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Research

Efficacy of a Citric Acid-based Organic Sanitizer against Salmonella enterica and Background Microflora on Fresh-cut Celery and Leeks.

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ABSTRACT: The efficacy of a citric acid-based organic sanitizer (organic Chico Wash™) against Salmonella enterica serovar Newport and background microflora on fresh-cut celery and leeks was investigated. Three concentrations (1:10, 1:20 and 1:40) of Chico Wash were used. For evaluating the efficacy against background microflora, fresh-cut celery or leeks were treated with sanitizers without an initial wash. For evaluating the efficacy against Salmonella, fresh-cut produce samples were initially washed and dip inoculated with 10⁷ CFU/ml S. Newport. Samples were immersed in the sanitizer solutions for 2 min. Samples were taken for enumeration of survivors immediately after treatment and after storage at 4°C for 1, 3 and 6 days. Compared to controls washed in water at day 6, Chico Wash reduced Salmonella and background microflora by 0.7-1.5 and 1.2-2.6 log CFU/g, respectively. All three concentrations of Chico Wash showed 0.2-1.1 log additional reductions in Salmonella population compared to 200 ppm chlorine. At day 6, background microflora population on celery and leeks were 1-1.7 and 1.5-2.2 logs lower, respectively, than that on chlorine treated samples. The results showed that Chico Wash could potentially serve as an alternative for chlorine sanitizer to wash produce.

KEY WORDS: sanitizer, citric acid, Salmonella, background microflora, celery, leeks

INTRODUCTION

The increased demand for fresh produce may raise the risk of foodborne illness outbreaks owing to the consumption of contaminated produce. The U.S. Centers for Disease Control (CDC) reported that almost 46% of all foodborne illnesses that led to hospitalization or death between 1998 to 2008 were attributable to fresh produce (Painter et al., 2013). The most common bacterial pathogenic agent in fresh produce related outbreaks is *Salmonella* (Callejón et al., 2015; Hanning et al., 2009). Background microflora naturally present on fresh produce can cause spoilage which could result in loss of quality and reduction of shelf-life (Ghidelli, Mateos, Rojas-Argudo, & Pérez-Gago, 2014).

Better control measures are needed to prevent outbreaks as well as food spoilage, and to increase the shelf-life of fresh produce. Post-harvest treatment with sanitizers is one way to reduce the pathogenic and background microorganisms on fresh produce. Chlorine is the most widely used disinfectant by the industry to wash fresh-cut produce, but its antimicrobial efficacy is limited, especially when the wash water contains heavy organic loads. There is a need to seek alternative sanitizers, such as organic acids to assure the safety and quality of produce (Ölmez & Kretzschmar, 2009).

Citric acid is generally recognized as safe (GRAS) for use as a food additive, and has also shown antimicrobial activities in previous studies. Akbas and Olmez (2007) compared the efficacy of citric acid and chlorine for inactivating non-pathogenic Escherichia coli on fresh-cut iceberg lettuce, and found that 2 min of dipping treatment in 0.5% citric acid was as effective as 100 ppm chlorine treatment in reducing the E. coli population. In another study (Park et al., 2011), citric acid was used to inactivate E. coli O157:H7, S. Typhimurium and Listeria monocytogenes on apples and lettuce, and significant log reductions (2.9-3.4) were observed for all 3 pathogens after 10 min treatment. Francis and O'Beirne (2002) showed that mesophilic microbial population on lettuce was reduced by about 1.5 log CFU/g after 5 min of 1.0% citric acid treatment.

Citric acid based sanitizer Chico Wash™ has been used by the food industry to reduce microbial contaminations, and has also been approved for use in organic food production. Citric acid based sanitizers do not form harmful byproducts (trihalomethanes) like chlorine based sanitizers and are more environmentally friendly. Also, unlike chlorine based sanitizers, the efficacy of Chico Wash is not affected by organic matter. The food industry may use Chico Wash as an alternative for chlorine. However, there have been no published studies that have investigated the antimicrobial effects of Chico Wash on fresh produce. The objective of this study was to investigate the efficacy of Chico Wash against S. enterica serovar Newport and background microflora on fresh-cut celery and leeks.

MATERIALS AND METHODS Bacterial culture and media

An antibiotic resistant strain of S. enterica serovar Newport was used in this study. S. enterica serovar Newport LAJ160311 (JJPX01.0014 PulseNet PFGE profile), was provided by Dr. Lynn Joens (University of Arizona, Tucson, AZ). This strain is resistant to amoxicillin-clavulanic acid, ampicillin, cefoxitin, chloramphenicol, streptomycin, and tetracycline (Ravishankar et al., 2010). Stock culture of the organism was frozen at -80 °C and activated by transferring 100 µL into tryptic soy broth with 0.6% yeast extract (TSBYE; Difco, Becton Dickinson, Sparks, MD, USA.), followed by incubation at 37°C for 18-24 h. The bacterial cultures were maintained in TSBYE at 4 °C with monthly transfers. For experimental use, an overnight culture of the organism was grown in TSBYE at 37 °C for 18-24 h. All dilutions were made in 0.1% peptone water (Difco). Enumerations for S. Newport and background microflora were done by plating on xylose lysine desoxycholate agar (XLD; Difco) and tryptic soy agar (TSA; Difco), respectively.

Produce and sanitizer preparation

Celery and leeks were obtained from a local grocery store in Tucson, AZ, USA. The roots of these vegetables were cut off, and any decayed parts of the vegetables were removed. Samples were then cut into 1 cm pieces. For evaluation on background microflora, ten grams of fresh-cut celery or leeks were weighed for each sample and kept ready for use. For testing the efficacy against Salmonella, the fresh-cut produce samples were washed in de-ionized water 3 times, and drained in a plastic basket. Ten grams of produce was weighed for each sample. The samples were kept in a bio hood with UV light turned on for 30 min. The samples were then immersed in 10⁷ CFU/ml S. Newport culture for 2 min. After inoculation, the samples were dried in the bio hood for 30 min to let the bacteria attach to the produce surface.

The sanitizer Chico Wash was provided by E3 Organics Inc. (Orland, CA, USA). Three different concentrations of Chico Wash solution (1:10, 1:20 and 1:40) prepared in de-ionized water (1 part of Chico Wash in 10, 20 or 40 parts of de-ionized water, to obtain 9.1%, 4.8% and 2.4% concentrations, respectively) were used in this study. De-ionized water and 200 ppm chlorine, which is a common sanitizer used in food industry, were also used as controls.

Sanitizer treatments

Each produce sample was immersed in 200 ml of Chico Wash solution of various concentrations (1:10, 1:20 or 1:40) and the sample bags were gently agitated for 2 min. The samples were then taken out and stored in stomacher bags at 4°C.

Enumeration of surviving bacteria

Samples were taken at day 0, 1, 3 and 6 for enumeration of survivors. Ninety ml of D/E neutralizing broth (Difco, Becton Dickinson, Sparks, MD, USA) was added into the stomacher bag with the produce sample, and then pummelled using a stomacher (Labblender 400; Seward, London, UK) at normal speed for 1 min. Serial dilutions were made and aliquots were plated onto XLD or TSA. The XLD and TSA plates were incubated at 37 °C for 24 h and 48 h, respectively. The colonies on the plates were counted for the enumeration of surviving *Salmonella* or background microflora.

Statistical analysis

Each experiment was repeated 3 times, and means and standard deviations of Log CFU/g were calculated. Analysis of variance (ANOVA) was performed using Minitab 17 (Minitab Inc., State College, PA, USA) to determine the differences among the treatments, followed by Tukey's test to compare the differences between the treatments with a level of significance at p < 0.05.

RESULTS AND DISCUSSION

Efficacy of sanitizers against antibiotic resistant Salmonella on celery and leeks

The survival of antibiotic resistant *S*. Newport on celery and leeks after sanitizer treatment is shown in Tables 1 and 2. Sanitizer treatments showed similar inactivation patterns on both celery and leeks. On celery and leek samples treated with de-ionized water, *Salmonella* populations decreased during the 6 days of storage at 4°C. Compared to day 0, there were 0.9 and 1.1 log CFU/g reduction by day 6 for celery and leeks, respectively.

Sanitizer	Day 0	Day 1	Day 3	Day 6
Water control	4.20±0.17 ^A	4.14 ± 0.18^{A}	3.93±0.47 ^A	3.34±0.46 ^A
200 ppm Cl	3.59±0.59 ^A	3.30±0.62 ^{AB}	3.02 ± 0.54^{AB}	3.27±0.43 ^A
1:10 Chico Wash	3.23±0.69 ^A	2.83±0.49 ^B	2.38±0.49 ^B	2.21±0.72 ^A
1:20 Chico Wash	3.25±0.44 ^A	2.90±0.38 ^B	2.49±0.20 ^B	2.40±0.29 ^A
1:40 Chico Wash	3.36±0.68 ^A	3.07±0.44 ^{AB}	2.60±0.29 ^B	2.57±0.30 ^A

Table 1. Surviving populations of S. Newport (Log CFU/g) on celery after sanitizer treatments

Data are shown as mean ± SD, N=3. Data with same superscript letters in the same column are not significantly different.

Table 2 Surviving populations of S. Newport (Log CFU/g) on leeks after sanitizer treatments

Sanitizer	Day 0	Day 1	Day 3	Day 6
Water control	4.71±0.20 ^A	4.41±0.36 ^A	4.15±0.15 ^A	3.58±0.16 ^A
200 ppm Cl	4.32±0.33 ^A	4.21±0.26 ^{AB}	4.05±0.19 ^{AB}	3.16±0.12 ^{AB}
1:10 Chico Wash	3.93±0.49 ^A	3.15±0.44 ^B	2.54±0.12 ^D	2.12±0.24 ^C
1:20 Chico Wash	3.92±0.33 ^A	3.30±0.45 ^{AB}	3.04±0.13 ^{CD}	2.65±0.20 ^{BC}
1:40 Chico Wash	4.07±0.29 ^A	3.62±0.65 ^{AB}	3.49±0.44 ^{BC}	2.92±0.29 ^B

Data are shown as mean ± SD, N=3. Data with same superscript letters in the same column are not significantly different.

For celery samples treated with 200 ppm chlorine, there was reduction 0.6 log (compared to de-ionized water control) at day 0, and Salmonella population slightly reduced during the storage. At day 1, the 1:10, 1:20 and 1:40 Chico Wash showed 1.3, 1.2 and 1.1 loa respectively. reductions, The Salmonella populations on 1:20 and 1:10 Chico Wash treated celery were significantly (p<0.05) lower than that on the control sample. All 3 Chico concentrations of Wash showed significant differences with water control in reducing Salmonella population on celery samples at day 3, while 200 ppm chlorine showed no significant differences with the control. After 6 days of storage, there were 1.1, 0.9 and 0.8 log reductions caused by 1:10, 1:20 and 1:40 Chico Wash, respectively.

On leek samples, antibiotic resistant S. Newport populations showed similar reduction trend as on celery samples treated with 200 ppm chlorine at day 0, 1 and 3, but at day 6, there was more than 1 log reduction compared to day 0. At day 1, there were 1.3, 1.1 and 0.8 log reductions on leeks caused by 1:10, 1:20 and 1:40 Chico Wash, respectively. At day 3, the 3 concentrations of Chico Wash reduced Salmonella population on leeks by 0.7-1.6 Log CFU/g, and the 1:10 and 1:20 Chico Wash treatments were significantly different from the control and chlorine (p<0.05) treatments. At day 6, the 1:10 Chico Wash showed 1.5 log reduction on leeks, and it was significantly different (p<0.05) from the chlorine treatment.

Park et al. (2011) showed that a 1 min treatment with 1 or 2% citric acid reduced Salmonella population by 1.5-1.8 and 3.2-3.9 logs on lettuce and apples, respectively, while water treatment caused 0.6 and 2.6 logs reduction on lettuce and apples, respectively. A previous study (Issa-Zacharia, Kamitani, Muhimbula, Iwasaki. Miwa. & 2011) demonstrated that 100 ppm chlorine reduced Salmonella population on celery by 2.4 log CFU/g (compared to de-ionized water control). The reduction was much higher than that in the present study. The reason might be that a

longer treatment time (5 min vs. 2 min) was used. Previous studies compared chlorine with organic acid based sanitizers for inactivation of microorganisms on fresh produce. Akbas and Olmez (2007) found that dipping of iceberg lettuce in 100 ppm chlorine solution for 2 min caused 1.0 and 2.0 log reductions in the population of L. monocytogenes and E. coli, respectively. The 0.5% citric acid treatments showed similar reductions in microbial populations chlorine; however, the as reductions caused by 1% citric acid were slightly higher (0.2 log). Another study (Rahman, Ding, & Oh, 2010) showed that after a 3 min treatment, 100 ppm chlorine and 1% citric acid reduced E.coli O157:H7 population on spinach by 2.0 and 1.5 log, respectively. Similar reductions in L. monocytogenes population on spinach were observed at 2.2 and 1.7 logs for chlorine and citric acid, respectively. The 100 ppm chlorine showed significantly (p<0.05) higher reductions than 1% citric acid in their study. In the present study, surviving Salmonella populations on produce were enumerated at day 0, 1, 3 and 6 after treatments to examine the residual effects of the sanitizers. The results showed that the reductions caused by 1:10 Chico Wash and 200 ppm chlorine on leeks were not significantly different at day 0 and 1, but 1:10 Chico Wash caused significantly higher Salmonella reductions on leeks at day 3 and 6 (p<0.05). Chico Wash showed a residual activity during the 6 days of storage for both celery and leeks.

Efficacy of sanitizers against background microflora on celery and leeks

Tables 3 and 4 show the survival of background microflora on celery and leeks after sanitizer treatments. On the control samples, higher background microbial loads were seen on leeks than on celery. This could be due to the multiple-layered leaves of leeks that make it easy for contaminants to harbour inside. The sanitizer treatments showed similar reductions of background microflora population on both celery and leek samples. After de-ionized water or sanitizer treatments, the population of background microflora increased during the 6 days of storage at 4°C due to the growth of psychrotrophic bacteria.

Table 3. Surviving populations of background microflora (Log CFU/g) on celery after sanitizer treatments

Sanitizer	Day 0	Day 1	Day 3	Day 6
Water control	4.00±0.52 ^A	4.32±0.50 ^A	5.59±0.51 ^A	6.02±0.17 ^A
200 ppm Cl	2.78±0.20 ^B	3.52±0.65 ^A	4.73±0.75 ^A	5.84±0.18 ^A
1:10 Chico Wash	2.01 ± 0.15^{B}	2.00±0.31 ^B	2.39±0.49 ^B	4.13±0.34 ^C
1:20 Chico Wash	2.45±0.27 ^B	2.19±0.07 ^B	2.58±0.21 ^B	4.60±0.19 ^{BC}
1:40 Chico Wash	2.37±0.29 ^B	2.36±0.13 ^B	2.67±0.30 ^B	4.86±0.02 ^B

Data are shown as mean ± SD, N=3. Data with same superscript letters in the same column are not significantly different.

Table 4. Surviving populations of background microflora (Log CFU/g) on leeks after sanitizer trea	atments
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Sanitizer	Day 0	Day 1	Day 3	Day 6
Water control	5.53±0.35 ^A	5.61±0.37 ^A	6.39±0.63 ^A	7.33±0.44 ^A
200 ppm Cl	4.97±0.36 ^A	5.01±0.30 ^A	6.09±0.53 ^A	6.93±0.49 ^A
1:10 Chico Wash	3.14±0.39 ^B	3.38±0.41 ^B	3.66±0.26 ^B	4.72±0.19 ^B
1:20 Chico Wash	3.15±0.31 ^B	3.31±0.41 ^B	3.64±0.29 ^B	4.97±0.41 ^B
1:40 Chico Wash	3.10±0.12 ^B	3.66±0.09 ^B	3.97±0.14 ^B	5.44±0.19 ^B

Data are shown as mean ± SD, N=3. Data with same superscript letters in the same column are not significantly different.

On celery samples, chlorine and 3 concentrations of Chico Wash caused 1.2-2.0 log reductions at day 0, and the surviving populations of background microflora on sanitizer treated samples were significantly lower than that on water treated samples. At day 1, 3, and 6, the Chico Wash treatments showed 2.0-2.3, 2.9-3.2 and 1.2-1.9 log reductions background microflora in populations, respectively, on celery samples, and these reductions were significantly different (p<0.05) from the reductions caused by chlorine treatment.

For leek samples, chlorine showed 0.6 log reduction at day 0 and 0.3-0.6 log reduction at the following sampling days. The 3 concentrations of Chico Wash caused 2.4, 2.0-2.3, 2.4-2.8 and 1.9-2.6 log reductions at day 0, 1, 3 and 6, respectively. Statistical analysis showed that all 3 Chico Wash treatments were significantly different (p<0.05) from chlorine treatment in reducing background microflora on leeks at each sampling day. Similar to the present study, Vandekinderen et al. (2009) reported that 200 ppm chlorine treatment reduced native microflora on fresh-cut leeks by 1.0 log CFU/g, which was not significantly higher than that achieved after washing with water.

Previous studies compared chlorine and a citric acid sanitizer for their efficacy on inactivation of background microflora on fresh produce. Allende et al. (2009) used 200 ppm chlorine and 0.6% citric acid to wash cilantro for 1 min, and achieved 1.0 and 0.8 log reductions in aerobic mesophilic bacterial

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populations for chlorine and citric acid, respectively. Another study (Gonzalez, Luo, Ruiz-Cruz, & McEvoy, 2004) reported that 200 ppm chlorine and a 0.66% citric acid based sanitizer reduced total aerobic bacterial counts on shredded carrots by 1 and 0.8 log CFU/a, respectively. Studies showed that after sanitizer treatments, even though produce samples were stored under refrigeration conditions, psychrotrophic or mesophilic bacteria in the produce can still grow to a high level. A study found that during a 7-day storage at 4°C, the psychrotrophic bacteria on lactic acid treated carrots increased to 4.6 log CFU/ml, from the initial level of 3.2 logs (Uyttendaele, Neyts, Vanderswalmen, Notebaert, & Debevere, 2004). Francis and O'Beirne (2002) found that citric acid reduced the initial total mesophilic populations on coleslaw by 1.5 logs following which the microbial counts remained lower than those on control samples throughout the storage period, while dipping in chlorine caused 0.8 log reductions on day 0, but these effects were lost by day 2 of storage. Final populations on samples dipped in citric acid were 1 log lower than that on samples dipped in chlorine. These findings were consistent with those of the present study, in which background microflora grew to similar levels on control and chlorine treated samples, while the populations were ca. 2 logs lower on Chico Wash treated samples.

CONCLUSIONS

results from the present study The demonstrated that the 1:10 concentration of Chico Wash showed the best antimicrobial activities against both S. Newport and background microflorg on celery and leeks, followed by Chico Wash at concentrations of 1:20 and 1:40. Inactivation of antibiotic resistant S. Newport by Chico Wash was storage time dependent, and the best reductions were observed at day 6 after treatments. On the contrary, the chlorine treatment didn't show time dependence, and the reductions were maintained constant over the 6 days of storage. Although the psychrotrophic bacteria

among the background microflora could still grow during storage after Chico Wash treatments, the microbial population on celery and leek samples were 1-1.7 and 1.5-2.2 logs lower than that on chlorine treated samples. Thus, Chico Wash could potentially serve as an alternative for conventional chlorine sanitizer for the treatment of celery and leeks. Sensory attributes such as taste, color and texture of the sanitizer treated samples need to be investigated in future studies.

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