

# Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas<sup>☆</sup>

Barakat S.M. Mahmoud\*, A.R. Bhagat, R.H. Linton

Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN 47907-2009, USA

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## Abstract

Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas at different concentrations (0.5, 1, 1.5, 3 and 5 mg l<sup>-1</sup>) for 10 min were studied. A cocktail of three strains of each targeted organism (100 µl) was spotted onto the surface of the strawberries (approximately 8–9 log ml<sup>-1</sup>) separately followed by air drying, and then treated with ClO<sub>2</sub> gas at 22 °C and 90–95% relative humidity. Approximately a 4.3–4.7 log CFU reduction per strawberry of all examined bacteria was achieved by treatment with 5 mg l<sup>-1</sup> ClO<sub>2</sub> for 10 min. The inactivation kinetics of *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* were determined using first-order kinetic models to establish *D*-values and *z*-values. The *D*-values of *E. coli*, *L. monocytogenes* and *S. enterica* were 2.6 ± 0.2, 2.3 ± 0.2 and 2.7 ± 0.7 min, respectively, at 5 mg l<sup>-1</sup> ClO<sub>2</sub>. The *z*-values of *E. coli*, *L. monocytogenes* and *S. enterica* were 16.8 ± 3.5, 15.8 ± 3.5 and 23.3 ± 3.3 mg l<sup>-1</sup>, respectively. Furthermore, treatment with ClO<sub>2</sub> gas significantly (*p* ≤ 0.05) reduced the initial microflora (mesophilic, psychrotrophic bacteria, yeasts and molds) on strawberries. Treatment with ClO<sub>2</sub> gas did not affect the color of strawberries and extended the shelf-life to 16 days compared to 8 days for the untreated control.

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## 1. Introduction

Fresh fruits are an important part of the human diet worldwide and consumers continue to eat more fruits partly because of reported health benefits (Beuchat, 1996; Sahari et al., 2004). In the US, consumption of fruit and vegetables increased 27% during 1970–1993 (NACMCF, 1999). Among these fruits, strawberries provide a desirable taste and flavor and account as one of most popular summer fruits (Ford et al., 1997; Moreno et al., 2000; Pelayo et al., 2003; Shamaila et al., 1992; Sturm et al., 2003; Vicente et al., 2002; Zabetakis and Holden, 1997; Van der

Steen et al., 2002). Strawberries are a good source for many vitamins, minerals and natural antioxidants, which give them high scavenging activity toward oxygen radicals, protecting tissues from stresses and disease (Ayala-Zavala et al., 2004; Azodanlou et al., 2003; Cao et al., 1996; Kallio et al., 2000; Ke et al., 1994; Wang et al., 1996, 2002; Velioglu et al., 1998; Zheng et al., 2007). However, strawberries have a short postharvest life, mostly due to high metabolic bacterial activities and fungal decay (Aguayo et al., 2006; Jiang et al., 2001; Nunes et al., 1995; Ragaert et al., 2006; Steen et al., 2002; Vargas et al., 2006).

Fruits can serve as a vehicle for many foodborne pathogenic microorganisms (Bean and Griffin, 1990; Beuchat, 1996; Sy et al., 2005). There are many reports about foodborne outbreaks associated with fruits including frozen strawberries contaminated with the hepatitis A virus

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\*Corresponding author. Tel.: +1 765 494 0831; fax: +1 765 494 7953.

E-mail address: bmahmoud@purdue.edu (B.S.M. Mahmoud).

(Behrsing et al., 2003; Flessa et al., 2005). The most frequent bacterial pathogens associated with fresh produce are *Escherichia coli* O157:H7, and *Salmonella* spp. (Bean and Griffin, 1990; Beuchat, 1996; Du et al., 2003).

There are many of forms traditional sanitizing agents, such as chlorinated water, electrolyzed water and hydrogen peroxide that are widely used to wash and decontaminate produce. However, their effects are limited in reducing pathogenic and spoilage bacteria to 3 log CFU or lower (Brackett, 1987; Cherry, 1999; Koseki et al., 2004; Ukuku, 2004; Yu et al., 2001). To ensure the safety of fresh produce, there is a continual need to identify a highly effective sanitation treatment that is suitable for industrial use. The Food and Drug Administration is currently recommended a 5 log reduction for pathogenic bacteria on produce (FDA, 1995).

Chlorine dioxide gas (ClO<sub>2</sub>) is a promising non-thermal technology for reducing pathogenic and spoilage bacteria on fresh produce. To date, limited studies of the powerful effect of ClO<sub>2</sub> for reducing microorganisms have been reported. A 5 log CFU reduction per strawberry of inoculated of *E. coli* O157:H7 and *Listeria monocytogenes* on strawberries were achieved after treatment with 4 mg l<sup>-1</sup> ClO<sub>2</sub> gas (batch treatment) for 30 min (Han et al., 2004). Meanwhile, a 4.4 log CFU g<sup>-1</sup> reduction in inoculated *Salmonella* spp. on strawberries was achieved after treatment with 8 mg l<sup>-1</sup> ClO<sub>2</sub> gas after 120 min (Sy et al., 2005). Studies on other produce showed that treatment of uninjured green peppers with 3 mg l<sup>-1</sup> ClO<sub>2</sub> gas reduced the population of *L. monocytogenes* by more than 6 log CFU 5 g<sup>-1</sup> after 30 min (Han et al., 2001). Additionally, Han et al. (2000) found that treatment of uninjured green peppers with 0.6 mg l<sup>-1</sup> ClO<sub>2</sub> gas reduced the population of *E. coli* by a 7.3 log CFU 5 g<sup>-1</sup> after 30 min at 22 °C and 90–95% relative humidity. Du et al. (2003) reported that treatment of apples with 3 mg l<sup>-1</sup> ClO<sub>2</sub> gas for 20 min resulted in a 5.9 log CFU 5 g<sup>-1</sup> reduction of the population of *E. coli* after 30 min. Treatment with 4 mg l<sup>-1</sup> ClO<sub>2</sub> gas for 30 min reduced the population of *L. monocytogenes* on apple pulp skin by 6.5 log CFU spotted per site (Du et al., 2002).

However, the inactivation kinetics (or rate of inactivation) of inoculated *E. coli* O157:H7, *L. monocytogenes* and *Salmonella enterica* on strawberries by chlorine dioxide gas have not been reported. The main goals of this study were to: (a) determine inactivation kinetics (*D* and *z*-values) for *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* inoculated onto the surface of strawberries and (b) to study the effect of ClO<sub>2</sub> gas on the quality and the shelf-life of strawberries during refrigerated storage.

## 2. Material and methods

### 2.1. Strawberries

Strawberries were purchased at a local supermarket the day before each experiment and stored at 4 °C until

use. Fresh unblemished strawberry of similar size and weight (25–30 g) were selected. Strawberry stems were removed and the strawberries were washed by dipping them in tap water for 2 min and air drying at 22 °C for 60 min in the biosafety cabinet (Labconco Corporation, Kansas City, Missouri, USA) to remove excessive moisture.

### 2.2. Bacterial strains and growing conditions

Three different bacteria were used including: (1) a cocktail mixture of *E. coli* O157:H7 (C7927, EDL933 and 204P), (2) a cocktail mixture of *L. monocytogenes* (Scott A, F5069 and LCDC 81-861) and (3) a cocktail mixture of *S. enterica* (*S. enteritidis*, *S. javiana* and *S. montevideo*). These strains were selected based on their prevalence in strawberries, and their ability to survive in strawberries over time. *E. coli* O157:H7 C7927, a human isolate from a cider-associated outbreak and an acid-resistant strain, was provided by Dr. M.P. Doyle (Center for Food Safety, University of Georgia). *L. monocytogenes* Scott A was provided by Dr. L.R. Beuchat (Center for Food Safety, University of Georgia). F5069 and LCDC 81-861 were provided by Dr. Arun Bhunia (Center for Food Safety Engineering, Purdue University). All other bacterial strains were obtained from our personal culture collection. Bacterial strains were grown in trypticase soy broth with 6% yeast extract (Difco, Becton Dickinson) and inoculated at 37 °C for 24 h. Three strains of each bacterium were mixed with an equal volume to give approximately 10<sup>8–9</sup> CFU ml<sup>-1</sup>.

### 2.3. Inoculation of strawberries

A spot inoculation method was used to inoculate the pathogenic bacteria on strawberries (Han et al., 2004). Briefly, 100 µl of each mixture culture was spotted (10 drops) on the surface of strawberries in a biosafety cabinet. Following, the strawberries were air-dried at 22 °C for 1 h (to allow bacterial attachment) in the biosafety cabinet prior to ClO<sub>2</sub> treatments.

### 2.4. Production of ClO<sub>2</sub> gas and relative humidity

Chlorine dioxide gas was prepared using a CDG technology generator (CDG Technology Inc., New York, USA). Production of ClO<sub>2</sub> was created based on the reaction of 4% chlorine gas (Matheson gas, Montgomeryville, Pa) with sodium chlorite. Afterwards, the ClO<sub>2</sub> gas was allowed to flow through a flask containing 8–10% sodium chlorite solution (Sigma-Aldrich, St. Louis, MO) to scrub chlorine gas residue. Then, the ClO<sub>2</sub> gas was mixed with filtered air for dilution and introduced into the Plexiglas treatment chamber. Flow of ClO<sub>2</sub> was controlled by a regulation valve to maintain the desired ClO<sub>2</sub> concentration in the treatment chamber. The ClO<sub>2</sub> gas content in the treatment chamber was constantly mixed

using two fans (UF12A series, Fulltech Electric Co. Ltd.; Taiwan) to keep the relative humidity and  $\text{ClO}_2$  concentration constant during treatment. Relative humidity in the chamber was generated and controlled using an ultrasonic humidifier (Electro-Tech Systems, Inc., Glenside PA). During each treatment, the percentage humidity in the chamber was monitored and recorded using a relative humidity meter (Thermo-Hygro recorder; Radioshack Corp., Fort Worth, TX). Concentration of  $\text{ClO}_2$  was monitored and recorded using an Interscan monitor (Interscan Corp., Chatsworth, CA).

### 2.5. Treatment of inoculated strawberries with $\text{ClO}_2$

Inoculated strawberries were placed in sterile plastic boats inside the treatment chamber. The inoculated area was exposed to the chamber environment. Samples were treated with 0.5, 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10 min at  $22^\circ\text{C}$ . The humidity was controlled at 90–95%. Samples were pulled from the treatment chamber every 2 min for microbial enumeration to determine surviving cell populations.

### 2.6. Microbial enumeration

A washing procedure was used to recover pathogens from treated strawberries (Han et al., 2004). Uninoculated and untreated; inoculated and untreated; and inoculated and treated samples were mixed with 100 ml of neutralizing buffer (Difco Laboratories, Sparks, MD) in a sterile 400-ml stomaching bag (Fisher Scientific, Pittsburgh, NJ) and shaken for 15 min using Innova 2100 platform shaker (New Brunswick Scientific Co., Inc., Edison, NJ) with 210 rpm at  $22^\circ\text{C}$ . Serial 10-fold dilutions were prepared in 0.1% peptone water (Difco Laboratories, Sparks, MD). Bacterial populations were enumerated using a membrane transferring method using trypticase soy agar (TSA, Difco, USA) and selective media, according to Han et al. (2002). Plates were incubated for 24 h at  $37^\circ\text{C}$ . Colonies were counted and results expressed as log CFU per strawberry.

### 2.7. D-value and z-value determination

A first-order kinetic model (linear model) was used to analyze each of the data for log of surviving organisms per treatment time. The efficacy of five different concentrations 0.5, 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$  of  $\text{ClO}_2$  treated for 0, 2, 4, 6, 8 and 10 min was determined. The z-value value was calculated by plotting data of the  $\log_{10}$  of the D-values for each  $\text{ClO}_2$  concentration. The z-value was also calculated using a first-order kinetic model for log D-value per  $\text{ClO}_2$  concentration by the same way as the D-value. Both analyses were performed using Excel software (Microsoft Windows XP).

### 2.8. Effect of $\text{ClO}_2$ on the quality and the shelf-life of strawberries

Fresh, unblemished, whole, intact strawberries (25–30 g) with stem intact were used in the shelf-life study. Strawberries were treated with the lowest ( $0.5 \text{ mg l}^{-1}$ ) and highest ( $5 \text{ mg l}^{-1}$ )  $\text{ClO}_2$  concentrations for 0, 2 and 10 min at  $22^\circ\text{C}$ , then placed into a plastic clamshell package (Monte Package company, Michigan, USA) then wrapped in PVC film (AEP industries Inc., NJ, USA) and then stored at  $4^\circ\text{C}$  for 16 days. PVC film (high oxygen and water vapor transmission) was used to prevent potential contamination from air in refrigerated storage. Samples were pulled from the refrigerator at 0, 4, 8, 12 and 16 days and several parameters were evaluated.

The appearance (overall) of strawberries was visually measured (by two trained judges) using a scale from 1–9; where 8–9 = excellent (fresh), 6–7 = very good, 5 = good, and 1–4 = not acceptable (visible mold growth). The surface color was also measured instrumentally using a Hunter colorimeter to obtain  $L^*$ ,  $a^*$ , and  $b^*$  values (LabScan XE Hunter Colorimeter, Hunter Associates Laboratory, Inc., Reston, VA).

The microbiological assay for mesophilic, psychrotrophic bacteria, and for yeasts and molds were examined. For each determination, samples of 25 g were homogenized, for 2 min using a Stomacher 80 Lab-blender (Steward Limited, London, UK) with 225 ml of neutralizing buffer (Difco Laboratories, Sparks, MD). Several dilutions ( $10^{-1}$ – $10^{-4}$ ) were prepared from the homogenate with 0.1% peptone water. For mesophilic microorganism counts, 0.1 ml of each dilution was plated in TSA and the plates were incubated at  $37^\circ\text{C}$  for 24–48 h. For psychrotrophic microorganism counts, 0.1 ml of each dilution was plated in TSA and the plates were incubated at  $4^\circ\text{C}$  for 10 days. For yeast and mold counts, 0.1 ml of each dilution was plated in PDA (potato dextrose agar, Difco, USA) and the plates were incubated at  $25^\circ\text{C}$  for 5 days. Viable counts were expressed as CFU  $\text{g}^{-1}$ .

### 2.9. Statistical analysis

All experiments were replicated three times using two strawberries per experiment for a total of 6 data points per treatment. Data were pooled and the mean values and standard deviations were determined. Differences between samples were determined by Student's *t*-test, using Excel software and were considered to be significant when  $p \leq 0.05$ .

## 3. Results

### 3.1. Inactivation of *E. coli* O157:H7

The inactivation of inoculated of *E. coli* O157:H7 on strawberries by 0.5, 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas at  $22^\circ\text{C}$  and 90–95% relative humidity for 10 min is shown in

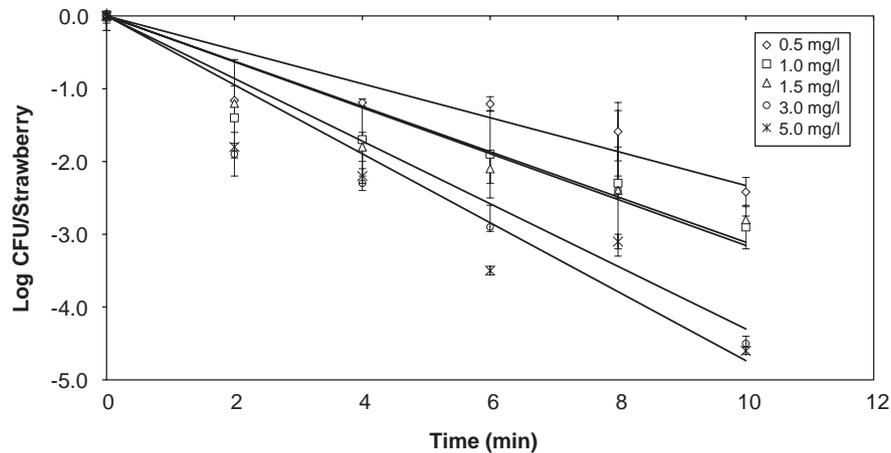


Fig. 1. Inactivation of inoculated *E. coli* O157:H7 on strawberries by different concentrations of  $\text{ClO}_2$  gas (0.5, 1.0, 1.5, 3.0 and  $5.0 \text{ mg l}^{-1}$ ) for 10 min.

Fig. 1. The initial (before air-drying for 60 min) level of all inoculated bacteria on strawberries was approximately  $8\text{--}9 \log \text{CFU ml}^{-1}$ , which lessened to  $7\text{--}8 \log \text{CFU ml}^{-1}$  after air-drying and before treatment with  $\text{ClO}_2$  gas.

The inactivation effect of  $\text{ClO}_2$  gas against *E. coli* O157:H7 increased with increasing treatment time and concentration. Approximately a  $1.2 \log \text{CFU}$  reduction of *E. coli* O157:H7 per strawberry was achieved by treatment with  $0.5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min, while a  $2.4 \log \text{CFU}$  reduction per strawberry was achieved by treatment at  $0.5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10 min. Furthermore, the populations were reduced by  $1.8 \log \text{CFU}$  by treatment with  $5.0 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min. A 3 log reduction of *E. coli* O157:H7 per strawberry was achieved by treatment with 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10, 10, 6 and 6 min, respectively. Approximately a 4.5 and  $4.6 \log \text{CFU}$  reduction per strawberry was achieved by treatment with 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10 min. The *D*-value of inoculated *E. coli* O157:H7 on strawberries was  $4.7 \pm 0.7 \text{ min}$  at  $0.5 \text{ mg l}^{-1}$  which was significantly ( $p \leq 0.05$ ) higher compared to a  $5 \text{ mg l}^{-1}$  where the *D*-value was  $2.6 \pm 0.2 \text{ min}$  (Table 1).

The linear model for estimation of *D*-value was used to calculate inactivation kinetics to compare different treatment conditions for the same organism, and for different organisms for the same treatments. This approach also allowed us to calculate *z*-values. Note that some microbial survival curves appear to have a rapid decline in surviving cells, followed by a constant decrease over time. Information related to *D*-value is helpful for comparing organisms and treatments; however, a direct comparison of actual data points might also provide a more useful comparison.

### 3.2. Inactivation of *L. monocytogenes*

The inactivation of *L. monocytogenes* on strawberries by 0.5, 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas at  $22^\circ\text{C}$  and 90–95% relative humidity for 10 min is shown in Fig. 2. The inactivation effect of  $\text{ClO}_2$  gas against *L. monocytogenes* also increased with increasing treatment time and concen-

Table 1

Inactivation kinetics of inoculated *Escherichia coli* O157:H7 (C7927, EDL933 and 204P); *L. monocytogenes* (Scott A, F5069 and LCDC 81-861) and *Salmonella* (*S. enteritidis* E190-88, *S. javiana* and *S. montevideo*) on strawberries by chlorine dioxide gas

$\text{ClO}_2$ concentration ( $\text{mg l}^{-1}$ )	<i>D</i> -value (min)		
	<i>E. coli</i> O157:H7	<i>L. monocytogenes</i>	<i>Salmonella enterica</i>
0.5	$4.7 \pm 0.7^{\text{aA}}$	$4.6 \pm 0.9^{\text{aA}}$	$4.2 \pm 0.0^{\text{aA}}$
1.0	$4.2 \pm 0.5^{\text{aA}}$	$4.7 \pm 1.1^{\text{aA}}$	$3.8 \pm 0.0^{\text{aA}}$
1.5	$3.9 \pm 0.1^{\text{aC}}$	$3.1 \pm 0.2^{\text{bA}}$	$3.5 \pm 0.0^{\text{aB}}$
3.0	$2.7 \pm 0.3^{\text{bB}}$	$2.5 \pm 0.1^{\text{cA}}$	$2.9 \pm 0.0^{\text{bB}}$
5.0	$2.5 \pm 0.2^{\text{bA}}$	$2.3 \pm 0.2^{\text{cA}}$	$2.7 \pm 0.0^{\text{bA}}$
<i>z</i> -value ( $\text{mg l}^{-1}$ )	$16.8 \pm 3.5^{\text{A}}$	$15.8 \pm 3.5^{\text{A}}$	$23.3 \pm 3.3^{\text{B}}$

Mean values with different lowercase letters in same column are significantly different ( $p < 0.05$ ). Mean values with different uppercase letters in same row are significantly different ( $p < 0.05$ ).

tration. Approximately a  $0.8 \log \text{CFU}$  reduction per strawberry was achieved by treatment with  $0.5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min, while a  $2.3 \log \text{CFU}$  reduction/strawberry was achieved by the treatment for 10 min. Furthermore, the populations were reduced by  $2.4 \log \text{CFU}$  when treated with  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min. More than a  $3 \log \text{CFU}$  reduction of *L. monocytogenes* per strawberry was achieved by treatment with 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 6 and 4 min, respectively. Approximately a 4.6 and  $4.7 \log \text{CFU}$  reduction/strawberry was achieved by treatment with 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10 min. The *D*-value (Table 1) of *L. monocytogenes* was  $4.6 \pm 0.9 \text{ min}$  at  $0.5 \text{ mg l}^{-1}$  which significantly ( $p \leq 0.05$ ) decreased to  $2.3 \pm 0.2$  as the concentration of  $\text{ClO}_2$  gas was increased to  $5 \text{ mg l}^{-1}$ .

### 3.3. Inactivation of *S. enterica*

The inactivation of inoculated *S. enterica* on strawberries by 0.5, 1, 1.5, 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas at  $22^\circ\text{C}$  and 90–95% relative humidity for 10 min is shown in Fig. 3.

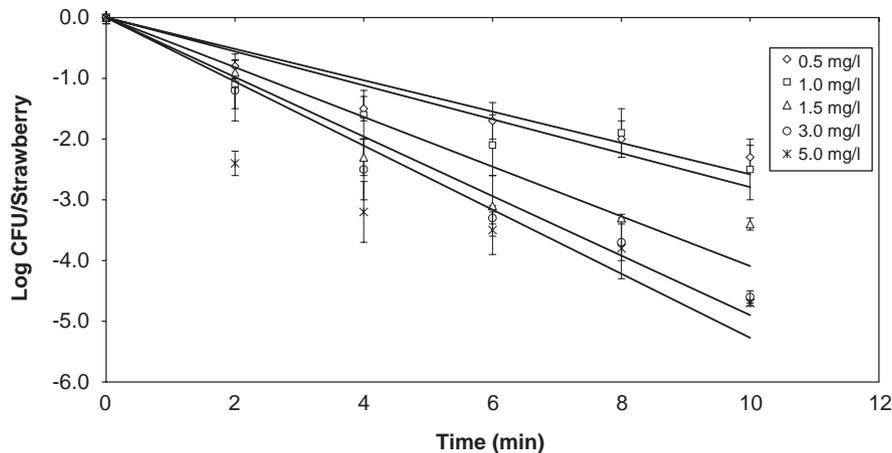


Fig. 2. Inactivation of inoculated *L. monocytogenes* on strawberries by different concentrations of  $\text{ClO}_2$  gas (0.5, 1.0, 1.5, 3.0 and  $5.0 \text{ mg l}^{-1}$ ) for 10 min.

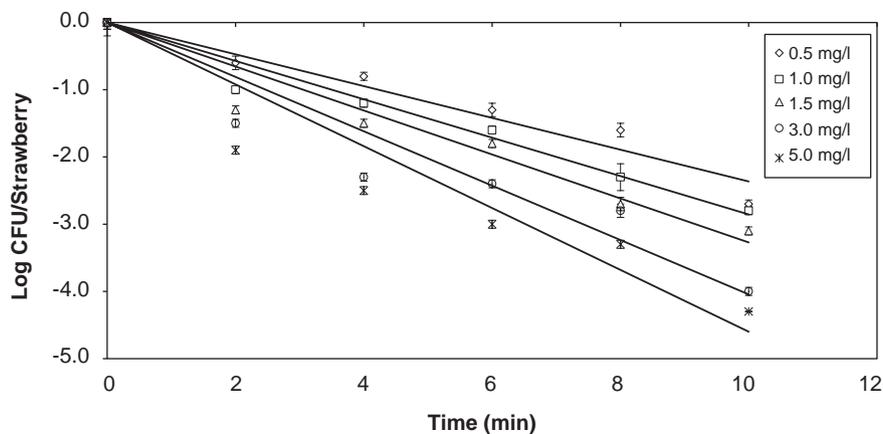


Fig. 3. Inactivation of inoculated *Salmonella enterica* on strawberries by different concentrations of  $\text{ClO}_2$  gas (0.5, 1.0, 1.5, 3.0 and  $5.0 \text{ mg l}^{-1}$ ) for 10 min.

Similar to *E. coli* and *L. monocytogenes*, the inactivation effect of  $\text{ClO}_2$  gas against *S. enterica* increased with increasing treatment time and concentration. Approximately a  $0.6 \text{ log CFU}$  reduction of *S. enterica* per strawberry was achieved by treatment with  $0.5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min, while a  $2.7 \text{ log CFU}$  reduction was achieved by the treatment for 10 min. Furthermore, the populations were reduced by a  $1.9 \text{ log CFU}$  when treated with  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 2 min. A  $3 \text{ log}$  reduction of *S. enterica* per strawberry was achieved by treatment with 5 and  $1.5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 6 and 10 min, respectively. Approximately a  $4.0$  and  $4.3 \text{ log CFU}$  reduction per strawberry was achieved by treatment with 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 10 min. The *D*-value (Table 1) of *S. enterica* on strawberries was  $4.2 \pm 0.0 \text{ min}$  at  $0.5 \text{ mg l}^{-1}$  which significantly ( $p \leq 0.05$ ) decreased to  $2.7 \pm 0.0$  as the concentration of  $\text{ClO}_2$  gas was increased to  $5 \text{ mg l}^{-1}$ .

### 3.4. The *z*-value determination

The *z*-values of *E. coli*, *L. monocytogenes*, and *S. enterica* on strawberries were  $16.8 \pm 3.5$ ,  $15.8 \pm 3.5$  and  $23.3 \pm 3.3$ , respectively (Table 1). The *z*-value for *S. enterica* was

significantly higher than the other bacteria tested ( $p \leq 0.05$ ). Therefore, the impact of changing  $\text{ClO}_2$  gas concentration will impact resistance of *S. enterica* differently from the other bacteria tested.

### 3.5. Effect of treatment with $\text{ClO}_2$ gas on the quality and shelf-life of strawberries

The quality, color, microflora counts, and visual appearance (overall) of treated strawberries with  $\text{ClO}_2$  ( $0.5 \text{ mg l}^{-1}/2 \text{ min}$ ,  $0.5 \text{ mg l}^{-1}/10 \text{ min}$ ,  $5 \text{ mg l}^{-1}/2 \text{ min}$  and  $5 \text{ mg l}^{-1}/10 \text{ min}$ ) gas were evaluated during storage at  $4^\circ \text{C}$  for 16 days.

Changes in the external color of strawberries were monitored by measuring lightness ( $L^*$ ), red–greenness ( $a^*$ ) and blue–yellowness ( $b^*$ ) by the Hunter colorimeter during storage at  $4^\circ \text{C}$  for 16 days as shown in Table 2 and visually in Table 3. Treatment with  $\text{ClO}_2$  gas did not significantly ( $p > 0.05$ ) affect the color (day 0). During storage, all samples including the untreated control showed a decrease in  $L^*$ ,  $a^*$  and  $b^*$  values ranging from 31–33, 31–34 and 20–21 to 26–27, 20–23 and 16–18, respectively, with no significant differences ( $p > 0.05$ ) among them.

Changes in the microflora on strawberries during storage at 4 °C for 16 days were evaluated using mesophilic, psychrotrophic and yeast and mold microbial counts. Treatment with 5 mg l<sup>-1</sup> ClO<sub>2</sub> for 5 and/or 10 min significantly decreased the initial populations of mesophilic, psychrotrophic and yeast and mold on strawberries from 3.9, 3.8, 4.8 log CFU g<sup>-1</sup> to the less than detectable

limit (2 log CFU g<sup>-1</sup>) as shown in Tables 4–6, respectively. In addition, treatment with 5 mg l<sup>-1</sup> ClO<sub>2</sub> for 10 min kept the populations of mesophilic, psychrotrophic and yeast and mold under the detectable limit (< 2.0 log CFU) to 12, 4 and 8 days, respectively.

Changes in appearance of treated strawberries during storage at 4 °C for 16 days were monitored visually (Table 7). There were no significant differences ( $p > 0.05$ ) between all samples including the untreated control at day 0 and day 4 of storage. The visual quality of strawberries gradually decreased over storage time. The score lessened from 9 to 4 after 8 days for the untreated control, while treatment with 5 mg l<sup>-1</sup>/10 min maintained a score of 7.3 after 16 days.

Table 2  
Changes in the color (Hunter parameters) of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time	L*	a*	b*
<i>Day 0</i>			
Control	31.8 ± 2.7	32.9 ± 2.5	20.6 ± 3.3
0.5 mg l <sup>-1</sup> /2 min	32.5 ± 1.2	34.8 ± 2.4	21.4 ± 2.7
0.5 mg l <sup>-1</sup> /10 min	31.2 ± 4.9	31.8 ± 3.2	20.0 ± 4.5
5 mg l <sup>-1</sup> /2 min	33.5 ± 1.5	34.6 ± 0.9	20.5 ± 2.4
5 mg l <sup>-1</sup> /10 min	33.7 ± 1.9	32.0 ± 2.1	20.5 ± 2.3
<i>Day 4</i>			
Control	31.1 ± 4.0	34.0 ± 2.9	20.9 ± 2.3
0.5 mg l <sup>-1</sup> /2 min	30.2 ± 3.1	35.3 ± 1.9	21.8 ± 3.7
0.5 mg l <sup>-1</sup> /10 min	30.9 ± 2.8	32.2 ± 1.9	20.5 ± 4.3
5 mg l <sup>-1</sup> /2 min	32.6 ± 2.1	34.7 ± 1.7	19.6 ± 0.7
5 mg l <sup>-1</sup> /10 min	31.9 ± 3.4	32.1 ± 2.0	19.5 ± 2.8
<i>Day 8</i>			
Control	27.3 ± 2.6	30.8 ± 1.5	18.3 ± 3.6
0.5 mg l <sup>-1</sup> /2 min	27.9 ± 0.4	31.6 ± 0.9	19.8 ± 1.7
0.5 mg l <sup>-1</sup> /10 min	25.9 ± 3.4	31.4 ± 2.6	17.9 ± 3.3
5 mg l <sup>-1</sup> /2 min	28.7 ± 0.9	32.2 ± 0.3	18.7 ± 1.7
5 mg l <sup>-1</sup> /10 min	28.1 ± 1.5	30.1 ± 1.6	17.8 ± 2.1
<i>Day 12</i>			
Control	26.8 ± 3.2	28.8 ± 4.7	16.4 ± 4.9
0.5 mg l <sup>-1</sup> /2 min	28.2 ± 2.2	30.0 ± 2.6	18.4 ± 2.4
0.5 mg l <sup>-1</sup> /10 min	26.7 ± 1.7	27.9 ± 2.5	16.7 ± 3.4
5 mg l <sup>-1</sup> /2 min	29.3 ± 0.9	30.7 ± 1.4	17.7 ± 1.5
5 mg l <sup>-1</sup> /10 min	28.3 ± 2.2	29.7 ± 0.6	17.5 ± 2.2
<i>Day 16</i>			
Control	27.8 ± 1.6	20.1 ± 2.6	16.8 ± 3.3
0.5 mg l <sup>-1</sup> /2 min	27.4 ± 0.7	20.7 ± 3.5	16.9 ± 3.2
0.5 mg l <sup>-1</sup> /10 min	26.4 ± 2.4	21.7 ± 1.4	16.7 ± 2.4
5 mg l <sup>-1</sup> /2 min	27.3 ± 2.0	21.7 ± 2.1	18.5 ± 0.7
5 mg l <sup>-1</sup> /10 min	27.6 ± 2.3	23.0 ± 0.2	18.9 ± 1.6

No significant differences ( $p > 0.05$ ) between samples (in the same column for each day) were detected. L\* = lightness, a\* = red-greenness, or b\* = blue-yellowness.

#### 4. Discussion

At most of the lower ClO<sub>2</sub> concentrations (0.5 and 1 mg l<sup>-1</sup>) and treatment times (2 and 4 min) all examined bacteria exhibited a similar log reduction of their populations. However, with higher concentrations (1.5, 3 and 5 mg l<sup>-1</sup>) and longer treatment times (6, 8 and 10 min), *L. monocytogenes* was only slightly more susceptible to ClO<sub>2</sub> gas treatment than *E. coli* O157:H7 and *S. enterica* (Table 1). These results are in agreement with those obtained by (Han et al., 2004; Du et al., 2002; Lee et al., 2004; Park et al., 2001).

Understanding the inactivation mechanism of this new sanitizer technology will be an important for both scientists and for the food industry. Chlorine dioxide gas is highly water soluble which may act similar to ClO<sub>2</sub> aqueous to inactivate microorganisms (Linton et al., 2006). The primary inactivation mechanism of aqueous ClO<sub>2</sub> may be to disrupt protein synthesis (Benarde et al., 1967) or increase the permeability of the outer membrane by reacting with the membrane protein and lipids (Aieta and Berg, 1986). Leakage of intracellular ions (such as potassium) due to the cellular damage by ClO<sub>2</sub> is the primary lethal physiological event (Berg et al., 1986). More research is necessary to understand the exact inactivation mechanisms of ClO<sub>2</sub> gas.

Kinetic parameters and models are used for the development of food preservation processes to ensure the safety and to understand the mechanism of microbial

Table 3  
Changes in the visual color of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time (days)	Treatments				
	Control	0.5 mg l <sup>-1</sup> /2 min	0.5 mg l <sup>-1</sup> /10 min	5.0 mg l <sup>-1</sup> /2 min	5.0 mg l <sup>-1</sup> /10 min
0	5.7 ± 0.5	5.7 ± 0.5	5.3 ± 0.6	5.3 ± 0.6	5.3 ± 0.6
4	5.3 ± 0.5	5.3 ± 0.5	5.6 ± 0.5	5.3 ± 0.5	5.3 ± 0.5
8	5.3 ± 0.6	5.3 ± 0.6	5.3 ± 0.6	5.6 ± 0.6	5.7 ± 0.6
12	5.6 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.7 ± 0.5	5.5 ± 0.7
16	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5	5.3 ± 0.5

No significant differences ( $p > 0.05$ ) between samples (in the same row) were detected ( $p > 0.05$ ).

Table 4  
Changes in the mesophilic bacterial counts (log CFU g<sup>-1</sup>) of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time (days)	Treatments				
	Control	0.5 mg l <sup>-1</sup> /2 min	0.5 mg l <sup>-1</sup> /10 min	5.0 mg l <sup>-1</sup> /2 min	5.0 mg l <sup>-1</sup> /10 min
0	3.9±0.07 <sup>a</sup>	2.4±0.15 <sup>b</sup>	2.0±0.00 <sup>b</sup>	ND <sup>c</sup>	ND <sup>c</sup>
4	4.0±0.10 <sup>a</sup>	2.7±0.17 <sup>b</sup>	2.1±0.13 <sup>b</sup>	2.0±0.00 <sup>b</sup>	ND <sup>c</sup>
8	4.4±0.10 <sup>a</sup>	2.9±0.10 <sup>b</sup>	2.4±0.37 <sup>b</sup>	2.0±0.00 <sup>b</sup>	ND <sup>c</sup>
12	4.7±0.12 <sup>a</sup>	3.2±0.05 <sup>b</sup>	3.1±0.10 <sup>b</sup>	2.8±0.20 <sup>b</sup>	ND <sup>c</sup>
16	5.2±0.13 <sup>a</sup>	3.8±0.05 <sup>b</sup>	3.0±0.05 <sup>b</sup>	3.3±0.12 <sup>b</sup>	2.0±0.0 <sup>c</sup>

Mean values with different letters in same row are significantly different ( $p < 0.05$ ).  
ND = no detectable survivors (less than 2.0 log CFU g<sup>-1</sup>).

Table 5  
Changes in the psychrotrophic bacterial counts (log CFU g<sup>-1</sup>) of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time (days)	Treatments				
	Control	0.5 mg l <sup>-1</sup> /2 min	0.5 mg l <sup>-1</sup> /10 min	5.0 mg l <sup>-1</sup> /2 min	5.0 mg l <sup>-1</sup> /10 min
0	3.8±0.02 <sup>a</sup>	ND <sup>b</sup>	ND <sup>b</sup>	ND <sup>b</sup>	ND <sup>b</sup>
4	4.1±0.02 <sup>a</sup>	2.3±0.03 <sup>b</sup>	2.1±1.20 <sup>b</sup>	2.0±0.00 <sup>b</sup>	ND <sup>c</sup>
8	4.5±0.10 <sup>a</sup>	3.3±0.60 <sup>b</sup>	3.2±0.07 <sup>b</sup>	3.2±0.13 <sup>b</sup>	2.8±0.05 <sup>c</sup>
12	4.7±0.10 <sup>a</sup>	4.2±0.06 <sup>a</sup>	3.3±0.10 <sup>b</sup>	3.4±0.11 <sup>b</sup>	2.9±0.06 <sup>c</sup>
16	4.9±0.05 <sup>a</sup>	4.4±0.11 <sup>a</sup>	4.1±0.10 <sup>a</sup>	3.6±0.06 <sup>b</sup>	3.2±0.20 <sup>b</sup>

Mean values with different letters in same row are significantly different ( $p < 0.05$ ).  
ND = no detectable survivors (less than 2.0 log CFU g<sup>-1</sup>).

Table 6  
Changes in the yeast and mold counts (log CFU g<sup>-1</sup>) of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time (days)	Treatments				
	Control	0.5 mg l <sup>-1</sup> /2 min	0.5 mg l <sup>-1</sup> /10 min	5.0 mg l <sup>-1</sup> /2 min	5.0 mg l <sup>-1</sup> /10 min
0	4.8±0.05 <sup>a</sup>	2.9±0.10 <sup>b</sup>	2.3±1.30 <sup>c</sup>	ND <sup>d</sup>	ND <sup>d</sup>
4	4.9±0.10 <sup>a</sup>	3.1±0.06 <sup>b</sup>	2.6±0.30 <sup>c</sup>	2.0±0.00 <sup>c</sup>	ND <sup>d</sup>
8	5.2±0.10 <sup>a</sup>	3.4±0.15 <sup>b</sup>	3.0±0.20 <sup>b</sup>	2.7±0.10 <sup>c</sup>	ND <sup>d</sup>
12	5.3±0.06 <sup>a</sup>	4.6±0.10 <sup>b</sup>	3.6±0.15 <sup>c</sup>	3.3±0.10 <sup>c</sup>	2.5±0.20 <sup>d</sup>
16	5.7±0.10 <sup>a</sup>	5.2±0.06 <sup>b</sup>	4.6±0.06 <sup>c</sup>	3.8±0.06 <sup>d</sup>	3.3±0.01 <sup>d</sup>

Mean values with different letters in same row are significantly different ( $p < 0.05$ ).  
ND = no detectable survivors (less than 2.0 log CFU g<sup>-1</sup>).

Table 7  
Changes in the visual overall quality of treated strawberries with ClO<sub>2</sub> during storage at 4 °C

Storage time (days)	Treatments				
	Control	0.5 mg l <sup>-1</sup> /2 min	0.5 mg l <sup>-1</sup> /10 min	5.0 mg l <sup>-1</sup> /2 min	5.0 mg l <sup>-1</sup> /10 min
0	9.0±0.0 <sup>a</sup>	8.7±0.5 <sup>a</sup>	8.7±0.6 <sup>a</sup>	8.7±0.6 <sup>a</sup>	9.0±0.0 <sup>a</sup>
4	8.3±0.5 <sup>a</sup>	8.3±0.5 <sup>a</sup>	8.7±0.5 <sup>a</sup>	8.3±0.5 <sup>a</sup>	8.7±0.5 <sup>a</sup>
8	4.3±1.1 <sup>a</sup>	4.7±2.8 <sup>a</sup>	7.3±1.0 <sup>b</sup>	7.7±0.5 <sup>b</sup>	8.3±1.1 <sup>c</sup>
12	3.3±1.5 <sup>a</sup>	3.0±0.1 <sup>a</sup>	5.6±1.5 <sup>b</sup>	7.3±0.9 <sup>b</sup>	8.0±1.0 <sup>c</sup>
16	1.3±0.5 <sup>a</sup>	2.7±0.5 <sup>b</sup>	3.6±0.6 <sup>c</sup>	4.3±1.2 <sup>c</sup>	7.3±1.5 <sup>d</sup>

Mean values with different letters in same row are significantly different ( $p < 0.05$ ).

inactivation (Parish, 2006). The inactivation kinetics of *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* were determined using first-order kinetic model ( $D$  and

$z$ -values). The  $D$ -values of *E. coli*, *L. monocytogenes* and *S. enterica* were approximately 4 min at 0.5 mg l<sup>-1</sup> ClO<sub>2</sub>. The  $D$ -values of all bacteria decreased with increasing ClO<sub>2</sub>

concentration and treatment time. The maximum reductions of the  $D$ -values were obtained after treatment with  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas. The  $D$ -values of *E. coli*, *L. monocytogenes* and *S. enterica* were approximately 2 min at  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$ .

It is advisable for the industry to use sanitizers that reduce microbial populations of pathogenic bacteria on produce by a 5 log CFU that to ensure their safety (FDA, 1995). In this study, treatment with 3 and  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas for 6 and 10 min, respectively, reduced the population by more than a 3 log CFU per strawberry. These results are similar to those reported by (Han et al., 2004; Sy et al., 2005).

From the results obtained for determining  $D$ -values, we can estimate the necessary  $\text{ClO}_2$  gas concentrations and treatment times to achieve a 5 log CFU reduction of the pathogenic bacteria on strawberries. The 5 log CFU reduction of *E. coli*, *L. monocytogenes* and *S. enterica* may be achieved with  $5 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas at 12.5, 11.5 and 13.5 min, respectively.

The  $z$ -value is another parameter commonly used to determine the inactivation kinetics. The  $z$ -value provides information about the relative resistance of the microorganisms to antimicrobial agents over different concentrations. The  $z$ -value describes the rate of death in terms of  $\text{ClO}_2$  concentration necessary to achieve a 90% log bacterial reduction. The  $z$ -values were  $16.8 \pm 3.5$ ,  $15.8 \pm 3.5$  and  $23.3 \pm 3.3 \text{ mg l}^{-1}$ , for *E. coli*, *L. monocytogenes* and *S. enterica*, respectively. Differences in  $z$ -values should be taken into account when using different gas concentrations.

The shelf-life of strawberries is limited by 5–7 days at refrigerator temperature and can be extended by several techniques combined with refrigerator temperature (Rivera and Tong, 2006; Almenar et al., 2006). In this study, treatment with  $\text{ClO}_2$  gas did not affect quality parameters including surface color measured visually (the most important factor for consumers) and surface color measured with the Hunter colorimeter compared with the untreated control sample at day 0. However,  $L^*$ ,  $a^*$  and  $b^*$  values for all samples did gradually decrease during storage. These results are in agreement with those obtained by (Almenar et al., 2006; Vicente et al., 2002; Zheng et al., 2007). Taste is one of the most important factors for evaluating the quality of any food products. However, we could not evaluate taste in this study because this technology has not been approved for use on strawberries. At present, the Food and Drug Administration has approved the use of aqueous  $\text{ClO}_2$  ( $3 \text{ mg l}^{-1}$ ) to wash fruits and vegetables (FDA, 1998), but this technology has not yet been approved for direct use on fruit and vegetable products.

From the microbial view, however, the treatment with  $\text{ClO}_2$  gas significantly reduced the initial microflora on strawberries. The short shelf-life of strawberries is mostly caused by fungal decay (Almenar et al., 2006; Wszelaki and Mitcham, 2000). From day 8, fungal decay (mycelia

became visible) was observed in the control and treated sample with  $0.5 \text{ mg l}^{-1}/2 \text{ min}$ . While, treatment with  $5 \text{ mg l}^{-1}/10 \text{ min}$  significantly maintained the quality and delayed the fungal decay of strawberries to 16 days during storage at  $4^\circ\text{C}$ .

Our group's previous work (Han et al., 2004) reported that the residual  $\text{ClO}_2$  and chlorite on the strawberry surfaces (treated with  $3 \text{ mg l}^{-1}$   $\text{ClO}_2$  gas) were  $0.19 \text{ mg ClO}_2$  per kg and  $1.17 \pm 2.0 \text{ mg Cl}_2$  per kg immediately after treatment. However, no residual of  $\text{ClO}_2$  was detected on treated strawberries, after storage for 1 week, and the amount of residual chlorite decreased to a lower level of  $0.07 \pm 0.12 \text{ mg Cl}_2$  per kg of strawberries. We recognize that there may be a potential safety risk related to toxic by-products formed by high levels of  $\text{ClO}_2$  gas treatment. More information is need on the chemical byproducts on fruit and vegetable surfaces after  $\text{ClO}_2$  treatment.

In summary, this is the first report that describes inactivation kinetics of inoculated *E. coli* O157:H7, *L. monocytogenes* and *S. enterica* on strawberries by  $\text{ClO}_2$  gas. The  $\text{ClO}_2$  gas showed strong inactivation effect against selected pathogenic bacteria and natural microflora on strawberries. This information may be useful to establish processing parameters if/when  $\text{ClO}_2$  gas is approved for use.

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