96 ± 0.15 Da	6.16 ± 0.14 Da	5.75 ± 0.23 Ca	6.12 ± 0.13 Da	4.84 ± 0.29 Da	5.23 ± 0.33 Da
10	6.74 ± 0.07 Aa	6.86 ± 0.06 Aa	6.38 ± 0.26 Ba	6.82 ± 0.21 Ca	5.49 ± 0.18 Ea	5.87 ± 0.16 Ea	5.08 ± 0.18 Db	5.69 ± 0.29 Ea	4.16 ± 0.16 Ea	4.48 ± 0.13 Ea

[a] Data represent means ± standard deviations from three replications.
[b] Means with the same uppercase letter in the same column are not significantly different ($P > 0.05$).
[c] Within each HPV concentration columns, means with the same lowercase letter in the same row are not significantly different ($P > 0.05$).
[d] OAB, Oxford Agar Base; OV OAB, overlay OAB agar on TSA.

Table 4
L*, a*, and b* values[a] of lettuce stored at 4 °C for 7 days following treatment with vaporized distilled water (0%) or 1%, 3%, 5% and 10% HPV for 10 min.

Parameter[b]	Treatment concentration (%)	Storage time (day)				
		0	1	3	5	7
L*	Non-treated	72.70 ± 0.76[NS]	72.36 ± 0.45	72.55 ± 0.23	72.36 ± 0.77	72.16 ± 0.24
	0	72.73 ± 0.29	72.23 ± 0.28	72.38 ± 0.32	72.29 ± 0.31	72.45 ± 0.69
	1	72.32 ± 0.48	72.43 ± 0.47	72.49 ± 0.16	72.35 ± 0.80	72.33 ± 0.41
	3	72.72 ± 0.84	72.45 ± 0.30	71.94 ± 0.78	71.98 ± 0.38	72.35 ± 0.62
	5	72.75 ± 0.82	72.47 ± 0.08	72.59 ± 0.79	72.09 ± 0.68	72.30 ± 0.07
	10	72.10 ± 0.41	72.55 ± 0.19	72.09 ± 0.31	71.96 ± 0.87	71.88 ± 0.06
a*	Non-treated	−18.47 ± 0.31	−18.33 ± 0.13	−18.46 ± 0.44	−18.42 ± 0.29	−18.39 ± 0.12
	0	−18.01 ± 0.40	−18.24 ± 0.16	−18.46 ± 0.31	−18.39 ± 0.52	−18.45 ± 0.30
	1	−18.44 ± 0.50	−18.36 ± 0.27	−18.49 ± 0.23	−18.17 ± 0.06	−18.46 ± 0.28
	3	−17.80 ± 0.22	−17.96 ± 0.50	−17.91 ± 0.40	−17.94 ± 0.12	−17.96 ± 0.51
	5	−18.38 ± 0.45	−18.02 ± 0.24	−18.45 ± 0.12	−18.25 ± 0.26	−18.30 ± 0.42
	10	−18.06 ± 0.03	−18.21 ± 0.16	−17.96 ± 0.29	−18.05 ± 0.24	−17.89 ± 0.17
b*	Non-treated	35.85 ± 0.68	35.94 ± 0.48	35.76 ± 1.00	36.07 ± 0.48	36.03 ± 0.54
	0	35.68 ± 0.85	36.02 ± 0.63	36.39 ± 0.42	36.12 ± 0.46	36.38 ± 0.68
	1	36.69 ± 0.18	36.84 ± 0.87	36.27 ± 0.20	36.51 ± 0.58	36.58 ± 0.59
	3	36.05 ± 0.32	36.76 ± 0.16	36.79 ± 0.49	36.62 ± 0.29	36.21 ± 0.78
	5	35.62 ± 0.83	35.74 ± 0.76	35.78 ± 0.63	35.95 ± 0.54	35.93 ± 0.64
	10	36.33 ± 0.30	36.51 ± 0.28	36.63 ± 0.58	36.71 ± 0.70	36.62 ± 0.14

[NS]: no significant difference within L*, a*, b* values.
[a] Mean of three replications ± standard deviation.
[b] Color parameters are L* (lightness), a* (redness), and b* (yellowness).

residues of 4.33 and 6.67 mg/kg, respectively. Hydrogen peroxide residues at all exposure levels declined to undetectable levels (<0.05 mg/kg) after 36 h storage.

4. Discussion

There have been several research studies which demonstrate that aqueous sanitizers have limited effect in reducing populations of pathogenic microorganisms on fruits and vegetables. Sapers, Miller, Jantscheke, and Mattrazzo (2000) demonstrated that the efficacy of washing with H_2O_2 as a means of decontaminating apples is limited by microbial adhesion to apple surfaces, attachment at inaccessible sites, and survival and growth in punctures. Lang, Harris, and Beuchat (2004) reported that levels of *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 on lettuce were reduced by 1.1–1.8 $\log_{10}$ CFU/lettuce sample when treated with 200 μg/ml chlorine. Zhang and Farber (1996) reported the efficacy of various disinfectants, such as chlorine (200 ppm), ClO_2 solution (5 ppm), Salmide (200 ppm), trisodium phosphate (0.01 N), lactic acid (1%), and acetic acid (1%) against *L. monocytogenes* on fresh cut vegetables. None of these sanitizer solutions reduced *L. monocytogenes* on fresh-cut lettuce and cabbage by more than 2-log after treatment for 10 min at 4 and 22 °C. Most of these studies indicate that using aqueous sanitizers was not sufficient to control pathogens on vegetables and fruits. This is because aqueous sanitizers may not permit sufficient contact between attached bacteria on fresh produce surfaces and the sanitizing agents (Sapers, 2001). In this study, therefore, we designed a vapor-phase (HPV) treatment system to control foodborne pathogens, including *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* on lettuce surfaces.

If pathogenic bacteria form biofilms after processing, their resistance to antibacterial compounds may be enhanced compared to planktonic bacteria (Nickel, Ruseska, Wright, & Costerton, 1985). Therefore, it is important to sanitize fruits and vegetables during transport and storage. Because HPV dispersed in air can penetrate remote surfaces of fresh produce enclosed in a confined area, HPV treatment might be applied as an alternative intervention during transportation and storage operations.

Following HPV treatment, sub-lethally injured foodborne pathogens could assume added significance, since injured cells are able to repair themselves to resume growth, and regain their pathogenicity under suitable conditions (Hurst, 1977; Wesche, Gurtler, Marks, & Ryser, 2009). Therefore, cell populations enumerated on selective media following treatment are likely not representative of the total surviving cells in foodstuffs. So, it is important to enumerate injured microorganisms in many applications, such as in the study of the preservation and spoilage of foods, the manufacture of safe foods, and evaluating the effectiveness of processing for reduction of microorganisms. In this study, HPV treatment

Table 5
Maximum load values[a] for texture of lettuce stored at 4 °C for 7 days following treatment with vaporized distilled water (0%) or 1%, 3%, 5% and 10% HPV for 10 min.

Treatment concentration (%)	Maximum load[b] (N)				
	Storage time (day)				
	0	1	3	5	7
Non-treated	78.53 ± 3.94[Ns]	78.59 ± 3.15	78.70 ± 4.30	78.03 ± 3.25	77.47 ± 3.41
0	78.21 ± 1.06	77.03 ± 4.80	76.91 ± 4.68	76.89 ± 4.24	76.92 ± 3.14
1	77.63 ± 4.88	79.23 ± 5.19	78.48 ± 4.63	77.26 ± 1.76	77.65 ± 1.74
3	77.75 ± 3.28	78.08 ± 5.26	77.87 ± 3.17	77.01 ± 5.04	77.57 ± 2.49
5	78.30 ± 5.84	77.31 ± 4.45	77.52 ± 1.93	77.39 ± 5.51	76.01 ± 4.38
10	77.80 ± 5.18	78.03 ± 3.85	78.20 ± 6.20	77.05 ± 3.94	77.69 ± 1.89

[NS]: no significant difference within maximum load values.
[a] Mean of five replications ± standard deviation.
[b] Maximum load is load at rupture point.

Table 6
Hydrogen peroxide residues[a] in leachate from lettuce treated with 1%, 3%, 5% and 10% HPV for 10 min during different storage times.

Hydrogen peroxide concentration	H_2O_2 (mg/kg) after storage (hr)			
	1	12	24	36
1%	100 ± 0.00	16.67 ± 5.78	<0.05	–
3%	250 ± 0.00	36.67 ± 11.55	<0.05	–
5%	500 ± 0.00	66.67 ± 28.87	4.33 ± 1.15	<0.05
10%	>1000	83.33 ± 28.87	6.67 ± 2.89	<0.05

[a] Mean of three replications ± standard deviation.

effectively inactivated *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* on lettuce without generating many injured cells which could resuscitate (Tables 1–3). On the other hand, HPV treatment could potentially affect the quality of lettuce during storage following treatment. To investigate this possibility, the quality of treated lettuce leaves was evaluated by colorimetric and texture analysis. However, significant influences on the color and texture of lettuce were not observed during 7 day storage after HPV treatments (Tables 4 and 5).

In conclusion, HPV treatment effectively controlled pathogenic microorganisms on lettuce without generating significant levels of sub-lethally injured cells. Additionally, HPV treatment did not significantly ($P > 0.05$) affect the quality (color and texture) of lettuce during 7 day storage following HPV treatments. Hydrogen peroxide residues were not detected after 36 h storage in any of the treated samples. Although, the amount of treated sample (25 g) in the lab-scale instrument (Fig. 1) cannot be extrapolated out to commercial application, the results of this study suggest that HPV treatment could be an alternative method to control foodborne pathogens on fresh produce during transportation and storage.

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