International Journal of Food Microbiology 207 (2015) 103–108

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Combination treatment of chlorine dioxide gas and aerosolized sanitizer for inactivating foodborne pathogens on spinach leaves and tomatoes

Sang-Hyun Park, Dong-Hyun Kang *

Department of Food and Animal Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
Institutes of Green BioScience & Technology, Seoul National University, Pyeongchang-gun Gangwon-do 232-916, Republic of Korea

ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form 24 April 2015
Accepted 27 April 2015
Available online 2 May 2015

Keywords:
Chlorine dioxide gas
Spinach
Tomato
Escherichia coli O157:H7
Salmonella Typhimurium
Listeria monocytogenes

ABSTRACT

The objective of this study was to evaluate the antimicrobial effect of chlorine dioxide (ClO_2) gas and aerosolized sanitizer, when applied alone or in combination, on the survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* inoculated onto spinach leaves and tomato surfaces. Spinach leaves and tomatoes were inoculated with a cocktail of three strains each of the three foodborne pathogens. ClO_2 gas (5 or 10 ppmv) and aerosolized peracetic acid (PAA) (80 ppm) were applied alone or in combination for 20 min. Exposure to 10 ppmv of ClO_2 gas for 20 min resulted in 3.4, 3.3, and 3.4 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves, respectively. Treatment with 80 ppm of aerosolized PAA for 20 min caused 2.3, 1.9, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) for 20 min caused 5.4, 5.1, and 4.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes experienced similar reduction patterns to those on spinach leaves. As treatment time increased, most combinations of ClO_2 gas and aerosolized PAA showed additive effects in the inactivation of the three pathogens. Combined treatment of ClO_2 gas and aerosolized PAA produced injured cells of three pathogens on spinach leaves while generally did not produce injured cells of these pathogens on tomatoes. Combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) did not significantly ($p > 0.05$) affect the color and texture of samples during 7 days of storage.

1. Introduction

Consumption of fresh produce has increased because of its health benefits (Perni et al., 2008). However, several foodborne outbreaks related to the presence of pathogenic bacteria in fresh produce have been reported in recent years (Fernández and Thompson, 2012). Fresh spinach and spinach-containing products were implicated in an outbreak of *Escherichia coli* O157:H7 (CDC, 2006; Maki, 2006) which infected a total of 205 persons and resulted in 4 deaths (Wendel et al., 2009). In 2012, a total of 33 persons infected with *E. coli* O157:H7 traced to organic spinach and spring mix blend was reported from 5 US states (CDC, 2012). Tomatoes were associated with more than 14 outbreaks of foodborne illness between 1996 and 2008, and accounted for 17% of all produce-associated outbreaks in the United States during that period (Gravani, 2009).

Chlorine dioxide (ClO_2) has emerged as a promising non-thermal sanitizing technology for fresh produce in recent years (Bhagat et al., 2010). Several factors such as gas concentration, relative humidity (RH), treatment time, and temperature could affect the antimicrobial effect of ClO_2 gas. Especially, the combination of gas concentration and RH shows a synergistic effect (Han et al, 2001a; Park and Kang, 2015). The antimicrobial effect of ClO_2 gas has been evaluated on fresh produce such as spinach (Neal et al., 2012), potatoes (Wu and Rioux, 2010), mung bean sprouts (Prodduk et al., 2014), lettuce (Mahmoud and Linton, 2008), onions, cabbage (Sy et al., 2005), cantaloupe (Mahmoud et al., 2008), and strawberries (Han et al., 2004). However, the concentration of ClO_2 gas used in previous studies was excessive (Morino et al., 2011).

Combinations of different technologies, known as hurdle technology, could be an alternative to the use of high ClO_2 gas concentrations. Combined treatments could achieve required levels of food safety and the maintenance of organoleptic qualities of foods, while decreasing the intensity of each hurdle, that is, the antimicrobial concentration (Leistner and Gorris, 1995). Studies which evaluated sanitizer–sanitizer or sanitizer–novel technique combinations have both drawn great attention (Huang and Chen, 2011; Singh et al., 2002).

Aerosolization, another non-thermal technology, is the dispersion of a liquid material as a fine mist in air (Oh et al., 2005a, 2005b). Some studies have investigated the efficacy of aerosolized sanitizers for inhibiting foodborne pathogens on fresh produce. Aerosolized peroxyacetic acid,

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea. Tel.: +82 2 880 4927; fax: +82 2 883 4928.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

http://dx.doi.org/10.1016/j.ijfoodmicro.2015.04.044
0168-1605/

hydrogen peroxide, and malic acid were effective for controlling *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on lettuce and spinach leaves (Choi et al., 2012; Huang et al., 2012; Oh et al., 2005a, 2005b). Not only the antimicrobial effect, but also the ability to control humidity is an advantage of aerosolized sanitizers for combination with ClO_2 gas. Since aerosolized sanitizer exists as a fine mist dispersed in air, it can be used to control RH of the ClO_2 gas treatment chamber. Thus, aerosolized sanitizers in combination with ClO_2 gas could enhance the inactivation efficacy of ClO_2 gas by maintaining conditions of high RH.

The objective of this study was to evaluate the antimicrobial effect of ClO_2 gas and aerosolized sanitizer, when applied alone or in combination, on the survival of inoculated *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves and tomato surfaces. Also, any changes in color and texture of samples were assessed.

2. Materials and methods

2.1. Bacterial strains and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19586, ATCC 43174, DT 104), and *L. monocytogenes* (ATCC 7644, ATCC 19114, ATCC 19115) were provided by the bacterial culture collection of the Food Hygiene Laboratory at Seoul National University (SNCC; Seoul, Korea), for this study. All strains of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were cultured individually in 5 ml of tryptic soy broth (TSB; Difco, Sparks, MD, USA) at 37 °C for 24 h, followed by centrifugation (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 7–8 log CFU/ml. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* strains were combined to make culture cocktails for use in experiments.

2.2. Sample preparation and inoculation

Spinach and whole tomatoes were purchased from a local market (Seoul, South Korea). Spinach leaves were trimmed to approximately 5 cm × 3 cm in size, and the outer surface of tomatoes was cut into 5 cm × 2 cm size pieces. Prepared spinach leaves and tomato surface samples were placed on aluminum foil in a laminar flow biosafety hood, and 0.1 ml of culture cocktail was inoculated onto one side of each prepared sample by depositing droplets with a micropipettor at 15–20 locations. Samples were dried in the hood for 1 h at 22 ± 2 °C with the fan running.

2.3. Combined treatment system of ClO_2 gas and aerosolized sanitizer

The combined treatment of ClO_2 gas and aerosolized sanitizer was conducted in the treatment apparatus described previously (Park and Kang, 2015). ClO_2 gas produced by the ClO_2 gas generator (Daehan E&B, Goyang-si, South Korea) was introduced into the polyvinyl chloride treatment chamber (length × width × height, 0.7 m × 0.5 m × 0.6 m). A ClO_2 gas transmitter (ATi F12, Analytical Technology, UK) was used to monitor and control the concentration of ClO_2 gas in the treatment chamber. A ring blower (HRB-101, Hwanghae electronic, Incheon, South Korea) was used to continuously circulate ClO_2 gas in the treatment chamber. A commercial ultrasonic nebulizer (H-C976, Osungsa, Changwon-si, South Korea) was used to control RH in the treatment chamber by generating aerosolized sanitizer or distilled water. RH and temperature in the treatment chamber were monitored with a thermohygrometer (YTH-600, Uins, Seoul, South Korea).

2.4. Procedures for treating samples

Peracetic acid (PAA) (Omega Chemical, Gyeongbuk, Korea) was used as an aqueous sanitizer and diluted with distilled water to a concentration of 80 ppm. The U.S. Food and Drug Administration (FDA) approved the use of PAA for sanitizing fruits and vegetables at concentrations that do not exceed 80 ppm in wash water (Anonymous, 2000). Inoculated spinach leaves and tomatoes were placed in the treatment chamber and covered with a plastic lid. For treatments with ClO_2 gas alone, samples were subjected to 5 or 10 ppmv ClO_2 gas for 20 min. The RH of the treatment chamber was adjusted with distilled water to 90% with an accuracy of ± 2%. For treatment with only aerosolized PAA, samples were exposed to 80 ppm of aerosolized PAA for 20 min. During treatment, the RH of the treatment chamber was adjusted with aerosolized PAA to 90 ± 2%. For combined treatments, samples were subjected to ClO_2 gas (5 or 10 ppmv) and 80 ppm of aerosolized PAA for 20 min. The RH of the treatment chamber was adjusted with aerosolized PAA to 90 ± 2% during treatment. All experiments were performed at 22 ± 2 °C. When the desired ClO_2 gas concentration and RH were achieved, the plastic lid was removed and the inoculated side of spinach leaves and tomatoes were exposed to ClO_2 gas. Samples were withdrawn after 5, 10, 15, and 20 min exposure to each treatment, and treated samples were used to determine surviving bacterial populations. These experiments were repeated three times.

2.5. Bacterial enumeration

Treated and untreated (control) spinach leaves (10 ± 0.2 g) and one piece of tomato were transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 90 or 30 ml of neutralizing buffer (Difco), respectively. Stomacher bags were homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml sample aliquots were tenfold serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto selective media. Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Desoxycholate agar (XLD; Difco), and Modified Oxford Medium (MOX; Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Where low numbers of surviving cells were anticipated, 250 μl of undiluted sample was plated onto each of four plates to lower the detection limit. The plates were incubated at 37 °C for 24–48 h, and colonies were counted after incubation.

For the resuscitation of injured *E. coli* O157:H7, phenol red agar base (Difco) with 1% sorbitol (SPRAB) was used (Rhee et al., 2003). One hundred microliter of sample or diluent was spread-plated onto SPRAB and incubated at 37 °C for 24 h. Injured cells of *S.* typhimurium and *L. monocytogenes* were enumerated using the overlay (OV) method proposed by Kang and Fung (1999, 2000). One hundred microliter of sample or diluent was spread-plated onto TSA and incubated at 37 °C for 2 h to allow injured cells to resuscitate before overlaying with 7 mL of XLD (OV-XLD) or MOX (OV-MOX) for *S.* Typhimurium and *L. monocytogenes*, respectively. The plates were incubated at 37 °C for 22 h after the overlay solidified. Where low numbers of surviving cells were anticipated, 250 μl of undiluted cell suspension was plated onto four plates of each respective medium.

2.6. Measurement of color and texture of samples

Treated spinach leaves and tomatoes (uninoculated) were stored at 7 °C for 7 days to identify quality changes during storage following each treatment. Color values (Hunter's L, a, b) of spinach leaves and tomatoes were measured with a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan) at 3 locations on each sample. The texture of spinach leaves and tomatoes was evaluated with a texture analyzer (TA-CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a blade set and cylinder probe with a 4 mm diameter, respectively. Twenty grams of spinach leaves was placed onto the press holder with the stems positioned perpendicular to the path of the blade, and a blade was moved down at 2 mm/s (path length 10 mm). For tomatoes, the loading rate and path length were set at 2 mm/s and 10 mm. Three

Table 1
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves treated with aerosolized PAA (80 ppm), 5 ppmv ClO_2 gas, and both technologies simultaneously (PAA-ClO_2 gas)[a].

Treatment time	Log reduction (log CFU/g)											
	E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
	Aerosolized PAA (SMAC)	ClO_2 gas (5 ppmv) (SMAC)	PAA-ClO_2 gas		Aerosolized PAA (XLD)	ClO_2 gas (5 ppmv) (XLD)	PAA-ClO_2 gas		Aerosolized PAA (MOX)	ClO_2 gas (5 ppmv) (MOX)	PAA-ClO_2 gas	
			SPRAB	SMAC			OV-XLD	XLD			OV-MOX	MOX
5 min	0.6(0.3)A[b]	1.3(0.2)A	1.0(0.5)Aa	1.0(0.1)Aa	1.1(0.1)A	1.1(0.2)A	0.8(0.1)Aa	1.2(0.3)Aa	0.6(0.2)A	0.7(0.4)A	0.3(0.11)Aa	0.8(0.3)Ab
10 min	1.5(0.2)B	1.9(0.4)AB	1.7(0.2)Ba	1.8(0.3)Ba	1.6(0.1)B	1.6(0.1)B	1.3(0.1)Ba	1.7(0.2)Ab	0.8(0.2)A	0.8(0.4)A	0.8(0.1)Ba	1.2(0.4)Aa
15 min	1.6(0.2)B	2.0(0.2)AB	1.9(0.3)Ba	2.6(0.5)Ca	1.7(0.4)B	1.8(0.3)BC	1.8(0.2)Ca	2.3(0.1)Bb	0.8(0.2)A	1.3(0.3)A	1.3(0.2)Ca	2.3(0.6)Bb
20 min	2.3(0.1)C	2.3(0.5)B	3.2(0.3)Ca	4.2(0.5)Db	1.9(0.2)B	2.2(0.3)C	3.0(0.1)Da	3.9(0.4)Cb	0.8(0.3)A	2.0(0.4)B	2.1(0.1)Da	2.6(0.3)Bb

[a] Log reduction = population (log CFU/g) before treatment − population (log CFU/g) after treatment. SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; MOX, modified Oxford agar; OV-MOX, overlay MOX agar on TSA.
[b] Means with different uppercase letters in the same column are significantly different ($p < 0.05$). Within the PAA-ClO_2 gas columns, means with different lowercase letters in the same row are significantly different ($p < 0.05$).

measurements were performed with independently-prepared samples for each treatment. Texturepro CT software (Brookfield Engineering Laboratories, Inc.) was used to record maximum force.

2.7. Statistical analysis

All experiments were done in triplicate. Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC, USA), and separation of means by Duncan's multiple range test at a probability level of $p < 0.05$.

3. Results

3.1. Effects of ClO_2 gas, aerosolized sanitizer, and combination treatment on populations of E. coli O157:H7, S. Typhimurium, and L. monocytogenes

Microbial reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves by ClO_2 gas, aerosolized PAA, and combined treatment of both technologies simultaneously are shown in Tables 1–2. In general, antimicrobial effects of combined treatments of ClO_2 gas (5 ppmv) and aerosolized PAA (80 ppm) were not superior to those of individual treatments during 10 min exposure. After 15 min treatment, the combination of both treatments resulted in more significant ($p < 0.05$) microbial reduction of the three foodborne pathogens than each treatment alone. Levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells following 15 min treatment with ClO_2 gas were reduced by 2.0, 1.8, and 1.3 log, respectively. Exposure to aerosolized PAA for 15 min resulted in 1.6, 1.7, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (5 ppmv) and aerosolized PAA for 15 min resulted in 2.6, 2.3, and 2.3 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (5 ppmv) and aerosolized PAA showed an additive effect after 20 min treatment: the total microbial inactivation of the combined treatment was not significantly ($p > 0.05$) different from the sum of individual treatments. Exposure to 5 ppmv of ClO_2 gas for 20 min resulted in 2.3, 2.2, and 2.0 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Treatment with aerosolized PAA for 20 min caused 2.3, 1.9, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (5 ppmv) and aerosolized PAA for 20 min resulted in 4.2, 3.9, and 2.6 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Similarly, most combinations of ClO_2 gas (10 ppmv) and aerosolized PAA showed additive effects in the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* after 15 min treatment. Exposure to 10 ppmv of ClO_2 gas for 20 min resulted in 3.4, 3.3, and 3.4 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA for 20 min produced 5.4, 5.1, and 4.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively.

Tables 3–4 shows microbial reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes following ClO_2 gas, aerosolized PAA, and combined treatment of both technologies simultaneously. *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes showed similar reduction patterns to those on spinach leaves. As treatment time increased, most combinations of ClO_2 gas and aerosolized PAA showed additive effects in the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, with inactivation generally superior to that of each treatment applied individually. Levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* cells following 20 min treatment of ClO_2 gas (10 ppmv) were reduced by 3.9, 3.5, and 3.4 log, respectively. Treatment with aerosolized PAA for 20 min caused 1.3, 1.3, and 0.8 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. Combined

Table 2
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves treated with aerosolized PAA (80 ppm), 10 ppmv ClO_2 gas, and both technologies simultaneously (PAA-ClO_2 gas)[a].

Treatment time	Log reduction (log CFU/g)											
	E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
	Aerosolized PAA (SMAC)	ClO_2 gas (10 ppmv) (SMAC)	PAA-ClO_2 gas		Aerosolized PAA (XLD)	ClO_2 gas (10 ppmv) (XLD)	PAA-ClO_2 gas		Aerosolized PAA (MOX)	ClO_2 gas (10 ppmv) (MOX)	PAA-ClO_2 gas	
			SPRAB	SMAC			OV-XLD	XLD			OV-MOX	MOX
5 min	0.6(0.3)A[b]	1.1(0.4)A	0.9(0.4)Aa	1.6(0.1)Ab	1.0(0.2)A	0.7(0.2)A	0.9(0.1)Aa	1.4(0.3)Aa	0.6(0.2)A	1.0(0.2)A	0.5(0.2)Aa	1.4(0.4)Ab
10 min	1.5(0.2)B	2.0(0.1)B	2.2(0.4)Ba	2.5(0.4)Ba	1.6(0.2)B	1.6(0.2)B	1.3(0.1)Ba	2.1(0.1)Bb	0.8(0.2)A	1.4(0.1)A	1.1(0.2)Ba	1.9(0.4)Ab
15 min	1.6(0.2)B	2.5(0.4)C	3.1(0.6)Ca	3.9(0.3)Ca	1.8(0.3)B	2.7(0.2)C	2.5(0.3)Ca	3.6(0.4)Cb	0.8(0.2)A	2.5(0.5)B	2.0(0.6)Ca	3.5(0.2)Bb
20 min	2.3(0.1)C	3.4(0.1)D	5.0(0.1)Da	5.4(0.3)Da	1.9(0.1)B	3.3(0.2)D	4.8(0.1)Da	5.1(0.3)Da	0.8(0.3)A	3.4(0.3)C	4.0(0.1)Da	4.1(0.2)Ca

[a] Log reduction = population (log CFU/g) before treatment − population (log CFU/g) after treatment. SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; MOX, modified Oxford agar; OV-MOX, overlay MOX agar on TSA.
[b] Means with different uppercase letters in the same column are significantly different ($p < 0.05$). Within the PAA-ClO_2 gas columns, means with different lowercase letters in the same row are significantly different ($p < 0.05$).

Table 3
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes treated with aerosolized PAA (80 ppm), 5 ppmv ClO_2 gas, and both technologies simultaneously (PAA-ClO_2 gas)[a].

Treatment time	Log reduction (log CFU/cm^2)											
	E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
	Aerosolized PAA (SMAC)	ClO_2 gas (5 ppmv) (SMAC)	PAA-ClO_2 gas		Aerosolized PAA (XLD)	ClO_2 gas (5 ppmv) (XLD)	PAA-ClO_2 gas		Aerosolized PAA (MOX)	ClO_2 gas (5 ppmv) (MOX)	PAA-ClO_2 gas	
			SPRAB	SMAC			OV-XLD	XLD			OV-MOX	MOX
5 min	0.5(0.1)A[b]	1.3(0.3)A	1.0(0.4)Aa	1.2(0.3)Aa	0.4(0.3)A	1.0(0.2)A	0.9(0.3)Aa	1.1(0.3)Aa	0.7(0.2)A	0.9(0.1)A	0.4(0.1)Aa	0.9(0.5)Aa
10 min	0.9(0.3)B	1.6(0.2)AB	1.3(0.1)Aa	2.0(0.2)Bb	0.5(0.1)AB	1.2(0.2)AB	1.3(0.4)Aa	1.9(0.3)Ba	0.7(0.4)A	1.0(0.4)AB	0.7(0.1)Aa	1.3(0.1)ABb
15 min	1.0(0.2)B	1.9(0.2)BC	2.5(0.1)Ba	2.7(0.3)Ca	1.1(0.6)AB	1.6(0.5)B	2.4(0.1)Ba	2.7(0.2)Ca	0.9(0.1)A	1.4(0.1)BC	1.4(0.1)Ba	1.6(0.1)BCa
20 min	1.2(0.1)B	2.3(0.2)C	3.5(0.4)Ca	3.8(0.1)Da	1.2(0.3)B	2.2(0.3)C	3.5(0.5)Ca	3.7(0.4)Da	0.9(0.2)A	1.6(0.2)C	1.9(0.6)Ba	1.9(0.1)Ca

[a] Log reduction = population (log CFU/cm^2) before treatment − population (log CFU/cm^2) after treatment. SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; MOX, modified Oxford agar; OV-MOX, overlay MOX agar on TSA.
[b] Means with different uppercase letters in the same column are significantly different ($p < 0.05$). Within the PAA-ClO_2 gas columns, means with different lowercase letters in the same row are significantly different ($p < 0.05$).

treatment of ClO_2 gas (10 ppmv) and aerosolized PAA for 20 min resulted in 5.1, 5.2, and 4.5 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively.

Combined treatment of ClO_2 gas and aerosolized PAA produced injured cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves. In the case of tomatoes, combined treatment of ClO_2 gas and aerosolized PAA generally did not produce injured cells of three pathogens on tomatoes.

3.2. The quality changes of spinach leaves and tomatoes during storage

During storage, the a^* value of spinach leaves decreased while the b^* value increased. However, there were no significant ($p > 0.05$) differences in Hunter's color values (L^*, a^*, b^*) between untreated samples (control) and those treated with combined ClO_2 gas (10 ppmv) and aerosolized PAA during 7 days storage at 7 °C (Table 5). In the case of tomatoes, the L^* and a^* values increased during storage. However, there were also no significant ($p > 0.05$) differences in Hunter's color values (L^*, a^*, b^*) between controls and those treated with combined ClO_2 gas (10 ppmv) and aerosolized PAA during storage.

Table 6 shows the effects of combined treatment of ClO_2 gas (10 ppmv) and aerosolized PAA on the texture of samples. There were no significant ($p > 0.05$) differences in texture between control and treated samples during storage at 7 °C for 7 days.

4. Discussion

Several studies have reported antimicrobial effects of ClO_2 gas on produce including spinach and tomatoes. Neal et al. (2012) reported that *Salmonella* and *E. coli* O157:H7 on spinach leaves exposed to 2.1 mg/l ClO_2 gas (generated by a sachet) for 1 h were reduced by 0.6 and 0.7 log CFU/g, respectively. Treatment with 10 mg/l ClO_2 gas for 180 s reduced the levels of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on Roma tomatoes (*Lycopersicon esculentum*) by 4.8, 3.6, and 3.0 log CFU/cm^2, respectively (Trinetta et al., 2013). Bhagat et al. (2010) reported that more than a 5 log reductions in *Salmonella* and *L. monocytogenes* were observed on tomato skin surfaces after treatment with 0.5 mg/l ClO_2 gas for 12 min. However, the concentration of ClO_2 gas used in previous reports was excessive (about 180–3600 ppmv). These concentrations were much higher than a LC50 value (32 ppmv, 90 mg/m^3) determined for rats as a single exposure (Dobson et al., 2002).

In the present study, aerosolized PAA was applied as hurdle technology to reduce the concentration of ClO_2 gas. Some studies have evaluated the antimicrobial effect of aerosolized sanitizers on produce decontamination. Oh et al. (2005a) reported that aerosolized peroxyacetic acid resulted in a 3–4 log reductions in populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on lettuce leaves. *E. coli* O157:H7 on spinach and lettuce leaves exposed to 2% malic acid for 30 min was reduced by 2.1 and 2.5 log CFU/g, respectively. Huang et al. (2012) reported that treatments of aerosolized sanitizers (2.5% lactic acid + 2% allyl isothiocyanate) resulted in >4.8 log reduction of *E. coli* O157:H7 on spinach leaves. Treatment of inoculated lettuce with aerosolized malic acid (2%) for 30 min caused 2.6 and 2.5 log reductions of *S.* Typhimurium and *E. coli* O157:H7, respectively (Choi et al., 2012).

The combination of ClO_2 gas (5 ppmv) and aerosolized PAA (80 ppm) was more effective than 10 ppmv ClO_2 gas alone for inactivating the three foodborne pathogens, except for *L. monocytogenes*, on spinach leaves and tomato surfaces after 20 min treatment. Our recent initial study (Park and Kang, 2015) revealed that exposure to 30 ppmv of ClO_2 gas for 20 min at 90% RH resulted in 5.8, 5.7, and 4.9 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves, respectively. In this study, a combination treatment of ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) for 20 min yielded 5.4, 5.1, and 4.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach

Table 4
Log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes treated with aerosolized PAA (80 ppm), 10 ppmv ClO_2 gas, and both technologies simultaneously (PAA-ClO_2 gas)[a].

Treatment time	Log reduction (log CFU/cm^2)											
	E. coli O157:H7				*S.* Typhimurium				*L. monocytogenes*			
	Aerosolized PAA (SMAC)	ClO_2 gas (10 ppmv) (SMAC)	PAA-ClO_2 gas		Aerosolized PAA (XLD)	ClO_2 gas (10 ppmv) (XLD)	PAA-ClO_2 gas		Aerosolized PAA (MOX)	ClO_2 gas (10 ppmv) (MOX)	PAA-ClO_2 gas	
			SPRAB	SMAC			OV-XLD	XLD			OV-MOX	MOX
5 min	0.6(0.2)A[b]	1.2(0.6)A	1.0(0.1)Aa	1.0(0.4)Aa	0.5(0.3)A	1.3(0.2)A	1.0(0.5)Aa	1.2(0.4)Aa	0.7(0.2)A	1.0(0.1)A	0.4(0.2)Aa	0.7(0.2)Ab
10 min	1.0(0.2)B	2.5(0.3)B	2.3(0.1)Ba	2.6(0.5)Ba	0.6(0.1)A	1.9(0.3)AB	1.7(0.3)Ba	1.9(0.3)Ba	0.7(0.3)A	1.4(0.1)A	1.4(0.1)Ba	1.5(0.1)Ba
15 min	1.1(0.1)B	3.2(0.1)BC	3.5(0.1)Ca	3.7(0.4)Ca	1.2(0.5)B	2.6(0.6)B	3.5(0.2)Ca	3.8(0.1)Ca	0.9(0.1)A	2.3(0.5)B	2.6(0.1)Ca	3.0(0.3)Ca
20 min	1.3(0.2)B	3.9(0.4)C	4.9(0.5)Da	5.1(0.4)Da	1.3(0.2)B	3.5(0.3)C	5.2(0.4)Da	5.2(0.3)Da	0.8(0.1)A	3.4(0.3)C	4.5(0.1)Da	4.5(0.2)Da

[a] Log reduction = population (log CFU/cm^2) before treatment − population (log CFU/cm^2) after treatment. SMAC, sorbitol MacConkey agar; SPRAB, phenol red agar base with 1% sorbitol; XLD, xylose lysine desoxycholate agar; OV-XLD, overlay XLD agar on TSA; MOX, modified Oxford agar; OV-MOX, overlay MOX agar on TSA.
[b] Means with different uppercase letters in the same column are significantly different ($p < 0.05$). Within the PAA-ClO_2 gas columns, means with different lowercase letters in the same row are significantly different ($p < 0.05$).

Table 5
Changes in color values[a] of spinach leaves and tomatoes combinatation-treated with ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) during storage at 7 °C for 7 days.

Day	Treatment			
	Spinach leaves		Tomatoes	
	Control	ClO_2 + aerosolized PAA	Control	ClO_2 + aerosolized PAA
L^*				
0	40.51 ± 1.27A[b]	39.93 ± 1.31A	44.07 ± 0.42A	43.15 ± 0.58A
2	40.68 ± 1.12A	40.50 ± 1.54A	44.55 ± 1.03A	44.29 ± 0.55A
4	40.51 ± 1.02A	40.21 ± 1.04A	42.52 ± 0.64A	41.41 ± 1.25A
7	39.47 ± 1.35A	38.85 ± 0.64A	41.03 ± 1.02A	41.78 ± 0.65A
a^*				
0	−6.91 ± 0.57A	−6.99 ± 0.70A	18.37 ± 2.10A	19.63 ± 0.68A
2	−6.93 ± 0.83A	−7.74 ± 0.47A	20.27 ± 2.19A	18.83 ± 0.35A
4	−7.84 ± 0.66A	−8.40 ± 0.57A	19.38 ± 1.13A	19.35 ± 1.01A
7	−8.86 ± 0.76A	−9.02 ± 0.62A	20.33 ± 0.93A	20.02 ± 0.29A
b^*				
0	9.14 ± 0.60A	9.46 ± 0.97A	19.43 ± 1.93A	22.23 ± 1.42A
2	8.97 ± 1.19A	10.01 ± 1.00A	24.50 ± 0.57A	25.37 ± 0.61A
4	10.32 ± 1.25A	10.54 ± 0.98A	25.26 ± 2.23A	24.30 ± 0.77A
7	12.76 ± 1.64A	12.53 ± 0.63A	24.22 ± 0.58A	24.17 ± 0.20A

[a] Color parameters are lightness (L^*), redness (a^*), and yellowness (b^*).
[b] Means ± standard deviations from three replications. Within the same storage time and type of vegetable, means with the same uppercase letters within a row are not significantly different ($p > 0.05$).

leaves, respectively. Also, treatment with 20 ppmv of ClO_2 gas for 15 min at 90% RH resulted in greater than 5.9, 5.7, and 5.1 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on tomatoes, respectively (unpublished). In this study, the combination treatment of ClO_2 gas (10 ppmv) and aerosolized PAA for 20 min resulted in 5.1, 5.2, and 4.5 log reductions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. These results suggest that the combination treatment of ClO_2 gas and aerosolized PAA could reduce the concentration of ClO_2 gas required, while still ensuring microbial safety.

The combination of gas concentration and RH represents a synergistic effect (Han et al., 2001a, 2001b; Park and Kang, 2015). Reductions of *E. coli* O157:H7 on green peppers increased from 1.9 to 4.0 log CFU/5 g as RH increased from 55 to 95% when green peppers were treated with 0.3 mg/l ClO_2 gas at 15 °C (Han et al., 2001a, 2001b). Several studies have also evaluated the antimicrobial effect of ClO_2 gas under conditions of high RH (>80%) (Bhagat et al., 2011; Gómez-López et al., 2008; Popa et al., 2007; Vandekinderen et al., 2009). Its own antimicrobial effect as well as the ability to control RH is an advantage of utilizing aerosolized sanitizers in combination with ClO_2 gas. Combination treatment could easily be constructed by substituting an aqueous sanitizer for distilled water in an ultrasonic nebulizer without adding any additional treatment step. Also, combined treatment of ClO_2 gas and aerosolized sanitizers may be more effective in reducing foodborne pathogens internalized or present in low numbers in inaccessible areas of produce, because both technologies have better penetration properties than aqueous sanitizers (Han et al., 2001b; Hiom et al., 2003).

Table 6
Maximum force (N) required for breakage of spinach leaves and tomatoes combination-treated with ClO_2 gas (10 ppmv) and aerosolized PAA (80 ppm) during storage at 7 °C for 7 days.

Day	Maximum force (N)			
	Spinach leaves		Tomatoes	
	Control	ClO_2 + aerosolized PAA	Control	ClO_2 + aerosolized PAA
0	48.16 ± 1.87A[a]	47.12 ± 2.71A	10.85 ± 0.73A	10.75 ± 0.54A
2	49.16 ± 2.33A	49.27 ± 2.10A	10.77 ± 0.79A	10.34 ± 0.66A
4	47.12 ± 2.78A	48.22 ± 2.56A	9.90 ± 1.01A	10.53 ± 0.74A
7	46.85 ± 3.16A	44.34 ± 2.26A	9.12 ± 0.06A	9.90 ± 0.91A

[a] Means ± standard deviations from three replications. Within the same storage time and type of vegetable, means with the same uppercase letters within a row are not significantly different ($p > 0.05$).

In the present study, most combinations of ClO_2 gas and aerosolized PAA showed additive effects in the inactivation of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. It has been reported that the application of technologies in hurdles with complementary modes of action may have an impact on microbial inactivation (Gallo et al., 2007; Sobrino-López and Martín-Belloso, 2008). However, ClO_2 gas and PAA have a similar mode of action, as they are both strong oxidizing agents (Benarde et al., 1965; Kitis, 2004). This might explain results of this study which demonstrate that the combination treatment of ClO_2 gas and aerosolized PAA represents only an additive effect on inactivation.

As injured cells of foodborne pathogens might be repaired under suitable conditions, it is a very important aspect that needs to be taken into account regarding food safety (García et al., 2005). In the present study, the occurrence of sublethally injured pathogens was assessed after combined treatment of ClO_2 gas and aerosolized PAA. Combined treatment of ClO_2 gas and aerosolized PAA produced more injured cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on spinach leaves than those on tomatoes. This difference may due to different surface characteristics of spinach leaves and tomatoes. Foodborne pathogens on tomatoes might be more easily exposed to ClO_2 gas and aerosolized PAA than spinach leaves as tomatoes have smoother surfaces than spinach leaves.

In conclusion, this study showed that the combination treatment of ClO_2 gas and aerosolized PAA showed additive effects in the inactivation of three foodborne pathogens with inactivation generally superior to that of each treatment applied individually, as treatment time increased. The color and texture of samples were maintained during 7 days of storage after combined treatment with ClO_2 gas (10 ppmv) and aerosolized PAA. The results of this study suggest that the combination treatment of ClO_2 gas and aerosolized PAA could be an alternative technology to reduce the concentration of ClO_2 gas while ensuring microbial safety.

Acknowledgments

This research was supported by Agriculture, Food and Rural Affairs Research Center Support Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea. This research was also supported by the Public Welfare & Safety research program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT and Future Planning (NRF — 2012M3A2A1051679).

References

Anonymous, 2000. Chemicals used in washing or to assist in the peeling of fruits and vegetables. Code of Federal Regulations 21 CFR 173.315.OYce of the Federal Register. US Government Printing OYce, Washington,DC.
Benarde, M.A., Israel, B.M., Olivieri, V.P., Granstrom, M.L., 1965. Efficiency of chlorine dioxide as a bactericide. Appl. Environ. Microbiol. 13, 776–780.
Bhagat, A., Mahmoud, B.S.M., Linton, R.H., 2010. Inactivation of *Salmonella enterica* and *Listeria monocytogenes* inoculated on hydroponic tomatoes using chlorine dioxide gas. Foodborne Pathog. Dis. 7, 677–685.
Bhagat, A., Mahmoud, B.S.M., Linton, R.H., 2011. Effect of chlorine dioxide gas on *Salmonella enterica* inoculated on navel orange surfaces and its impact on the quality attributes of treated oranges. Foodborne Pathog. Dis. 8, 77–85.
CDC, 2006. Update on multi-state outbreak of *E. coli* O157:H7 infections from fresh spinach, October 6, 2006. MMWR Morb. Mortal. Wkly. Rep. 55, 1045–1046.
CDC, 2012. Multistate Outbreak of Shiga Toxin-Producing *Escherichia coli* O157:H7 Infections Linked to Organic Spinach and Spring Mix Blend. (http://www.cdc.gov/ecoli/2012/O157H7-11-12).
Choi, M.R., Lee, S.Y., Park, K.H., Chung, M.S., Ryu, S., Kang, D.H., 2012. Effect of aerosolized malic acid against *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 on spinach and lettuce. Food Control 24, 171–176.
Dobson, S., Dobson, S., Cary, R., 2002. Effects on laboratory mammals and in vitro test systems. In: Dobson, S., Cary, R. (Eds.), Previous Evaluations by International Bodies. Concise International Chemical Assessment Document 37—Chlorine Dioxide (Gas). World Health Organization, Geneva, pp. 1–26.

Fernández, A., Thompson, A., 2012. The inactivation of *Salmonella* by cold atmospheric plasma treatment. Food Res. Int. 45, 678–684.
Gallo, L.I., Pilosof, A.M.R., Jagus, R.J., 2007. Effect of the sequence of nisin and pulsed electric fields treatments and mechanisms involved in the inactivation of *Listeria innocua* in whey. J. Food Eng. 79, 188–193.
Gómez-López, V.M., Ragaert, P., Jeyachchandran, V., Debevere, J., Devlieghere, F., 2008. Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. Int. J. Food Microbiol. 121, 74–83.
García, D., Gómez, N., Mañas, P., Condón, S., Raso, J., Pagan, R., 2005. Occurrence of sublethal injury after pulsed electric fields depending on the micro-organism, the treatment medium pH and the intensity of the treatment investigated. J. Appl. Microbiol. 99, 94–104.
Gravani, R.B., 2009. The role of good agricultural practices in produce safety. In: Fan, X., Niemira, B.A., Doona, C.J., Feeherry, F.E., Gravani, R.B. (Eds.), Microbial Safety of Fresh Produce. Wiley-Blackwell Publishing, Ames, IA, pp. 101–117.
Han, Y., Floros, J.D., Linton, R.H., Nielsen, S.S., Nelson, P.E., 2001a. Response surface modeling for the inactivation of *Escherichia coli* O157:H7 on green peppers (*Capsicum annuum* L.) by chlorine dioxide gas treatments. J. J. Food Prot. 64, 1128–1133.
Han, Y., Linton, R.H., Nielsen, S.S., Nelson, P.E., 2001b. Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 °C. J. J. Food Prot. 64, 1730–1738.
Han, Y., Shelby, T.L., Schultze, K.K., Nelson, P.E., Linton, R.H., 2004. Decontamination of strawberries using batch and continuous chlorine dioxide gas treatments. J. Food Prot. 67, 2450–2455.
Hiom, S.J., Lowe, C., Oldcorne, M., 2003. Assessment of surface bioburden during hospital aseptic processing. Int. J. Pharm. Pract. 11, R62.
Huang, Y., Chen, H., 2011. Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. Food Control 22, 1178–1183.
Huang, Y., Ye, M., Chen, H., 2012. Efficacy of washing with hydrogen peroxide followed by aerosolized antimicrobials as a novel sanitizing process to inactivate *Escherichia coli* O157:H7 on baby spinach. Int. J. Food Microbiol. 153, 306–313.
Kang, D.H., Fung, D.Y., 1999. Thin agar layer method of heat-injured *Listeria monocytogenes*. J. Food Prot. 62, 1346–1349.
Kang, D.H., Fung, D.Y., 2000. Application of thin layer method for recovery of injured *Salmonella* Typhimurium. Int. J. Food Microbiol. 54, 127–132.
Kitis, M., 2004. Disinfection of wastewater with peracetic acid: a review. Environ. Int. 30, 47–55.
Mahmoud, B.S.M., Linton, R.H., 2008. Inactivation kinetics of inoculated *Escherichia coli* O157:H7 and *Salmonella enterica* on lettuce by chlorine dioxide gas. Food Microbiol. 25, 244–252.
Mahmoud, B.S.M., Vaidya, N.A., Corvalan, C.M., Linton, R.H., 2008. Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. Food Microbiol. 25, 857–865.
Maki, D.G., 2006. Don't eat the spinach-controlling foodborne infectious disease. N. Engl. J. Med. 355, 1952–1955.
Leistner, L., Gorris, L.G.M., 1995. Food preservation by hurdle technology. Trends Food Sci. Technol. 6, 41–46.
Morino, H., Fukuda, T., Miura, T., Shibata, T., 2011. Effect of low-concentration chlorine dioxide gas against bacteria and viruses on a glass surface in wet environments. Lett. Appl. Microbiol. 53, 628–634.
Neal, J.A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L.M., O'Bryan, C.A., Crandall, P.G., Ricke, S.C., Castillo, A., 2012. Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157:H7 on spinach (*Spinacia oleracea*) leaves. Food Res. Int. 45, 1123–1128.
Oh, S.W., Dancer, G.I., Kang, D.H., 2005a. Efficacy of aerosolized peroxyacetic acid as a sanitizer of lettuce leaves. J. Food Prot. 68, 1743–1747.
Oh, S.W., Gray, P.M., Dougherty, R.H., Kang, D.H., 2005b. Aerosolization as novel sanitizer delivery system to reduce food-borne pathogens. Lett. Appl. Microbiol. 41, 56–60.
Park, S.H., Kang, D.H., 2015. Antimicrobial effect of chlorine dioxide gas against foodborne pathogens under differing conditions of relative humidity. LWT Food Sci. Technol 60, 186–191.
Perni, S., Shama, G., Kong, M.G., 2008. Cold atmospheric plasma disinfection of cut fruit surfaces contaminated with migrating microorganisms. J. Food Prot. 8, 1619–1625.
Popa, I., Hanson, E.J., Todd, E.C.D., Schilder, A.C., Ryser, E.T., 2007. Efficacy of chlorine dioxide gas sachets for enhancing the microbiological quality and safety of blueberries. J. Food Prot. 70, 2084–2088.
Prodduk, V., Annous, B.A., Liu, L., 2014. Evaluation of chlorine dioxide gas treatment to inactivate Salmonella enterica on mungbean sprouts. J. Food Prot 11, 1876–1881.
Rhee, M.S., Lee, S.Y., Hillers, V.N., Mccurdy, S.M., Kang, D.H., 2003. Evaluation of consumer-style cooking methods for reduction of *Escherichia coli* O157:H7 in ground beef. J. Food Prot. 66, 1030–1034.
Singh, N., Singh, R.K., Bhunia, A.K., Stroshine, L., 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oils or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. LWT Food Sci. Technol. 35, 720–729.
Sobrino-López, A., Martín-Belloso, O., 2008. Use of nisin and other bacteriocins for preservation of dairy products. Int. Dairy J. 18, 329–343.
Sy, K.V., Murray, M.B., Harrison, M.D., Beuchat, L.R., 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, yeasts, and molds on fresh and fresh-cut produce. J. Food Prot. 68, 1176–1187.
Trinetta, V., Linton, R.H., Morgan, M.T., 2013. The application of high-concentration short-time chlorine dioxide treatment for selected specialty crops including Roma tomatoes (*Lycopersicon esculentum*), cantaloupes (*Cucumis melo* ssp. *melo* var. *cantaloupensis*) and strawberries (*Fragaria* × *ananassa*). Food Microbiol. 34, 296–302.
Vandekinderen, I., Devlieghere, F., Van Camp, J., Kerkaert, B., Cucu, T., Ragaert, P., De Bruyne, J., De Meulenaer, B., 2009. Effects of food composition on the inactivation of foodborne microorganisms by chlorine dioxide. Int. J. Food Microbiol. 131, 138–144.
Wendel, A.M., Johnson, D.H., Sharapov, U., Grant, J., Archer, J.R., Monson, T., 2009. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August–September 2006: the Wisconsin investigation. Clin. Infect. Dis. 48, 1079–1086.
Wu, V.C.H., Rioux, A., 2010. A simple instrument-free gaseous chlorine dioxide method for microbial decontamination of potatoes during storage. Food Microbiol. 27, 179–184.