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Evaluation of Organic Acid-Based Sanitizers for Reduction of *Escherichia coli* O157:H7 during Flume-Washing of Organic Leafy Greens

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Abstract

Antimicrobial efficacy of three novel organic sanitizers, CHICO Wash™, C8C10 and CG100, was evaluated for the reduction of *Escherichia coli* O157:H7 during flume-washing of organic leafy greens. Organic formulations at various concentrations: CHICO ($C_3H_8I_0C_3O_7$) Wash™ (1:20 ratio) and C8C10 and CG100 (0.2 and 0.4%), along with the controls: hydrogen-peroxide and water, were used for washing organic baby and mature spinach and romaine and iceberg lettuce. Leafy greens were inoculated with a 2-strain cocktail of *E. coli* O157:H7 (6 logs CFU/mL) and washed in each treatment for 1 or 2 minutes. The treated leafy greens were stored at 4˚C and surviving pathogen populations determined on days 0, 1, and 3 of storage. Organic sanitizers, for both treatment times, significantly ($P < 0.05$) reduced *E. coli* O157:H7 on all the leafy greens during storage. Highest reduction (3.4 logs CFU/g) was observed after treatment with CG100 (0.4%) in romaine lettuce, while CHICO Wash™ showed greater than 2 logs CFU/g reduction on all the leafy greens, by day 3. This study demonstrates the potential application of organic sanitizers in flume-washing of organic leafy greens for the reduction of *E. coli* O157:H7.

Keywords

Organic Sanitizers, *Escherichia coli* O157:H7, Organic Leafy Greens, Antimicrobials

1. Introduction

Over the past two decades, fresh produce has become an important part of the modern diet. At the same time, increased consumer demand for organic fresh produce in the United States (US), has resulted in it being the top selling catego-

ry of organically-grown food. In a recent report by the Organic Trade Association (OTA) and US Department of Agriculture-Economic Research Services (USDA-ERS), fresh produce accounted for 43 percent of organic food sales in 2012, followed by other organic food products [1] [2] [3]. However, fresh produce represents a high-risk food commodity due to the high number of reported outbreaks in the last two decades. It has been estimated that over 46% of foodborne illness outbreaks are caused by fresh produce [4] [5]. In recent years, foodborne outbreaks and recalls associated with organic fresh produce have also been reported. According to the Centers for Disease Control and Prevention (CDC), foodborne pathogens implicated in these outbreaks and recalls include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* and Hepatitis A [6] [7] [8]. *Escherichia coli* O157:H7 is a serious public health concern due to the illnesses caused by this foodborne pathogen. Hemolytic uremic syndrome (HUS) is one of the most severe infections caused by *E. coli* O157:H7, which can lead to kidney failure, predominantly in children, elderly, and immunocompromised individuals [9]. More than 96,534 foodborne illness cases and 61 deaths, each year in the US, are linked to this pathogen [4]. From 1998 to 2008, 49 states reported 350 *E. coli* O157:H7 outbreaks, of which 52% were foodborne and 22% of these cases were associated with fresh produce [5] [10].

Fresh produce is a highly susceptible food commodity for pathogen manifestation, throughout the production chain. Various factors such as contaminated soil, manure, irrigation water and wild birds or animal dropping can mediate pathogen transmission to fresh produce [11] [12]. As a result, several intervention techniques, for maintaining food safety, have been employed by the fresh produce industry. However, strict regulations imposed on organic foods by the USDA-National Organic Program (USDA-NOP), prevent the use of certain antimicrobials, when sanitizing, handling or packaging organic produce [13] [14]. The USDA-NOP-approved disinfectants or sanitizers allowed in the wash water for organic fresh produce include, chlorine (4 ppm, residual), peroxy-acetic acid, hydrogen-peroxide, and ozone [15]. Hydrogen-peroxide is an effective antimicrobial due to its strong oxidative activity [16] [17] [18]. Sapers and Sites [19] reported that 1% hydrogen-peroxide had an equal effect against *E. coli* O157:H7 than 200 ppm chlorine when applied on apples. However, its efficacy is limited to certain produce and pathogens types [16] [20] [21]. In a study by Beuchat [16], less than 1 log reduction in *Salmonella* population was observed on cantaloupe cubes treated with 2% hydrogen-peroxide. Additionally, Denton *et al.* [22] reported that 3% hydrogen-peroxide treatment reduced *E. coli* O157:H7 populations by less than 2 logs on baby spinach compared to that on romaine lettuce. Furthermore, in shredded lettuce and mushrooms, hydrogen-peroxide has shown to cause severe browning [23] [24] and therefore, may not be suitable for all produce types. It is therefore necessary to seek effective alternatives to control foodborne pathogens in organic fresh produce.

For several decades, organic acids and compounds have been used as food additives, preservatives and antimicrobial treatments [25]. Organic acids, such as citric

acid-based sanitizers, fall under the GRAS (Generally Recognized As Safe) category and have shown antimicrobial activity against foodborne pathogens [26]. Several research studies have looked at the effects of organic acids and compounds for disinfecting fresh produce [23] [26] [27]. Their use as sanitizers in the organic fresh produce industry is therefore becoming widely popular. Citric acid-based sanitizer, CHICO Wash™ ($C_3H_8I_0C_3O_7$), is approved organic sanitizer by Food and Drug Administration (FDA), and has been used by the food industry to minimize microbial contamination in fresh or minimally processed fruits and vegetables. Additionally, studies have shown that the efficacy of CHICO Wash™ is minimally affected by the presence of organic matter in food products [28], and hence could be used as an alternative to conventional sanitizers. However, very few sanitizers with an organic make-up have been tested against *E. coli* O157:H7 in organic leafy greens. The present study investigated the efficacy of three organic acid-based, novel sanitizers (CHICO Wash™, C8C10, and CG100), in reducing *E. coli* O157:H7 on organic leafy greens during flume-washing and subsequent storage at 4˚C. Due to their chemical makeup, these organic sanitizers can be added to the list of approved organic sanitizers currently used in the industry.

2. Materials and Methods

2.1. Bacterial Culture Preparation

Two *E. coli* O157:H7 strains (ATCC 43888, and ATCC 43895) were used to prepare a cocktail for the study. Each strain was revived from the frozen (−80˚C) stock-culture and maintained on tryptic soy agar (TSA; Acumedia, Lansing, MI) at 4˚C. One day prior to the experiment, one to two colonies from the TSA plate were inoculated into 9 mL tryptic soy broth (TSB; Bacto™, BD, Sparks, MD) and incubated at 37˚C for 18 - 24 hours. To obtain an overnight culture, 1 mL of the resulting culture was transferred to 9 mL TSB and incubated at 37˚C for 18 - 20 hours. On the day of the experiment, a cocktail (1:1) from the overnight cultures of the two *E. coli* O157:H7 strains was prepared and further diluted in buffered peptone water (BPW; Oxoid Ltd., Basingstoke, Hampshire, England) to obtain the dip inoculum (6 logs CFU/mL) for leafy greens.

2.2. Antimicrobial Treatment Preparation

The following organic-acid based sanitizers (provided by E3 Organics, Orland, CA) were tested: CHICO ($C_3H_8I_0C_3O_7$) Wash™ (pH 1.35), C8C10 (pH 1.93) and CG100 (pH 1.64). The CHICO Wash™ was prepared at a 1:20 ratio (CHICO:water; v:v), while the C8C10 and CG100 treatments were prepared at 0.2 and 0.4% concentrations (v:v) in sterile distilled water. Hydrogen-peroxide (3%) and sterile distilled water were used as control treatments with each experiment.

2.3. Organic Leafy Greens Preparation

The organic leafy greens tested were, bunched mature spinach, bagged baby spinach, and romaine and iceberg lettuce heads. Organic leafy greens were bought

on the day of the experiment from a local store, transported on ice, and stored at 4˚C until use. Running tap water (room temperature (RT); 23˚C - 25˚C) was used to wash the greens thoroughly for 2 minutes to remove any soil or organic matter. The outer leaves of romaine and iceberg lettuce and the core of iceberg lettuce were removed aseptically. Using sterile scissors, the lettuce leaves were cut into 2 × 2 sq. inch pieces. Whole leaves of baby spinach (approximately 1.5 to 2.0 sq. inch leaves) were used. Bunched mature spinach samples were prepared by separating individual leaves, trimming off the stalks, and cutting the leaves into 2 × 2 sq. inch pieces, using aseptic techniques. Leafy greens samples were then weighed (400 g); washed with sterile distilled water; and prepared for the experiments, as previously described [22] [29].

2.4. Antimicrobial Treatment of Organic Leafy Greens

Prior to the antimicrobial treatments, a 20 g sample of each leafy green was removed and placed in a 24 oz Whirl-Pak™ bag (Nasco, Fort Atkison, WI) as the negative (un-inoculated) control. The remaining leafy greens were then dip inoculated [29] [30] [31] [32] for 2 minutes in the *E. coli* O157:H7 inoculum (6 logs CFU/mL). Inoculated leafy greens were then placed under the bio-safety cabinet for 30 minutes to facilitate bacterial adherence [22]. A 20 g sample was set aside as the positive (inoculated) control, while the remaining greens were subjected to the antimicrobial treatments for 1 minute or 2 minutes, as described by Denton *et al.* [22]. Additional neutralization step was performed for the treated samples with 180 mL Dey/Engley (D/E) neutralizing broth (Remel Inc., Lenexa, KS) for 1 minute [32]. Immediately following, excess liquid was shaken off and the leafy greens transferred to Whirl-Pak™ bags and stored at 4˚C for 3 days. Samples for each treatment, along with the negative and positive controls, were collected on days 0 (immediately after treatment), 1, and 3 to determine surviving *E. coli* O157:H7 populations [32]. Surviving pathogen populations, for the respective treatments and storage days, were determined by serially diluting the samples in BPW and plating on Sorbitol MacConkey agar (SMAC; Remel Inc., Lenexa, KS). Plates were incubated at 37˚C for 22 - 24 hours, colonies of *E. coli* O157:H7 (CFU/g) counted after incubation, and converted to $\log_{10}$ CFU/g. Log reductions in *E. coli* O157:H7 populations, for each treatment, were calculated by subtracting the log values from those obtained for the positive control.

2.5. Statistical Analysis

All experiments were repeated three times. Surviving *E. coli* O157:H7 populations, recovered after the antimicrobial treatments at each sampling period, were converted to $\log_{10}$ CFU/g and mean values of the three replicates obtained. The limit of detection was 0.5 $\log_{10}$ CFU/g. Data were analyzed to determine the analysis of variance (ANOVA) using PROC GLM and PROC MIXED procedures (SAS v. 9.3 software; SAS Inst., Cary, NC, USA) for main and interaction effects of treatments, wash times and storage times for all the leafy greens. Sig-

nificant differences between treatments were estimated at $P < 0.05$.

3. Results

The results of surviving *E. coli* O157:H7 populations, after each treatment, are shown in Tables 1-4, with log reductions calculated on the basis of values obtained for the positive control. Compared to the positive control (4.5 - 5.0 logs CFU/g), all the organic sanitizer treatments significantly ($P < 0.05$) reduced *E. coli* O157:H7 population in all the tested organic leafy greens, during the 3-day storage period at 4˚C. Effects of the treatments on each leafy green are described below.

3.1. Baby Spinach

The antimicrobial effects of organic sanitizers on organic baby spinach are shown in Table 1. The untreated positive control had a pathogen population of 5.0 logs CFU/g on day 0 of storage at 4˚C. Compared to the positive control, all the organic sanitizers showed immediate reduction in pathogen population on

Table 1. Antimicrobial effects of CHICO Wash™, C8C10 and CG100 against *Escherichia coli* O157:H7 population on organic baby spinach with 1-minute or 2-minutes treatment exposure time.

Treatments	Time (minutes)	Surviving *E. coli* O157:H7 population* (Log_{10} CFU/g)			*E. coli* O157:H7 Log_{10} reduction**		
		Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC	-	5.0 ± 0.5^{a}	4.7 ± 1.0^{a}	4.2 ± 0.3^{a}			
W	1	4.1 ± 0.1^{a}	3.5 ± 0.1^{b}	3.1 ± 0.7^{b}	0.9	1.2	1.1
HP	1	1.8 ± 0.7^{b}	2.4 ± 0.1^{c}	2.0 ± 0.7^{c}	3.3	2.4	2.2
CHICO Wash™	1	2.2 ± 0.7^{bc}	2.4 ± 0.2^{cd}	2.1 ± 0.3^{c}	2.9	2.3	2.1
C8C10 (0.2%)	1	2.5 ± 0.3^{bc}	2.7 ± 0.3^{cd}	2.8 ± 0.4^{d}	2.5	2.0	1.4
C8C10 (0.4%)	1	3.2 ± 0.5^{cd}	2.8 ± 0.2^{cd}	3.2 ± 0.8^{d}	1.9	1.9	1.0
CG100 (0.2%)	1	2.9 ± 0.3^{cd}	2.5 ± 0.3^{cd}	3.0 ± 0.1^{d}	2.1	2.3	1.2
CG100 (0.4%)	1	2.3 ± 0.2^{bc}	2.7 ± 0.6^{cd}	2.6 ± 0.5^{c}	2.7	2.0	1.6
W	2	3.7 ± 0.3^{a}	3.1 ± 0.1^{d}	3.1 ± 0.6^{b}	1.4	1.6	1.1
HP	2	1.9 ± 0.2^{bc}	2.7 ± 0.4^{cd}	1.9 ± 0.4^{c}	3.2	2.1	2.3
CHICO Wash™	2	2.6 ± 0.7^{bc}	2.4 ± 0.6^{c}	1.6 ± 0.4^{c}	2.5	2.3	2.6
C8C10 (0.2%)	2	3.1 ± 0.2^{d}	2.9 ± 0.6^{cd}	2.5 ± 0.2^{d}	1.9	1.8	1.7
C8C10 (0.4%)	2	3.1 ± 0.3^{d}	3.3 ± 0.6^{d}	2.8 ± 0.0^{d}	1.9	1.5	1.4
CG100 (0.2%)	2	2.7 ± 0.3^{cd}	2.5 ± 0.6^{cd}	2.6 ± 0.6^{d}	2.4	2.3	1.6
CG100 (0.4%)	2	2.8 ± 0.1^{cd}	2.7 ± 0.9^{cd}	1.9 ± 0.3^{c}	2.3	2.0	2.3

PC: Positive Control; W: Water; HP: Hydrogen Peroxide. *Values represent the average of three replications. Standard deviation (±) for surviving *E. coli* O157:H7 population (Log_{10} CFU/g) follows mean value. Letters *a, b, c, d* provide evidence of statistical significance ($P < 0.05$), where different letters represent statistical difference between treatments for the same sampling day. **Calculations based on surviving populations obtained for PC on day 0, 1, and 3.

day 0, with CHICO Wash™ and CG100 (0.4%) being the most effective (2.9 and 2.7 logs CFU/g reduction, respectively) after 1-minute treatment. Other organic sanitizers showed a reduction of 1.9 - 2.5 logs CFU/g on day 0. These reductions were maintained over the 3-day storage period at 4˚C. For the 2-minutes wash treatment of baby spinach, highest reduction in *E. coli* O157:H7 population was observed with CHICO Wash™ (2.6 logs CFU/g) on day 3. Hydrogen-peroxide also decreased surviving *E. coli* O157:H7 populations on day 0 (3.2 and 3.3 logs CFU/g for 1- and 2-minutes treatments, respectively), however, this reduction was not maintained during the 3-day storage period and an increase in pathogen counts was observed on day 1 and day 3. Water-treated baby spinach leaves showed a reduction of 0.9 and 1.4 logs CFU/g in bacterial counts on day 0, at 1- and 2-minutes treatment times, which was significantly less than the organic sanitizer treatments.

3.2. Mature Spinach

Organic sanitizers also significantly ($P < 0.05$) reduced *E. coli* O157:H7 population on organic mature spinach throughout the storage period at 4˚C (Table 2).

Table 2. Antimicrobial effects of CHICO Wash™, C8C10 and CG100 against *Escherichia coli* O157:H7 population on organic mature spinach with 1-minute or 2-minutes treatment exposure time.

Treatments	Time (minutes)	Surviving *E. coli* O157:H7 population* (Log_{10} CFU/g)			*E. coli* O157:H7 Log_{10} reduction**		
		Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC	-	4.5 ± 0.3^{a}	4.5 ± 0.3^{a}	4.2 ± 0.3^{a}			
W	1	3.4 ± 0.2^{b}	3.5 ± 0.1^{b}	3.8 ± 0.4^{a}	1.1	1.0	0.4
HP	1	2.3 ± 0.2^{c}	2.6 ± 0.2^{c}	2.6 ± 0.1^{b}	2.1	2.0	1.6
CHICO Wash™	1	3.2 ± 0.4^{c}	2.6 ± 0.3^{c}	2.9 ± 0.7^{b}	1.3	1.9	1.3
C8C10 (0.2%)	1	3.2 ± 0.1^{c}	3.2 ± 0.2^{d}	3.3 ± 0.3^{a}	1.3	1.3	0.9
C8C10 (0.4%)	1	3.4 ± 1.3^{b}	3.2 ± 0.4^{d}	3.1 ± 0.3^{b}	1.1	1.3	1.1
CG100 (0.2%)	1	3.7 ± 0.2^{a}	3.3 ± 0.4^{d}	3.1 ± 0.3^{b}	0.8	1.2	1.1
CG100 (0.4%)	1	3.3 ± 0.6^{bc}	3.0 ± 0.5^{cd}	3.1 ± 0.4^{b}	1.2	1.5	1.0
W	2	3.3 ± 0.5^{b}	3.7 ± 0.6^{b}	3.6 ± 0.5^{a}	1.1	0.9	0.6
HP	2	2.6 ± 0.7^{c}	2.8 ± 0.3^{c}	2.0 ± 0.1^{c}	1.9	1.7	2.1
CHICO Wash™	2	3.2 ± 0.8^{c}	2.5 ± 1.0^{c}	2.2 ± 0.5^{c}	1.3	2.0	2.0
C8C10 (0.2%)	2	3.4 ± 0.3^{b}	3.5 ± 0.4^{b}	3.3 ± 0.3^{a}	1.1	1.0	0.9
C8C10 (0.4%)	2	3.5 ± 0.3^{b}	3.3 ± 0.4^{b}	3.2 ± 0.4^{b}	1.1	1.0	0.4
CG100 (0.2%)	2	3.4 ± 0.4^{b}	3.5 ± 0.5^{b}	3.6 ± 0.1^{a}	2.1	2.0	1.6
CG100 (0.4%)	2	3.1 ± 0.8^{c}	2.6 ± 0.4^{c}	3.1 ± 0.8^{b}	1.3	1.9	1.3

PC: Positive Control; W: Water; HP: Hydrogen Peroxide. *Values represent the average of three replications. Standard deviation (±) for surviving *E. coli* O157:H7 population (Log_{10} CFU/g) follows mean value. Letters *a, b, c, d* provide evidence of statistical significance ($P < 0.05$), where different letters represent statistical difference between treatments for the same sampling day. **Calculations based on surviving populations obtained for PC on day 0, 1, and 3.

The initial concentration of *E. coli* O157:H7 on positive control leaves was 4.5 logs CFU/g. Treatment with all the tested organic sanitizers showed immediate reduction (1 - 1.3 logs CFU/g) in bacterial counts on day 0, which was maintained over a period of 3-day storage (Table 2). CHICO Wash™ at 2-minutes exposure time was the most effective of the three sanitizers and resulted in significant reductions (2.0 logs CFU/g) in *E. coli* O157:H7 populations during storage of mature spinach. A reduction of 0.6 - 1.6 logs CFU/g by day 3 was seen in mature spinach washed for 1 or 2 minutes with C8C10 and CG100. Hydrogen-peroxide reduced pathogen populations on day 0 but this reduction was not maintained during the 3 days of storage on mature spinach washed for 1 minute.

3.3. Romaine Lettuce

Antimicrobial effects of CHICO Wash™, C8C10 or CG100 against *E. coli* O157:H7 on organic romaine lettuce is shown in Table 3. The untreated positive control had a pathogen population of 4.7 logs CFU/g on day 0, followed by 4.3 and 4.6 logs CFU/g on day 1 and 3 of storage at 4˚C, respectively. Compared to

Table 3. Antimicrobial effects of CHICO Wash™, C8C10 and CG100 against *Escherichia coli* O157:H7 population on organic romaine lettuce with 1-minute or 2-minutes treatment exposure time.

Treatments	Time (minutes)	Surviving *E. coli* O157:H7 population* (Log_{10} CFU/g)			*E. coli* O157:H7 Log_{10} reduction**		
		Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC	-	4.7 ± 0.8^{a}	4.3 ± 0.7^{a}	4.6 ± 1.3^{a}			
W	1	3.6 ± 0.8^{b}	3.1 ± 0.2^{b}	3.4 ± 1.0^{b}	1.2	1.3	1.1
HP	1	1.6 ± 0.9^{c}	1.8 ± 0.1^{c}	1.3 ± 0.2^{c}	3.2	2.6	3.3
CHICO Wash™	1	2.5 ± 0.5^{cd}	2.2 ± 0.5^{c}	2.1 ± 0.1^{d}	2.2	2.2	2.4
C8C10 (0.2%)	1	3.5 ± 0.0^{b}	2.7 ± 0.2^{d}	2.6 ± 0.2^{de}	1.3	1.7	2.0
C8C10 (0.4%)	1	2.7 ± 0.3^{d}	2.6 ± 0.1^{d}	2.2 ± 0.1^{d}	2.0	1.8	2.3
CG100 (0.2%)	1	3.1 ± 0.7^{d}	2.6 ± 0.0^{d}	2.3 ± 0.1^{d}	1.7	1.8	2.3
CG100 (0.4%)	1	2.6 ± 0.8^{cd}	1.8 ± 0.3^{c}	1.2 ± 0.6^{c}	2.1	2.6	3.4
W	2	3.6 ± 0.9^{b}	2.7 ± 0.4^{d}	2.4 ± 0.3^{de}	1.1	1.6	2.2
HP	2	2.2 ± 0.9^{c}	1.8 ± 0.1^{c}	1.6 ± 1.0^{c}	2.5	2.5	2.9
CHICO Wash™	2	1.9 ± 0.1^{c}	2.2 ± 0.5^{c}	2.0 ± 0.3^{c}	2.8	2.1	2.6
C8C10 (0.2%)	2	2.0 ± 0.1^{c}	2.8 ± 0.9^{d}	2.2 ± 0.1^{cd}	2.8	1.6	2.4
C8C10 (0.4%)	2	1.8 ± 0.2^{c}	2.3 ± 0.6^{c}	2.5 ± 0.8^{d}	3.0	2.1	2.1
CG100 (0.2%)	2	2.1 ± 0.4^{c}	2.3 ± 0.6^{c}	2.3 ± 0.6^{d}	2.6	2.1	2.3
CG100 (0.4%)	2	1.9 ± 0.8^{c}	2.0 ± 0.8^{c}	1.7 ± 1.1^{c}	2.8	2.4	2.8

PC: Positive Control; W: Water; HP: Hydrogen Peroxide. *Values represent the average of three replications. Standard deviation (±) for surviving *E. coli* O157:H7 population (Log_{10} CFU/g) follows mean value. Letters *a, b, c, d* provide evidence of statistical significance ($P < 0.05$), where different letters represent statistical difference between treatments for the same sampling day. **Calculations based on surviving populations obtained for PC on day 0, 1, and 3.

the positive control, CHICO Wash™, CG100 (0.4%) and C8C10 (0.4%) were the most effective treatments, with an immediate reduction of 2.0-2.2 logs CFU/g in pathogen population after 1-minute treatment. Further reductions in bacterial populations were observed with these sanitizers (after 1-minute treatment) over the 3-day storage period, with CG100 (0.4%) showing a log reduction of 3.4 by day 3. At an exposure time of 2 minutes, all the organic sanitizers were equally effective, showing an immediate reduction (2.6 to 2.8 logs CFU/g on day 0) in pathogen population on romaine lettuce. By day 3, CG100 at 0.4% resulted in the highest reduction in *E. coli* O157:H7 population (2.8 logs CFU/g) on romaine lettuce washed for 2 minutes, followed by CHICO Wash™ (2.6 logs CFU/g). Compared to hydrogen-peroxide, the organic sanitizers were more effective when romaine lettuce was washed for 2 minutes. On the other hand, romaine lettuce washed with water (1 or 2 minutes) did not show a significant reduction in pathogen populations.

3.4. Iceberg Lettuce

Results similar to those with romaine lettuce were observed with iceberg lettuce (**Table 4**). The initial concentration of *E. coli* O157:H7 on positive control leaves was 4.0 logs CFU/g. Compared to the positive control and other organic sanitizers, CG100 at both concentrations (0.2% and 0.4%) and exposure times (1 or 2 minutes) was the most effective, reducing *E. coli* O157:H7 populations by 1.7 to 2 logs CFU/g on day 0. By day 3, further reductions (1.9 to 2.7 logs CFU/g) in pathogen populations were observed on iceberg lettuce washed with CG100. CHICO Wash™ at 2 minutes showed similar results as CG100, reducing pathogen counts by 2.3 logs CFU/g on day 3. Other sanitizers reduced *E. coli* O157:H7 population by 1.7 logs CFU/g at both treatment times, by day 3. Hydrogen-peroxide treatment showed similar results except that it was more effective at 1-minute than the 2-minutes treatment. At the same time, iceberg lettuce washed with water (1 or 2 minutes) did not show a significant reduction in pathogen populations.

4. Discussion

All the tested organic sanitizers showed immediate reduction in *E. coli* O157:H7 populations on all the leafy greens tested, when compared to the positive control. To our knowledge, no other studies have been reported with the organic compounds, C8C10 and CG100, and this study is the first to test the antimicrobial activity of these compounds against *E. coli* O157:H7 on organic leafy greens. These two sanitizers, at both concentrations (0.2 and 0.4%) showed immediate reduction in *E. coli* O157:H7 populations on the leafy greens.

Compared to other treatments, CHICO Wash™ was the most effective on baby spinach, resulting in up to 3 logs CFU/g reduction on day 0. Consistent with other studies [28] [33], CHICO Wash™ also showed immediate reduction in pathogen populations on iceberg lettuce. Studies [33] conducted with iceberg

Table 4. Antimicrobial effects of CHICO Wash™, C8C10 and CG100 against *Escherichia coli* O157:H7 population on organic iceberg lettuce with 1-minute or 2-minutes treatment exposure time.

Treatments	Time (minutes)	Surviving *E. coli* O157:H7 population* (Log_{10} CFU/g)			*E. coli* O157:H7 Log_{10} reduction**		
		Day 0	Day 1	Day 3	Day 0	Day 1	Day 3
PC	-	4.0 ± 0.1^{a}	4.0 ± 0.5^{a}	4.1 ± 0.2^{a}			
W	1	2.7 ± 0.1^{b}	3.0 ± 0.3^{b}	2.2 ± 0.3^{b}	1.2	1.0	1.9
HP	1	1.9 ± 0.6^{c}	1.7 ± 0.9^{c}	1.0 ± 0.2^{c}	2.1	2.3	3.0
CHICO Wash™	1	2.6 ± 0.9^{bd}	1.5 ± 1.1^{c}	2.5 ± 1.1^{d}	1.4	2.5	1.6
C8C10 (0.2%)	1	2.5 ± 0.3^{bd}	3.1 ± 0.7^{b}	2.3 ± 0.2^{d}	1.5	1.0	1.7
C8C10 (0.4%)	1	2.7 ± 0.2^{b}	2.8 ± 1.7^{b}	2.4 ± 0.2^{d}	1.3	1.3	1.6
CG100 (0.2%)	1	2.0 ± 0.2^{c}	1.9 ± 0.2^{c}	2.0 ± 0.4^{b}	1.9	2.1	2.0
CG100 (0.4%)	1	2.0 ± 0.3^{c}	1.7 ± 0.6^{c}	1.4 ± 0.9^{c}	2.0	2.4	2.7
W	2	3.4 ± 0.5^{a}	3.2 ± 0.4^{b}	2.3 ± 0.2^{d}	0.6	0.8	1.7
HP	2	2.4 ± 1.1^{bd}	1.5 ± 0.6^{c}	1.4 ± 0.6^{c}	1.5	2.6	2.7
CHICO Wash™	2	2.1 ± 0.7^{c}	1.8 ± 0.9^{c}	1.8 ± 0.9^{cd}	1.9	2.2	2.3
C8C10 (0.2%)	2	2.9 ± 0.8^{b}	3.1 ± 0.4^{b}	2.4 ± 0.3^{d}	1.0	1.0	1.7
C8C10 (0.4%)	2	2.5 ± 0.3^{b}	2.5 ± 0.4^{bd}	2.4 ± 0.2^{d}	1.4	1.5	1.7
CG100 (0.2%)	2	2.3 ± 0.2^{bd}	2.3 ± 0.2^{d}	2.2 ± 0.3^{b}	1.7	1.8	1.9
CG100 (0.4%)	2	2.1 ± 0.5^{c}	1.5 ± 0.8^{c}	1.4 ± 0.6^{c}	1.9	2.5	2.6

PC: Positive Control; W: Water; HP: Hydrogen Peroxide. *Values represent the average of three replications. Standard deviation (±) for surviving *E. coli* O157:H7 population (Log_{10} CFU/g) follows mean value. Letters *a, b, c, d* provide evidence of statistical significance ($P < 0.05$), where different letters represent statistical difference between treatments for the same sampling day. **Calculations based on surviving populations obtained for PC on day 0, 1, and 3.

lettuce contaminated with *E. coli* K-12 showed 1.6 logs CFU/g reductions with 2-minute CHICO Wash™ on day 0. The present study achieved slightly higher reductions (1.9 logs CFU/g) on iceberg lettuce treated with CHICO Wash™ for 2 minutes, on day 0. This could be due to the difference in bacterial strains used in the two studies where the present study involved pathogenic strains of *E. coli* O157:H7 whereas the other study included the surrogate (K-12) for *E. coli* O157:H7. Studies suggest that *E. coli* K-12 can develop acid resistance, which could explain its survival on lettuce treated with CHICO Wash™ [34]. In another study, Ravishankar and Zhu [28] revealed that CHICO Wash™ reduced *Salmonella* Newport population by 0.8 and 1.0 logs CFU/g on leek and celery, respectively, on day 0. These results indicate that, compared to *S.* Newport, *E. coli* O157:H7 may be more susceptible to organic sanitizers. Additionally, the current study tested these organic sanitizers on leafy greens, which could account for the differences observed in pathogen reduction due to differences in produce types.

The tested sanitizers fall under organic acid-based compounds and were

found to be more effective against *E. coli* O157:H7, when compared with other organic acids evaluated in previous studies [31] [35]. Park *et al.* [35] demonstrated that lettuce leaves washed for 1 minute with 1% or 2% propionic, acetic, lactic, malic or citric acid reduced *E. coli* O157:H7 populations by 0.8 - 1.4 logs CFU/g. However, in the present study, 1-minute wash treatment of romaine and iceberg lettuce with CHICO Wash™ and CG100 (0.4%) showed immediate reduction (up to 2.2 logs CFU/g) in *E. coli* O157:H7 population. In a similar study by Choi *et al.* [31], treatment of lettuce with 2% malic acid for 10 minutes resulted in 1.4 logs CFU/g reductions in *E. coli* O157:H7 counts. In the current study, immediate reduction of 3.0 logs CFU/g in *E. coli* O157:H7 population was observed after 2-minutes treatment with C8C10 (0.4%).

Results from the present study clearly show that tested organic sanitizers may be more effective in reducing *E. coli* O157:H7 populations on leafy greens than other organic acids. It is well established that low pH of organic acids is one of the primary factors responsible for their antibacterial activity. Additionally, several other factors such as the ratio of un-dissociated ions, chain length and degree of branching in the organic acid, along with bacterial cell physiology and metabolism, can also affect the antimicrobial activity of organic acids [36]. It is also well known that foodborne pathogens are more susceptible to acidic environment, and prefer slightly higher pH values for optimal growth [37] [38]. In this study, among all the organic sanitizers tested, CHICO Wash™ had the lowest pH value (1.34), which could explain the extended antibacterial effect of this particular sanitizer in all the leafy greens during the 3-day storage at 4˚C. Hydrogen-peroxide (3%) had similar outcomes, however, slight increase in surviving pathogen populations was observed in hydrogen-peroxide-treated organic baby and mature spinach leaves during storage (**Table 1** and **Table 2**). Similar results were reported in previous studies [22] [29] [32] [39] where organic leafy greens treated with hydrogen-peroxide showed an increase in pathogen populations by the end of the storage period. Hydrogen-peroxide exhibits antibacterial activity by producing oxidizing molecules, such as hydroxyl radicals, with the help of available peroxidase enzyme, which can be toxic to the bacterial cell [40]. Different produce types contain varying levels of peroxidase which in turn can affect the residual levels of hydrogen-peroxide and its effectiveness on fresh produce [41] [42]. Results from the present study suggest that hydrogen-peroxide does possess antimicrobial properties, but may not be able to maintain long-term antimicrobial effects in certain produce types.

Although the extent of *E. coli* O157:H7 inactivation varied among the different organic sanitizers, differences were also observed on the various leafy greens tested. Higher reductions in pathogen populations were observed on romaine lettuce (3.0 logs CFU/g), followed by baby spinach (2.9 logs CFU/g) and iceberg lettuce (2.0 logs CFU/g), when compared to mature spinach (1.3 logs CFU/g), on day 0. These results are consistent with other studies where differences in efficacy of antimicrobials have been observed on various produce types. A study by

Ravishankar and Zhu [28] suggested that the efficacy of CHICO Wash™ may vary based on the type of food surface. In their study, *S.* Newport population was reduced to 0.8 logs CFU/g on leeks when compared to celery, where a reduction of up to 1.0 logs CFU/g was observed on day 0. Previous studies with organic compounds [22] and plant-extracts [29] [39] have also suggested differences in efficacies of antimicrobial wash treatments with different leafy greens surfaces. In the present study, the antimicrobials were least effective on mature spinach leaves, which could be explained by their leaf topography. Studies have shown that spinach cultivar can affect *E. coli* O157:H7 movement, colonization, and internalization on the leaf surface [43] [44]. A study by Macarisin *et al.* [44] revealed that spinach leaf morphology, which varies with the cultivar, affected the attachment and persistence of *E. coli* O157:H7 on rough organic spinach leaf surface. The cultivar that had the greatest leaf roughness, supported significantly higher *E. coli* O157:H7 populations than the other cultivars. This could explain the observed higher *E. coli* O157:H7 populations on mature spinach in the present study.

No significant differences were observed between the 1-minute and 2-minutes treatment exposure times in the current study, except on romaine lettuce, where 2-minutes treatment time was more effective. This indicates that washing for 1 minute would be sufficient to reduce pathogen populations on leafy greens such as baby and mature spinach, and iceberg lettuce. Our results are similar to those obtained by Akbas and Ölmez [26] where no significant differences were observed between treatment times (2 and 5 minutes) to reduce *E. coli* on iceberg lettuce on day 0. Lin *et al.* [23] also demonstrated that increasing hydrogen-peroxide exposure time from 60 seconds to 90 seconds did not significantly affect *S.* Enteritis, *L. monocytogenes* and *E. coli* O157:H7 populations on lettuce leaves on day 0. Romine lettuce treated for 1 minute with the organic sanitizers showed a reduction between 1.3 to 2.2 logs CFU/g whereas that treated for 2 minutes had log reductions between 2.6 to 3.0 logs CFU/g on day 0. The differences observed in romaine lettuce could be due to the leaf surface where it has more ridges and grooves that could provide a niche for bacterial attachment and colonization, thereby protecting them from sanitizer washes. Washing times longer than 1 minute may therefore be required to effectively remove or reduce *E. coli* O157:H7 populations on the romaine leaf surface.

5. Conclusion

Organic sanitizers effectively reduced *E. coli* O157:H7 populations on organic leafy greens. CHICO Wash™ and CG100 at 0.4% were found to be the most effective flume-tank wash treatments compared to other formulations. Organic sanitizers therefore have the potential to be used as an alternative antimicrobial wash treatment for organic leafy greens. However, future studies call for sensory analysis of the organic leafy greens washed with these organic sanitizers, to determine consumer acceptability.

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