FOOD QUALITY and CULTURE
Food Quality Culture 3(1): 34~39 (2009)

Effect of Chlorine Dioxide and Commercial Chlorine Sanitizer on Inhibiting Foodborne Pathogens and on Preventing the Formation of Chemically Injured Cells on Radish Sprouts

Mi-Ran Choi, Dong-Hyun Kang[1,2], Sunggi Heu[3], and Sun-Young Lee*
Department of Food and Nutrition, Chung-Ang University, Anseong-si, 456-756, Korea
[1]Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376, USA
[2]Department of Food and Animal Biotechnology, Seoul National University, Seoul 151-742, Korea
[3]Microbial Safety Division, Department of Agro-food Safety, National Academy of Agricultural Science, Rural Development Administration, Suwon, 441-857, Korea

ABSTRACT This study assessed the efficacy of aqueous chlorine dioxide (ClO_2) and commercial chlorine sanitizer in terms of its ability to eliminate *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 on radish sprouts (*Raphanus sativus* L.). Radish sprouts were inoculated with a cocktail containing one each of three strains of three different foodborne pathogens, then treated with distilled water (control) or chemical sanitizers (100 ppm commercial chlorine, and 50, 100, 200 ppm ClO_2) for 1, 5, and 10 min at room temperature (22±2°C). Populations of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* were counted at 4.64, 6.05, and 4.29 log CFU/g, respectively, after inoculation. Treatment with water did not significantly reduce the levels of any of the three foodborne pathogens. The levels of all three pathogens were reduced by treatment with chemical sanitizers; however, the observed levels of reduction of *E. coli* O157:H7 and *L. monocytogenes* were not significant as compared with the controls. The levels of the three pathogens were reduced most profoundly when treated for 10 min with 200 ppm of ClO_2, and the reduction levels of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* were 1.17, 1.63, and 0.96 log CFU/g, respectively. When chemically injured cells were investigated using SPRAB for *E. coli* O157:H7 and by selective overlay methods for *S.* Typhimurium and *L. monocytogenes*, respectively, it was noted that commercial chlorine sanitizer generated more numbers of injured pathogens than did ClO_2. These data indicate that ClO_2 treatment may prove useful in reducing the numbers of pathogenic bacteria in radish sprouts.

KEYWORDS: chlorine dioxide, commercial chlorine sanitizer, foodborne pathogens, radish sprout, chemically injured cells

INTRODUCTION

Several recent major outbreaks of foodborne illness have been linked to the consumption of fresh fruits and vegetables -these incidents have gained an increasing amount of attention as a potentially serious public health threat (Stopforth et al 2008). In particular, outbreaks of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* caused by sprouts have become a specific concern (Beuchat 1995). Taormina et al (1999) reported that the largest outbreak, in Japan, involved more than 6000 persons, and was associated with the consumption of radish sprouts. White radish sprouts were again implicated in another Japanese outbreak of *E. coli* O157:H7 infection, that affected 126 people.

During the cultivation of sprouts, bacterial loads can be fairly substantial, as the conditions in which seeds are sprouted (2-7 days of sprouting, temperatures of 20-40°C, and optimum water activity of near 1.0) are ideal for bacterial proliferation. Also, because sprouts are generally consumed raw, the risk of consuming viable pathogens is also fairly substantial (Fu et al 2001). The growth of human foodborne pathogens in these large microbial communities is of major concern due to its potentially deleterious effects on human health, particularly for very elderly people, very

*Corresponding author
Tel: +82-31-670-4587
Fax: +82-31-676-8741
E-mail: nina6026@cau.ac.kr or nina6026@gmail.com

Received April 19, 2009; Revised May 21, 2009;
Accepted May 21, 2009

young people, and immunocompromised individuals (Matos et al 2002). Therefore, in order to reduce the proliferation of foodborne pathogens, appropriate sanitization techniques for fresh fruits and vegetables need to be developed.

In August 1998, a sprouts task force initiated research at the National Center for Food Safety and Technology in Illinois, in an effort to evaluate the effects of commercial sprouting conditions on pathogens, and to evaluate potential seed treatment interventions (including chemical, heat, and irradiation treatments) (Lee et al 2002). One of the sanitizers, chlorine, has raised health concerns due to the existence of trihalomethanes generated in the presence of organic materials (Beuchat et al 1998). Most produce is washed with chlorinated water to reduce the population of microorganisms, but this effects a microbial reduction of less than 2 log CFU/g when applied to fruits and vegetables (Beuchat 1999; Cherry 1999). In an effort to minimize the risk of food poisoning, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has recommended a 5-log reduction in pathogens on seeds used for sprout production, and it was also demonstrated that treatment with 20,000 ppm calcium hypochlorite can achieve this level of decontamination (NACMCF 1999). Because seed sprouting provides an excellent environment for the growth of many types of microorganisms, they are likely to grow to significant levels in the finished sprouts if pathogens are present on or within the seeds. Therefore, the appropriate sanitation processes for reducing pathogens in finished commercial sprouts must be applied after sprouting.

Chlorine dioxide (ClO_2) is a strong oxidizing and sanitizing agent, which may have practical applications for the sanitizing of surfaces in the food industry (Lee et al 2004). It has an oxidation capacity approximately 3.5 times that of chlorine (Benarde et al 1965). The United States Food and Drug Administration (US FDA) has permitted the use of aqueous ClO_2 for the washing of fresh raw produce, such as fruits and vegetables; recently, it the use of ClO_2 for the same purposes has also been approved by the Korean FDA. Therefore, the application of ClO_2 has received increased attention for its potential advantages over chlorine-based sanitizers.

Therefore, this study was conducted in order to assess the effects of commercial chlorine sanitizer and ClO_2 against *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* on radish sprouts. After sanitizing treatment, sublethally-injured foodborne pathogens potentially remain as dangerous as their uninjured counterparts, because they can survive and grow over the storage time of products, and can also induce foodborne disease. Therefore, injuries to foodborne pathogens on radish sprouts after treatment with sanitizers (commercial chlorine sanitizer and ClO_2) were also evaluated in this study.

MATERIALS AND METHODS

Cultures and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43174 and DT 104), *L. monocytogenes* (ATCC 7644, ATCC 19114 and ATCC 19115) were obtained from the Food Nutrition Department at Chung-Ang University (Anseong-si, South Koera) and used for inoculation on radish sprouts. Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was cultured for 24 h on 5 mL of Tryptic Soy Broth (TSB: Difco, Becton-Dickinson, Sparks, MD, US) at 37°C, harvested via 20 min of centrifugation at 4,000×g at 4°C and washed three times in buffered peptone water (pH 7.0, Difco). The final cell concentration in the buffer was approximately 10^{8-9} CFU/mL. After that, each of the three strains of each of the foodborne pathogens was mixed to construct the culture cocktails. The mixed culture cocktails were utilized for subsequent experiments.

Sample preparation and inoculation

Radish sprouts were purchased from a local store (Anseong-si, South Korea) on the day before the experiment. The radish sprouts were inoculated with *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* as follows. Prepared culture cocktails of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* were diluted in 5-liter sterile distilled water to a concentration of 10^5 to 10^6 CFU/mL. Radish sprouts were immersed in 5 liters of aqueous suspension containing *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* for 20 min at room temperature. The radish sprouts were then placed on sterile aluminum foil under a hood. The inoculated radish sprouts were dried under the hood for 60 min with the fan running.

Preparation and treatment of sanitizers

Commercial chlorine sanitizer (Daisung Chemical Co. LTD., Korea) was diluted to 100 ppm with distilled water, in accordance with the manufacturer's instructions. ClO_2 was prepared via the addition of 8% commercial ClO_2 sanitizer (Youngchoen Kristi Co., Korea) in sterile distilled water and solutions containing 50, 100, and 200 ppm. Sterile distilled water was used as a control. All solutions were used within 30 min after preparation at room temperature (22°C). All solutions were determined using a measuring instrument for chlorine concentration (HI 95771 Chlorine Ultra HR ISM, Hanna Instruments, Hungary) in accordance with the manufacturer's instructions. The concentration levels of 100 ppm commercial chlorine sanitizer, 50, 100, and 200 ppm ClO_2 were 94±3.49, 65±3.39, 136±8.58 and 252±10.95 ppm, respectively.

Each of the inoculated and dried radish sprouts were immersed in sanitizers (water, commercial chlorine, and ClO_2) for 1, 5, and 10 min. After the treatments, the samples (25 g each) were placed on sterile aluminum foil under a hood. The inoculated radish sprouts were dried under the hood for 60 min with the fan running.

Bacterial enumeration

After 1, 5, and 10 min of treatment, radish sprouts (25 g) were placed in stomacher bags containing 50 mL of buffered peptone water (Difco) and homogenized for 2 min using a stomacher (BagMixer 400, Interscience Laboratory Inc., St. Nom, France). After homogenization, the samples were serially 10-fold diluted with 9 mL of sterile buffered peptone water and 0.1 mL of sample or diluents was plated onto each selective agar. Sorbitol MacConkey agar (SMAC; Difco), xylose lysine desoxycholate agar (XLD; Difco), and Oxford agar base (OAB; Difco) with antimicrobial supplement (Bacto Oxford antimicrobic supplement, Difco) were used as selective media for the enumeration of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively. All plates were incubated at 37°C for 24 to 48 h, and then typical colonies characteristic of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were enumerated.

Enumeration of injured cells

The injured pathogens cannot survive on selective media, because the selective agents or dyes in selective agar can inhibit the injured pathogens; therefore, significant differences between selective agar for the recovery of healthy cells and non-selective agar for the recovery of healthy and injured cells could represent the levels of injured cells generated by treatments (Lee and Kang 2001). Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to enumerate the chemically injured *E. coli* O157:H7 cells (Table 2). Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7, because SPRAB is not typically employed as a selective agar for the enumeration of *E. coli* O157: H7. The overlay (OV) method was utilized to enumerate the injured *S.* Typhimurium and *L. monocytogenes* cells on XLD and OAB, respectively (Table 1 and 3). Tryptic soy agar (Difco) was used as a nonselective medium for the repair and enumeration of injured cells. Following the solidification of tryptic soy agar in a petri dish, chemically injured *S.* Typhimurium or *L. monocytogenes* was directly inoculated into the medium. After 3 h of incubation at 37°C, in order to allow injured microorganisms to resuscitate, 7 mL of selective medium (XLD or OAB agar) was overlaid on the petri dishes. After solidification, the plates were further incubated for an additional 24 to 48 h at 37°C. After incubation, typical colonies were enumerated.

Statistical analysis

All experiments were repeated three times with duplicate samples and averages of duplicate plate counts from three replications were converted to units of $\log_{10}$ CFU/g. Data were analyzed via the ANOVA procedure of SAS software (Version 8.1. SAS Institute Inc., Cary, NC, USA) for a completely randomized design. When the main effect was significant ($p \leq 0.05$), the means were separated via Duncan's multiple range test.

RESULTS AND DISCUSSION

Table 1 shows the *S.* Typhimurium populations on radish sprouts before and after treatment with water, commercial chlorine sanitizer, and chlorine dioxide. Initial levels of *S.* Typhimurium were 4.64 log CFU/g and were unchanged by 10 min of treatment with distilled water. However, 10 min of treatment with 100 ppm commercial chlorine sanitizer did significantly reduce the population of *S.* Typhimurium ($p \leq 0.05$). When radish sprouts were treated with 50 ppm or 100 ppm ClO_2 for 5 min, or 200 ppm ClO_2 for 1 min, the levels of *S.* Typhimurium declined significantly ($p \leq 0.05$). In

Table 1. Populations (Log_{10} CFU/g)[1)] of *Salmonella* Typhimurium on radish sprouts treated with water or chemical sanitizers (commercial chlorine or chlorine dioxide) for 1, 5, and 10 min at room temperature

Time (min)	Culture medium	Treatment				
		Water	100 ppm Chlorine	50 ppm ClO_2	100 ppm ClO_2	200 ppm ClO_2
0	XLD	4.64±0.30$^{A2)}$	4.64±0.30^{A}	4.64±0.30^{A}	4.64±0.30^{A}	4.64±0.30^{A}
1		4.30±0.16$^{Aab3)}$	4.22±0.19^{ABab}	4.36±0.16^{ABa}	4.22±0.09^{ABab}	3.96±0.23^{Bb}
5		4.33±0.24^{Aa}	4.20±0.24^{ABa}	4.23±0.06^{Ba}	4.10±0.19^{Ba}	3.67±0.18^{Bb}
10		4.43±0.29^{Aa}	4.11±0.21^{Ba}	4.12±0.02^{Ba}	4.02±0.36^{Ba}	3.47±0.40^{Bb}
0	OV-XLD	5.19±0.20^{A}	5.19±0.20^{A}	5.19±0.20^{A}	5.19±0.20^{A}	5.19±0.20^{A}
1		4.90±0.19^{Aa}	4.90±0.21^{ABa}	4.62±0.17^{Ba}	4.48±0.38^{ABa}	4.48±0.18^{Ba}
5		5.06±0.38^{Aa}	4.92±0.14^{ABa}	4.68±0.16^{Bab}	4.26±0.58^{Bb}	4.18±0.24^{Bb}
10		5.02±0.51^{Aa}	4.63±0.14^{Bab}	4.60±0.21^{Bab}	4.29±0.31^{Bb}	4.10±0.23^{Bb}

[1)]Data represent means±standard deviations of three measurements.
[2)]Means with the same letter within a column are not significantly different ($p>0.05$).
[3)]Means with the same letter within a row are not significantly different ($p>0.05$).

Table 2. Populations (Log_{10} CFU/g)[1] of *Escherichia coli* O157:H7 on radish sprouts treated with water or chemical sanitizers (commercial chlorine or chlorine dioxide) for 1, 5, and 10 min at room temperature

Time (min)	Culture medium	Treatment				
		Water	100 ppm Chlorine	50 ppm ClO_2	100 ppm ClO_2	200 ppm ClO_2
0	SMAC	6.05±0.14$^{A2)}$	6.05±0.14^{A}	6.05±0.14^{A}	6.05±0.14^{A}	6.05±0.14^{A}
1		5.53±0.63$^{Aa3)}$	5.11±0.52^{Ba}	5.37±0.79^{Aa}	5.05±0.57^{Aa}	4.82±0.67^{Ba}
5		5.64±0.58^{Aa}	5.01±0.43^{Ba}	5.40±0.92^{Aa}	5.14±0.76^{Aa}	4.60±0.66^{Ba}
10		5.58±0.71^{Aa}	4.90±0.35^{Ba}	5.34±0.93^{Aa}	5.13±0.63^{Aa}	4.42±0.87^{Ba}
0	SPRAB	7.05±0.29^{A}	7.05±0.29^{A}	7.05±0.29^{A}	7.05±0.29^{A}	7.05±0.29^{A}
1		6.86±0.21^{Aa}	5.93±0.26^{Bab}	6.14±0.55^{Aab}	5.74±0.77^{Ab}	6.04±0.56^{ABab}
5		6.78±0.07^{Aa}	6.07±0.43^{Ba}	6.00±0.68^{Aa}	6.07±0.82^{Aa}	5.92±0.58^{Ba}
10		6.82±0.12^{Aa}	6.06±0.59^{Ba}	5.92±0.80^{Aa}	5.93±0.68^{Aa}	5.78±0.67^{Ba}

[1)]Data represent means±standard deviations of three measurements.
[2)]Means with the same letter within a column are not significantly different ($p>0.05$).
[3)]Means with the same letter within a row are not significantly different ($p>0.05$).

Table 3. Populations (Log_{10} CFU/g)[1] of *Listeria monocytogenes* on radish sprouts treated with water or chemical sanitizers (commercial chlorine or chlorine dioxide) for 1, 5, and 10 min at room temperature

Time (min)	Culture medium	Treatment				
		Water	100 ppm Chlorine	50 ppm ClO_2	100 ppm ClO_2	200 ppm ClO_2
0	OAB	4.29±1.01$^{A2)}$	4.29±1.01^{A}	4.29±1.01^{A}	4.29±1.01^{A}	4.29±1.01^{A}
1		4.18±1.03$^{Aa3)}$	3.50±0.84^{Aa}	3.98±0.87^{Aa}	4.13±0.61^{Aa}	3.61±0.78^{Aa}
5		4.18±1.02^{Aa}	3.65±0.82^{Aa}	4.12±0.85^{Aa}	4.03±0.66^{Aa}	3.49±0.96^{Aa}
10		4.19±0.93^{Aa}	3.52±0.91^{Aa}	3.96±0.88^{Aa}	4.57±0.41^{Aa}	3.33±1.05^{Aa}
0	OV-OAB	5.08±0.86^{A}	5.08±0.86^{A}	5.08±0.86^{A}	5.08±0.86^{A}	5.08±0.86^{A}
1		5.18±0.52^{Aa}	4.05±1.00^{Aa}	4.56±1.38^{Aa}	4.66±0.22^{Aa}	4.24±0.56^{Aa}
5		5.22±0.53^{Aa}	3.90±1.16^{Aa}	4.94±0.97^{Aa}	4.70±0.86^{Aa}	4.06±0.66^{Aa}
10		5.13±0.75^{Aa}	4.03±1.25^{Aa}	4.80±1.15^{Aa}	4.50±0.93^{Aa}	3.82±0.82^{Aa}

[1)]Data represent means±standard deviations of three measurements.
[2)]Means with the same letter within a column are not significantly different ($p>0.05$).
[3)]Means with the same letter within a row are not significantly different ($p>0.05$).

particular, treatment with 200 ppm ClO_2 was the most effective tested modality in terms of reducing *S.* Typhimurium levels, and resulted in a higher reduction (1.17 log reduction) compared to the other treatments. Additionally, 10 min of treatment with 100 ppm ClO_2 more effectively reduced the levels of *S.* Typhimurium (0.62 log reduction) than did treatment with 100 ppm commercial chlorine sanitizer (0.53 log reduction), although the level of reduction was not high (less than 1 log).

The effects of distilled water, commercial chlorine sanitizer, and ClO_2 on the survival of *E. coli* O157:H7 on radish sprouts are depicted in Table 2. The radish sprouts contained 6.05 log CFU/g, and that number was not reduced as the result of 10 min of treatment with distilled water, 50 ppm ClO_2, and 100 ppm ClO_2. Whereas treatment with 100 ppm commercial chlorine sanitizer and 200 ppm ClO_2 reduced the population significantly ($p\leq0.05$), and resulted in reductions of 1.15 and 1.63 log CFU/g after 10 min, respectively. In particular, populations of *E. coli* O157:H7 were affected by treatment with 200 ppm ClO_2, which reduced the levels of *E. coli* O157:H7 to 1.23, 1.45, and 1.63 log CFU/g for 1, 5 and 10 min, respectively.

L. monocytogenes populations as affected by different treatments (distilled water, commercial chlorine, and ClO_2) were illustrated in Table 3. Populations of *L. monocytogenes* were not affected by treatment with distilled water, and no significant differences were observed in the levels of *L. monocytogenes* after 10 min of treatment with commercial chlorine sanitizer or ClO_2 ($p>0.05$). However, among the applied treatments, treatment with 200 ppm ClO_2 was particularly effective in inhibiting the levels of *L. monocytogenes* in radish sprouts. When 200 ppm ClO_2 was applied for 1, 5 and 10 min, the levels of *L. monocytogenes* were reduced by 0.68, 0.80 and 0.96 log CFU/g, respectively.

This study investigated the effects of commercial chlorine sanitizer and ClO_2 in terms of the elimination of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* on radish sprouts. From the results, treatment with 200 ppm

ClO_2 proved most effective in the reduction of three foodborne pathogens contaminating radish sprouts. However, the reduction levels were not high, and were less than 2 log CFU/g for all three tested pathogens. Previous studies have reported the inhibitory effects of ClO_2 against foodborne pathogens on sprouts. Park et al (2007) reported that 10 min of treatment with 100 ppm chlorine water was effective in reducing the levels of aerobic counts on radish sprouts, resulting in a 0.39 log reduction. Lee et al (2002) noted that 10 min of treatment with 200 ppm sodium hypochlorite reduced the levels of *S.* Typhimurium and *L. monocytogenes* on mung bean sprouts from 4.96 and 5.25 log to 2.73 and 4.23 log, respectively. Taormina PJ and Beuchat (1999) reported that chlorine was ineffective at a concentration of approximately 1,000 ppm, and after treatment with 2,000 ppm a significant reduction of *E. coli* O157:H7 was observed on alfalfa seeds. Whereas Singh et al (2003) treated alfalfa seeds with 50 ppm ClO_2 for 3, 5, and 10 min and achieved *E. coli* O157:H7 reductions of 0.91, 1.14, and 1.22 log, respectively. Park et al (2008) found that 10 min of treatment with 50 and 200 ppm chlorine dioxide resulted in 0.55 and 1.08 log reductions in populations of *S.* Typhimurium on radish seeds. Kim et al (2009) reported that 5 min of treatment with 50 ppm chlorine dioxide reduced the numbers of coliforms on broccoli sprouts by 0.63 log. These studies reported very different levels of pathogens after treatment with sanitizers, thus indicating that the efficacy of different sanitizers might vary considerably depending on the type of fresh produce being treated.

After the antimicrobial treatments, sublethally injured foodborne pathogens assume added importance, as they are potentially as dangerous as their uninjured counterparts owing to their ability to survive and grow over the storage time of products, and to cause foodborne disease (Lee and Kang 2001). Thus, in this study, chemically injured *S.* Typhimurium and *L. monocytogenes* were investigated via the overlay method (Lee and Kang 2001), and SPRAB was used to evaluate chemically injured *E. coli* O157:H7 (Rhee et al 2003).

Tables 1, 2, and 3 also show populations of surviving and injured *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* in radish sprouts treated with water and chemical sanitizers (commercial chorine sanitizer and ClO_2). The differences between OV-XLD, SPRAB, & OV-OAB (non-selective medium), and XLD, SMAC, & OAB (selective medium), respectively, could represent levels of chemically injured *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes*, respectively. Prior to treatment, the populations of surviving cells including healthy and chemically injured *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* in radish sprouts were enumerated at 5.19, 7.05, and 5.08 log, respectively. We noted no significant differences in the levels of pathogens enumerated on selective agar and non-selective agar, thus indicating that treatment with water did not injure pathogens ($p>0.05$). In the case of OV-XLD, treatment with 100 ppm ClO_2 applied to radish sprouts significantly reduced the population of *S.* Typhimurium after 5 min ($p\leq0.05$). Significant reductions were also observed as the result of 1 min of treatment with 50 or 200 ppm ClO_2 ($p\leq0.05$). In particular, 10 min of treatment with 200 ppm ClO_2 proved effective in killing *S.* Typhimurium without generating injured cells. Treatment with 100 ppm commercial chlorine sanitizer and 100 ppm ClO_2 for 10 min resulted in 0.51 and 0.27 log populations of chemically-injured *S.* Typhimurium, respectively (Table 1). We noted no significant differences between the groups treated with 50 or 100 ppm ClO_2 for 10 min and initial levels of *E. coli* O157:H7 (Table 2) ($p>0.05$). After chemical treatment (commercial chlorine sanitizer and ClO_2) for 10 min at the same concentration (100 ppm), 1.16 and 0.80 log of chemically injured *E. coli* O157:H7 cells were counted, respectively. These results show that commercial chlorine sanitizer generated greater numbers of injured *E. coli* O157:H7 than did ClO_2. Similar results were observed for *L. monocytogenes* on OAB and OV-OAB. However, none of the tested treatments induced significant reductions in *L. monocytogenes* populations on radish sprouts ($p>0.05$). Lee et al (2002) reported that levels of injured *S.* Typhimurium and *L. monocytogenes* on mung bean sprouts were 1.3 log and 0.4 log when treated with 10 min of 200 ppm sodium hypochlorite, and these levels were higher than the levels observed after treatments with chlorous acid. However, they also detected no difference between levels of injured *E. coli* O157:H7 after treatments with ClO_2 and sodium hypochlorite. Therefore, levels of chemically injured cells could differ depending on the pathogen type. However, in general, ClO_2 proved more effective in reducing pathogens without chemically injuring cells than commercial chlorine sanitizer in this study.

In conclusion, ClO_2 treatment proved effective in reducing the levels of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* contaminating radish sprouts. In particular, 10 min of treatment with 200 ppm ClO_2 proved the most effective in inhibiting *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* populations, reducing the original levels by 1.17, 1.63, and 0.96 log, respectively. When the chemically injured cells were assessed via SPRAB for *E. coli* O157:H7 and selective overlay methods for *L. monocytogenes* and *S.* Typhimurium, respectively, it was noted that commercial chlorine sanitizer generated greater numbers of injured *L. monocytogenes* than did ClO_2. These results show that ClO_2 may prove useful as an alternative sanitizer for reducing pathogens on fresh produce. However, the effectiveness of ClO_2 could differ considerably depending on the type of raw fresh produce being treated; therefore, additional studies will be necessary to determine the efficacy of ClO_2 on various types of raw fresh produce. Additionally, the efficacy of ClO_2 in reducing pathogens on radish sprouts was not particularly high, although it was

more effective than was commercial chlorine sanitizer. Therefore, further studies will focus on improving the effectiveness of sanitizing pathogens on radish sprouts.

ACKNOWLEDGMENT

This study was carried out with the support of the Cooperative Research Program for Agricultural Science & Technology Development (Project no. 200906AFT13368 1013), RDA, Republic of Korea.

REFERENCES

Benarde MA, Israel BM, Olivieri VP, Granstrom ML. 1965. Efficiency f chlorine dioxide as a bactericide. Appl Microbiol. 13:776-780.

Beuchat, LR. 1995. Pathogenic microorganisms associated with fresh produce. J Food Prot. 59:204-216.

Beuchat LR, Nail BV, Adler BB, Clavero MR. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. J Food Prot. 61:1305-1311.

Beuchat LR. 1999. Survival of Enterohemorrhagic *Escherichia coli* O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant. J Food Prot. 62:845-849.

Cherry JP. 1999. Improving the safety of fresh produce with antimicrobials. Food Thechnol. 53:54-59.

Fu T, Stewart D, Reineke K, Ulaszek J, Schlesser J, Tortorello M. 2001. Use of Spent irrigation water for microbiological analysis of alfalfa sprouts. J Food Prot. 64:802-806.

Kim YJ, Kim MH, Song KB. 2009. Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on broccoli sprouts. Food Cont. 20:1002-1005.

Lee SY, Kang DH. 2001. Suitability of overlay method for recovery of heat-injured Listeria *monocytogenes* and *Salmonella typhimurium*. Food Sci Biotechnol. 10: 323-326.

Lee SY, Yun KM, Fellman J, Kang DH. 2002. Inhibition of *Salmonella* Typhimurium and *Listeria monocytogenes* in mung bean sprouts by chemical treatment. J Food Prot. 65:1088-1092.

Lee SY, Gray PM, Dougherty RH, Kang DH. 2004. The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. 92:121-127.

Matos A, JAY L. Garland JL, Fett WF. 2002. Composition and Physiological Profiling of Sprout-Associated Microbial Communities. J Food Prot. 65:1903-1908.

NACMCF, National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. Int J Food Microbiol. 52:123-153.

Park KJ, Lim JH, Kim JH, Jeong JW, Jo JH, Jeong SW. 2007. Reduction of microbial load on radish (*Raphanus sativus L.*) seeds by aqueous chlorine dioxide and hot water treatments. Korean J Food Preserv. 14:487-491.

Park KJ, Lim JH, Kim BK, Kim JC, Jeong JW, Jeong SW. 2008. Effect of Aqueous Chlorine Dioxide and Citric Acid on Reduction of Salmonella typhimurium on Sprouting Radish Seeds. Korean J Food Preserv. 15:754-759.

Rhee MS, Lee SY, Hillers VN, Mccury SM, Kang DH. 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 RK, Bhunia AK. 2003. Sequential disinfection of *Escherichia coli* O157:H7 inoculated alfalfa seeds before and during sprouting using aqueous chlorine dioxide, ozonated water, and thyme essential oil. Lebensm Wiss U-Technol. 36:235-243.

Stopforth JD, Mai T, Kottapalli B, Samadpour M. 2008. Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157:H7, Salmonella, and *Listeria monocytogenes* inoculated onto leafy greens. J Food Prot. 71:625-628.

Taormina PJ, Beuchat LR. 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J Food Prot. 62:318-324.

Taormina PJ, Beuchat LR, Slutsker L. 1999. Infections associated with eating seed sprouts: an international concern. Emer Infect Dis. 5:626-634.