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Effect of Sanitizers and Oregano Essential Oils to Reduce the Incidence of *Salmonella* and Other Pathogens in Vegetables

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1. Introduction

Consumption of fruits and vegetables has increased in the last years because form an important component in human nutrition, being rich sources of phytochemicals compounds that play a significant role in the health to helps prevent many degenerative diseases (González-Aguilar et al., 2008). However, as a consequence of inappropriate manipulation during their growth, development, harvesting, processing, distribution, retail sale, and final preparation, pathogenic microorganisms, may contaminate a product, thus increasing the risk of microbial diseases. In fact, the number of outbreaks and cases of illness caused by consumption of these produce has increased in the last years.

One of the most common disease-causing pathogens in fruits and vegetables is *Salmonella*. This reside in the intestinal tracts of animals, including humans, and are more likely to contaminate raw fruits and vegetables through contact with feces, sewage, untreated irrigation water or surface water (Beuchat, 1996; Wells & Butterfield, 1997). Outbreaks of salmonellosis have been linked to a diversity of fruits and vegetables including tomatoes (Hedberg et al., 1994; Wood et al., 1991), bean sprouts (Mahon et al., 1997; Van Beneden et al., 1999) and melons (Blostein 1991).

Washing is an important step that has been widely adopted by the industry to remove soils and microorganism from the surface (Sapers, 2003). Given that fresh and fresh-cut products are marketed as pre-washed and ready to eat, and not subject to further microbial killing steps, the development and proper application of sanitizing agents or antimicrobial

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compounds to remove microorganism's pathogen effectively is critical to ensure the safety of these produce (Ruiz-Cruz et al., 2006).

Chlorine has been widely used as a sanitizer during produce washing (Sapers, 2003). However, numerous studies have shown that chlorine used at concentrations permitted by the FDA lacks efficacy in removing human pathogens and spoilage microorganisms (Zhang & Farber, 1996). Additionally, chlorine may react with organic matter in water to form carcinogenic products (Paris et al., 2003). The inadequacies of chlorine as a sanitizer have stimulated interest in finding safer, more effective sanitizers (Ruiz-Cruz et al., 2006). Alternatives to chlorine, such as chlorine dioxide, peroxyacetic acid, acidified sodium chlorite and some essential oils have been already proposed (Beuchat, 1998). Acidified sodium chlorite as shown strong antimicrobial activity against *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes* and spoilage microorganisms on fresh-cut produce (González et al., 2004; Ruiz-Cruz et al., 2007). Peroxyacetic acid has also been shown to be effective against spoilage and pathogenic microorganisms (Weissinger & Beuchat, 2000). Others sanitizers such as chlorine dioxide and essential oils have been to be very effective in reducing the microbial populations of fresh-cut fruit and vegetable (Burt & Reinders, 2003; Selma et al., 2008).

Moreover, since water wash is often recycled, high organic matter content reduces the activity of sanitizers and increases the likelihood for contamination of fresh vegetables. For this case not all washing methods and washing solutions are equally effective. In order to ensure the safety of fresh produce, it is necessary to evaluate the efficacy of chemical sanitizers and natural antimicrobial compounds in water containing concentrations of organic matter to simulate commercial practices. In view of the importance of *Salmonella* as a cause of food-borne disease and vegetables as a vehicle for its transmission, in our researches are going to look alternatives chemical and naturals to eliminated *Salmonella* and others pathogens in different fruits and vegetables. The objective of this chapter is to evaluate the effectiveness of chemical sanitizers, such as chlorine (Cl, 200 ppm), chlorine dioxide (DC, 5%), peroxyacetic acid (PA, 80 ppm), and acidified sodium chlorite (ASC, 100, 250 and 500 ppm) and oregano essentials oils (OEO, 1 and 2.5 mM) for reducing populations of *Salmonella* and *E. coli* O157:H7 from inoculated cilantro, spinach, lettuce and jalapeño peppers under laboratory and simulated commercial processing wash water conditions.

2. Material and methods

2.1 Bacterial strains and media

Salmonella Typhimurium (ATCC 14028) and *E. coli* O157:H7 (ATCC 43890) were used in this study. Cultures of *Salmonella* and *E. coli* O157:H7 from freezer stocks were grown in tryptic soy broth (Difco Laboratories, Detroit, MI). To suppress growth of microorganisms naturally present on different vegetables, nalidixic acid-resistant strains were obtained and used in this study (Inatsu et al., 2005a; Inatsu et al., 2005b; Ruiz-Cruz et al., 2007). *Salmonella* and *E. coli* O157:H7 strains were adapted to grow on Luria-Bertani broth (LBB; Difco, Becton Dickinson, Sparks, MD) supplemented with 50 µg/mL nalidixic acid (LBB-Nal) and incubated at 37°C. To obtain pure cultures, a loop of *Salmonella* was streaked on Bismuth Sulfite agar (BS; Difco Laboratories, Detroit, MI), *E. coli* O157:H7 on Sorbitol MacConkey agar (SMAC; Difco Becton Dickinson, Sparks, MD). Each agar medium was supplemented with nalidixic acid (50 µg/mL) and plates were incubated at 37°C. After incubation, a single colony from each plate was

selected and inoculated into 10 mL of LBB-Nal (*E. coli* O157:H7 and *Salmonella*). Individual strains were grown in each broth at room temperature (30°C with constant agitation at 175 rpm. Cultures were transferred to each broth by loop at two successive 24 h intervals and one overnight (16-18 h) before they were used as inoculants. Cells of each strain were harvested by centrifugation (4,000 X g, 15 min) and washed with 2 vol of sterile phosphate-buffered saline and resuspended at a cell density of approximately 10⁹ cfu/mL. Volumes of cell suspensions of *Salmonella* or *E. coli* O157:H7 were combined to create a two-strain mixture. The cells were added proportionally to tap water (laboratory conditions) and simulated commercial processing to obtain a dip inoculum solution of approximately 10⁷ cfu/mL. The inoculum level was confirmed by replica plating onto selective agar after serial dilution in sterile phosphate-buffered saline, followed by incubation at 37°C for 24 h.

2.2 Vegetable preparation

Fresh cilantro, spinach, lettuce and jalapeño peppers were purchased from a local wholesale market in Cd. Obregón, Sonora, México, transported to the laboratory and used within 24 h following storage at 5°C. Produce were sorted to eliminate damaged, poor quality produce. Vegetable were washed in tap water at ambient temperature to remove residual soil. After washing, cilantro, lettuce and spinach were cut manually in 2 cm pieces and jalapeño peppers into longitudinal strips of 1 cm width and divided into individual 40 g portions contained in nylon mesh bags.

2.3 Inoculation procedure

Since the immersion process is a possible point of contamination in the food industry, dip inoculation is the most suitable method that can be used to simulate such a process (Beuchat et al., 2001). Spinach, lettuce, cilantro and jalapeño peppers were immersed in the inoculum solution (sample:inoculums ratio = 1:7 w/v) and kept under constant agitation for 30 min. After dipping, the samples were drained for 30 s. Samples were then placed into plastic containers and maintained for 1 h at room temperature until washed with the different treatments. Previous studies indicate that bacteria attach to leaf surfaces within 1 h of inoculation (Yang et al., 2003).

2.4 Treatment procedure

The following sanitizer treatments were evaluated for their efficacy in killing or reducing *E. coli* O157:H7 and *Salmonella* on fresh produce cilantro, jalapeño peppers, lettuce and spinach. The treatments were 200 ppm sodium hypochlorite of a commercial bleach preparation, with pH adjusted to 6.5 with HCl (Cloralex®, NL, México, 6% NaOCl), 80 ppm PA; pH 3.5 (Ecolab, St. Paul, Minn., U.S.A.), 100, 250 and 500 ppm ASC; pH 2.8, 2.6 and 2.4, respectively (Sigma-Aldrich, USA), DC 5%; pH 5.4 and 1 and 2 mM OEO (Sigma-Aldrich, USA). Untreated produce samples were used as control. For the simulated commercial water processing, was obtained by repeatedly dipping freshly shredded produce of known mass in a fixed volume of tap water (González et al., 2004; Ruiz-Cruz et al., 2007). Each mesh bag of inoculated produce were dipped into one sanitizer solution (sample to wash water ratio of 1:5 w/v) with contact time of 1 (ASC and OEO) and 2 min (Cl, PA and DC) with constant stirring. After dipping samples were drained for 30 s to remove excess water. Samples of produce weighing 30 g were packaged in ziploc bags and stored at 5°C for 7 days.

2.5 Procedures for microbial enumeration

Samples (10 g) were transferred aseptically into sterile stomacher bags, 90 mL of Dey-Engley (DE) neutralizing broth was added and samples were macerated. Homogenized samples were serially diluted by a factor of ten in sterile phosphate buffer saline. For each dilution, 1 mL was plated on each of sorbitol MacConkey agar and bismuth sulfite agar for *E. coli* O157:H7 and *Salmonella*, respectively and incubated at 37°C for 24 or 36 h. Agars for pathogenic bacterial growth were supplemented with 50 µg/mL nalidixic acid.

3. Results and discussions

Analysis of the fresh produce that had not been inoculated revealed the absence of *Salmonella* and *E. coli* O157:H7. Preliminary studies were conducted to determine the level of inoculum that could be retained on the surface of different produce. The amount of *Salmonella* and *E. coli* O157:H7 attached to the surface of spinach was 6.53 and 6.35 log cfu/g (Fig. 1), jalapeño peppers 6.52 and 6.4 log cfu/g (Fig. 2), cilantro 5.81 and 5.92 log cfu/g (Fig. 3) and lettuce 5 and 5.35 log cfu/g (Fig. 4), respectively.

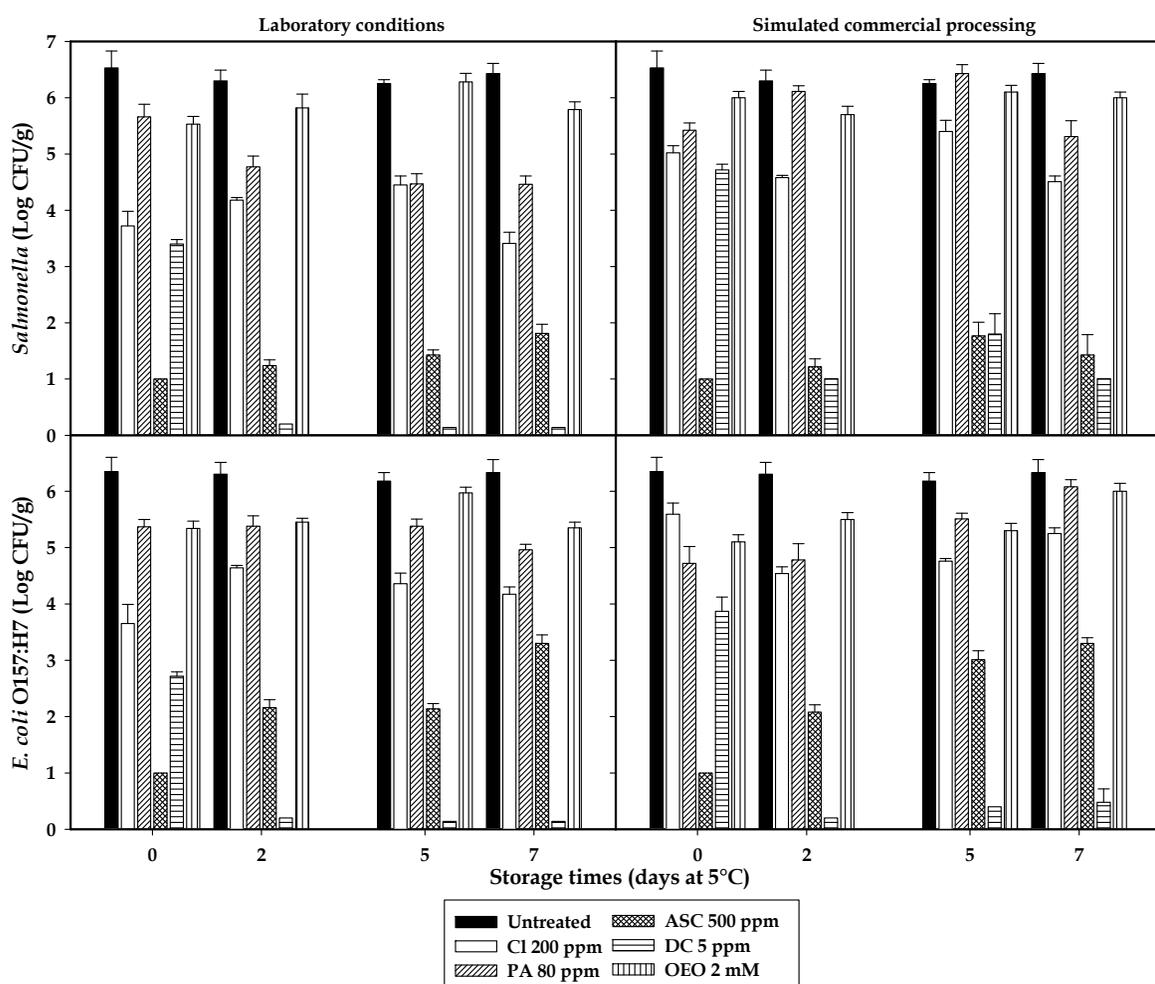


Fig. 1. Efficacy of sanitizers on the reduction of *Salmonella* and *E. coli* O157:H7 populations from artificially inoculated spinach. Bars represent the standard errors of the mean resulting from triplicate experiments. The limit of detection was 0.2 log cfu/g of produce.

All sanitizers reduced significantly ($P \leq 0.05$) *Salmonella* and *E. coli* O157:H7 compared with the control (untreated) on day 0, with reduction of 1 – 6 log cfu/g and all produce evaluated. The results of the decontamination for *Salmonella* and *E. coli* O157:H7 on spinach are shown in Fig. 1. The organic matter content in simulated commercial processing reduces the activity of the Cl and DC treatment. However, the effectiveness of Cl was significantly higher than PA and OEO with reductions of 2.5 – 2.7 log cfu/g, but lower than ASC and DC. ASC was the most effective treatment in reducing *Salmonella* and *E. coli* O157:H7. This sanitizer reduced the both pathogen populations to undetectable levels (with 10 cfu/g detection limit), achieving reductions of 5.53 and 5.35 log cfu/g of *Salmonella* and *E. coli* O157:H7, respectively, under both water conditions. The results are similar to those reported by other researchers (González et al., 2004; Ruiz-Cruz et al., 2007). Cells of both pathogens increased by 1.5-3 log cfu/g at the end of storage. On the other hand, interestingly sample treated with DC on tap water conditions lower after 2 days of storage at levels of 0.2-1 log cfu/g. This reduction could be due to damage to the cell by sanitizer and coupled with the cooling temperature, the cells failed to grow.

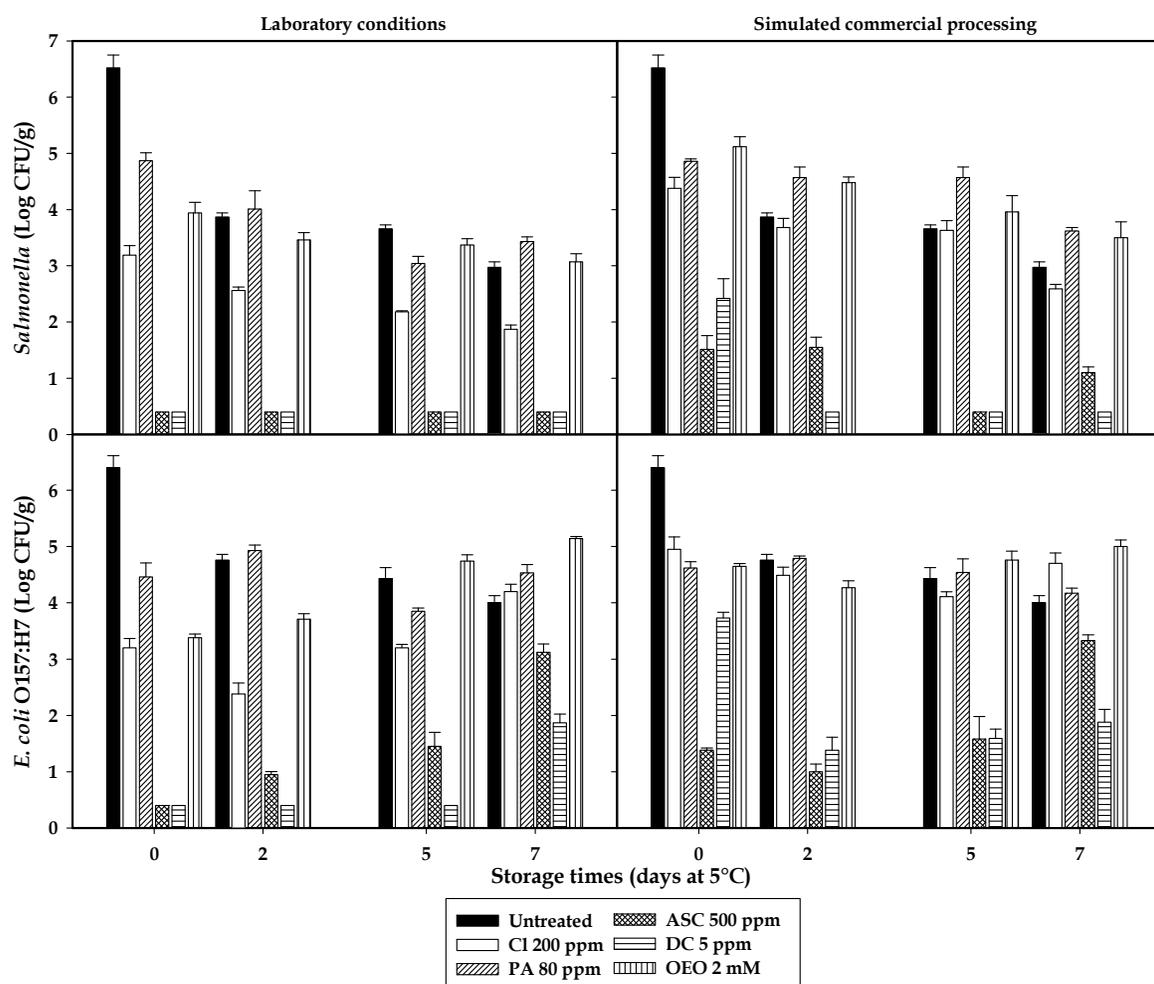


Fig. 2. Efficacy of sanitizers on the reduction of *Salmonella* and *E. coli* O157:H7 populations from artificially inoculated jalapeño peppers. Bars represent the standard errors of the mean resulting from triplicate experiments. The limit of detection was 0.2 log cfu/g of produce.

The use of ASC and DC showed better in inactivating *Salmonella* and *E. coli* O157:H7 on jalapeño peppers that the others sanitizers (Fig. 2). Populations were reduce to undetectable levels (0.4 log cfu/g) at day 0 only under laboratory conditions. Followed by CI, OEO and PA with reductions of 2-3.2 log cfu/g. The efficacy of DC was affected significantly when was used in simulated commercial processing; however, also caused a higher reduction with 2.7-5 log cfu/g. In general the PA treatment was the least effective in reducing the pathogens; however it effectivity was not affected by the presence of organic matter and this treatment resulted in 1.6 to 2 log cfu/g reduction both pathogens. The cells of *Salmonella* were not recovered during storage time with DC were used under both water conditions. This indicates that the cell could be not retrieved in the agar plate, which caused a reduction of 6 log cfu/g. ASC caused the same effect but only under laboratory conditions. The results are similar to those reports by us previously researched with 250 and 500 ppm of ASC on shredded carrots (Ruiz-Cruz et al., 2007).

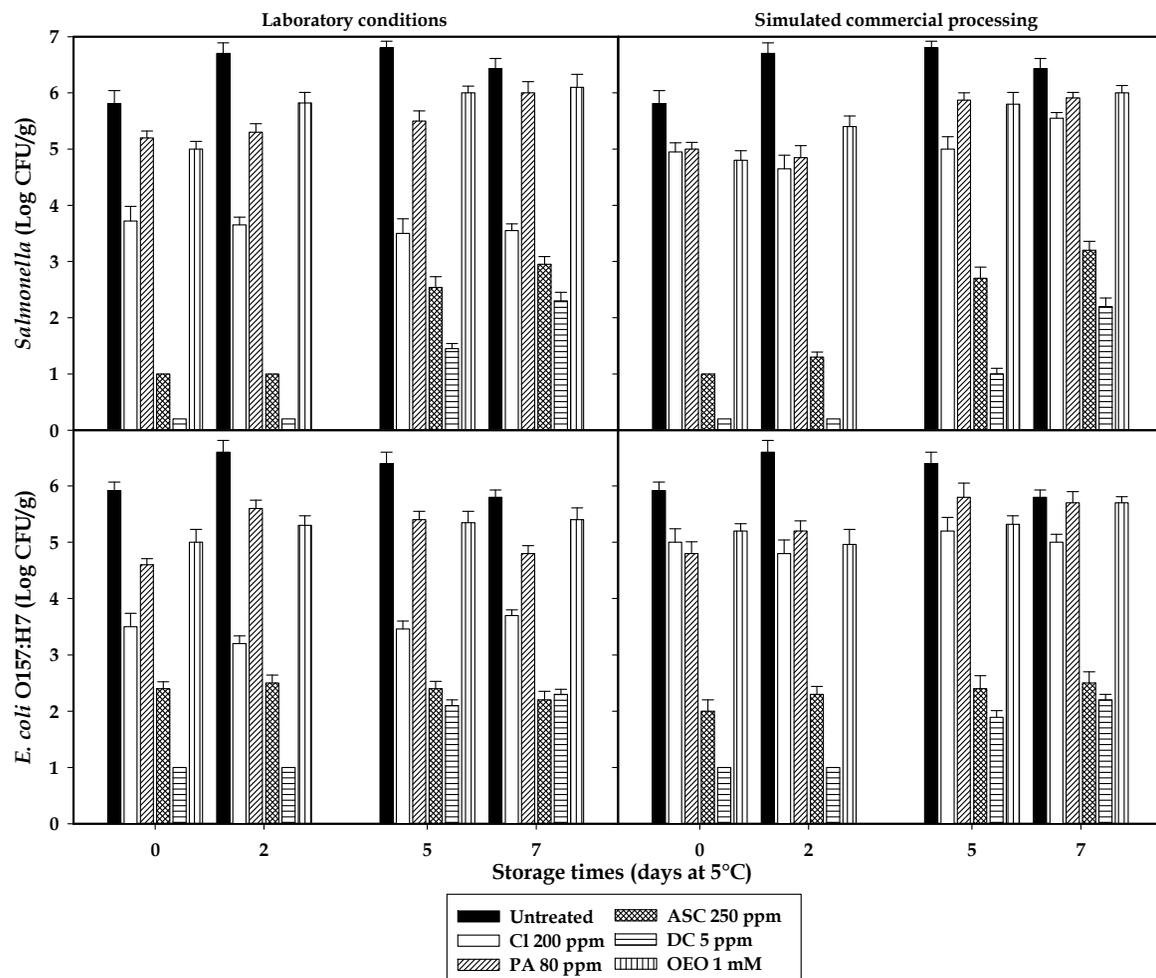


Fig. 3. Efficacy of sanitizers on the reduction of *Salmonella* and *E. coli* O157:H7 populations from artificially inoculated cilantro. Bars represent the standard errors of the mean resulting from triplicate experiments. The limit of detection was 0.2 log cfu/g of produce.

The efficacies of CI, ASC and DC in reduced *Salmonella* and *E. coli* O157:H7 in cilantro were statistically different ($P \leq 0.05$), when compared with untreated and other treatments (Fig. 3). The effectiveness of CI treatment was significantly higher than PA and OEO, but lower

than ASC and DC in both pathogens. DC and ASC were the most effective treatments in reducing pathogens on day 0. DC treatments reduced *Salmonella* to undetectable levels (with 0.2 log cfu/g, detection limit), achieving reductions of 5.61 and 4.92 log cfu/g by *Salmonella* and *E. coli* O157:H7, respectively. ASC also caused a strong reduction of both pathogens with values of 4.81 and 3.52 log cfu/g of *Salmonella* and *E. coli* O157:H7, respectively. Allende et al. (2009) reported on reduction of more than 3 log cfu/g were used 100 ppm of ASC on fresh-cut cilantro. Ruiz-Cruz et al. (2007) observed that ASC treatment at 100, 250 and 500 ppm reduced the *Salmonella* and *E. coli* O157:H7 population on shredded carrots in a similar manner to the reduction of *Salmonella* and *E. coli* O157:H7 achieved with 250 ppm, in this study on fresh-cut cilantro. Our study previously, found that 1000 ppm of ASC affected overall quality of shredded carrots (Ruiz-Cruz et al., 2006). Moreover, we found that concentrations up of 250 ppm of ASC affected the quality of fresh-cut cilantro and the concentrations used in this study (250 ppm) maintained the quality of cilantro by 16 days at 5°C (data no show). Moreover, concentrations of ASC above 500 ppm affected the quality of different vegetable (spinach, jalapeño peppers and lettuce). These results confirmed that the effectiveness of ASC to maintain quality and reduce pathogen counts is influenced by the concentration of the sanitizer and the contact time.

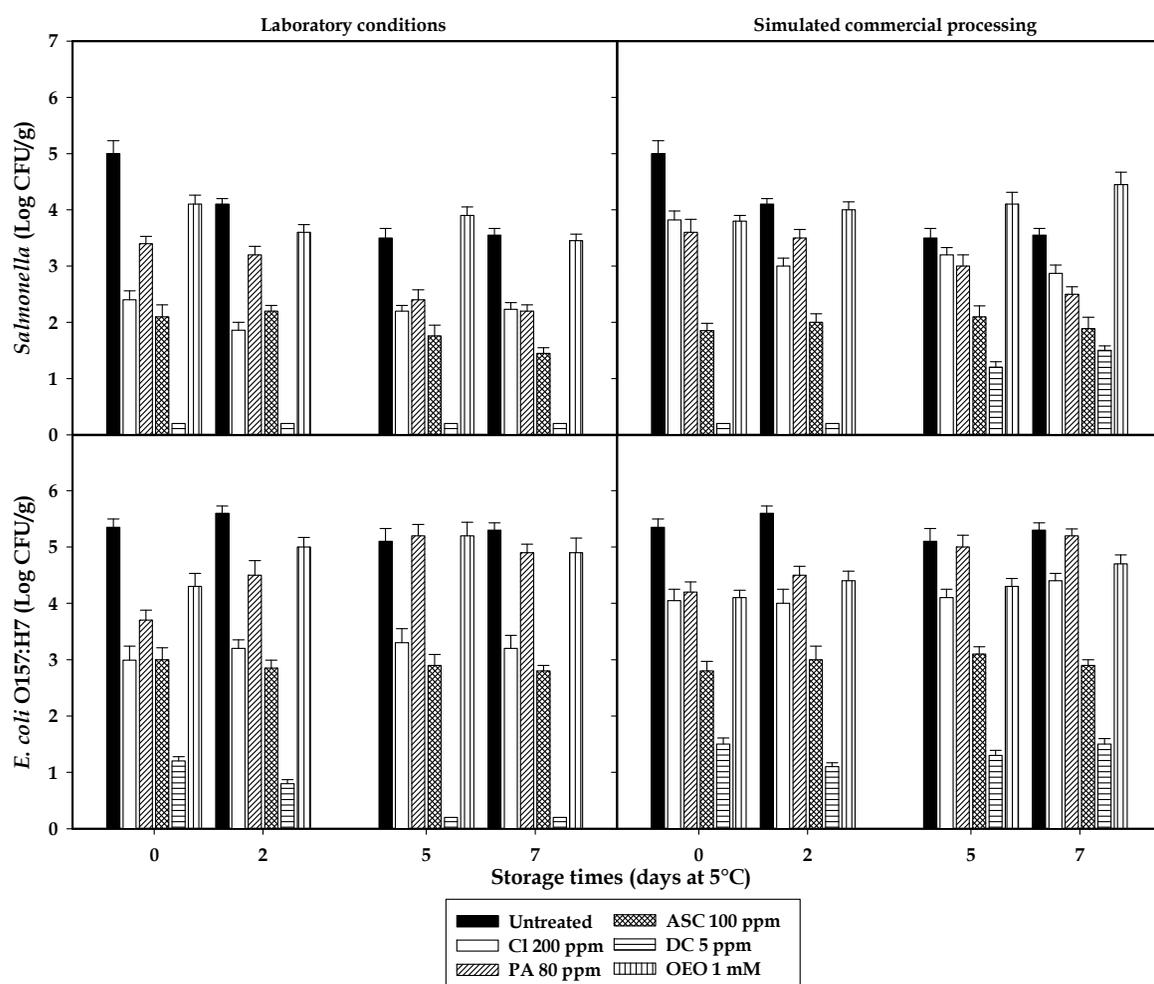


Fig. 4. Efficacy of sanitizers on the reduction of *Salmonella* and *E. coli* O157:H7 populations from artificially inoculated lettuce. Bars represent the standard errors of the mean resulting from triplicate experiments. The limit of detection was 0.2 log cfu/g of produce.

The reduction of *Salmonella* and *E. coli* O157:H7 on lettuce after washing with the different sanitizers were shown in Fig. 4. All treatments caused significant reductions in *Salmonella* and *E. coli* O157:H7 on day 0 under both water conditions compared with the control. Reduction of 1.2 log cfu/g in both pathogens in both water conditions were observed for the OEO treatment, this indicated that its effectivity was not affected by the organic matter. This reduction was maintained throughout the storage. The effectiveness of Cl treatment was significantly higher than PA and OEO, but lower than ASC and DC in reducing *Salmonella* and *E. coli* O157:H7, were used in tap water, causing a reduction of 2.6 and 2.35 log cfu/g, respectively. However, its effectivity was affected in reduced *Salmonella* under simulated commercial processing reducing only 1.18 log cfu/g. PA caused a reduction of 2.4 and 2.6 log cfu/g of *Salmonella* and 1.65 and 1.15 log cfu/g on *E. coli* O157:H7, under tap water and simulated commercial processing, respectively. Similar to the results on the DC treated cilantro, after treated lettuce with DC resulted in a high reduction of *Salmonella* to undetectable levels (0.2 log cfu/g, limit detection), achieving reductions of 4.8 log cfu/g.

4. Conclusions

ASC and DC were the most effective treatments in reducing *Salmonella* and *E. coli* O157:H7 in all produces at all concentrations evaluated. No viable cells of *Salmonella* and *E. coli* O157:H7 were recovered at concentrations of 5% of DC in spinach and lettuce. As well as *Salmonella* in jalapeño peppers treated with DC 5% and ASC 500 ppm, producing a bactericidal effect. However, cells were able to grow during storage, therefore this indicates the ability of the pathogens to adapt to adverse environments present in food is an interesting area that requires more investigation.

The results show that all sanitizers were capable of controlling growth of *Salmonella* and *E. coli* O157:H7 during storage time and can be used by washed these produce. ASC and DC was the most effective sanitizer and have the advantage of being more stable and preserve its efficacy in the presence of organic matter. However, further studies are needed to determine whether these sanitizers might have more lethal effects when lower levels of bacteria are present on produce.

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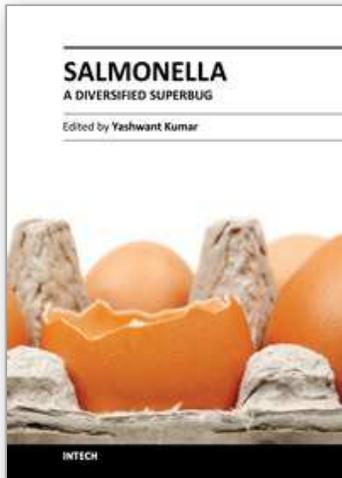
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Salmonella - A Diversified Superbug

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Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

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