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ORIGINAL ARTICLE

Effect of relative humidity on inactivation of foodborne pathogens using chlorine dioxide gas and its residues on tomatoes

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Significance and Impact of the Study: This study reported on the correlation between the amount of ClO_2 residues on produce surfaces and the level of inactivation of pathogens after ClO_2 gas treatment. Variations in RH have great effect on the solubilization of ClO_2 gas on tomato surfaces considering that ClO_2 residues on tomatoes increased with increasing RH. Also, the amount of ClO_2 residues on tomatoes is positively correlated with the level of inactivation of pathogens. The results of this study provide insights for predicting inactivation patterns of foodborne pathogens by ClO_2 gas for practical application by the fresh produce industry.

Keywords
disinfection, enterohaemorrhagic *E. coli*, food safety, *Listeria*, *Salmonella*.

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Abstract

The effect of relative humidity (RH) on the antimicrobial efficacy of chlorine dioxide (ClO_2) gas against foodborne pathogens on tomatoes was evaluated. Also, levels of ClO_2 residues on tomatoes after exposure to ClO_2 gas under different RH conditions were measured to determine the quantity of solubilized ClO_2 gas on tomato surfaces. *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* were inoculated on tomatoes and exposed to ClO_2 gas (5, 10, 20 and 30 ppmv) under different RH conditions (50, 70 and 90%). As ClO_2 gas concentration and treatment time increased, significant differences ($P < 0{\cdot}05$) were observed between inactivation levels under different RH conditions. Exposure to 30 ppmv of ClO_2 gas (50% RH) for 20 min resulted in 1·22–1·52 log reductions of the three foodborne pathogens. Levels of the three foodborne pathogens were reduced to below the detection limit (0·48 log CFU per cm^2) within 15 min when exposed to 30 ppmv of ClO_2 gas at 70% RH and within 10 min at 90% RH. At a given ClO_2 gas concentration, ClO_2 residues on tomatoes significantly ($P < 0{\cdot}05$) increased with increasing RH, and there were close correlations between log reductions of pathogens and ClO_2 residues on tomatoes.

Introduction

Consumption of tomatoes has been frequently linked to foodborne outbreaks (Behraveshm *et al.* 2012; Mukhopadhyay *et al.* 2018). A total of 15 multistate outbreaks associated with raw tomatoes were reported from 1990 to 2010, and all outbreaks were caused by *Salmonella enterica* (Bennetti *et al.* 2015). Tomatoes can become contaminated with foodborne pathogens during production due to contact with animal faeces, irrigation water, wash water, handling by workers and contact with contaminated surfaces (Allen *et al.* 2005; Hanning *et al.* 2009; Ma *et al.* 2010).

Recently, several sanitizers have been evaluated to inactivate foodborne pathogens on tomatoes, including electrolysed water (Wang and Ryser 2014), alkaline water (Islam *et al.* 2015), ozone (Mukhopadhyay *et al.* 2015), aqueous chlorine dioxide (ClO_2) (São José and Vanetti

2012), hydrogen peroxide (São José and Vanetti 2012) and peracetic acid (Williams *et al.* 2012). However, washing of postharvest produce with sanitizers is insufficient for reducing foodborne pathogens without incurring sensory loss (Beuchat 2006). Thus, preventing microbial contamination at all stages in the field-to-fork continuum is preferable to treatment to eliminate contamination after it has occurred (FDA 2009).

The antimicrobial effect of gaseous sanitizers, such as ClO_2 gas, against foodborne pathogens on tomatoes has also been evaluated (Bhagat *et al.* 2010; Trinetta *et al.* 2013; Olanya *et al.* 2015). ClO_2 has emerged as a promising nonthermal sanitizing technology for fresh produce in recent years (Bhagat *et al.* 2010; Yeap *et al.* 2016). It has been well known that the antimicrobial effect of ClO_2 gas increases with increasing relative humidity (RH) (Han *et al.* 2001), but little information is available on the inactivation tendency of ClO_2 gas according to levels of RH and treatment time. In our previous study, we observed significant differences ($P < 0{\cdot}05$) in reduction levels of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated on spinach leaves exposed to ClO_2 gas under different RH conditions (Park and Kang 2015).

The objectives of this study were to evaluate the effect of RH conditions on the antimicrobial effect of chlorine dioxide (ClO_2) gas against *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on tomatoes. The quantity of solubilized ClO_2 gas on tomato surfaces was measured after exposure to ClO_2 gas under different RH conditions to determine how they affect inactivation patterns of pathogens.

Results and discussion

The effects of ClO_2 gas treatment against the three pathogens on smooth surfaces of tomato pieces are shown in Tables 1–4. Exposure to 5 ppmv of ClO_2 gas for 20 min caused 0·97–1·17 (50% RH), 1·54–1·81 (70% RH) and 2·14–2·37 (90% RH) log reductions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* (Table 1). Treatment with 10 ppmv of ClO_2 gas for 20 min resulted in 1·10–1·33 (50% RH), 1·46–1·97 (70% RH) and 3·30–4·33 (90% RH) log reductions of the three foodborne pathogens (Table 2). Treatment with 20 ppmv of ClO_2 gas for 20 min caused 1·07–1·35 (50% RH) and 3·06–4·39 (70% RH) log reductions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* (Table 3). The levels of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* were reduced to below the detection limit (0·48 log CFU per cm^2) within 15 min when treated with 20 ppmv of ClO_2 gas at 90% RH. Treatment with 30 ppmv of ClO_2 gas for 20 min resulted in 1·22–1·52 (50% RH) log reductions of the three foodborne pathogens (Table 4). Levels of the three foodborne pathogens were reduced to below the detection limit within 15 min when treated with 30 ppmv of ClO_2 gas at 70% RH. Exposure to 30 ppmv of ClO_2 gas under conditions of 90% RH reduced *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* to below the detection limit within 10 min treatment.

Figure 1 shows levels of residual ClO_2 on tomatoes treated with 10, 20 and 30 ppmv of ClO_2 gas under conditions of 50, 70 and 90% RH for 20, 15 and 10 min respectively. At a given ClO_2 gas concentration, ClO_2 residues on tomatoes significantly ($P < 0{\cdot}05$) increased with increasing RH, except for those treated with 10 ppmv of ClO_2 gas at 50 and 70% RH. No significant differences ($P > 0{\cdot}05$) in levels of ClO_2 residues were observed between samples treated with ClO_2 gas under conditions of 50% RH and the control. At 70% RH, there were no significant differences ($P > 0{\cdot}05$) between levels of ClO_2 residues on tomatoes treated with 20 ppmv of ClO_2 gas

Table 1 Log reductions* of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on tomatoes after 5 ppmv of ClO_2 gas treatment

Bacteria	Relative humidity (%)	Log reduction (log CFU per cm^2)				
		1 min	5 min	10 min	15 min	20 min
E. coli O157:H7	50	0·53 ± 0·13Aa†	0·57 ± 0·10Aa	0·58 ± 0·07Aa	0·85 ± 0·10Ba	1·17 ± 0·16Ca
	70	0·78 ± 0·14Aa	0·99 ± 0·26Aab	1·06 ± 0·11Ab	1·55 ± 0·35Ba	1·81 ± 0·08Bb
	90	0·69 ± 0·48Aa	1·15 ± 0·29ABb	1·28 ± 0·27ABb	1·74 ± 0·67BCa	2·15 ± 0·38Cb
S. Typhimurium	50	0·62 ± 0·20Aa	0·79 ± 0·02ABa	0·82 ± 0·04ABa	0·91 ± 0·21ABa	0·97 ± 0·17Ba
	70	0·53 ± 0·17Aa	0·77 ± 0·21ABa	0·85 ± 0·08Ba	1·26 ± 0·20Ca	1·54 ± 0·11Cb
	90	0·50 ± 0·09Aa	0·72 ± 0·08Ba	1·22 ± 0·03Cb	1·81 ± 0·09Db	2·14 ± 0·16Ec
L. monocytogenes	50	0·68 ± 0·07Aa	0·81 ± 0·05Aa	0·93 ± 0·40Aa	0·99 ± 0·07Aa	1·03 ± 0·34Aa
	70	0·86 ± 0·16Aa	1·20 ± 0·10ABc	1·34 ± 0·27BCa	1·51 ± 0·30BCb	1·62 ± 0·19Cb
	90	0·64 ± 0·16Aa	1·03 ± 0·03Bb	1·45 ± 0·09Ca	1·93 ± 0·18Dc	2·37 ± 0·07Ec

*Log reduction = population (log CFU per cm^2) before treatment − population (log CFU per cm^2) after ClO_2 gas treatment. Populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* before treatment were 7·02, 7·54 and 6·39 log CFU per cm^2 respectively.
†Means followed by different uppercase letters within a row were significantly different ($P < 0{\cdot}05$). Means followed by different lowercase letters within a column were significantly different ($P < 0{\cdot}05$).

Table 2 Log reductions* of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on tomatoes after 10 ppmv of ClO_2 gas treatment

Bacteria	Relative humidity (%)	Log reduction (log CFU per cm^2)				
		1 min	5 min	10 min	15 min	20 min
E. coli O157:H7	50	0·68 ± 0·23Aa†	0·80 ± 0·28Aa	0·87 ± 0·25Aa	1·07 ± 0·26Aa	1·11 ± 0·24Aa
	70	0·86 ± 0·15Aa	0·97 ± 0·21ABab	1·29 ± 0·19Ba	1·64 ± 0·18Cb	1·97 ± 0·20Cb
	90	0·97 ± 0·05Aa	1·56 ± 0·42Ab	2·34 ± 0·32Bb	2·98 ± 0·25Bc	4·26 ± 0·65Cc
S. Typhimurium	50	0·62 ± 0·02Aa	0·82 ± 0·35ABa	1·11 ± 0·38ABa	1·22 ± 0·36ABa	1·33 ± 0·42Ba
	70	0·62 ± 0·18Aa	0·95 ± 0·05ABa	1·19 ± 0·31BCa	1·43 ± 0·24Ca	1·92 ± 0·17 Da
	90	0·89 ± 0·34Aa	1·36 ± 0·32ABa	1·92 ± 0·36Bb	2·61 ± 0·24Cb	4·33 ± 0·40Db
L. monocytogenes	50	0·69 ± 0·38Aa	0·74 ± 0·03Aa	0·98 ± 0·48Aa	1·07 ± 0·36Aa	1·10 ± 0·52Aa
	70	0·70 ± 0·29Aa	0·78 ± 0·24Aa	0·94 ± 0·24ABa	1·27 ± 0·24BCa	1·46 ± 0·25Ca
	90	0·61 ± 0·28Aa	0·82 ± 0·24Aa	1·44 ± 0·27Ba	2·47 ± 0·30Cb	3·30 ± 0·47Db

*Log reduction = population (log CFU per cm^2) before treatment – population (log CFU per cm^2) after ClO_2 gas treatment. Populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* before treatment were 7·29, 6·68 and 6·32 log CFU per cm^2 respectively.
†Means followed by different uppercase letters within a row were significantly different ($P < 0·05$). Means followed by different lowercase letters within a column were significantly different ($P < 0·05$).

Table 3 Log reductions† of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on tomatoes after 20 ppmv of ClO_2 gas treatment

Bacteria	Relative humidity (%)	Log reduction (log CFU per cm^2)				
		1 min	5 min	10 min	15 min	20 min
E. coli O157:H7	50	0·59 ± 0·09Aa[b]	0·57 ± 0·13Aa	0·79 ± 0·18ABa	0·84 ± 0·09BCa	1·07 ± 0·14Ca
	70	0·43 ± 0·12Aa	1·09 ± 0·28Bb	1·99 ± 0·43Cb	3·13 ± 0·55Db	4·39 ± 0·08Eb
	90	0·45 ± 0·15Aa	1·41 ± 0·21Bb	3·60 ± 0·53Cc	>6·74Dc	>6·74Dc
S. Typhimurium	50	0·64 ± 0·17Aa	0·70 ± 0·05Aa	0·88 ± 0·06ABa	1·10 ± 0·21BCa	1·35 ± 0·29Ca
	70	0·47 ± 0·23Aa	1·06 ± 0·30Bab	1·68 ± 0·13Cb	2·51 ± 0·23Db	3·88 ± 0·30Eb
	90	0·55 ± 0·09Aa	1·38 ± 0·31Bb	3·50 ± 0·49Cc	>6·93Dc	>6·93Dc
L. monocytogenes	50	0·66 ± 0·19Aa	0·94 ± 0·13ABa	0·96 ± 0·09ABa	1·07 ± 0·43ABa	1·23 ± 0·09Ba
	70	0·43 ± 0·43Aa	0·87 ± 0·51ABa	1·61 ± 0·57BCa	2·17 ± 0·19Cb	3·06 ± 0·55Db
	90	0·49 ± 0·52Aa	1·51 ± 0·30Ba	3·15 ± 0·45Cb	>5·87Dc	>5·87Dc

*Log reduction = population (log CFU per cm^2) before treatment – population (log CFU per cm^2) after ClO_2 gas treatment. Populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* before treatment were 7·18, 7·21 and 6·75 log CFU per cm^2 respectively.
†Means followed by different uppercase letters within a row were significantly different ($P < 0·05$). Means followed by different lowercase letters within a column were significantly different ($P < 0·05$).

for 15 min *vs* those treated with 30 ppmv of ClO_2 gas for 10 min. Also, at 90% RH, ClO_2 residues on tomatoes treated with 20 ppmv of ClO_2 gas for 15 min were not significantly ($P > 0·05$) different from those treated with 30 ppmv of ClO_2 gas for 10 min.

The L^* value of tomatoes decreased while the a^* and b^* values increased during storage (data not shown). However, no significant ($P > 0·05$) differences in Hunter's colour values (L^*, a^*, b^*) between untreated samples (control) and those treated with ClO_2 gas (30 ppmv) for 20 min were observed during 7 days storage at 12°C. There were no significant ($P > 0·05$) differences in firmness between control and treated tomatoes during storage at 12°C for 7 days (data not shown). Also, there were no significant ($P > 0·05$) differences in sensory evaluation (colour, flavour, texture and overall acceptability) between untreated and ClO_2 gas-treated (30 ppmv for 20 min) samples after storage at 12°C for 7 days (data not shown).

Han *et al.* (2001) reported the antimicrobial effect of ClO_2 gas increases with increasing RH. After exposure to 0·3 mg l^{-1} ClO_2 gas at 15°C, reductions of *E. coli* O157:H7 on green peppers increased from 1·93 to 4·00 log CFU/5 g as RH increased from 55 to 95%. Survival of *Lactobacillus buchneri* on stainless steel strips after exposure to 8 mg l^{-1} ClO_2 gas for 10 min decreased as RH increased from 56 to 94% (Han *et al.* 1999). Regarding this synergistic relation between ClO_2 gas and RH, most studies have evaluated the antimicrobial effect of ClO_2 gas under high RH conditions (>80%) (Bhagat *et al.* 2011;

Table 4 Log reductions* of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* on tomatoes after 30 ppmv of ClO_2 gas treatment

Bacteria	Relative humidity (%)	Log reduction (log CFU per cm^2)				
		1 min	5 min	10 min	15 min	20 min
E. coli O157:H7	50	0·70 ± 0·09Aa†	0·75 ± 0·03Aa	1·14 ± 0·12Ba	1·19 ± 0·24Ba	1·39 ± 0·24Ba
	70	0·36 ± 0·26Aa	0·85 ± 0·34Aa	2·83 ± 0·56Bb	>6·85Cb	>6·85Cb
	90	0·89 ± 0·03Aa	2·02 ± 0·18Bb	>6·85Cc	>6·85Cb	>6·85Cb
S. Typhimurium	50	0·75 ± 0·11Aa	0·92 ± 0·17ABa	1·13 ± 0·10ABa	1·08 ± 0·15ABa	1·22 ± 0·38Ba
	70	0·73 ± 0·44Aa	1·17 ± 0·37Aa	2·48 ± 0·50Bb	>7·01Cb	>6·01Cb
	90	0·89 ± 0·03Aa	2·02 ± 0·18Bb	>6·90Cc	>6·90Cb	>6·90Cb
L. monocytogenes	50	0·44 ± 0·10Aab	0·71 ± 0·22ABa	0·89 ± 0·26Ba	1·07 ± 0·23Ba	1·52 ± 0·29Ca
	70	0·24 ± 0·02Aa	0·70 ± 0·15Aa	2·28 ± 0·59Bb	>5·49Cb	>5·49Cb
	90	0·68 ± 0·29Ab	1·95 ± 0·19Bb	>5·78Cc	>5·78Cb	>5·78Cb

*Log reduction = population (log CFU per cm^2) before treatment – population (log CFU per cm^2) after ClO_2 gas treatment. Populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* before treatment were 7·33, 7·36 and 6·45 log CFU per cm^2 respectively.
†Means followed by different uppercase letters within a row were significantly different ($P < 0·05$). Means followed by different lowercase letters within a column were significantly different ($P < 0·05$).

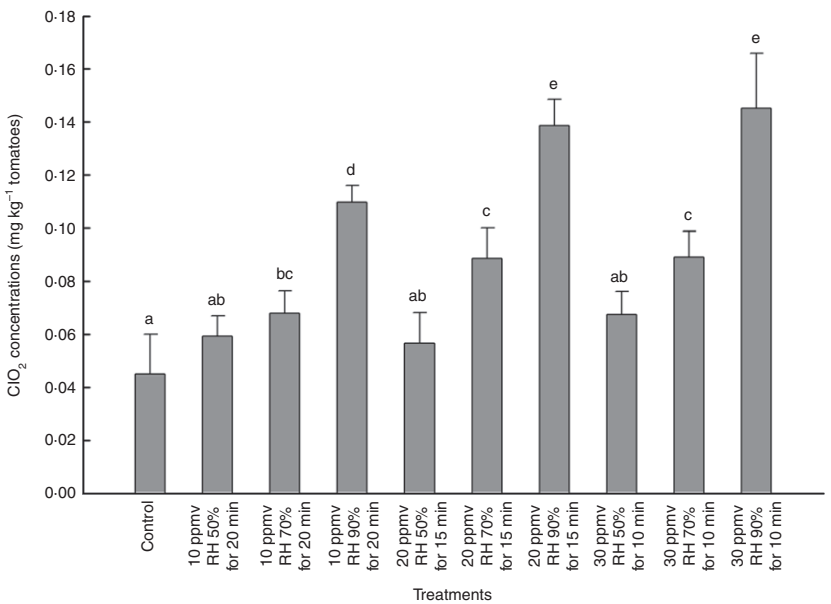

Figure 1 Residual ClO_2 on tomatoes immediately following treatment with 10, 20 or 30 ppmv ClO2 gas under 50, 70 or 90% RH for 20, 15 or 10 min respectively. Values with different lowercase letters were significantly different ($P < 0.05$). Values are expressed as the mean values of triplicate experiments, with error bars.

Nam *et al.* 2014; Kim and Song 2017; Park and Kang 2017).

However, little information is available on the inactivation tendency of ClO_2 gas according to levels of RH and treatment time. In the present study, significant differences ($P < 0·05$) were observed between inactivation levels under different RH conditions as ClO_2 gas concentration increased. Park and Kang (2015) reported that populations of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on spinach leaves subjected to ClO_2 gas treatments were reduced more under conditions of 90% RH than under 50 and 70% RH. Generally, no significant differences ($P > 0·05$) in reduction levels of the three foodborne pathogens were observed between 50 and 70% RH. However, significant differences ($P < 0·05$) in inactivation levels were observed between 50 and 70% RH as ClO_2 gas concentration and treatment time increased. These results are in agreement with the results of this study.

Differences in inactivation levels may due to the different solubility of ClO_2 gas under different levels of RH. ClO_2 gas acts similar to aqueous ClO_2 for inactivating micro-organisms due to its high solubility in water (Linton *et al.* 2006). To determine the quantity of solubilized ClO_2 gas on the surface of tomatoes, the *N*, *N*-diethyl-*p*-phenylenediamine (DPD) method used. ClO_2 residues on

tomatoes significantly ($P < 0{\cdot}05$) increased with increasing RH at a given ClO_2 gas concentration. This tendency follows the inactivation patterns of foodborne pathogens under different RH conditions. For example, under 70 and 90% RH conditions, inactivation levels of the three pathogens by 20 ppmv ClO_2 gas treatment for 15 min was not significantly different ($P > 0{\cdot}05$) from that of 30 ppmv of ClO_2 for 10 min. Also, as shown in Fig. 1, no significant differences were observed ($P > 0{\cdot}05$) between ClO_2 residues on tomatoes treated with 20 ppmv of ClO_2 gas under 70 and 90% RH for 15 min and those treated with 30 ppmv of ClO_2 gas at 70 and 90% RH for 10 min respectively. These results indicate that there are positive correlations between log reductions of pathogens and ClO_2 gas residues on tomatoes.

In the present study, there were generally no significant differences in the sensitivity of the three pathogens to ClO_2 gas. Some researchers have reported *L. monocytogenes* was only slightly more susceptible to ClO_2 gas treatment than *E. coli* O157:H7 and *S. enterica* (Han *et al.* 2004; Lee *et al.* 2004; Mahmoud *et al.* 2007). However, Bhagat *et al.* (2010) reported the *D*-values for *S. enterica* and *L. monocytogenes* after ClO_2 gas treatment did not differ significantly ($P > 0{\cdot}05$). The different results in the sensitivity to ClO_2 gas treatment may be due to strain of pathogens used in each study.

The results of this study provide insights for predicting inactivation patterns of foodborne pathogens by ClO_2 gas for practical application by the fresh produce industry. However, further research is needed to validate the efficacy of ClO_2 gas treatment in inactivating foodborne pathogen on tomatoes in packed or palletized cartons for the practical application of ClO_2 gas.

Materials and methods

Bacterial cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971 and DT 104) and *L. monocytogenes* (ATCC 19111, ATCC 19115 and ATCC 15313) were obtained from the bacterial culture collection of the School of Food Science, Seoul National University (Seoul, South Korea). Each strain was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA), incubated for 24 h at 37°C, collected 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 sterile BPW, corresponding to approximately 10^7–10^8 CFU per ml. Afterwards, suspended pellets of the three pathogens were combined to comprise a mixed culture cocktail of each strain of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*.

Sample inoculation

Globe tomatoes (90–110 g) were purchased from a local market (Seoul, South Korea) and stored at 12°C for a maximum of 2 days before use. Tomatoes used in this study were supplied by the same producer in order to maximize the reproducibility of the results. Tomatoes were washed in running water and dried in a laminar flow biosafety hood (22 ± 2°C) for 1 h before experiments. Tomatoes used in this study were previously confirmed to ensure no presumptive *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* like colonies were grown from uninoculated tomatoes. The outer surface of tomatoes was cut into 5 × 2 cm pieces. Prepared tomato surfaces were placed on aluminium foil in a laminar flow biosafety hood, and 0·1 ml of culture cocktail was inoculated onto one side of the sample by depositing droplets with a micropipettor at 15–20 locations. After inoculation, samples were dried in the hood at 22 ± 2°C for 1 h with the fan running.

ClO_2 gas treatment

ClO_2 gas treatment was conducted in a treatment system described previously (Park and Kang 2015). Tomatoes samples were placed in the treatment chamber with the inoculated surfaces facing upwards and covered with a plastic lid. Samples were treated with 1, 5, 10, 20 and 30 ppmv of ClO_2 gas at 22 ± 1°C for 1, 5, 10, 15 and 20 min. The RH of the treatment chamber was adjusted to 50, 70 and 90% with an accuracy of ±2% with a ultrasonic nebulizer (H-C976, Osungsa, Changwon-si, South Korea). RH in the treatment chamber was monitored with a thermohygrometer (YTH-600, Uins, Seoul, South Korea). When the desired ClO_2 gas concentration and RH were achieved, the plastic lid was removed and samples were exposed to the treatment.

Bacterial enumeration

One treated piece of tomato was immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 30 ml of neutralizing buffer (Difco) to quench the remaining ClO_2 residues in the sample. Samples were homogenized with a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. After homogenization, 1 ml aliquots withdrawn from stomacher bags were 10-fold serially diluted in BPW, and 0·1 ml of appropriate diluents were spread-plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco) and Oxford agar base (OAB; Difco) with antimicrobic supplement (Difco) were used as selective media for

enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* respectively. Where low levels of surviving cells were expected, 1 ml of aliquots withdrawn from stomacher bags were divided between four plates of each medium and spread-plated to lower the detection limit. The plates were incubated at 37°C for 24–48 h. Colonies were counted after incubation and calculated as log CFU per cm^2.

Residual ClO_2 on tomatoes after treatment

Whole tomatoes were exposed to 10, 20 and 30 ppmv of ClO_2 gas under different RH conditions (50, 70 and 90%) for 20, 15 and 10 min respectively. After treatment, tomato surfaces were immediately rinsed with 100 ml of sterile distilled water in sterile stomacher bags and massaged by hand for 5 min. A quantity of 10 ml of sample was removed from each stomacher bag and tested by the DPD method using a Hach DR/820 Colorimeter (Hach, Loveland, Co) (Trinetta *et al.* 2011). The limit of detection for this method is 0·04 mg l^{-1}. ClO_2 residues in rinse water were reported as mg l^{-1} and subsequently converted to mg kg^{-1} of tomatoes. Untreated tomatoes were used as a control.

Colour and texture measurement

Treated and untreated (control) whole tomatoes (uninoculated) were stored at 12°C for 7 days to identify quality changes during storage following each treatment. Colour values (Hunter's L, a, b) of tomatoes were measured with a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) at three locations on each sample. The texture of tomatoes was evaluated with a texture analyser (TA-CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA) with a cylinder probe with a 4 mm diameter. The loading rate and path length were set at 2 mm s^{-1} and 10 mm. Three measurements were performed with independently prepared samples for each treatment. Texturepro CT software (Brookfield Engineering Laboratories, Inc.) was used to record maximum force.

Sensory evaluation

A sensory evaluation was conducted to assess the changes in quality of treated and untreated whole tomato samples following storage at 12°C for 7 days. Samples were analysed for their colour, flavour, texture and overall acceptability. The sensory qualities of the samples were evaluated using a nine-point scoring method as follows: 8–9, very good; 6–7, good; 4–5, fair; 2–3, poor; and 1, very poor. Evaluations were conducted by 10 untrained sensory panel and they was instructed prior to analysis on applying the score system.

Statistical analysis

Microbial reductions were given as means ± standard deviations of three independent determinations with duplicate samples at each trial. Residual ClO_2, colour and texture measurement were done in triplicates with each measurement repeated thrice. Data were analysed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC), and separation of means by the Duncan's multiple range test at a probability level of $P < 0{\cdot}05$.

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Conflict of Interest

No conflict of interest declared.

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