Assessment of Sodium Hypochlorite and Acidified Sodium Chlorite as Antimicrobial Agents to Inhibit Growth of *Escherichia coli* O157:H7 and Natural Microflora on Shredded Carrots

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ABSTRACT. Acidified sodium chlorite (ASC) is an alternative to chlorine in reducing microbial populations to maintain food quality and safety on fresh-cut produce. However the most effective concentration for microbial reduction on shredded carrots is unknown. In this study the influence of tap water, sodium hypochlorite (SH; 200 $mg \cdot L^{-1}$) and acidified sodium chlorite (ASC; 100, 250, 500 and 1,000 $mg \cdot L^{-1}$) washes on natural microflora, and survival and growth of *Escherichia coli* O157:H7 inoculated onto shredded carrots, was determined after treatment and 7 and 14 days of storage. The carrots were stored under passive

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modified atmosphere at 5°C. While a tap water wash did not reduce growth of *E. coli* O157:H7, total plate count or yeast and molds, spoilage and pathogenic microorganisms were reduced by two logs by using SH or ASC at 200 and 100 mg·L^{-1}, respectively. ASC at concentrations above 100 mg·L^{-1} was very effective in reducing microbial growth by 6 logs. During storage, total mesophilic growth increased in samples washed with tap water, SH or ASC at 100 and 250 mg·L^{-1}. However, shredded carrots washed with ASC at 1000 mg·L^{-1} did not show any microbial growth even after 14 days of storage at 5°C. doi:10.1300/J512v13n03_05

KEYWORDS. Acidified sodium chlorite, disinfection, fresh-cut produce, foodborne pathogens, sodium hypochlorite, spoilage microorganisms, washing

INTRODUCTION

In general, fresh-cut fruit and vegetables are very perishable food products that need special processing and preservation technologies to maintain their quality while extending shelf-life. Maintaining food safety of fresh-cut produce remains a major challenge to industry. Since the early 1990s, the number of foodborne illnesses associated with fresh fruits and vegetables has doubled in the United States mainly due to the globalization of the food supply and the development of extensive food distribution networks (FDA, 2006; Naimi et al., 2003). Contamination by foodborne pathogenic bacteria and their growth during storage is a serious health concern, especially since fresh-cut fruit and vegetables are consumed without a major killing step (Bharathi et al., 2001). It is important to note that pathogenic microorganisms are not part of the naturally occurring microflora found on or in fresh-cut produce. Any human pathogens present on fruits and vegetables are there because of inadvertent contamination, which can occur in the field or at any point in the food supply chain from production to table (CAST, 2003). Additionally, foodborne human pathogens such as *Escherichia coli* O157:H7, are capable of growing on vegetables stored at 8-12°C, the temperatures that fresh-cut produce could be exposed to during marketing or distribution (Abdul-Raouf et al., 1993).

Several chemical and physical methods have been reported for decontamination of fresh-cut produce, but published data suggests that most of the available washing and sanitizing methods, including some of the newest sanitizing agents, are not capable of reducing microbial populations by more than 90 or 99% and even more rarely 99.9% (Beltrán et al., 2005; Beuchat et al., 1998; Brackett, 1999; Sapers, 2003). These treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms, but not always producing fruits and vegetables free of pathogens (Beuchat, 1998). The type of produce, the type of microorganisms present, the cell type, and the location of microorganisms on the surface, and in subsurface tissues, cause sanitizers to differ greatly in their ability to disinfect raw produce (Kreske et al., 2006). One of the newest sanitizing agents is acidified sodium chlorite (ASC; SANOVA®, Ecolab Inc., MN), which has proven to be a highly effective antimicrobial mixture for decontamination of fresh fruit and vegetable products (Allende et al., 2006; Conner, 2001; Caldwell et al., 2003; Gonzalez et al., 2004; Inatsu et al., 2005; Lukasik et al., 2003; Park and Beuchat, 1999; Ruiz-Cruz et al., 2006a). Ecolab markets the Sanova's spray system that is currently used in more than 100 large food processing plants across the U.S. that prepare poultry, red meat, fruits, and vegetables for distribution to consumers (Kemp et al., 2001). The chemistry of sodium chlorite is related to that of chlorine dioxide; with the acidification of a sodium chlorite solution, chlorous acid is formed. ASC typically operates in a pH range of 2.3 to 3.2, and acts as a broad spectrum disinfectant by oxidizing the microbial cell wall, and attacking the sulfide and disulfide linkage of proteins (Bashor, 2002). When ASC was used at 100-250 $mg \cdot L^{-1}$ the quality of shredded carrots was maintained while populations of spoilage microflora were reduced, but use of ASC above 500 $mg \cdot L^{-1}$ could be detrimental to carrot tissue quality (Ruiz-Cruz et al., 2006b).

The aim of this study was to evaluate the efficacy of tap water, sodium hypochlorite and different concentrations of acidified sodium chlorite in inactivating natural microflora and *E. coli* O157:H7 inoculated on shredded carrots (*Daucus carota* L.), as well as determining the survival rate of the pathogen during storage in modified atmospheres at 5°C.

MATERIALS AND METHODS

Plant material: Fresh carrots were purchased from a produce wholesale market (Jessup, MD) 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) and treated within 24 hr after being placed in storage at 5°C. Fresh carrots were shredded with a food processor (Cuisinart, East Windsor, NJ) in a fresh-cut preparation room at 10°C. Samples of 150 g of shredded carrots were placed in nylon mesh bags (Linens N' Things, Clifton, NJ). All samples were stored at 5°C for about 2 hr before the inoculation process was carried out.

Inoculation: The same mix of three nalidixic acid-resistant (Nal^R) strains of *E. coli* O157:H7 used by Allende et al. (2006), and which were derived from the outbreak strains, F6460, F15110, H26696, was used. Cultures were kept at −80°C in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, MI) containing 25% (v/v) glycerol. *E. coli* O157:H7 strains were grown at 37°C, shaken in LB broth supplemented with nalidixic acid (Nal) (50 $\mu g \cdot L^{-1}$) until stationary phase (20 hr growth) and cultured onto LB-Nal agar at 37°C for 24 hr. *E. coli* O157:H7 Nal^R strains were consecutively subcultured twice in 100 mL of LB-Nal broth at 37°C for 24 hr with constant agitation at 175 rpm (1.3 g) to obtain a final OD_{600} reading of about 0.5. The second culture was allowed to adapt to a final temperature of 12°C for 4 hr. Cultures were washed twice by centrifugation (4,000 × g, 15 min, 4°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 et al. (2004). The strain cocktail was proportionally diluted in deionized water at 12°C to achieve a final concentration of about 10^7 $cfu \cdot ml^{-1}$ of *E. coli* O157:H7 Nal^R (confirmed by plating on selective media). The mesh bags of shredded carrots were completely immersed in the inoculum solution and kept under constant agitation for 30 min. The product was removed from the inoculum solution and maintained at 5°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 sec. The entire experiment was carried out in a Biosafety Level 2 Laboratory.

Wash treatments: Shredded carrots were not washed (NW) or washed with refrigerated tap water (WW), sodium hypochlorite (SH) (200 $mg \cdot L^{-1}$, pH 6.5) and ASC at 100, 250, 500 and 1,000 $mg \cdot L^{-1}$ (Aldrich Chemical Co., Inc., Milwaukee, WI) prepared according to the manufacturer's directions. The free chlorine concentration present in the chlorinated solutions was determined using a Chlorine Photometer (CP-15, HF Scientific Inc., Ft. Myers, FL). Washing solutions were

prepared in 3 L of tap water at 5°C and used within 30 min. Inoculated product was dipped in the washing solutions and agitated for 1 min using a product to sanitizer ratio of 1 to 20 (wt/vol). Finally, the excess water was removed by centrifugation with a hand operated enclosed spinner (OXO Good Grips) for 30 sec.

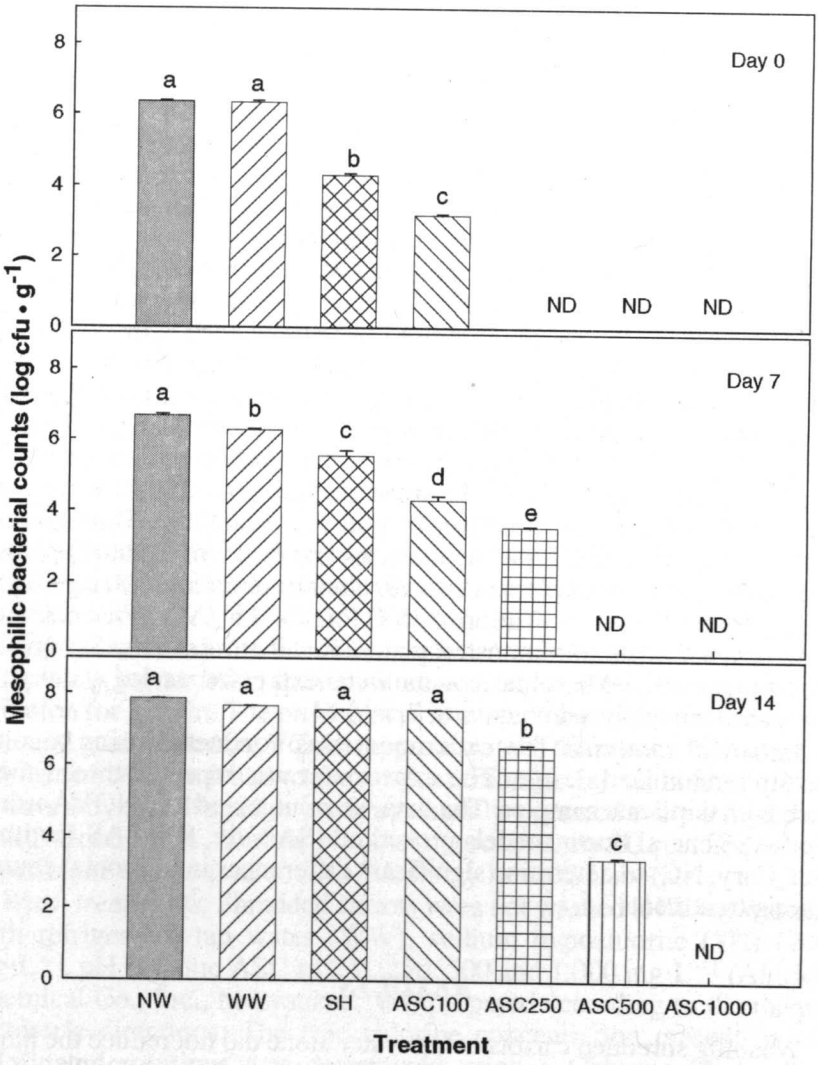
Antimicrobial activity of washing solutions: Shredded carrot samples (30 g) were homogenized in a 1:10 dilution of sterile peptone water (8.5 $g \cdot L^{-1}$ of NaCl [S9625, Sigma-Aldrich, Inc., St. Louis, MO] plus 1 $g \cdot L^{-1}$ of neutralized bacteriological peptone [Difco, Detroit, MI]) using a stomacher 400 Biomaster (Seward Limited, London, UK). Filter stomacher bags (Seward Limited) were used to eliminate solid particles from homogenates. An enrichment step was carried out at day 0 by adding 225 mL of sterile tryptic soy broth (TSB; Difco) supplemented with nalidixic acid (50 $\mu g \cdot L^{-1}$, Sigma-Aldrich) to each stomacher bag, followed by incubation at 37°C for 24 hr and subsequent homogenization in the same medium. Tenfold dilution series were made in peptone buffered water as needed for plating. Samples (100 µL) of each carrot filtrate or its corresponding dilutions, were logarithmically spread on agar plates (Wasp II Spiral Plater, DW Scientific, West Yorkshire, UK). Sorbitol MacConkey agar (SMAC, Difco) supplemented with Nal (50 $\mu g \cdot L^{-1}$) and sodium pyruvate (0.1%) was used to determine the survival of *E. coli* O157:H7 incubated at 37°C for 24 hr (Strockbine et al., 1998). Total plate counts (TPC) were enumerated on Tryptic Soy Agar (TSA, Difco) plates after incubation at 30°C for 48 hr and yeast and molds on Potato Dextrose Agar (PDA, Difco) supplemented with chloramphenicol (200 $\mu g \cdot mL^{-1}$; Sigma-Aldrich) after incubation at 30°C for 48-72 hr. Microbial colonies were counted with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK). Microbial counts were expressed as $\log_{10}$ $cfu \cdot g^{-1}$. Samples were analyzed on days 0, 7, and 14.

Statistical analysis: The experiment was conducted using a completely randomized design. The experiment was repeated three times, each with duplicate samples. The data were subjected to ANOVA using the SAS general linear models procedure (SAS ver. 8.2, SAS Institute Inc., Cary, NC) to determine significant differences in microbial counts among treatments.

RESULTS

Washing shredded carrots in tap water alone did not reduce the number of recoverable bacteria (Figure 1). The incorporation of 200 $mg \cdot L^{-1}$ of free chlorine or 100 $mg \cdot L^{-1}$ ASC in the wash water resulted in a de-

FIGURE 1. Aerobic mesophilic bacterial counts on shredded carrots stored at 5°C. NW = not washed, WW = water wash, SH = sodium hypochlorite (200 $mg \cdot L^{-1}$), ASC = acidified sodium chlorite (100, 250, 500 and 1,000 $mg \cdot L^{-1}$). Bars represent means of three replications. Different letters above bars indicate data that are significantly different at $P \leq 0.05$. ND = not detectable at 10 $cfu \cdot g^{-1}$.



crease in the number of total plate counts (TPC). Increasing the concentration of ASC through 100 mg·L^{-1} increased the growth inhibition effect. The average reduction of total mesophilic counts by using concentrations of ASC above 100 mg·L^{-1} was about 6 log units, since no recovery was observed after the enrichment step. During storage, TPC of NW and WW treated shredded carrots were similar. No changes were observed after 7 days of storage but a significant increased was observed at the end of storage (14 days), reaching values close to 7.5 log cfu·g^{-1} (Figure 1). Sodium hypochlorite and ASC (100 mg·L^{-1}) were able to retard the mesophilic growth through 7 days of storage, where TPC were 5.55 and 4.29 log cfu·g^{-1}, respectively. However, the final mesophilic counts were equal to those of the NW and WW treated shredded carrots. ASC at the highest concentrations was efficient in reducing TPC. No growth was observed in samples washed with 1,000 mg·L^{-1} of ASC after 14 days of storage at 5°C.

Washing shredded carrots with water did not reduce yeast and mold counts after treatment (Figure 2). Chlorinated water (200 mg·L^{-1}) and ASC at 100 mg·L^{-1} resulted in similar reductions of about 1 log unit. A washing solution containing more than 100 mg·L^{-1} of ASC completely inhibited growth of yeast and molds. Yeast and mold population reached 6.3-6.75 log cfu·g^{-1} after 14 days of storage in NW and WW treated shredded carrots, and with SH and ASC at 100 or 250 mg·L^{-1} (Figure 2). The yeast and mold counts of the product treated with the highest concentrations of 500 and 1,000 mg·L^{-1} of ASC were below the detection limit of 100 cfu·g^{-1}.

Growth of pathogenic bacteria was slightly reduced using chlorinated water at 100 and 250 mg·L^{-1} and ASC at 100 and 250 mg·L^{-1} (Figure 3). Treatment with 500 and 1,000 mg·L^{-1} of ASC significantly reduced populations (>6 log-reductions), compared to the number of *E. coli* O157:H7 remaining on carrots that were not washed. The population of *E. coli* O157:H7 in ASC treated shredded carrots did not increase during storage at 5°C (Figure 3). In fact, the pathogenic population retrieved from NW carrots and from produce treated with WW, SH and ASC was significantly lower after 14 days of storage. Additionally, *E. coli* O157:H7 was not recovered from product treated with ASC after 7 or 14 days of storage.

DISCUSSION

The producer-oriented disinfectant wash additive Sanova (ASC; acidified sodium chlorite) has been introduced and marketed as a potent

FIGURE 2. Yeast and mold counts on shredded carrots stored at 5°C. NW = not washed, WW = water wash, SH = sodium hypochlorite (200 mg·L^{-1}), ASC = acidified sodium chlorite (100, 250, 500 and 1,000 mg·L^{-1}). Bars represent means of three replications. Different letters above bars indicate data that are significantly different at $P \leq 0.05$. ND = not detectable at 10 cfu·g^{-1}.

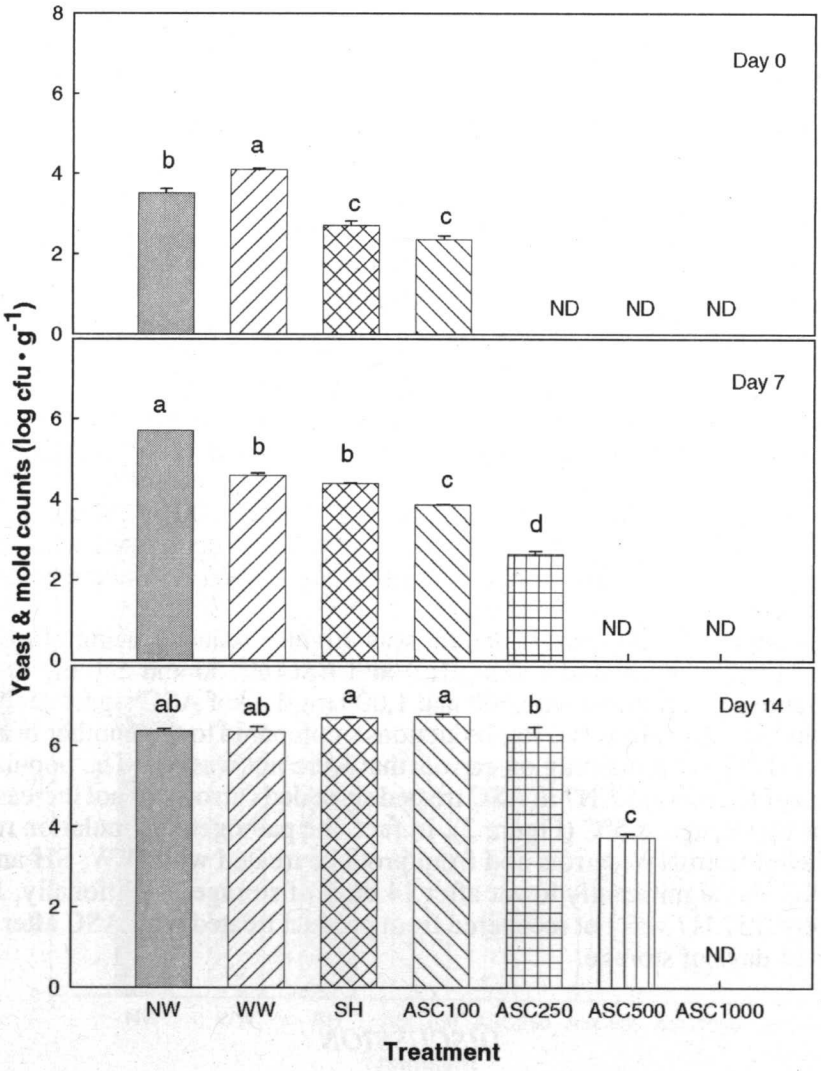
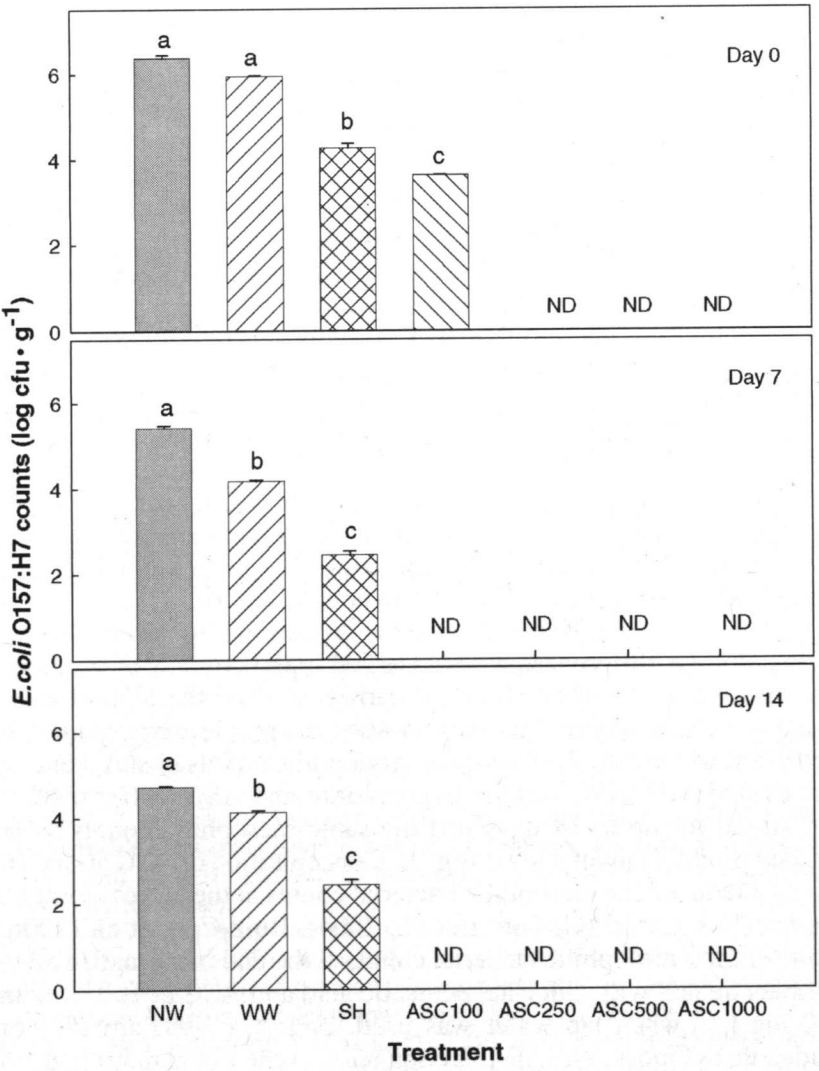


FIGURE 3. *Escherichia coli* O157:H7 counts on shredded carrots stored at 5°C. NW = not washed, WW = water wash, SH = sodium hypochlorite (200 $mg \cdot L^{-1}$), ASC = acidified sodium chlorite (100, 250, 500 and 1,000 $mg \cdot L^{-1}$). Bars represent means of three replications. Different letters above bars indicate data that are significantly different at $P \leq 0.05$. ND = not detectable at 10 $cfu \cdot g^{-1}$.



disinfectant (Lukasik et al., 2003). Use of this disinfectant at, or below, the concentrations approved by the FDA (1,200 $mg \cdot L^{-1}$) (CFR 2000) was effective in reducing both naturally occurring microflora and *E. coli* O157:H7. These data are in accordance with previous reports (Conner, 2001; Caldwell et al., 2003; Inatsu et al., 2005; Lukasik et al., 2003; Ruíz-Cruz et al., 2006b). However, microbial inhibition by using ASC appeared to be produce-dependent. The treatment of raw cabbage with ASC for 5 and 10 min was not found to be effective in reducing the population of *E. coli* O157:H7 (Inatsu et al., 2005). As previously found by Gonzalez et al. (2004), when shredded carrots were treated with 1,000 $mg \cdot L^{-1}$ of ASC, growth of *E. coli* O157:H7 was not detectable (with a 100 $cfu \cdot g^{-1}$ limit of detection) under both tap water and process water scenarios. Ruíz-Cruz et al. (2006b) treated shredded carrots with 100, 200 and 500 $mg \cdot L^{-1}$ of ASC, and found a similar efficacy in the reduction of *E. coli* O157:H7 at all evaluated concentrations. In the present study, the antimicrobial effect of ASC was checked in a wide range of concentrations, and reductions of 3 log units were found when the lowest ASC concentrations were used (100 and 250 $mg \cdot L^{-1}$), while reductions of 6 log units or higher were found when shredded carrots were washed in 500 and 1000 $mg \cdot L^{-1}$. However, concentrations higher than 250 $mg \cdot L^{-1}$ are not recommended because of phytotoxic effects on the carrots, i.e., bleaching (Lukasik et al., 2003; Ruiz-Cruz et al., 2006b). It was found that bacterial reductions following treatment with ASC solutions appeared to be related to the low pH values and the highly oxidative intermediates of ASC (Mehyar et al., 2005).

Due to effects of different treatments on mesophilic bacterial counts during storage, differences were observed in the efficacy of washings. As expected, not washed shredded carrots showed the highest mesophilic counts reaching the same values as previously reported by Barry-Ryan et al. (2000). However, mesophilic counts of shredded carrots treated with WW, sodium hypochlorite and ASC at 100 $mg \cdot L^{-1}$, and stored for up to 14 days had the same mesophilic counts as not washed product (about 8 log $cfu \cdot g^{-1}$). Concentration of ASC above 100 $mg \cdot L^{-1}$ reduced the mesophilic bacterial counts at the end of storage as previously reported (Gonzalez et al., 2004). Ruiz-Cruz et al. (2006a) found similar mesophilic bacterial counts at the end of storage in all the samples treated with chlorine, peracetic acid and ASC at 100, 250 and 500 $mg \cdot L^{-1}$ when tap water was used. Zagory (1999) already concluded that in most cases, disinfection reduces the initial microbial load of fresh-cut fruits and vegetables, but during subsequent storage at re-

frigeration temperatures, epiphytic bacteria grew rapidly on the disinfected leaves.

In a challenge study it is very important to be aware of the role that naturally occurring microflora play, because a large population of spoilage microorganisms could create an additional hurdle to the growth of the test microorganism (Sinigaglia et al., 2006). Therefore, the observed high total counts in shredded carrots after 7 and 14 days of storage might play an important role on the observed reduction of *E. coli* O157:H7 during storage. Gonzalez et al. (2004) and Ruiz-Cruz et al. (2006a) also observed a general decline in *E. coli* 0157:H7 population, whereas mesophilic bacterial counts increased over time, with the exception of samples treated with ASC at 500 and 1,000 mg·L^{-1}. Additionally, Abdul-Raouf et al. (1993) reported that *E. coli* O157:H7 on shredded carrots appeared to be inhibited, or killed, upon exposure to carrot tissue fluid, particularly at 5°C. They suggested that the presence of 6-methoxymellein in carrot tissue, known as carrot phytoalexin, may be inhibitory or toxic to *E. coli* O157:H7.

In summary, low concentrations (100 and 250 mg·L^{-1}) of ASC were as effective as SH in reducing *E. coli* O157:H7 populations on day 0. There was no *E. coli* O157:H7 recoverable during the rest of the storage period. Furthermore, ASC at 500 and 1,000 mg·L^{-1} completely eliminated recoverable *E. coli* O157:H7 cells starting at day 0 and throughout the storage period. This suggests that ASC is a promising alternative to chlorine in the treatment of fresh-cut carrots.

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