



## Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro

Ana Allende<sup>a,\*</sup>, James McEvoy<sup>b</sup>, Yang Tao<sup>c</sup>, Yaguang Luo<sup>b</sup>

<sup>a</sup> Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, E-30100 Espinardo, Murcia, Spain

<sup>b</sup> Produce Quality and Safety Laboratory, US Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Building. 002, Beltsville, MD 20705, USA

<sup>c</sup> Department of Biological Resources Engineering, University of Maryland, College Park, MD 20742-1427, USA

### ARTICLE INFO

#### Article history:

Received 27 February 2008

Received in revised form 15 April 2008

Accepted 13 May 2008

#### Keywords:

Cilantro quality  
Fresh-cut  
Microbial growth  
Pathogen  
Sanitizer  
Washing

### ABSTRACT

Fresh-cut cilantro is particularly susceptible to microbial growth and, therefore, use of an effective sanitizer on this product is of great importance. The objective of this study was to evaluate the efficacy of different sanitizing treatments on reducing *Escherichia coli* O157:H7 populations, aerobic mesophilic bacterial, yeast and mould counts on fresh-cut cilantro. Cut cilantro was treated with sodium hypochlorite (SH) at 0.2 g L<sup>-1</sup> free chlorine and acidified sodium chlorite (ASC) at 0.1, 0.25, 0.5 and 1 g L<sup>-1</sup>, along with the components of ASC, i.e., citric acid (CA) at 6 g L<sup>-1</sup> and sodium chlorite (SC) at 1 g L<sup>-1</sup>. In the present study, it was found that SH inactivated, at maximum, 1–1.3 logcfu g<sup>-1</sup> of background or pathogenic microflora present on cut cilantro. However, reductions of more than 3 logcfu g<sup>-1</sup> were observed after washing with 1 g L<sup>-1</sup> of ASC. Moreover, when lower concentrations of ASC were used (0.25 and 0.5 g L<sup>-1</sup>), microbial populations were reduced by about 2 logcfu g<sup>-1</sup>. SC was as effective as ASC at 1 g L<sup>-1</sup> in reducing aerobic mesophilic bacteria and *E. coli* O157:H7 populations, although it was not as effective as ASC in reducing yeast and mould populations.

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Food safety constitutes a growing concern for regulatory agencies, producers and the public due to the incidence of foodborne illness caused by enteric human pathogens in various foods at retail and commercial food service facilities (Bhagwat, Saftner, & Abbott, 2004; CAST, 1994; CDC, 2004). Fruits and vegetables are important components of a healthy diet. However, recent studies show that the occurrence of foodborne illness related to the consumption of fruit and vegetables has increased, such as the two outbreaks associated with the consumption of lettuce and spinach in the fall of 2006 (Behrsing, Winkler, Franz, & Premier, 2000; Beuchat et al., 2001; Erickson & Doyle, 2007; FDA, 2006). A wide variety of illnesses associated with fresh produce have involved herbs such as cilantro and parsley (Campbell et al., 2001). An analysis conducted by the FDA in 1999 and 2000, determined that cilantro was one of three imported produce items with a high incidence of pathogen contamination (FDA, 2001). Of significant concern are the human pathogens *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Washing produce with sanitizing solutions is the only step in the fresh-cut produce production chain where a reduction in spoilage microorganisms and potential pathogens can be achieved (Allende, Aguayo, & Artés, 2004; Allende, Selma, López-Gálvez, Villares, & Gil, 2008; Beuchat, Nail, Adler, & Clavero, 1998; Wiley, 1994). However, limited scientific information is available on the efficacy of many disinfection methods for reducing the populations of pathogenic bacteria on fruits and vegetables (Lukasik et al., 2003).

Sodium hypochlorite (NaOCl; SH) is commonly used to sanitize fresh-cut cilantro. However, the antimicrobial effectiveness of this chlorinated water is limited and at the consumer level, residual chlorine and its reaction products in the commodity shall be reduced to a quantity that is technologically unavoidable, has no persisting technological effect in the product, and is harmless to health (Delaquis, Stewart, Toivonen, & Moyls, 1999; Klaiber, Baur, Wolf, Hammes, & Carle, 2005; Nguyen-the & Carlin, 1994; Simons & Sanguansri, 1997). It was reported that if a pathogen can persist on the phylloplane, then the chance of an infectious dose remaining at consumption is increased and this microbial attachment to the hydrophobic plant surface is believed to limit contact between chlorinated water and contaminating microorganisms (Beuchat, 1992; Delaquis et al., 1999; Heaton & Jones, 2007). Furthermore, fresh-cut processing can lead to faster microbial growth by break-

\* Corresponding author. Tel.: +34 968396275; fax: +34 968396213.  
E-mail address: [aallende@cebas.csic.es](mailto:aallende@cebas.csic.es) (A. Allende).

ing protective surface structures and increasing the availability of nutrients and surface area (Brackett, 1994). Liao and Cooke (2001) reported that bacterial human pathogens bound to cut surfaces of green pepper were more difficult to kill with conventional sanitizers than those present on non-cut surfaces. Moreover, the reaction of active hypochlorite with nitrogen-containing compounds in foods resulting in the formation of toxic compounds, especially trihalomethanes, has incited research for alternative disinfection agents (Allende et al., 2008; Bower & Daeschel, 1999; Inatsu, Bari, Kawasaki, Isshiki, & Kawamoto, 2005).

Acidified sodium chlorite (ASC; Alcide Corp., Redmond, WA) is a highly effective antimicrobial that is produced by lowering the pH (2.5–3.2) of a solution of sodium chlorite ( $\text{NaClO}_2$ ; SC) with any GRAS acid (Warf, 2001). The FDA has recently approved ASC ( $0.5\text{--}1.2\text{ g L}^{-1}$ ) for spray or dip application on various food products, including fresh and fresh-cut produce (Code of Federal Regulations, 2000). Inatsu et al. (2005) demonstrated the same sanitation efficacy of different organic acid-activated acidified sodium chlorite solutions. Currently, ASC is commercially supplied as a kit containing citric acid (CA) and SC. These chemicals when combined produce active chlorine dioxide ( $\text{ClO}_2$ ), which is more soluble than sodium hypochlorite ( $\text{NaOCl}$ ) in water and has about 2.5 times greater oxidizing capacity than hypochlorous acid ( $\text{HOCl}$ ) (Inatsu et al., 2005). A number of reports have described the strong efficacy of ASC in the FDA approved application concentration range of  $0.5\text{--}1.2\text{ g L}^{-1}$  on inactivation of pathogens, including *E. coli* O157:H7 and *Salmonella* spp., (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004; Park & Beuchat, 1999; Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna, & González-Aguilar, 2007). However, a negative impact on organoleptic quality of red meat and shredded carrots occurred when ASC was used within the approved concentration range (Bosilevac, Shackelford, Fahle, Biela, & Koohmaraie, 2004). Therefore, it is critical to find the concentration of ASC that will optimize microbial safety while maintaining quality of fresh-cut cilantro.

The main objectives of this research were to compare the efficacy of ASC at various concentrations to that of SH on reducing microbial populations (including the human pathogen *E. coli* O157:H7) on cut cilantro, and to examine the roles of the individual components of ASC, i.e., SC and CA, in this inactivation phenomenon.

## 2. Materials and methods

### 2.1. Preparation of cilantro

Fresh cilantro (*Coriandrum sativum* L.) was obtained from a local wholesale market in Jessup, MD (USA), on the day of its arrival from the grower. The product was transported (within 30 min) under refrigerated conditions to the Product Quality and Safety Laboratory (Beltsville, MD, USA). The product was physically inspected and stems and defective leaves were removed. The product was stored overnight at  $5\text{ }^\circ\text{C}$ . The next morning, 3 kg of cilantro were processed in a fresh-cut preparation room at  $10\text{ }^\circ\text{C}$ . Selected cilantro leaves were cut into approximately 1.0 cm segments using a sharp knife. Samples of 100 g of fresh-cut cilantro were placed in nylon mesh bags (Linens N' Things, Clifton, NJ). All samples were stored at  $5\text{ }^\circ\text{C}$  for about 2 h before the inoculation process was carried out.

### 2.2. Natural microflora analyses of fresh-cut cilantro

Cut cilantro samples of 25 g each were homogenized in 225 mL sterile peptone water ( $8.5\text{ g L}^{-1}$  of  $\text{NaCl}$  [S9625, Sigma-Aldrich, Inc.] plus  $1\text{ g L}^{-1}$  of neutralized bacteriological peptone [Difco, Detroit, Mich.]) using a stomacher 400 Biomaster (Seward Limited, London, UK). Sterile filter stomacher bags (Seward Limited, Lon-

don, UK) were used to eliminate solid particles from the cilantro homogenate. Ten fold dilution series were made in peptone saline solution as needed for plating. Samples ( $100\text{ }\mu\text{L}$ ) of each cilantro filtrate or their corresponding dilutions were logarithmically spread on agar plates (Wasp II Spiral Plater, DW Scientific, West Yorkshire, UK). Aerobic mesophilic bacteria were enumerated on Tryptic Soy Agar (TSA, Difco) plates after incubation at  $30\text{ }^\circ\text{C}$  for 48 h and yeast and moulds on Potato Dextrose Agar (PDA, Difco) supplemented with chloramphenicol ( $200\text{ mg L}^{-1}$ ; Sigma-Aldrich, St. Louis, MO, USA) after incubation at  $30\text{ }^\circ\text{C}$  for 48–72 h. Microbial colonies were counted with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK).

### 2.3. *Escherichia coli* O157:H7

A cocktail of three nalidixic acid-resistant ( $\text{Nal}^R$ ) strains of *E. coli* O157:H7, which were derived from the outbreak strains, F6460, F15110, H26696, were used in this study. F6460 was isolated from patient fecal samples during a 1999 Nebraska lettuce outbreak and a gift from Timothy Barrett, Centers for Disease Control, Atlanta, Ga. (Wachtel & Charkowski, 2002). The strains F15110 and H26696 were clinical samples from an outbreak associated with fresh-cut watermelon in Wisconsin in 2000, and a gift from Milwaukee Children's Hospital. Cultures were kept at  $-80\text{ }^\circ\text{C}$  in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, Mich.) containing 25% (vol/vol) glycerol. *E. coli* O157:H7 strains were grown at  $37\text{ }^\circ\text{C}$ , shaken in LB broth supplemented with nalidixic acid ( $\text{Nal}$ ) ( $50\text{ }\mu\text{g L}^{-1}$ ) until stationary phase (20 h growth) and cultured onto LB-Nal agar at  $37\text{ }^\circ\text{C}$  for 24 h.

### 2.4. Inoculation

The inoculation process involved the use of a cocktail of three *E. coli* O157:H7  $\text{Nal}^R$  strains. The *E. coli* O157:H7  $\text{Nal}^R$  strains were consecutively subcultured twice in 100 mL of LB-Nal broth at  $37\text{ }^\circ\text{C}$  for 24 h with constant agitation at 175 rpm to obtain a final  $\text{OD}_{600}$  reading of about 0.4. After cultures were transferred the second time, they were allowed to adapt to a final temperature of  $12\text{ }^\circ\text{C}$  for 4 h. Cultures were washed twice by centrifugation ( $4000\text{g}$ , 15 min,  $4\text{ }^\circ\text{C}$ ) with 0.1% peptone water. The final pellets were resuspended in 5–10 mL of 0.1% peptone water containing 5% horse serum according to the method of Beuchat et al. (2001) and Burnett, Iturriaga, Escartin, Pettigrew, and Beuchat (2004). Equal volumes of cell suspensions were combined to give approximately equal populations of each culture. The strain cocktail was proportionally diluted in deionized water at  $12\text{ }^\circ\text{C}$  to achieve a final concentration of about  $10^7\text{ cfu mL}^{-1}$  of *E. coli* O157:H7  $\text{Nal}^R$ . Final concentrations of the inoculum solutions were confirmed by plating on Sorbitol MacConkey agar (SMAC) (Difco) supplemented with nalidixic acid ( $50\text{ }\mu\text{g L}^{-1}$ ). The pathogenic suspension was maintained at room temperature and applied to cilantro within 10 min of preparation. The mesh bags of cut cilantro were completely immersed in the inoculum solution and kept under constant agitation for 30 min. After inoculation, the product was maintained at  $4\text{ }^\circ\text{C}$  for approximately 60 min. to increase the number of cells attached to the product. Finally, excess inoculum was removed by centrifugation using a manually-operated enclosed spinner (OXO Good Grips, Elmira, NY) for approximately 20 s. The entire experiment was carried out in a Biosafety Level 2 Laboratory.

### 2.5. Wash treatments

Cut cilantro was washed with water solutions of sodium hypochlorite ( $\text{NaOCl}$ , SH, Aldrich Chemical Co., Inc., Milwaukee, Wis.) at  $0.2\text{ g L}^{-1}$  of free chlorine (pH 6.5), acidified sodium chlorite ( $\text{NaClO}_2$ , ASC, SANOVA<sup>®</sup>, Alcide Corp., Redmond, Wash) at 0.1, 0.25, 0.5 and

1 g L<sup>-1</sup>, citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>, CA, Aldrich Chemical Co., Inc., Milwaukee, Wis.) at 6 g L<sup>-1</sup> and sodium chlorite (NaClO<sub>2</sub>, SC, Aldrich Chemical Co., Inc., Milwaukee, Wis.) at 1 g L<sup>-1</sup>. The initial free chlorine concentration present in the chlorinated solutions was determined using a Chlorine Photometer (CP-15, HF Scientific Inc., Ft. Myers, FL). Three liters of tap water at 5 °C were used for the preparation of each wash. Washing solutions were prepared immediately before application and used within 30 min. Approximately 1 h after the inoculation step, each mesh bag was dipped into one sanitizer solution for 1 min. The excess wash solution was removed by centrifugation with a hand operated enclosed spinner (OXO Good Grips, Elmira, NY) for 30 s. The washing treatments were carried out in a Biosafety Level 2 Laboratory.

### 2.6. Antimicrobial activity of wash solutions

Cilantro samples of 25 g were collected from each disinfection treatment immediately after washing and homogenized in 225 mL sterile peptone water and plated on agar plates as previously indicated in Section 2.2. Sorbitol MacConkey agar (SMAC) (Difco) supplemented with NaI (50 µg L<sup>-1</sup>) and sodium pyruvate (0.1%) was used to determine the survival of *E. coli* O157:H7 incubated at 37 °C for 24 h (Strockbine, Wells, Bopp, & Barrett, 1998). The inclusion of sodium pyruvate (0.1%) was to aid in the recovering of injured *E. coli* O157:H7 cells (Mizunoe, Wai, Takade, & Yoshida, 1999). Aerobic mesophilic bacteria, yeasts and moulds were enumerated as indicated in Section 2.2. Microbial colonies were counted with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK).

### 2.7. Experimental design

The described experiment was repeated three times separately in time, each with duplicate samples. Statistical analysis of the data was carried out using the SAS general linear models procedure (SAS version 8.2, SAS Institute Inc., Cary, NC, USA) to determine significant differences in microbial counts for treatments.

## 3. Results and discussion

Unwashed and uninoculated cilantro showed typically high initial microbial loads. Aerobic mesophilic bacterial counts on unwashed cut cilantro were very similar in all the replications, with an average value of 7.00 ± 0.12 logcfu g<sup>-1</sup>, while the average value of yeasts and moulds was 4.57 ± 0.37 logcfu g<sup>-1</sup>. The obtained bacterial values agree with the initial aerobic mesophilic bacterial counts reported by Wang, Feng, and Luo (2004) (6.7 logcfu g<sup>-1</sup>), and is only slightly higher than counts reported by Fan, Niemira, and Sokorai (2003) (5.9 logcfu g<sup>-1</sup>). Babic and Watada (1996) attributed this elevated contamination to the fact that cilantro is a low-growing crop. The cilantro leaf pattern also contributes to its susceptibility to microbial growth by providing a large exposed surface area for microbial attachment and growth. Therefore, a sanitizing procedure is often used in the production of fresh-cut cilantro for improved quality and safety.

Washing cut cilantro in a SH solution resulted in reductions of aerobic mesophilic bacterial and *E. coli* O157:H7 counts of about 1 logcfu g<sup>-1</sup> (Figs. 1 and 2). This value agrees with previous reports in fresh-cut vegetable products (Beuchat et al., 1998; Foley, Euper, Caporaso, & Prakash, 2004). However, yeast and mould counts were not significantly (*P* < 0.01) reduced in fresh-cut cilantro after washing with SH, when compared to unwashed produce (Fig. 3). It can be concluded that SH inactivated, at maximum, 1–1.3 logcfu g<sup>-1</sup> of background microflora present in fresh-cut products. Therefore, according to Beuchat (1992), chlorine dips

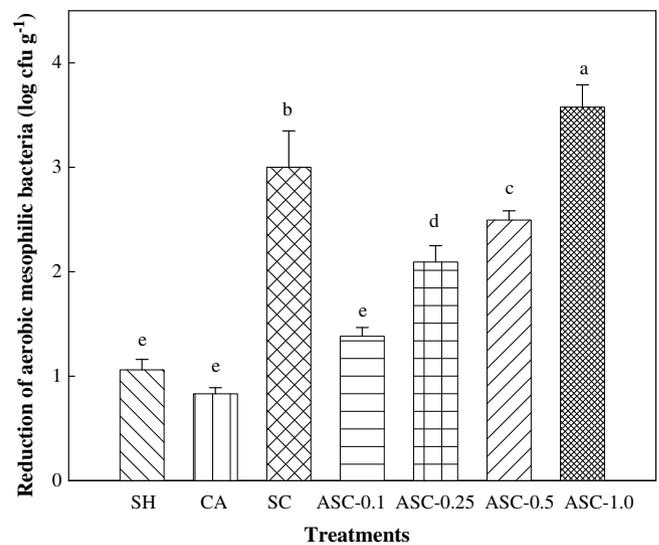


Fig. 1. Reduction of aerobic mesophilic bacterial populations on fresh-cut cilantro after washing with sodium hypochlorite (SH, 0.2 g L<sup>-1</sup>), citric acid (CA, 6 g L<sup>-1</sup>), sodium chlorite (SC, 1 g L<sup>-1</sup>) and acidified sodium chlorite (ASC 0.1–1.0 g L<sup>-1</sup>), relative to an unwashed control. Vertical bars represent means of three replications ±SE. Bars labeled with different letters indicate significant difference at *P* < 0.05.

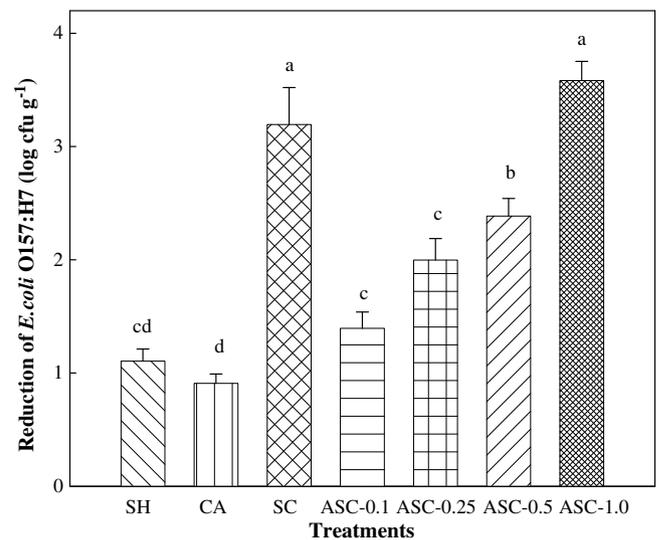
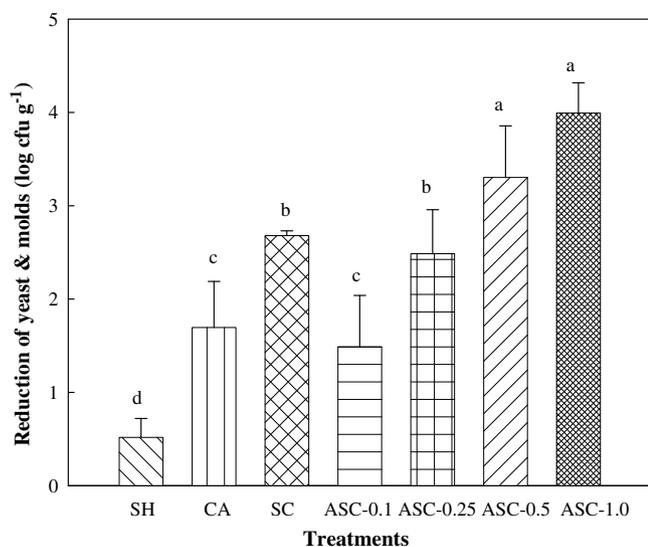


Fig. 2. Reduction of *Escherichia coli* O157:H7 (F6460, F15110N, H26696N) populations on fresh-cut cilantro after washing with sodium hypochlorite (SH, 0.2 g L<sup>-1</sup>), citric acid (CA, 6 g L<sup>-1</sup>), sodium chlorite (SC, 1 g L<sup>-1</sup>) and acidified sodium chlorite (ASC 0.1–1.0 g L<sup>-1</sup>), relative to an unwashed control. Vertical bars represent means of three replications ±SE. Bars labeled with different letters indicate significant difference at *P* < 0.05.

should not be relied on to kill pathogens on produce and they should be used to reduce viable microorganisms rather than eliminate them.

ASC at 1 g L<sup>-1</sup> has been found to effectively reduce aerobic bacterial growth in shredded carrots (Gonzalez et al., 2004; Ruiz-Cruz, Luo, Gonzalez, Tao, & González, 2006; Ruiz-Cruz et al., 2007). In the present study, the antimicrobial activity of ASC in cut cilantro was significantly (*P* < 0.01) influenced by the applied concentration. Thus, the reduction in microbial counts increased with the increase in ASC concentration (Figs. 1–3). Maximum bacterial reductions, of more than 3 logcfu g<sup>-1</sup>, were observed after washing with 1 g L<sup>-1</sup> of ASC and SC (Figs. 1 and 2). Moreover, when lower concentrations of ASC were used (0.25 and 0.5 g L<sup>-1</sup>), the obtained reductions in



**Fig. 3.** Reduction of yeast and mould populations on fresh-cut cilantro after washing with sodium hypochlorite (SH, 0.2 g L<sup>-1</sup>), citric acid (CA, 6 g L<sup>-1</sup>), sodium chlorite (SC, 1 g L<sup>-1</sup>) and acidified sodium chlorite (ASC 0.1–1.0 g L<sup>-1</sup>), relative to an unwashed control. Vertical bars represent means of three replications ±SE. Bars labeled with different letters indicate significant difference at  $P < 0.05$ .

aerobic mesophilic bacterial and yeasts and moulds were still significantly ( $P < 0.01$ ) higher than those obtained in SH treated samples (Figs. 1–3). Similar results were obtained in shredded carrots by Ruiz-Cruz et al., 2006, 2007. On the other hand, CA treatment achieved the lowest reduction in bacterial counts (Figs. 1 and 2).

Conner (2001) and Caldwell, Adler, Anderson, Williams, and Beuchat (2003) affirmed that ASC applied to inoculated fresh fruits and vegetables at 1.2 g L<sup>-1</sup> for 1 min, killed at least 99.9% of *Salmonella* serotypes, *E. coli* O157:H7, and *L. monocytogenes* on carrots, strawberries, tomatoes, cucumbers, lettuce, cantaloupe and apples. Gonzalez et al. (2004), Inatsu et al. (2005) and Ruiz-Cruz et al. (2007) found a strong *E. coli* O157:H7 reduction, even under process water conditions, when using 0.5 and 1 g L<sup>-1</sup> ASC on shredded carrots and Chinese cabbage. Lukasik et al. (2003) found that ASC at 0.1 and 0.2 g L<sup>-1</sup> was more effective at reducing *E. coli* O157:H7 and *Salmonella* Montevideo populations on strawberries than stabilized chlorine dioxide or free chlorine disinfectants at comparable concentrations. However, they did not recommend concentrations greater than 0.2 g L<sup>-1</sup> because of observed deleterious effects on the strawberries. In the present study, all tested washing solutions significantly ( $P < 0.01$ ) reduced *E. coli* O157:H7 populations on fresh-cut cilantro (Fig. 2) when compared to unwashed produce. However, clear differences ( $P < 0.01$ ) were observed among treatments. Thus, ASC at 1 g L<sup>-1</sup> achieved the greatest reduction ( $3.58 \pm 0.17$  log cfu g<sup>-1</sup>), followed by 1 g L<sup>-1</sup> SC ( $3.19 \pm 0.62$  log cfu g<sup>-1</sup>) and ASC at 0.5 g L<sup>-1</sup> ( $2.38 \pm 0.15$  log cfu g<sup>-1</sup>). A reduction of less than 2 log units was obtained by using CA at 6 g L<sup>-1</sup> and ASC at 0.1 and 0.25 g L<sup>-1</sup>. However, similar to the viable aerobic mesophilic bacterial and yeast and mould counts, ASC at 0.25 g L<sup>-1</sup>, still achieved a significantly ( $P < 0.01$ ) higher reduction than SH.

Warf (2001) hypothesized that the mode of action of ASC derives from the uncharged chlorous acid, which is formed by the acidification of chlorite. Chlorous acid gradually decomposes to form chlorate ions, chlorine dioxide, and chloride ions. These reactive intermediates are highly oxidative with broad-spectrum germicidal activity (FDA, 2007). Chlorous acid is also able to penetrate bacterial cell walls. This ability is thought to facilitate proton leakage into cells, which increases energy use by the cells

in order to maintain homeostasis (Warf, 2001). To determine whether or not the combination of SC with CA is needed for the effectiveness of the treatment, both ingredients were separately tested. It was observed that despite the low pH of the CA solution (pH  $2.2 \pm 0.1$ ), this treatment did not reduce growth of *E. coli*, aerobic mesophilic bacterial and yeasts and moulds to the same degree as either SC (pH  $9.4 \pm 0.3$ ) or ASC (pH range  $2.6 \pm 0.4$ – $2.5 \pm 0.2$ ). In fact, SC alone reduced microbial populations nearly as much as ASC (Figs. 1–3). This suggests that SC (and not CA or the pH of the treatment) was the major antimicrobial factor.

#### 4. Conclusions

In summary, the commercial ASC product exhibited strong efficacy on reduction of microorganisms, including *E. coli* O157:H7. Both ASC and SC significantly reduced aerobic mesophilic bacteria, yeast and moulds and *E. coli* O157:H7 populations, even when ASC applied at low concentrations. Since ASC at the current FDA approved range (0.5–1.2 g L<sup>-1</sup>) is known to cause tissue damage to some food products, our findings that ASC or SC at concentrations below the FDA approved range achieved better efficacy on microbial inhibition than SH, provide valuable insight regarding the optimization of ASC and SC applications to maintaining both food safety and quality.

#### Acknowledgements

The authors wish to thank Verneta Gaskins for excellent technical support, and Dr. Shengmin Lu and Ellen Turner for assistance in sample preparation. A. Allende is the recipient of a JaeDoc contract (CSIC). Use of a company name or product by the USDA does not imply approval or recommendation of the product to the exclusion of others that also may be suitable.

#### References

- Allende, A., Aguayo, E., & Artés, F. (2004). Quality of commercial minimally processed red lettuce throughout the production chain and shelf life. *International Journal of Food Microbiology*, 91, 109–117.
- Allende, A., Selma, M. V., López-Gálvez, F., Villacusa, R., & Gil, M. I. (2008). Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biology and Technology*, 49, 155–163.
- Babic, I., & Watada, A. E. (1996). Microbial populations of fresh-cut spinach leaves affected by controlled atmospheres. *Postharvest Biology and Technology*, 9, 187–193.
- Behring, J., Winkler, S., Franz, P., & Premier, R. (2000). Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. *Postharvest Biology and Technology*, 19, 187–192.
- Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation*, 12, 6–9.
- Beuchat, L. R., Farber, J. M., Garrett, E. H., Harris, L. J., Parish, M. E., Suslow, T. V., et al. (2001). Standardization of a method to determine the efficacy of sanitizers in inactivating human pathogenic microorganisms on raw fruits and vegetables. *Journal of Food Protection*, 64, 1079–1084.
- Beuchat, L. R., Nail, B. V., Adler, B. B., & Clavero, M. R. S. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection*, 61, 1305–1311.
- Bhagwat, A. A., Saftner, R. A., & Abbott, J. A. (2004). Evaluation of wash treatments for survival of foodborne pathogens and maintenance of quality characteristics of fresh-cut apple slices. *Food Microbiology*, 21, 319–326.
- Bosilevac, J. M., Shackelford, S. D., Fahle, R., Biela, T., & Koochmaria, M. (2004). Decreased dosage of acidified sodium chlorite reduces microbial contamination and maintains organoleptic qualities of ground beef products. *Journal of Food Protection*, 67, 2248–2254.
- Bower, C. K., & Daeschel, M. A. (1999). Resistance responses of microorganisms in food environments. *International Journal of Food Microbiology*, 50, 33–44.
- Brackett, R. E. (1994). Microbiological spoilage and pathogens in minimally processed refrigerated fruits & vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables* (pp. 269–312). New York, USA: Chapman and Hall.
- Burnett, A. B., Iturriaga, M. H., Escartin, E. F., Pettigrew, C. A., & Beuchat, L. R. (2004). Influence of variations in methodology on populations of *Listeria monocytogenes* recovered from lettuce treated with sanitizers. *Journal of Food Protection*, 67, 742–750.

- Caldwell, K. N., Adler, B. B., Anderson, G. L., Williams, P. L., & Beuchat, L. R. (2003). Ingestion of *Salmonella enterica* serotype *poona* by a free-living nematode, *Caenorhabditis elegans*, and protection against inactivation by produce sanitizers. *Applied and Environmental Microbiology*, *69*, 4103–4110.
- Campbell, J. V., Mohle-Boetani, J., Reporter, R., Abbott, S., Farrar, J., Brandl, M., et al. (2001). An outbreak of *Salmonella* serotype *thompson* associated with fresh cilantro. *Journal of Infectious Diseases*, *183*, 984–987.
- Council for Agriculture Science and Technology. (1994). Foodborne pathogens: risks and consequences. Report no. 122. Council for Agriculture Science and Technology, Ames, Iowa, USA.
- Centers for Disease Control and Prevention. (2004). Diagnosis and management of foodborne illnesses. A primer for physicians and other health care professionals. April 16, 53 / No. RR-4: <<http://www.cdc.gov/mmwr/PDF/RR/RR5304.pdf>>. Accessed 04.02.08.
- Code of Federal Regulations. (2000). Title 21, Part 173.325. Secondary direct food additives permitted in food for human consumption: acidified sodium chlorite solutions. <[http://www.access.gpo.gov/nara/cfr/waisidx\\_00/21cfr173\\_00.html](http://www.access.gpo.gov/nara/cfr/waisidx_00/21cfr173_00.html)>. Accessed 01.02.08.
- Conner, D.E. (2001). Effectiveness of acidified sodium chlorite as an antimicrobial treatment of fresh produce. In *2001 IFT Annual Meeting* (pp. 91–96).
- Delaquis, P. J., Stewart, S., Toivonen, P. M. A., & Moyls, A. L. (1999). Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Research International*, *32*, 7–14.
- Erickson, M. C., & Doyle, M. P. (2007). Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*. *Journal of Food Protection*, *70*, 2426–2449.
- Fan, X., Niemira, B. A., & Sokorai, K. J. B. (2003). Sensorial, nutritional and microbiological quality of fresh cilantro leaves as influenced by ionizing radiation and storage. *Food Research International*, *36*, 713–719.
- Food and Drug Administration. (2001). FDA survey of imported fresh produce. FY 1999 Field Assignment. <<http://vm.cfsan.fda.gov/~dms/prodsurv.html>>. Accessed 01.02.08.
- Food and Drug Administration. (2006). The FDA: Fresh Leafy Greens Grown in the United States Are Safe. <[http://www.fda.gov/fdac/features/2006/606\\_greens.html](http://www.fda.gov/fdac/features/2006/606_greens.html)>. Accessed 14.02.08.
- Food and Drug Administration. (2007). Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables. <<http://www.cfsan.fda.gov/~dms/prodgui3.html#ch8>>. Accessed 10.02.08.
- Foley, D., Euper, M., Caporaso, F., & Prakash, A. (2004). Irradiation and chlorination effectively reduces *Escherichia coli* O157:H7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. *Journal of Food Protection*, *67*, 2092–2098.
- Gonzalez, R. J., Luo, Y., Ruiz-Cruz, S., & McEvoy, J. L. (2004). Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection*, *67*, 2375–2380.
- Heaton, J. C., & Jones, K. (2007). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, *104*, 613–626.
- Inatsu, Y., Bari, M. L., Kawasaki, S., Isshiki, K., & Kawamoto, S. (2005). Efficacy of acidified sodium chlorite treatments in reducing *Escherichia coli* O157:H7 on Chinese cabbage. *Journal of Food Protection*, *68*, 251–255.
- Klaiber, R. G., Baur, S., Wolf, G., Hammes, W. P., & Carle, R. (2005). Quality of minimally processed carrots as affected by warm water washing and chlorination. *Innovative Food Science and Emerging Technologies*, *6*, 351–362.
- Liao, C. H., & Cooke, P. H. (2001). Response to trisodium phosphate treatment of *Salmonella chester* attached to fresh-cut green pepper slices. *Canadian Journal of Microbiology*, *47*, 25–32.
- Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W.-Y., et al. (2003). Reduction of poliovirus 1, bacteriophages, *Salmonella* Montevideo, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. *Journal of Food Protection*, *66*, 188–193.
- Mizunoe, Y., Wai, S. N., Takade, A., & Yoshida, S. (1999). Restoration of culturability of starvation-stressed and low-temperature-stressed *Escherichia coli* O157:H7 cells by using H<sub>2</sub>O<sub>2</sub>-degrading compounds. *Archives of Microbiology*, *172*, 63–67.
- Nguyen-the, C., & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *CRC, Critical Reviews in Food Science and Nutrition*, *34*, 371–401.
- Park, C. M., & Beuchat, L. R. (1999). Evaluation of sanitizers for killing *Escherichia coli* O157:H7, *Salmonella* and naturally occurring microorganisms on cantaloupes, honeydew melons, and asparagus. *Dairy, Food and Environmental Sanitation*, *19*, 842–847.
- Ruiz-Cruz, S., Luo, Y., Gonzalez, R. J., Tao, Y., & González, G. A. (2006). Acidified sodium chlorite as an alternative to chlorine to control microbial growth on shredded carrots while maintaining quality. *Journal of the Science of Food and Agriculture*, *86*, 1887–1893.
- Ruiz-Cruz, S., Acedo-Félix, E., Díaz-Cinco, M., Islas-Osuna, M. A., & González-Aguilar, G. A. (2007). Efficacy of sanitizers in reducing *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* populations on fresh-cut carrots. *Food Control*, *18*, 1383–1390.
- Simons, L. K., & Sanguansri, P. (1997). Advances in the washing of minimally processed vegetables. *Food Australia*, *49*, 75–80.
- Strockbine, N. A., Wells, J. G., Bopp, C. A., & Barrett, T. J. (1998). Overview of detection and subtyping methods. In J. B. Kaper & A. D. O'Brien (Eds.), *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains (pp. 331–356). Washington, DC, USA: ASM Press.
- Wachtel, M. R., & Charkowski, A. O. (2002). Cross-contamination of lettuce with *Escherichia coli* O157:H7. *Journal of Food Protection*, *65*, 465–470.
- Wang, H., Feng, H., & Luo, Y. (2004). Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Research International*, *37*, 949–956.
- Warf, C.C. (2001). The chemistry and mode of action of acidified sodium chlorite. In *2001 IFT Annual Meeting* (pp. 1–91).
- Wiley, R. C. (1994). Introduction to minimally processed fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables* (pp. 1–14). New York, London, UK: Chapman and Hall.