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ORIGINAL ARTICLE

Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide

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advanced oxidative process, decontamination, *Escherichia coli* O157:H7, hydrogen peroxide, lettuce, *Salmonella,* ultraviolet, vegetables.

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Abstract

Aims: To evaluate the efficacy of ultraviolet (UV) light (254 nm) combined with hydrogen peroxide (H_2O_2) to inactivate bacteria on and within fresh produce.

Methods and Results: The produce was steep inoculated in bacterial cell suspension followed by vacuum infiltration. The inoculated samples were sprayed with H_2O_2 under constant UV illumination. The log count reduction (LCR) of *Salmonella* on and within lettuce was dependent on the H_2O_2 concentration, temperature and treatment time with UV intensity being less significant. By using the optimized parameters (1·5% H_2O_2 at 50°C, UV dose of 37·8 mJ cm^{-2}), the surface *Salmonella* were reduced by 4·12 ± 0·45 and internal counts by 2·84 ± 0·34 log CFU, which was significantly higher compared with H_2O_2 or UV alone. Higher LCR of *Escherichia coli* O157:H7, *Pectobacterium carotovora, Pseudomonas fluorescens* and *Salmonella* were achieved on leafy vegetables compared with produce, such as cauliflower. In all cases, the surface LCR were significantly higher compared with the samples treated with 200 ppm hypochlorite. UV–H_2O_2-treated lettuce did not develop brown discolouration during storage but growth of residual survivors occurred with samples held at 25°C.

Conclusions: UV–H_2O_2 reduce the bacterial populations on and within fresh produce without affecting the shelf-life stability.

Significance of the Study: UV–H_2O_2 represent an alternative to hypochlorite washes to decontaminate fresh produce.

Introduction

Foodborne illness outbreaks linked to fresh produce, such as lettuce, continue to represent a significant food safety issue (Gorny 2006). To reduce the contamination levels on fresh produce, it is common practice throughout the industry to apply a biocidal wash typically using sodium or calcium hypochlorite (Sapers 2006). However, because of the low efficacy of hypochlorite-based washes, there is a sustained effort to find alternative decontamination technologies (Beuchat *et al.* 2004; Rodgers *et al.* 2004; Sapers 2005; Sy *et al.* 2005; Hellstrom *et al.* 2006). In a previous report, a process based on ultraviolet/hydrogen peroxide (UV–H_2O_2) has been developed with enhanced efficacy for inactivating MS2 F(+) bacteriophage on lettuce surfaces [log count reduction (LCR) 4·26 ± 0·05 log PFU, plaque forming units] compared with standard hypochlorite washes, which only reduced the levels by 1·67 ± 0·18 log PFU (Xie *et al.* 2007). The process is based on the principle of advanced oxidative process (AOP), whereby highly reactive, but short-lived, hydroxyl radicals are formed during the interaction of H_2O_2 with UV photons (Rosenfeldt *et al.* 2006). Radical formation from H_2O_2 is enhanced at temperatures above 50°C in the presence of oxidization agents, such as ozone or UV light at 254 nm (Rosenfeldt *et al.* 2006). To date, processes based on AOP

have been applied to degrade pesticides and other toxic chemicals in waste water (Suty *et al.* 2004; Rosenfeldt *et al.* 2006; Thiruvenkatachari *et al.* 2006; Toor and Mohseni 2007). AOP has also been applied for inactivating bacterial endospores on carton packaging materials (Warriner *et al.* 2004). The decontamination approach was developed from the works of Bayliss and Waites (1979), who demonstrated that a 2% H_2O_2 solution in combination with UV (2·6 mW cm^{-2}) light at 254 nm could achieve a 5 log reduction in *Bacillus subtilis* endospores within 2-s exposure. The researchers also reported that the synergistic action of UV and H_2O_2 was optimal using 1–2% v/v H_2O_2 but decreased when higher or lower concentrations were applied (Bayliss and Waites 1979).

Although UV and H_2O_2 combination has been demonstrated to be effective against bacteriophage on lettuce and *Bacillus* spores on carton material, there have been few reports on using AOP for inactivating bacterial cells. It may be expected that vegetative cells would be more susceptible to hydroxyl radicals given the higher intrinsic resistance of bacteriophage and endospores (Koivunen and Heinonen-Tanski 2005; Mamane-Gravetz *et al.* 2005). However, Crowe *et al.* (2007) reported that a combination of UV and H_2O_2 did not result in a significant decrease in the bacterial numbers associated with blueberries compared with those when H_2O_2 was applied alone. Therefore, the decontamination efficacy of UV–H_2O_2 for bacterial inactivation is currently unclear.

In the following, the efficacy of UV–H_2O_2 to inactivate model human pathogens (*Salmonella*, *Escherichia coli* O157:H7) and spoilage bacteria (*Pectobacterium carotovora*, *Pseudomonas fluorescens*) associated with fresh produce has been evaluated. Of specific interest was to confirm the efficacy of UV–H_2O_2 treatment to reduce the microflora associated with fresh produce and also evaluate if the treatment could inactivate bacteria internalized into the inner tissue of produce. It is becoming established that the limited decontamination efficacy of sanitizer treatments can be attributed to internalization of bacteria into the subsurface of plant material during pre or postharvest operations (Seo and Frank 1999; Warriner *et al.* 2003). Therefore, any sanitizer applied to decontamination produce should demonstrate at least a reduction in the internal bacterial counts to enhance the microbiological safety of fresh produce.

Materials and methods

Bacteria and preparation of cell suspensions

The different bacterial strains used in the study are listed in Table 1. The test bacteria were cultivated overnight in 500 ml tryptic soy broth (TSB; Difco, Sparks, MD, USA) at either 30 or 37°C. Bacterial cells were harvested by centrifugation (5500 ***g*** for 10 min, 4°C) and washed once in 0·8% w/v saline. The final cell pellet was re-suspended in saline to a cell density of 8 log CFU ml^{-1}.

Table 1 Bacteria used in the study and cultivation conditions

Bacteria	Source
Escherichia coli O157:H7 ph1 Lux CDABE Amp	Ground beef*
Pseudomonas fluorescens P30 *lux*CDABE, Km^R	Vegetable-processing facility*
Pectobacterium carotovora	Tomato†
Salmonella Montevideo P2 *lux*CDABE, Km^R	Tomato*

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Inoculation of fresh produce

The produce types tested were iceberg (crisp head) lettuce (*Lactuca sativa*), Romaine lettuce (*L. sativa* L. var. *longifolia*), baby spinach (*Spinacia oleracea*), cauliflower florets (*Brassica oleracea*), broccoli florets (*Brassica oleracea* var. *italica*), sliced Spanish onions (*Allium cepa*) and ripened whole tomatoes (*Solanum lycopersicum* L). All the produce was donated by Pride Pak Salads Ltd. (Mississauga, ON, Canada).

Suspensions (7 log CFU ml^{-1}) of each test bacterium in saline were prepared from overnight cultures. Batches (100 g) of produce were submerged in 500-ml bacterial suspension for 20 min and subsequently transferred to a vacuum chamber. Three vacuum and release cycles (2 min per cycle) were applied to facilitate internalization of the bacteria into the inner tissue (Young 1974). The inoculated samples were stored at 4°C and tested within 4 h.

UV–H_2O_2 treatment chamber

The treatment chamber consisted of a foil-lined box (1·5 × 1 × 0·5 m) with an access door and a vent to remove H_2O_2 vapour at the end of the treatment process. A Heraeus low-pressure lamp (12 W; Heraeus, Hanau, Germany) was used as the UV (254 nm) source and positioned 35 cm above the sample tray. The intensity of UV at the point of the sample tray was 0·632 ± 0·015 mW cm^{-2} as measured using a UVX Radiometer (Cole-Parmer, Mississauga, ON, Canada). To determine the effect of UV intensity on decontamination efficacy, the lamp was positioned at different distances from the sample surface.

H_2O_2 (Fisher Scientific, Whitby, ON, Canada) was held within a 2-l container placed within a water bath

maintained at the appropriate temperature and delivered through Tygon L/S tubing (Cole-Palmer,) into the treatment chamber *via* a peristaltic pump operating at 480 ml min^{-1}. The H_2O_2 was delivered as a fine mist *via* passage through three-screen filtered nozzle heads with an aperture of 1 mm that generated 1·7 bar pressure and a 110 degree dispersion of liquid.

Samples were introduced as a single layer on a holding tray positioned under the UV lamp and spray heads. After delivering the designated treatment, the sample was manually turned over and the opposing side treated using the same regime.

Microbiological analysis

The bacteria on the surface and those internalized within produce were enumerated separately. Bacteria were released from the surface of samples by submerging individual batches (25 g for leafy vegetables, onion slices, broccoli and cauliflower florets) in 30-ml D/E (Dey/Engley) neutralizing broth (Cole-Palmer). For tomatoes, individual units were submerged in 30 ml D/E neutralizing broth. The bacteria were released from produce surfaces by gentle rubbing for approximately 30 s. The rinse was removed and produce samples submerged in 2% w/v calcium hypochlorite (Fisher Scientific) solution for 20 min to inactivate the residual surface microflora (Hora *et al.* 2005; Jablasone *et al.* 2005). Residual hypochlorite was removed by rinsing the sample three times in 100-ml sterile water. The sample was then macerated using a sterile knife prior to suspending 25 g in 30 ml D/E neutralizing broth and stomaching for 2 min. Dilution series were prepared from the sample rinse and macerate prior to plating 0·1-ml aliquots. *Salmonella* P2 and *E. coli* O157:H7 ph1 were plated onto tryptic soy agar (TSA; Oxoid) supplemented with 32 μg ml^{-1} kanamycin (Fisher) or 200 μg ml^{-1} ampicillin (Fisher), respectively, and incubated at 37°C for 24 h. When no colonies were recovered on plates, the samples were enriched at 37°C for 24 h using TSA containing the appropriate antibiotic. The enriched cultures were streaked onto TSA_{KM} or TSA_{AMP} and incubated at 37°C for 24 h.

P. fluorescens P30 was enumerated on TSA_{KM} plates incubated at 30°C for 48 h.

Pe. carotovora was enumerated on PT (polygalacturonic acid tergitol) medium (Burr and Schroth 1977) incubated at 30°C for 48 h.

Shelf-life stability and recovery of human pathogens on UV–H_2O_2-treated iceberg lettuce

Iceberg lettuce, inoculated with either *Salmonella* Montevideo P2 or *E. coli* O157:H7 ph1, was treated with UV–H_2O_2 and 60-g batches packed into sterile stomacher bags, which were subsequently stored at either 4 or 25°C. Samples were removed periodically and colour measurements using a Hunter Lab colormeter (Hunter Associates Laboratory, Inc. Reston, VA, USA) performed at five randomly selected areas on the lettuce leaf surface. The a^* (green–red), b^* (blue–yellow) and L^* (lightness) values were recorded and hue angle calculated using the equation:

$$\text{Hue angle} = \text{Tan}^{-1} b^*/a^*$$

An increase in hue or a^* from negative to positive values is indicative of lettuce discolouration (Castaner *et al.* 1996; Hosoda *et al.* 2000).

A further set of samples were removed to enumerate *Salmonella* P2 or *E. coli* O157:H7 ph1 levels. On this occasion, 25 g lettuce sample was suspended in 225 ml D/E neutralizing broth and stomached for 30 s. A dilution series was prepared from the homogenate and plated onto either TSA_{Km} or TSA_{Amp} prior to incubating at 37°C for 24 h. For comparison, lettuce samples washed for 3 min in calcium hypochlorite solution (1 : 5 lettuce : sanitizer ratio, 200 ppm) were also sampled.

Experimental design and statistics

Each trial was repeated twice with triplicate samples tested for each treatment. Count data was converted to log values from which LCR were calculated. The LCR were then statistically analysed using *t*-test, ANOVAR or Tukey's test (S-Plus; Insightful Corp, NY, USA). In all cases, the significance level was set at $P \leq 0{\cdot}05$.

Results

Optimization of UV light and H_2O_2 treatment

The effect of UV intensity on the inactivation of *Salmonella* on and within lettuce was determined (Fig. 1). The total dose applied in all cases was 37·8 mJ cm^{-2} with continuous H_2O_2 spray been delivered over the treatment period. The LCR of *Salmonella* on or within lettuce was found to be independent of UV intensity between (0·63–1·47 mW cm^{-2}). However, significantly ($P < 0{\cdot}05$) higher LCR of both surface and internalized *Salmonella* was achieved when the temperature of the H_2O_2 delivered into the treatment chamber was increased from 20 to 50°C (Fig. 2). When H_2O_2 (at 50°C) or UV were delivered alone, the LCR of *Salmonella* on and within lettuce was significantly lower compared with that when applied in combination (Fig. 2). When 50°C sterile distilled water was applied in combination with UV, the LCR

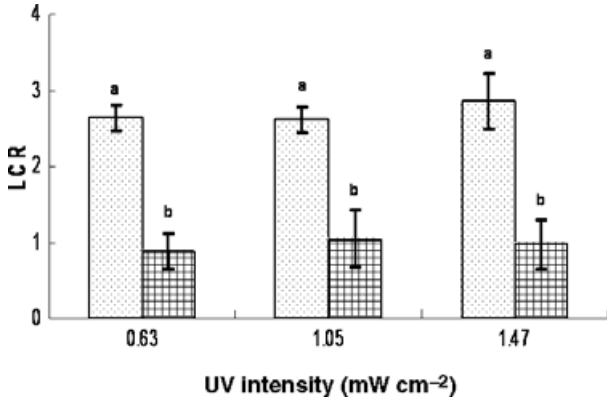

Figure 1 Effect of ultraviolet (UV) intensity on the inactivation of *Salmonella* Montevideo P2 on (▨) and within (▦) iceberg lettuce using 1·5% v/v hydrogen peroxide at 20°C. Different UV intensities were set be varying the distance of the lamp relative to the sample. In all cases, the total dose applied was 37·8 mJ cm^{-2}. Means with the same letter are not significantly different.

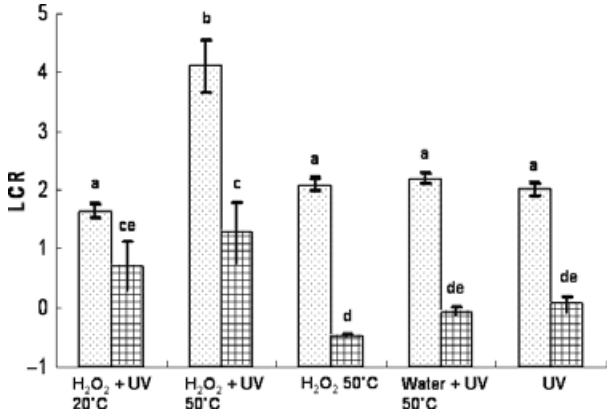

Figure 2 Effect of hydrogen peroxide temperature, used in combination with 37·8 mJ cm^{-2} ultraviolet (UV) on the inactivation of *Salmonella* Montevideo P2 on and within iceberg lettuce. Hydrogen peroxide (1·5% v/v) was introduced into the treatment chamber at either 20 or 50°C with continuous UV illumination. The *Salmonella* survivors present on (▨) and within (▦) lettuce were determined and used to calculate log count reductions. Values followed by the same letter are not significantly different.

reductions achieved were not significantly ($P > 0·05$) different from H_2O_2 alone or when UV–H_2O_2 was applied at 20°C. The LCR of *Salmonella* using a combination of hot water and UV were significantly lower than that achieved using 50°C H_2O_2 in combination with UV (Fig. 2). The results would suggest that the enhanced lethality of UV–H_2O_2 delivered at 50°C is the possible result of free radical generation as opposed to that by thermal affects alone.

Surface LCR of *Salmonella* on iceberg lettuce were significantly ($P < 0·05$) lower when H_2O_2 (at 50°C) was applied, in combination with UV, at 1% v/v compared with 1·5 or 2% v/v (Table 2). The surface LCR was further significantly ($P < 0·05$) enhanced by extending the treatment time from 30 to 60 s at 1·5% or 2% v/v H_2O_2 but not at 1% v/v concentration (Table 2). The LCR of internalized *Salmonella* was significantly ($P < 0·05$) higher when H_2O_2 (with UV) was applied at 1·5% v/v compared with 1% or 2% v/v (Table 2). Increasing the treatment time from 30 to 60 s (corresponding to 18·9 and 37·8 mJ cm^{-2}) did not significantly ($P > 0·05$) enhance the inactivation of internalized *Salmonella* within lettuce (Table 2).

To further enhance the decontamination efficacy of UV–H_2O_2, trials were performed with Tween 20 (0·1–0·5% v/v) supplemented into the H_2O_2 solution. It was hypothesized that the Tween would reduce the surface tension of the lettuce surface, thereby facilitating the penetration of free radicals into the inner leaf structure. However, no significant ($P > 0·05$) increase in the LCR for *Salmonella* on or within lettuce was observed with the inclusion of Tween (results not shown).

Effect of initial H_2O_2 spray followed by extended UV illumination time on the inactivation kinetics of *Salmonella* on and within iceberg lettuce

Trials were performed whereby the H_2O_2 and UV were delivered simultaneously in the first part of the treatment followed by an extended UV illumination period (Table 3). The underlying principle here was to form a layer of H_2O_2 on the sample surface and illuminate with UV to generate radicals in close proximity to the target bacterial cells.

From the LCR of *Salmonella* on the surface of iceberg lettuce, there was no significant ($P > 0·05$) difference using an extended illumination period of 30 or 60 s. However, the H_2O_2 spray time did have a significant ($P < 0·05$) effect on the level of *Salmonella* inactivation. Specifically, a 30-s spray time achieved significantly ($P < 0·05$) higher LCR of surface *Salmonella* compared with that when shorter treatment periods were applied (Table 3). The LCR for the 30-s spray was not significantly ($P > 0·05$) different compared with that when a continuous spray was delivered throughout the UV treatment period (Table 3). Therefore, the results would suggest that extended UV illumination period did not significantly enhance the lethality of the UV–H_2O_2 combination.

With internal *Salmonella* counts of treated lettuce, the 30-s spray time followed by a further UV illumination period resulted in significantly ($P < 0·05$) higher log reductions compared with those when shorter (10 s) treatment times were applied (Table 3). However, again,

Table 2 Effect of hydrogen peroxide concentration (delivered at 50°C) and ultraviolet dose on the inactivation of *Salmonella* Montevideo P2 on the surface and internal tissue of iceberg lettuce

% Hydrogen peroxide	*18·9 mJ cm^{-2}		*37·8 mJ cm^{-2}	
	LCR		LCR	
	Surface	Internal	Surface	Internal
1·0	1·33 ± 0·22^{Aa}	−0·03 ± 0·40^{Xa}	1·44 ± 0·35^{Aa}	0·34 ± 0·34^{Xa}
1·5	2·47 ± 0·41^{Ab}	1·83 ± 0·86^{Xb}	4·12 ± 0·45^{Bb}	1·30 ± 0·51^{Xb}
2·0	2·49 ± 0·40^{Ab}	0·70 ± 0·37^{Xa}	4·60 ± 0·27^{Bb}	0·68 ± 0·31^{Xa}

Initial loading: surface 7·48 ± 0·08 log CFU; internal 4·40 ± 0·13 log CFU g^{-1}
Means within columns followed by the same lower case letters are not significantly different.
Means within rows followed by the same capital letters are not significantly different.
LCR, log count reduction.
*Treatment time: 30 s (18·9 mJ cm^{-2}) and 60 s (37·8 mJ cm^{-2}).

Table 3 Log count reductions (LCR) of *Salmonella* Montevideo P2 introduced on and within iceberg lettuce using different treatment durations of ultraviolet–hydrogen peroxide (UV–H_2O_2)

Treatment*	Surface LCR	Internal LCR
Continuous H_2O_2 and UV 37·8 mJ cm^{-2}	4·12 ± 0·45^{a}	2·84 ± 0·34^{a}
10 s H_2O_2 UV 25·2 mJ cm^{-2}	2·72 ± 0·13^{b}	0·11 ± 0·09^{b}
10 s H_2O_2 UV 44·1 mJ cm^{-2}	2·58 ± 0·04^{b}	0·24 ± 0·08^{b}
20 s H_2O_2 UV 44·1 mJ cm^{-2}	2·88 ± 0·1^{b}	0·28 ± 0·02^{bc}
20 s H_2O_2 UV 50·4 mJ cm^{-2}	3·01 ± 0·09^{b}	0·60 ± 0·2^{cd}
30 s H_2O_2 UV 37·8 mJ cm^{-2}	4·07 ± 0·61^{a}	0·96 ± 0·1de
30 s H_2O_2 UV 56·7 mJ cm^{-2}	3·46 ± 0·15^{ab}	1·10 ± 0·22^{e}

Initial loading: surface 7·64 ± 0·01 log CFU; internal 4·31 ± 0·10 log CFU g^{-1}.
*Hydrogen peroxide (1·5% 50°C) was delivered for 10–30 s under continuous UV illumination. The peroxide spray was stopped and the samples were UV illuminated for a further time period.
Means within columns followed by the same letter are not significantly different.

the continuous spray and UV illumination resulted in a significantly ($P < 0·05$) higher LCR of internalized *Salmonella* (Table 3).

Decontamination of different produce types using UV light in combination with H_2O_2

The optimized UV–H_2O_2 treatment (1·5% v/v H_2O_2 at 50°C, 37·8 mJ cm^{-2} UV dose) was evaluated for decontaminating a diverse range of produce types (Table 4). In all cases, the surface LCR were significantly ($P < 0·05$) higher for UV–H_2O_2-treated samples compared with those when standard hypochlorite washes (200 ppm) were applied. Significantly ($P < 0·05$) higher surface log reductions were obtained using UV–H_2O_2 on lettuce, spinach and sliced Spanish onions compared with cauliflower, broccoli or tomatoes (Table 4).

Counts of internalized bacteria within leafy vegetables were also significantly ($P < 0·05$) reduced by UV–H_2O_2 compared with samples treated with hypochlorite. However, the LCR of bacteria internalized into cauliflower, sliced Spanish onion and broccoli were not significantly ($P > 0·05$) different between hypochlorite-treated samples and those subjected to UV–H_2O_2.

In the majority of cases, there were no differences in the level of inactivation amongst the different bacterial types tested on specific produce types. However, it was noted that the inactivation of *E. coli* O157:H7 was significantly ($P < 0·05$) greater on the surface of Romaine lettuce compared with the other bacterial types tested. *Escherichia coli* O157:H7 and *P. fluorescens* P30 LCR on the surface of cauliflower were significantly ($P < 0·05$) higher compared with *Salmonella* and *Pe. carotovora*. However, the LCR of *E. coli* O157:H7 on the surface of sliced Spanish onions was significantly ($P < 0·05$) lower than the other bacterial types tested. The inactivation of internal populations was less dependent on the bacterial type, although it was noted that the reductions of *Salmonella* P2 within iceberg lettuce was higher than *P. fluorescens* P30 and *E. coli* O157:H7 ph1. The LCR of *E. coli* O157:H7 ph1 within tomatoes was significantly lower than *Salmonella* P2 (Table 4).

Post-treatment stability and recovery of *Salmonella* Montevideo P2 and *Escherichia coli* ph1 on iceberg lettuce treated with UV light in combination with H_2O_2

Iceberg lettuce was treated with UV (37·8 mJ cm^{-2}) and H_2O_2 (1·5% v/v 50°C), and subsequently stored aerobically at either 4 or 25°C. Lettuce samples treated with UV–H_2O_2 and stored at 4°C exhibited an increase in the a^* value (loss of greenness), although this was insignificantly ($P > 0·05$) different from nontreated samples or those treated with calcium hypochlorite (Fig. 3a). In the same manner, the L^* and hue angle did not significantly ($P > 0·05$) change or differ between the different samples throughout the storage period (results not shown). Similar results were obtained with lettuce stored at 25°C

Table 4 Inactivation of model bacteria on and within different produce types using a combination of ultraviolet (UV) (37·8 mJ cm^{-2}) and hydrogen peroxide (H_2O_2) (1·5% v/v, 50°C). The different produce types were inoculated with the individual bacterial types and treated with UV–H_2O_2 for 60 s. For comparison, the samples were washed for 3 min in calcium hypochlorite solution (200 ppm)

	Initial loading CFU g^{-1}		Log count reductions			
			UV/H_2O_2		Calcium hypochlorite	
Produce type/organism	Surface	Internal	Surface*	Internal	Surface*	Internal
Iceberg lettuce						
Salmonella	6·05 ± 0·08	3·34 ± 0·19	$4·12 \pm 0·45^{Aa}A$	$2·84 \pm 0·34^{Xa}A$	$1·85 \pm 0·02^{Ba}A$	$0·20 \pm 0·10^{Ya}AB$
Pseudomonas fluorescens	5·16 ± 0·06	2·63 ± 0·04	$4·21 \pm 0·40^{Aa}$	$0·80 \pm 0·23^{Xb}$	$2·10 \pm 0·28^{Ba}$	$-0·40 \pm 0·22^{Ya}$
Escherichia coli O157:H7	5·52 ± 0·04	3·07 ± 0·15	$3·87 \pm 0·96^{Aa}AC$	$0·48 \pm 0·16^{Xb}A$	$1·55 \pm 0·32^{Ba}A$	$0·2 \pm 0^{Ya}A$
Romaine lettuce						
Salmonella	5·23 ± 0·10	2·54 ± 0·17	$3·75 \pm 0·48^{a}Ac$	$0·52 \pm 0·21^{a}B$	NT	NT
P. fluorescens	4·97 ± 0·21	3·01 ± 0·16	$4·01 \pm 0·15^{a}$	$0·77 \pm 0·31^{a}$	NT	NT
E. coli O157:H7	5·99 ± 0·07	2·83 ± 0·14	$4·94 \pm 0·31^{b}C$	$0·71 \pm 0·37^{a}$	NT†	NT†
Pectobacterium carotovora	5·10 ± 0·08	2·13 ± 0·42	$3·79 \pm 0·31^{a}$	$0·53 \pm 0·27^{a}$	NT†	NT†
Spinach						
Salmonella	6·35 ± 0·05	3·89 ± 0·20	$3·65 \pm 0·12^{A}AC$	$0·89 \pm 0·18^{X}B$	$0·48 \pm 0·08^{B}B$	$-0·34 \pm 0·34^{Y}A$
E. coli O157:H7	6·14 ± 0·05	4·01 ± 0·12	$4·75 \pm 0·85^{A}C$	$0·63 \pm 0·15^{X}A$	$0·46 \pm 0·07^{B}B$	$-0·05 \pm 0·05^{Y}A$
Cauliflower						
Salmonella	3·06 ± 0·09	3·76 ± 0·04	$2·02 \pm 0·55^{Aa}BC$	$0·70 \pm 0·21^{Xa}B$	$0·28 \pm 0·09^{Ba}B$	$0·45 \pm 0·17^{Xa}B$
P. flourescens	3·97 ± 0·23	2·12 ± 0·25	$3·32 \pm 0·15^{Ab}$	$0·63 \pm 0·45^{Xa}$	$0·23 \pm 0·41^{Ba}$	$0·26 \pm 0·24^{Xa}$
E. coli O157:H7	4·20 ± 0·10	3·64 ± 0·08	$3·11 \pm 0·43^{Aab}A$	$0·68 \pm 0·13^{Xa}A$	$0·77 \pm 0·15^{Ba}B$	$0·28 \pm 0·07^{Ya}A$
Pe. carotovora	3·76 ± 0·33	2·92 ± 0·30	$2·67 \pm 0·74^{Aa}$	$0·84 \pm 0·73^{Xa}$	$0·36 \pm 0·40^{Ba}$	$0·05 \pm 0·49^{Xa}$
Broccoli						
Salmonella	4·64 ± 0·38	3·59 ± 0·11	$2·18 \pm 0·65^{Aa}BC$	$0·79 \pm 0·13^{Xa}B$	$0·25 \pm 0·17^{Ba}B$	$0·31 \pm 0·29^{Xa}B$
P. fluorescens	4·45 ± 0·51	2·94 ± 0·64	$2·97 \pm 0·25^{Aa}$	$0·85 \pm 0·45^{Xa}$	$0·45 \pm 0·23^{Ba}$	$0·54 \pm 0·12^{Ya}$
E. coli O157:H7	4·73 ± 0·20	3·46 ± 0·21	$2·56 \pm 0·82^{Aa}A$	$0·61 \pm 0·19^{Xa}A$	$0·44 \pm 0·25^{Ba}B$	$0·34 \pm 0·27^{Xa}A$
Spanish onion						
Salmonella	4·81 ± 0·08	3·76 ± 0·05	$3·66 \pm 0·30^{Aa}AC$	$0·97 \pm 0·17^{Xa}B$	$0·34 \pm 0·10^{Ba}B$	$0·05 \pm 0·06^{Ya}B$
P. fluorescens	4·70 ± 0·10	2·59 ± 0·17	$3·06 \pm 0·51^{Aa}$	$1·40 \pm 0·28^{Xa}$	$0·21 \pm 0·19^{Ba}$	$0·05 \pm 0·20^{Ya}$
E. coli O157:H7	4·61 ± 0·09	3·76 ± 0·04	$2·09 \pm 0·49^{Ab}B$	$0·70 \pm 0·21^{Xa}A$	$0·28 \pm 0·09^{Ba}B$	$0·45 \pm 0·17^{Xa}A$
Tomato						
Salmonella	4·87 ± 0·38	2·64 ± 0·31	$2·22 \pm 1·51^{a}BC$	$1·95 \pm 0·11^{a}C$	NT†	NT†
E. coli O157:H7	4·82 ± 0·27	1·88 ± 0·55	$3·55 \pm 1·35^{a}B$	$0·89 \pm 0·54^{b}A$	NT†	NT†

*On occasions when no colonies on plates were recorded positive by enrichment, the lower detection limit (0·78 log CFU) was used to calculate the log count reductions. With samples testing negative following enrichment, the initial loading was taken as the log count reduction.
†NT. not tested.
Means within columns of the individual produce types followed by the same lower case letter are not significantly different. Means within rows followed by the same superscript capital letter are not significantly different. Means for reductions in *Salmonella* and *E. coli* O157:H7 levels followed by the same capital letter are not significantly different.

(Fig. 3b). Therefore, collectively, the results confirm that applying UV–H_2O_2 combination at 50°C does not lead to significant discolouration of lettuce.

The post-treatment recovery of *Salmonella* P2 or *E. coli* O157:H7 ph1 on UV–H_2O_2 or calcium hypochlorite-treated lettuce was assessed at different storage temperatures. At 4°C, the initial levels of either *E. coli* O157:H7 ph1 or *Salmonella* P2 on UV–H_2O_2-treated lettuce were significantly ($P < 0·05$) lower compared with the samples treated with calcium hypochlorite (Fig. 4a). At the end of the 8-day storage period, there was no significant difference in the levels of *E. coli* O157:H7 or *Salmonella* compared with that at day 0 (Fig. 4a).

When corresponding experiments were performed using a storage temperature of 25°C, residual survivors on the lettuce samples increased (Fig. 4b). With UV–H_2O_2-treated lettuce, the levels of *Salmonella* P2 or *E. coli* O157:H7 ph1 recovered on storage day 4 were insignificantly ($P > 0·05$) different from samples treated with calcium hypochlorite (Fig. 4b).

Discussion

A treatment using a combination of UV and H_2O_2 has been developed, which can enhance the inactivation of bacteria located on and within fresh produce. From

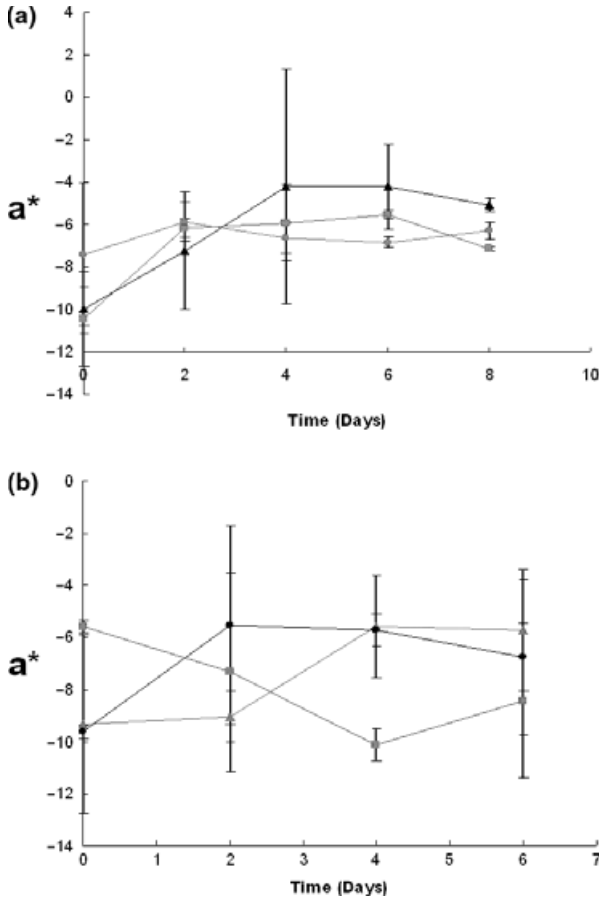

Figure 3 Colour (*a**) changes in iceberg lettuce treated with ultraviolet–hydrogen peroxide (UV–H_2O_2) (△1·5% v/v H_2O_2 at 50°C, 37·8 mJ cm^{-2}) compared with nontreated (●) or samples washed in calcium hypochlorite solutions (▣). Treated lettuce was packed into nonsealed sterile plastic bags and stored at 4°C (a) or 25°C (b). Periodically, samples were removed and the colour determined at five randomly selected areas on the lettuce surface.

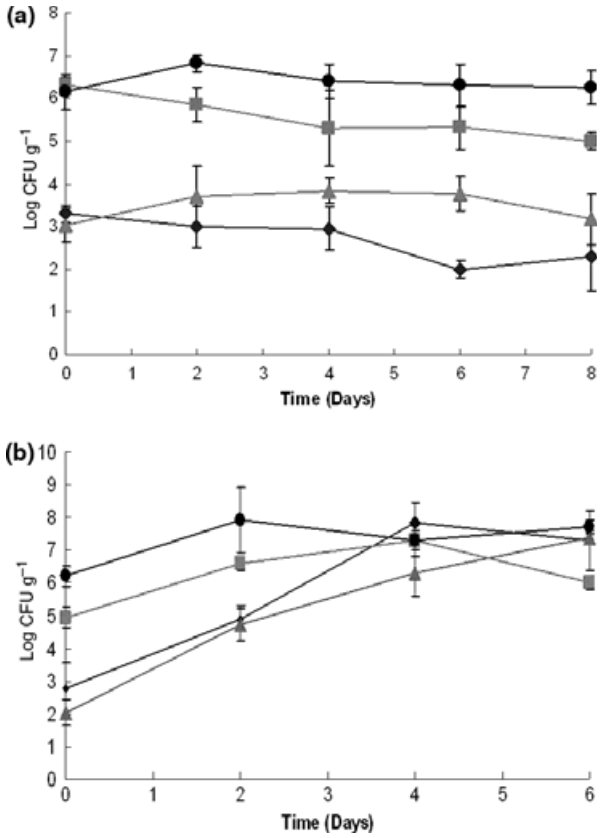

Figure 4 Levels of *Escherichia coli* O157:H7 ph1 (◆, ▣) and *Salmonella* Montevideo P2 (△, ●) associated with iceberg lettuce during storage following treatment with ultraviolet–hydrogen peroxide (UV–H_2O_2) (◆, △1·5% at 50°C, UV dose 37·8 mJ cm^{-2}) or calcium hypochlorite (▣, ● 200 ppm for 3 min). Treated lettuce was packed into unsealed sterile plastic bags and stored at either 4°C (a) or 25°C (b). Samples were withdrawn periodically and levels of introduced pathogens determined.

optimization studies, it was apparent that the efficacy of UV–H_2O_2 treatment was dependent on the concentration and temperature of the H_2O_2 solution delivered to the treatment chamber with UV intensity being less significant. It is well established that the inactivation of microbes directly by UV is dependent of the lamp intensity (Yaun *et al.* 2004). However, for the AOP-based degradation of trihalomethanes by UV–H_2O_2, the lamp intensity was found to be less significant (Toor and Mohseni 2007). This would suggest that the UV primarily acts to generate free radicals from H_2O_2 as opposed to inactivating microbes directly. This is supported by the fact that the combination of UV and H_2O_2 primarily enhances the H_2O_2 killing effect as opposed to any direct contribution from UV photons (Reidmiller *et al.* 2003).

It has previously been reported that the generation of free radicals from H_2O_2 by interaction with UV photons is optimal at 50°C (Reidmiller *et al.* 2003; Suty *et al.* 2004; Yaun *et al.* 2004). This fact can explain the enhanced efficacy of UV–H_2O_2 to inactivate *Salmonella* on or within iceberg lettuce when the H_2O_2 was delivered at 50°C compared with 20°C. It has been reported by Crowe *et al.* (2007) that using a combination of UV and H_2O_2 did not result in greater LCR of the microflora associated with blueberries compared with that when H_2O_2 was applied alone. In a further report, Koivunen and Heinonen-Tanski (2005) did not report synergism between UV and H_2O_2 for the inactivation of pathogens in waste water. In both cases, it is possible that the lack of an observed synergistic effect was a consequence of applying H_2O_2 at ambient temperature, where the

generation of free radicals would be lower. It is also possible that, because the free radicals formed are short lived, there would be restricted diffusion in bulk solution. In the current study, the generation of free radicals in the mist or vapour phase would have facilitated greater distribution of free radicals onto the surface and internal structures of lettuce leaves thereby enhancing decontamination efficacy.

It is possible that the enhanced lethality of applying UV–H_2O_2 at 50°C could be attributed to thermal inactivation of *Salmonella* as opposed to that through the action of generated free radicals. However, this is unlikely, given that, when H_2O_2 at 50°C without UV achieved LCR that were significantly lower compared with those when UV and H_2O_2 were applied simultaneously. When UV was applied alone, the LCR of *Salmonella* achieved were again significantly lower compared with the combined treatment. From studies performed by others, the LCR of *Salmonella* on lettuce using H_2O_2 (2% v/v at 50°C) (Lin *et al.* 2002) and UV (Yaun *et al.* 2004) alone were 3·71 and 2·79, respectively. The levels of inactivation were higher than those obtained in the current study when UV and H_2O_2 were applied alone. Such differences may be attributed to the method used to inoculate samples and also the higher UV intensity (24 mW cm^{-2}) used in the reported study (Yaun *et al.* 2004).

The concentration of H_2O_2 also had an impact on the efficacy of the UV–H_2O_2 decontamination treatment. In the current study, the 1·5% v/v H_2O_2 resulted in a significantly higher LCR than when 2% H_2O_2 was applied. Other workers (Bayliss and Waites 1979; Reidmiller *et al.* 2003) have also reported that an optimal H_2O_2 concentration, when used in combination with UV, inactivate *Bacillus* endospores. The reduced decontamination efficacy at high H_2O_2concentrations can be attributed to excess radical formation that ultimately leads to neutralization owing to the interaction of hydroxyl radicals (Reidmiller *et al.* 2003).

The inclusion of Tween in the H_2O_2 solution did not enhance the efficacy of the UV–H_2O_2 treatment. This may have been unexpected given that the surfactant would have reduced the surface tension thereby facilitating the penetration of the free radicals into the inner leaf structures and also physically detaching bacteria from the surface (Hassan and Frank 2003). In addition, it has been reported that Tween contains hydroperoxides that enhance the free radical formation from H_2O_2 (Nuchi *et al.* 2001). It is possible that the main penetration of free radicals into the inner lettuce leaf structures was through the vapour as opposed to the liquid phase. Consequently, surface tension effects would be less significant and may explain the negligible effect of Tween on the overall efficacy of the process.

Attempting to compare the efficacy of the UV–H_2O_2 with that of other reported sanitizers is problematic owing to the differences in the bacterial strains applied and the inoculation or enumeration protocols. However, from reviewing the literature, typical log reductions achieved for *Salmonella* on lettuce are reported to be peroxyacetic acid 1·7 (Hellstrom *et al.* 2006), acidified sodium chlorite 3·1 (Inatsu *et al.* 2005), chlorine dioxide 1·53 (Sy *et al.* 2005), ozone 5·6 (Rodgers *et al.* 2004) and electrolysed water 1·0 (Koseki *et al.* 2003). Therefore, the LCR obtained for lettuce treated with a combination of UV–H_2O_2 in the current study (4·12 reduction in surface counts) is more effective than most of the sanitizers tested to date although comparable with ozone.

Relatively few reports have been published with respect to the efficacy of sanitizers to inactivate internalized pathogens. The complete inactivation of *Salmonella* and *E. coli* O157:H7 (0·8–2·1 log CFU g^{-1}) within tomatoes has been reported using 1·5% lactic acid spray (Ibarra-Sanchez *et al.* 2004). However, in the reported study, the researchers did not vacuum infiltrate the pathogens deep into the inner tomato tissue, suggesting that the bacterial cells were located close to the surface. Consequently, pathogens could be relatively easily inactivated on the surface than when drawn deeper within the tomato tissue. Clearly, further studies are required to establish standardized protocols to enable comparisons between sanitizers to be determined.

The optimized UV–H_2O_2 treatment exhibited variable efficacy for decontaminating different produce types. However, in all cases, the surface LCR of the different bacterial types was significantly greater than those obtained using calcium hypochlorite washes. UV–H_2O_2 treatment was more effective at decontaminating leafy vegetables compared with produce, such as cauliflower, broccoli and tomatoes. This was likely attributed to the location of bacteria being at greater depths in the latter produce types inaccessible to the generated hydroxyl radicals. Sliced Spanish onions also were difficult to decontaminate owing to the multiple layers. Therefore, although the free radicals generated from H_2O_2 could penetrate the subsurface of vegetables, bacteria located deep within the plant tissue were protected and retained viability. The limited penetration of free radicals may have been expected given their reactive nature and instability (Sommer *et al.* 2004). In practical terms, the results indicate that the efficacy UV–H_2O_2 needs to be determined and process optimized for individual produce types.

In general, the lethality of UV–H_2O_2 treatment was independent of the bacterial type. However, it was evident that *E. coli* O157:H7 did exhibit greater sensitivity

towards UV–H_2O_2 on certain produce compared with the other bacteria tested. The relative tolerance to UV–H_2O_2 can be attributed to the intrinsic resistance of the bacterium and also the spatial location on the produce surface. Lin *et al.* (2002) reported no significant difference the LCR obtained with 2% v/v H_2O_2 at 50°C for *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes*. Therefore, it is possible that the spatial location of bacteria contributes the level of pathogen inactivation. In this respect, it has been reported that *E. coli* O157:H7 weakly attaches to the surface of plant material compared with *Salmonella* (Barak *et al.* 2002). Therefore, the greater susceptibility of *E. coli* O157:H7 might have been the result of the weak attachment on produce surfaces, although it is unclear why this was produce type specific.

Previous studies using H_2O_2 frequently report browning during post-treatment storage (Lin *et al.* 2002). The browning of fresh produce exposed to H_2O_2 can be attributed to the formation of polyphenols *via* the combined action of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) (Degl'Innocenti *et al.* 2005; Fujita *et al.* 2006). The physiological role of PAL–PPO system is to protect the plant from oxidative stress by scavenging free radicals. In this respect, it is likely that the combination of UV and H_2O_2 could potentially enhance leaf browning.

Leaf browning can be readily assessed using a^* values, although L^* and hue angle have also been used previously (Castaner *et al.* 1996; Hosoda *et al.* 2000). The inhibition of browning was likely owing to the inactivation of the PPO by applying H_2O_2 at elevated temperatures. The results are in agreement with those reported by others who also observed negligible browning of lettuce when H_2O_2 or ozonated water was applied at 50°C (Fukumoto *et al.* 2002; Lin *et al.* 2002; McWatters *et al.* 2002; Koseki and Isobe 2006). The visual appearance of UV–H_2O_2-treated lettuce was comparable with that of the control, although no evidence was found to suggest extended shelf-life. The same finding has been reported for lettuce treated with H_2O_2 at 50°C, where the sensory characteristics of treated lettuce stored under modified atmosphere packaging (MAP) for 15 days was similar to the controls (McWatters *et al.* 2002). Therefore, although UV–H_2O_2 can reduce the levels of pathogens and spoilage bacteria associated with the produce, the treatment does not contribute in extending the shelf-life of fresh-cut produce.

It was noted that although UV–H_2O_2 reduced bacterial levels on and within produce, there were always residual survivors present. Therefore, there is a possibility that survivors could repair damage induced by UV–H_2O_2 and proliferate during the post-treatment storage of lettuce. This was not observed with inoculated lettuce treated with UV–H_2O_2 prior to storing at 4°C. This can be attributed to the low storage temperature which was not conducive to the growth of either *Salmonella* or *E. coli* O157:H7.

It was also noted that no reduction in the pathogen levels occurred in treated lettuce stored at 4°C, which would suggest the UV–H_2O_2 treatment does not leave antimicrobial residues on lettuce. This may have been expected given that the radicals formed are only short lived and rapidly sequestered (Sommer *et al.* 2004).

When UV–H_2O_2-treated lettuce was stored at 25°C, there was a relatively rapid growth of the residual *Salmonella* and *E. coli* O157:H7 to the extent that benefits in terms of LCR were lost within 4 days. Therefore, although applying UV–H_2O_2 results in an initial decrease in the pathogen numbers, the potential for the survivors to grow under temperature abuse conditions is high. Similar results for the growth of microflora during post-treatment storage have been reported for produce treated with hot water at 50°C (Delaquis *et al.* 2002; Stringer *et al.* 2007). Here, it was hypothesized that the decrease in competitive microflora resulted in enhanced growth of residual populations (Delaquis *et al.* 2002; Stringer *et al.* 2007).

In conclusion, the study has demonstrated the enhanced efficacy of UV–H_2O_2 to decontaminate a diverse range of produce types compared with hypochlorite-based washes. However, the treatment would only be effective if combined with other interventions to reduce the growth of pathogens during distribution and storage.

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