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Use of a silver-based sanitizer to accelerate *Escherichia coli* die-off on fresh-cut lettuce and maintain produce quality during cold storage: Laboratory and pilot-plant scale tests

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ABSTRACT

Outbreaks and product recalls involving romaine and iceberg lettuce are frequently reported in the United States. Novel technologies are needed to inactivate pathogens without compromising product quality and shelf life. In this study, the effects of a process aid composed of silver dihydrogen citrate, glycerin, and lactic acid (SGL) on *Escherichia coli* and *Listeria monocytogenes* concentrations on lettuce immediately after washing and during cold storage were evaluated. Sensory and quality attributes of fresh-cut iceberg lettuce were also evaluated. Laboratory results indicated that application of SGL solution for 30 s as a first step in the washing process resulted in a 3.15 log reduction in *E. coli* O157:H7 immediately after washing. For *E. coli* O157:H7 a significant difference between SGL treatment and all other treatments was maintained until day 7. On day zero, SGL led to a 2.94 log reduction of *L. monocytogenes*. However, there was no significant difference between treatments with or without SGL regardless of storage time. Pilot-plant results showed that samples receiving SGL spray followed by chlorinated flume wash exhibited a greater reduction (1.48 log) in nonpathogenic *E. coli* populations at the end of shelf life than other treatments ($p < 0.05$). Additional pilot plant tests were conducted to investigate the hypothesis that SGL residues could continue to impact microbial survival on the final washed lettuce. Results show that pathogens introduced subsequent to flume washing of lettuce pretreated with SGL solution were not affected by antimicrobial residues. The final quality and shelf life of flume washed lettuce were also unaffected by pretreatment with SGL. In conclusion, the results of this study demonstrate that this new technology has the potential to accelerate *E. coli* die-off on fresh-cut lettuce during cold storage and improve product safety, while not affecting quality throughout the shelf life of the finished products.

1. Introduction

Fresh produce provides consumers with tremendous health benefits, yet it has also emerged as the leading source of foodborne illness outbreaks (Carstens et al., 2019; CDC, 2016a, 2016b, 2017, 2018, 2019, 2020). *E. coli* O157:H7 and *L. monocytogenes* are major pathogens of concern for leafy green vegetables given their associations with numerous outbreaks (CDC, 2016a, 2018, 2019). Additionally, *L. monocytogenes* can grow at low temperature, the condition that is commonly used to store leafy vegetables (McManamon et al., 2019).

Contamination of fresh produce with foodborne pathogens can occur at multiple points along the farm-to-fork supply chain significantly impacting public health and the produce industry's economic wellbeing (Alegbeleye et al., 2018; Machado-Moreira et al., 2019). Thus, it is imperative to develop interventions to prevent these outbreaks and maintain consumer confidence in these nutritious fresh foods.

Produce washing with sanitizers is the primary intervention method to reduce microbial populations on fresh produce post-harvest (Gibson et al., 2019). Over the past decades, chlorine has been used throughout the fresh-cut produce industry to enhance the microbial safety and

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increase the shelf life of these products (Gil et al., 2009; Meireles et al., 2016). The typical concentration applied to leafy greens varies between 10 and 150 mg/l of hypochlorous acid and hypochlorite ion for a maximum period of 5 min exposure (Delasquis et al., 2002; Meireles et al., 2016; Sucheta et al., 2020). However, concerns have been raised regarding the formation of carcinogenic disinfection by-products (DBPs) such as trihalomethanes in wash water when an excessive amount of free chlorine reacts with organic compounds (Fan & Sokorai, 2015).

While many alternative wash water antimicrobials have shown efficacy at reducing microbial populations on fresh-cut produce, such as peracetic acid solution (Banach et al., 2020), ozone (Baur et al., 2004; Gibson et al., 2019), chlorine dioxide (ClO_2) (Gómez-López et al., 2009; Banach et al., 2018), calcium lactate (Martin-Diana et al., 2005), acidified electrolyzed water (AEW) and neutral electrolyzed water (NEW) (Abadias et al., 2008; Koseki et al., 2004; Park et al., 2001, 2002; Yang et al., 2003), few have proven to be as effective and economical as chlorine for washing fresh-cut produce despite the drawbacks of chlorine.

Silver ions are known to have antibacterial activity against a variety of microorganisms (Bovenkamp et al., 2013; Feng et al., 2000; Hussain Dar et al., 2020; Jung et al., 2008), and have been employed in numerous medical applications (Alexander, 2009; Beattie, 2011; Becker & Spadaro, 1978; Chopra, 2007; Lansdown, 2004; Liedberg & Lundeberg, 1990; Liu et al., 2016; Wahab et al., 2021; Walker & Parsons, 2014). Silver ions inactivate microorganisms by damaging their cytoplasmic membranes, cell walls, and DNA. Sequential and independent events have been reported elucidating their mode of antimicrobial activity (Chamakura et al., 2011; Feng et al., 2000; Jiang & Ran, 2018). Silver ions may potentially improve the antimicrobial efficacy of washing systems for fresh-cut products and help reduce the incidence of produce-related outbreaks.

Lactic acid has known antibacterial and antifungal activity against spoilage microbes, as well as pathogenic microbes, both gram-negative and gram-positive bacteria and has a low minimal inhibitory concentration and minimal bactericidal concentration for some critical pathogens that cause food poisoning (Stanojević-Nikolić et al., 2016). Lactic acid can pass through cell membranes and create an acidic pH inside the cell, which damages proteins, including enzymes and DNA structure, thus destroying the extracellular membrane (Mani-López et al., 2012). Authors have reported that lactic acid is a more efficient antibacterial agent than acetic, citric or propionic acids (Bjornsdottir et al., 2006; Daskalov, 2012; Park et al., 2011). While lactic acid is able to inactivate gram-positive bacteria in the presence of a surfactant, it is able to inactivate gram-negative bacteria without a surfactant (Boomsma et al., 2015). Glycerin may serve to make the SGL formulation more effective for inactivating gram-positive bacteria (Peterson & Schlievert, 2006).

For the fresh-cut produce industry, it is important to develop effective post-harvest interventions capable of reducing the microbial population in fresh-cut products, without compromising final product quality. In addition, while laboratory studies are abundant, more research is needed at pilot plant or industrial scale on the washing process of fresh-cut produce. Therefore, the purpose of this study was to evaluate the effects of a new process aid, composed of silver dihydrogen citrate, glycerin, and lactic acid (SGL), on *Escherichia coli* O157:H7 and *Listeria monocytogenes* reduction on fresh-cut lettuce immediately after washing and during cold storage. This study consisted of two stages: (1) laboratory scale testing of SGL effectiveness as a processing aid to enhance chlorine efficacy against foodborne pathogens inoculated onto romaine lettuce, and (2) pilot-plant confirmation, with investigation into the possibility of SGL residual and its potential effects on lettuce quality and shelf life, as well as antimicrobial activities.

2. Materials and methods

2.1. Lettuce and chemicals for treatments

Romaine lettuce (*Lactuca sativa* var. *longifolia*) was purchased from a local produce wholesale market for use in laboratory testing. For the pilot plant trials, freshly harvested and cored iceberg lettuce (*Lactuca sativa L.*) heads were provided by a commercial collaborator and stored overnight at 5 °C and used within 24 h. Silver dihydrogen citrate is manufactured by Pure Bioscience, Inc. SGL is a proprietary formulation by SmartWash Solutions and was provided through a collaboration agreement. The SGL product was diluted 1:10 with distilled water resulting in a silver concentration of 30 ppm, as measured using Silver test strips (Pure Bioscience, El Cajon, CA). Clorox brand household bleach purchased from a local supermarket was used as a source of free chlorine (hypochlorous acid, HOCl) for wash water disinfection.

2.2. Stage 1: Laboratory scale testing of SGL efficacy

The laboratory scale testing of SGL involved the treatment of inoculated fresh-cut romaine lettuce with either a sequential SGL-chlorine wash (SGL-Cl), or a sequential water-chlorine wash (W-Cl). An unwashed control was also included for comparison. The laboratory scale experiment was repeated three times, and three independent samples were collected from each treatment for microbial analysis. Populations of *E. coli* O157:H7 and *L. monocytogenes* inoculated onto romaine lettuce were evaluated prior to washing, immediately after washing, and on days 0, 3, 7 and 14 of the storage.

2.2.1. Bacterial strain preparation and sample inoculation

Two GFP-labeled ampicillin-resistant *E. coli* O157:H7 strains EC4115 (associated with 2006 spinach outbreak), and EDL933, and two GFP-labeled *L. monocytogenes* strains of serotype 4b (FDA, LS 806, 4b), and *L. monocytogenes* serotype 1/2b (MGLT: 1.30, 1/2b, associated with 2011 Colorado cantaloupe outbreak), obtained from the Environmental Microbial and Food Safety Laboratory (EMFSL) culture collection, were used for laboratory trials. Cultures were grown in tryptic soy broth (TSB) (Becton Dickenson, Sparks, MD) for 20 h at 37 °C. Cells were harvested by centrifugation (14,300*g*, 2 min; Sorvall Legend Micro 21 microcentrifuge, Thermo Fischer Scientific, Waltham, MA) and washed once in sterile phosphate buffered saline (PBS, pH 7.2, Corning, Corning, NY). Cell suspensions were combined to form the inoculum cocktail and further diluted in sterile distilled water to a final concentration of 10^6 CFU/ml. Initial bacterial concentrations were estimated by measuring the absorbance of the bacterial suspension at 600 nm with a spectrophotometer (Genesys 20, Thermo Fisher Scientific, Waltham, MA) and confirmed by plating 0.1 ml aliquots of appropriately diluted culture onto MacConkey Agar (Becton Dickenson, Sparks, MD) supplemented with 100 mg/l of ampicillin (Fisher Scientific, Fair Lawn, NJ) for *E. coli* strains and Harlequin Agar (Neogen, Lansing, MI) for *L. monocytogenes* strains. The concentration of the inoculum cocktail in the water was also confirmed by diluting it 1000x and spiral plating (IUL Eddy Jet 2 Spiral Plater; Neutec Group, Inc., Farmingdale, NY) 0.1 ml onto the appropriate agar plates. Plates were incubated at 37 °C for 24 h (for *E. coli*) and 48 h (for *L. monocytogenes*).

Romaine lettuce stems, and outer and damaged leaves were discarded, and the remaining leaves were inoculated by submerging in the pathogen cocktail for 15 s. Then, the leaves were spread out and allowed to dry on paper towels in a BSL-2 biosafety cabinet operating with airflow for 1 h. The leaves were then transferred to a plastic bag and stored at 5 °C for 20 h.

2.2.2. Laboratory scale washing and bacterial enumeration

Inoculated romaine lettuce leaves were manually cut into 2.54 cm squares using a sterile knife. The freshly cut lettuce leaves (300 g/ 3 L) were rinsed with SGL or tap water for 30 s, rinsed in tap water for 5 s,

followed by washing in 15 mg/l free chlorine for 30 s. These two treatments are henceforth referred to as SGL-Cl and W-Cl, respectively. Excess wash liquid was removed using a household salad-spinner (20 rotations). Then lettuce was packaged (25 g of lettuce pieces/bag) and sealed (Model H-963, Uline, Allentown, PA) in 110 OTR bags (19 cm × 11 cm) and stored at 5 °C for 14 days. For each treatment, the experiment was performed with three independent replicates, and three samples per replicate were evaluated on days 0, 3, 7 and 14. A chlorine photometer (CP-15, HF Scientific, Inc., Ft. Myers, FL) was used to measure free and total chlorine. The chloramine content was calculated by subtracting free chlorine from total chlorine.

Triplicate 15-g samples of lettuce were weighed into filter bags (Whirl-Pak®, Nasco, Modesto, CA) and macerated with 75 ml of sterile PBS for 2 min at high speed with a stomacher blender (Model 400, Seward™ Stomacher™, Bohemia, NY). Surviving *E. coli* O157:H7 and *L. monocytogenes* were enumerated using appropriate dilutions and spiral plating 0.1 ml of filtrate on selective and differential agar plates as described in 2.2.1.

2.3. Stage 2: Pilot-plant confirmation

2.3.1. Stage 2a: Pilot-plant confirmation of E. coli reduction on lettuce after wash

2.3.1.1. Lettuce inoculation. Three generic *E. coli* strains, originally isolated from surface irrigation water (TVS 353), romaine lettuce (TVS 354), and sandy-loam soil (TVS 355), and rendered rifampicin resistant (Harrand et al., 2019; Tomás-Callejas et al., 2011) were used for pilot plant trials. *Listeria* was not used in the pilot plant study because SGL-Cl did not show significant improvement over Cl in the laboratory study. Each *E. coli* strain was grown in TSB at 37 °C with agitation at 110 rpm for 18 h, harvested by centrifugation at 2600 g (Sorvall Legend X1R Centrifuge, Thermo Fisher Scientific, Waltham, MA) for 5 min, and resuspended in PBS. The three strains were combined in equal proportion and further diluted in sterile distilled water to form the cocktail inoculum with an initial concentration of 10^7 CFU/ml. Iceberg lettuce heads were placed in 5-kg batches in large plastic bags and sprayed

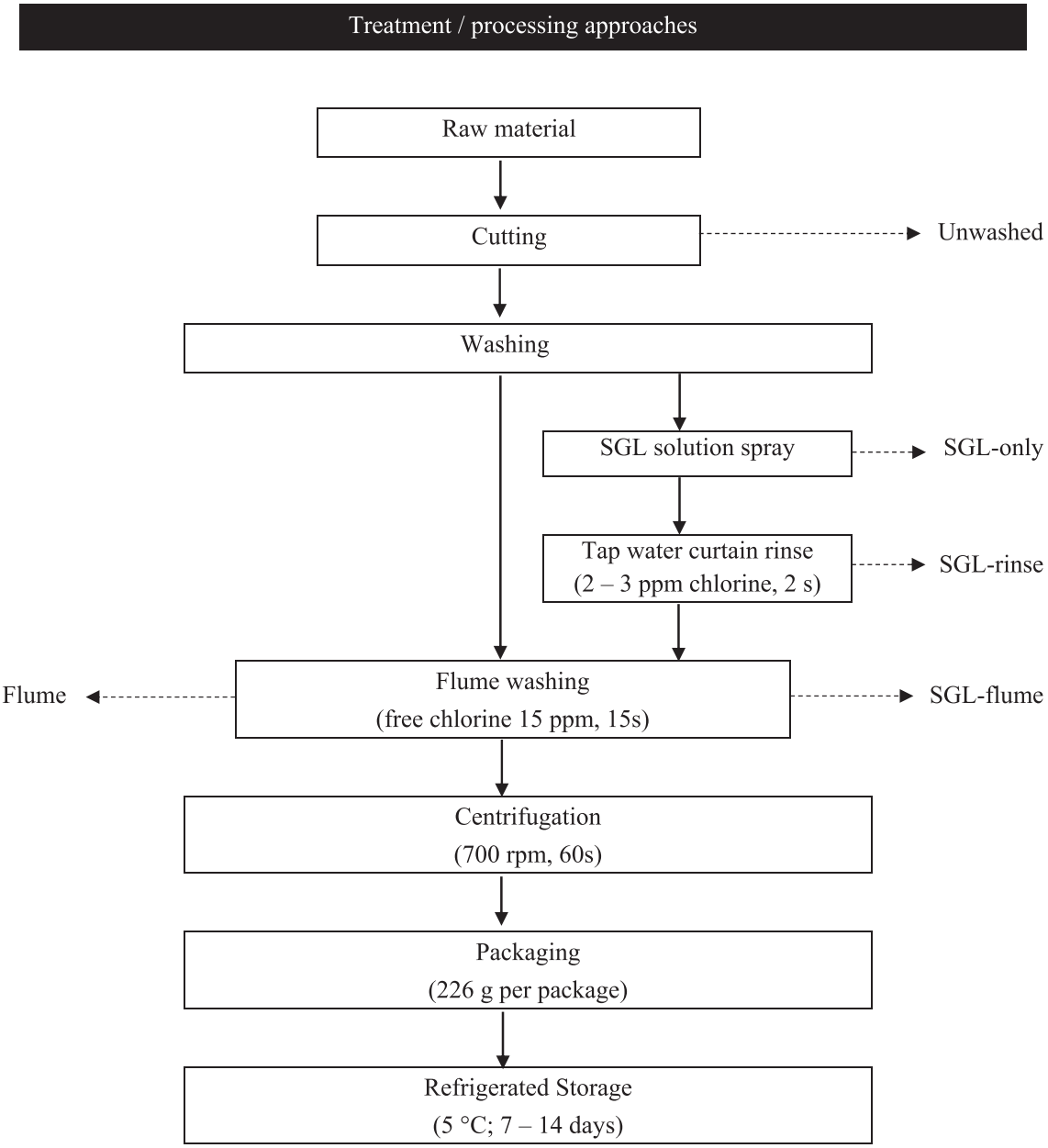

Fig. 1. Flow diagram of fresh-cut produce processing following five different treatments operating at a pilot plant scale.

with ~ 235 ml inoculum using a sprayer with fine-mist nozzle (HDX by The Home Depot, Atlanta, GA). Inoculated lettuce heads were stored at 5 °C overnight before processing.

2.3.1.2. Washing treatment conditions. Our fresh-cut pilot processing plant consisted of two conveyors, an Urschel slicer (Urschel Laboratories, Inc., Chesterton, IN), a propelled flume with a dewatering shaker bed (157.48 cm W × 426.72 cm L × 91.44 cm H; Heinzen Manufacturing International, Gilroy, CA), a MC-44 Vario centrifugal dryer (Somarc, Veno, Netherlands), An Automated Analytic Platform™ (SmartWash-Solutions Inc., Salinas, CA), and a packaging machine. Wash water chlorination and pH were monitored and maintained with the target pH of 6.2 and target chlorine concentration of 15 mg/l. Inoculated iceberg lettuce heads were sliced into 0.6 cm strips and washed at a throughput of approximately 40 kg/min, with dwell time of ~15 s.

Five treatments evaluated included: 1) washing with SGL solution sprayed during cutting (SGL-only); 2) SGL spray wash followed by an overhead water rinse (SGL-rinse); 3) SGL spray wash followed by chlorinated flume wash (SGL-flume); 4) chlorinated flume wash only (flume); and 5) unwashed lettuce as a control. The process is outlined in a flow diagram shown in Fig. 1. To enable SGL spray, the Urschel slicer was retrofitted with three nozzles to enable SGL spray onto fresh-cut products immediately after cutting. A water spray was applied just before the cut lettuce was discharged into the washing flume to minimize SGL introduction. The conveyor belt (Coastal Manufacturing, Inc., Watsonville, CA) between the cutter and the flume was set at a speed allowing 30 s of SGL contact time before the water spray was applied.

Washed lettuce was centrifugally dried at 700 rpm in 2 consecutive 30 s cycles, spinning in clockwise and counterclockwise directions. Samples (226 g) were placed in bags of selected O_2 transmission rate with bag configurations and product weight matching the industry settings. Bags were hermetically sealed using a horizontal continuous band sealer (CBS-8801, Sealer Sales Inc., Northridge, CA) and stored at 5 °C for subsequent evaluation. Experiments were repeated three times with five samples of fresh-cut lettuce collected per treatment per replication.

2.3.2. Stage 2b: Investigation of post-washing effects of SGL on pathogens - treatments and inoculation procedure

To further investigate whether the final washed lettuce would retain any SGL solution, a second pilot-plant trial was conducted using four treatments: 1) Unwashed; 2) SGL-rinse; 3) SGL-flume; and 4) Water-flume. Treatments 1–3 were performed as described in section 2.3.1.2. Treatment 4, Water-flume samples, were sprayed with tap water during cutting, followed by flume washing. This second pilot plant trial was repeated five times, and three replicate samples of each treatment condition were collected and analyzed to assess whether SGL treatment had any long-lasting antimicrobial effects that were still present post-washing.

In order to determine whether there were any residual effects of SGL that might impact pathogen survival, packaged SGL-treated lettuce samples that had been flume washed were transferred to a BSL-2 laboratory and inoculated with pathogenic strains of *E. coli* O157:H7 and *L. monocytogenes* and subsequently assayed for pathogen survival.

Prior to sealing, three bags of lettuce for each treatment and for all three evaluation times were spot-inoculated with the pathogenic strains. A total of 1 ml inoculum (10^8 CFU/ml) was added to each bag of 226 g lettuce in 0.1 ml aliquots dispersed in 10 locations within the package. Following inoculation, lettuce bags were gently mixed to distribute inoculum uniformly, and then sealed. For each experiment, one set of bags were inoculated this way for subsequent microbiological analyses, and another set remained un-inoculated for quality analyses. Both sets of bags were stored at 5 °C for up to 14 days.

2.3.3. Microbial enumeration

Microbial populations on inoculated lettuce samples were evaluated immediately after processing on day 0 and after 7- and 14-days of storage. Samples (25 g) were stomached (Bag Mixer 400, Interscience Laboratory Inc., Woburn, MA) for 2 min at 230 rpm in 125 ml PBS in Whirl-Pak filter bags. The rinsate and its decimal serial-dilutions were spiral plated using an Eddy Jet 2 (Neutec, Farmingdale, NY) onto MacConkey agar supplemented with 80 mg/l rifampicin (R-MAC), Sorbitol MacConkey Agar (Becton Dickenson, Sparks, MD) supplemented with 100 mg/l ampicillin, 0.05 mg/l cefixime, and 2.5 mg/l potassium tellurite (ACT-SMAC; Applied Biosystems™, Thermo Fisher Scientific, Vilnius, Lithuania), and Listeria Brilliance Agar (LBA; OXOID Ltd, Basingstoke, United Kingdom) in duplicate, and incubated at 37 °C for up to 48 h. Characteristic colonies were counted using a Flash & Go automatic colony counter (Neutec Group, Inc., Farmingdale, NY). R-MAC, ACT-SMAC, and LBA were used to select for the populations of rifampicin-resistant *E. coli* (pre-washing inoculation), ampicillin resistant *E. coli* O157:H7, and *L. monocytogenes* (post-washing inoculation) on lettuce, respectively.

2.3.4. Product quality and shelf life evaluation

Product quality on samples immediately after processing and during cold storage was assessed using instrumental and sensory (off-odor and visual only) techniques. This evaluation was conducted in the course of the pilot-plant confirmation of *E. coli* reduction on lettuce (Stage 2a) in a BSL-1 laboratory. Sensory attributes were determined via a 6-member trained panel (female = 3, male = 3). Before evaluation, the participants were trained to determine the intensity of parameters for off-odor and visual quality using commercial fresh-cut iceberg lettuce as reference (Bornhorst et al., 2018; Kou et al. 2014).

2.3.4.1. Packaging conditions and gas composition. The O_2 and CO_2 partial pressures in the headspace of iceberg lettuce packages were measured using a Dansensor CheckMate 3 gas analyzer (Ametek mocon, Brooklyn Park, MN) by inserting the needle of the measuring assembly into the package headspace through a rubber septum adhered to the packaging film (Baur et al., 2004). The CO_2 and O_2 partial pressures were expressed as percentages of the total gas composition.

2.3.4.2. Loss of tissue integrity. Tissue integrity was assessed by measuring the change in solution electrical conductivity due to electrolyte leakage. Lettuce (50 g) was immersed in 500 ml of deionized water for 30 min, after which free electrical conductivity from free electrolytes was measured after inserting probe for 10 s with swirling. The same lettuce sample in solution was then frozen at −20 °C overnight and thawed for 24 h, and conductivity was measured again to determine total electrical conductivity. Electrolyte leakage is expressed as a ratio of free conductivity to total conductivity, which is negatively correlated to lettuce tissue integrity (Kim et al., 2005a).

2.3.4.3. Off-odor. Off-odor was evaluated immediately after opening the packages and scored on a 1 to 99 scale, where 99 = severe, 55 = moderate, 22 = slight, and 1 = absence of off-odor. In between samples, panelists sniffed coffee beans as an olfactory palate cleanser, followed by smelling fresh-cut lettuce as a reference control (Bornhorst et al., 2018). For off-odor, a decay score of 40 or below was considered to be acceptable (Kou et al., 2014).

2.3.4.4. Overall visual quality. Visual quality was evaluated by comprehensively considering lettuce appearance, freshness, and color, and scored using a 1–99 hedonic scale where 99 = like extremely, 80 = like very much, 70 = like moderately, 60 = like slightly, 50 = neither like or dislike, 40 = dislike slightly, 30 = dislike moderately, 20 = dislike very much, and 10 = dislike extremely. A score of 60 or above is considered an acceptable or marketable range for visual quality (Kou et al., 2014). All samples were identified by randomly generated 3-digit codes to mask the treatment information and to

minimize subjectivity of the tests (Agüero et al., 2011; Kim et al., 2005b).

2.4. Statistical analysis

Data were analyzed using Sigma Stat (Sigma Plot 14.0; SYSTAT Software, Inc., San Jose, CA, USA). Microbial populations were subjected to $\log_{10}$ transformations before statistical analysis. Sensory and microbial data sets were analyzed using analysis of variance (ANOVA). For all studies, the assumptions of normality and equal variance were checked using the Shapiro-Wilk and Brown-Forsythe tests, respectively. When treatment effects were significant, post-hoc analysis of means was performed with Holm-Sidak adjusted *p*-values to maintain experiment-wise error of ≤ 0.05.

3. Results

3.1. Stage 1: Laboratory scale testing of SGL efficacy

E. coli O157:H7 populations differed significantly between the SGL-Cl treatment and the control (Unwashed) as well as the W-Cl treatment (Fig. 2A) ($p < 0.05$). The application of SGL solution as a first step in the washing process resulted in an initial 3.15 log reduction in *E. coli* O157:H7 compared to the control, while the W-Cl treatment caused a 2.50 log reduction. A significant difference between SGL-Cl treatment and the control (day 0) was maintained until day 7, on which a 2.05 log difference was observed, whereas there was no significant difference between W-Cl treatment and the control. On day 14, the SGL-Cl treated samples showed the lowest population of *E. coli* O157:H7 of 4.20 log CFU/g, which was a significant 0.95 log reduction compared to that observed for the W-Cl washed samples.

The application of SGL-Cl contributed to an initial 2.94 log reduction in *L. monocytogenes* compared to the control for which a significant difference between the SGL-Cl and the Unwashed lettuce was maintained until day 14 when a 1.69 log difference was recorded (Fig. 2B). Although a 2.72 log reduction was observed for W-Cl treated samples on day 0, the significant difference between this treatment and the control was maintained until day 7. Finally, while not statistically significantly different, adding SGL to the washing process decreased *L. monocytogenes* populations by 0.71 log by the end of lettuce shelf life.

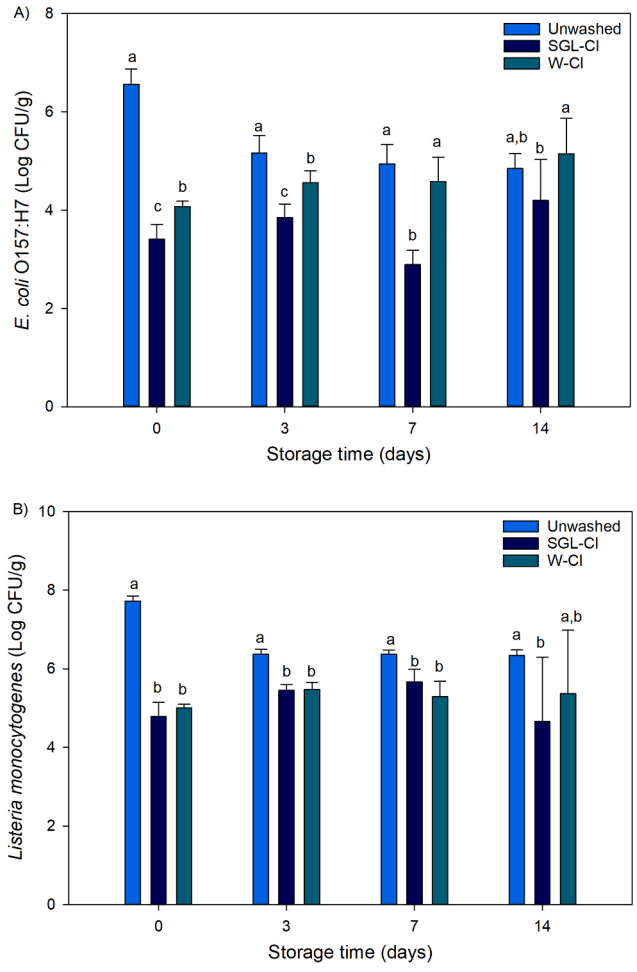

Fig. 2. Effect of treatment conditions on A) *Escherichia coli* O157:H7 and B) *L. monocytogenes* populations on fresh-cut lettuce samples immediately after treatment (day 0), and after 3, 7 and 14 days of storage at 5 °C. Different letters indicate significant differences between treatments within each evaluation day at the $\alpha = 0.05$ significance level, calculated using Holm-Sidak estimates.

3.2. Stage 2: Pilot-plant confirmation

3.2.1. Stage 2a: Pilot-plant confirmation of E. coli reduction on lettuce after wash

The effect of applying a novel process aid (SGL solution) to supplement washing of iceberg lettuce in four experimental treatment scenarios was tested on a pilot-plant scale that simulates the operations used by the leading processors in the fresh-cut industry in the United States. On day 0, unwashed samples had an initial *E. coli* population of 5.27 ± 0.05 log CFU/g of iceberg lettuce. A significant difference ($p < 0.05$) was observed between unwashed samples and washed samples (Fig. 3) taken immediately after processing. No significant difference was found between SGL-flume and flume samples, with *E. coli* populations after wash of 4.69 ± 0.08 and 4.79 ± 0.05 log CFU/g, respectively ($p > 0.05$). After 7 days storage, although a decline in the *E. coli* population was observed for all samples, mean values for SGL-flume washed samples were significantly lower than those for unwashed samples ($p < 0.05$). Following 14 days storage, however, *E. coli* population means for SGL-flume samples at 3.79 ± 0.12 log CFU/g were significantly lower than those for all other treatments. Furthermore, the SGL-flume samples exhibited the greatest population reduction (1.48 log reduction) at the end of shelf life, in contrast to the 0.70 log reduction observed for flume washed samples. This statistically significant difference implies that the SGL components may have acted synergistically with the chlorinated water in the flume system to enhance *E. coli* reduction on iceberg lettuce.

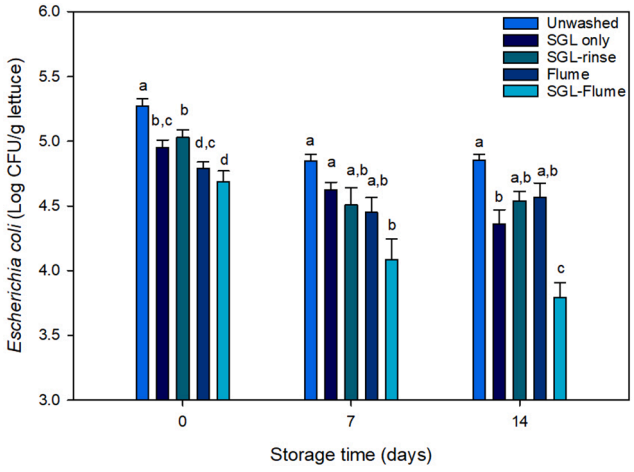

Fig. 3. Effect of treatments on *E. coli* populations on fresh-cut lettuce samples immediately after treatment (day 0), and after storage for 7 and 14 days at 5 °C. Different letters indicate significant differences between treatments within each evaluation day at the $\alpha = 0.05$ significance level.

Additional quantitative analysis was conducted to evaluate the benefit of adding SGL solution to the washing process. Subtracting the *E. coli* population in SGL-flume treated samples from that of the flume washed samples, shows a progressive increase in microbial inactivation for SGL-flume samples during storage. The difference on day 0 was 0.10 log CFU/g, and this increased to 0.37 and to 0.78 log CFU/g on days 7 and 14, respectively. This finding suggests that the application of SGL may have the potential to accelerate *E. coli* die-off during storage, whereas this delayed effect was not observed for the samples that were only chlorine treated (i.e., flume only washed samples). Equivalent results are reported here for the chlorinated flume only washed samples.

Although not statistically different, it is notable that *E. coli* populations in samples that were only flume washed increased slightly from day 7 to day 14 (~0.12 log CFU/g), possibly suggesting that *E. coli* cells that were initially injured were able to recover and grow during storage. This effect was not observed for SGL-flume washed samples which exhibited persistent bacterial die-off throughout storage, indicating that this treatment had stronger bactericidal activity.

3.2.2. Stage 2b: Investigation of post-washing effects of SGL on pathogens

The hypothesis that the SGL solution may have post-wash effects in reducing microbial populations was tested by carrying out a second pilot plant study. For this trial, *E. coli* O157:H7 and *L. monocytogenes* were inoculated onto lettuce samples after washing, and stored for 7 and 14 days. Results are shown in Fig. 4. In regards to the treatment procedure, washing or not washing the samples did not impact the pathogen populations. In addition, there were no significant differences ($p > 0.05$) between SGL-flume and Water-flume washed samples regardless of the storage time for both bacterial strains. Notably, the *E. coli* O157:H7 population declined over storage time following a similar trend for all treatment conditions, including for unwashed samples (Fig. 4A). This result indicates that pre-treatment with SGL solution prior to inoculation with *E. coli* O157:H7 did not significantly increase the die-off of these pathogenic strains. The natural decline of the *E. coli* O157:H7 population during cold storage is confirmed by the absence of significant differences between unwashed and SGL-flume, or between unwashed and flume only washed samples. *L. monocytogenes* populations remained at the 4.5–5 log CFU/g level throughout storage time, regardless of the treatment condition applied (Fig. 4B). There was no significant difference in the reduction of *L. monocytogenes* population for any treatment during storage for up to 14 days. This result suggests that the population concentration of *L. monocytogenes* was stable during storage, regardless of the treatment condition.

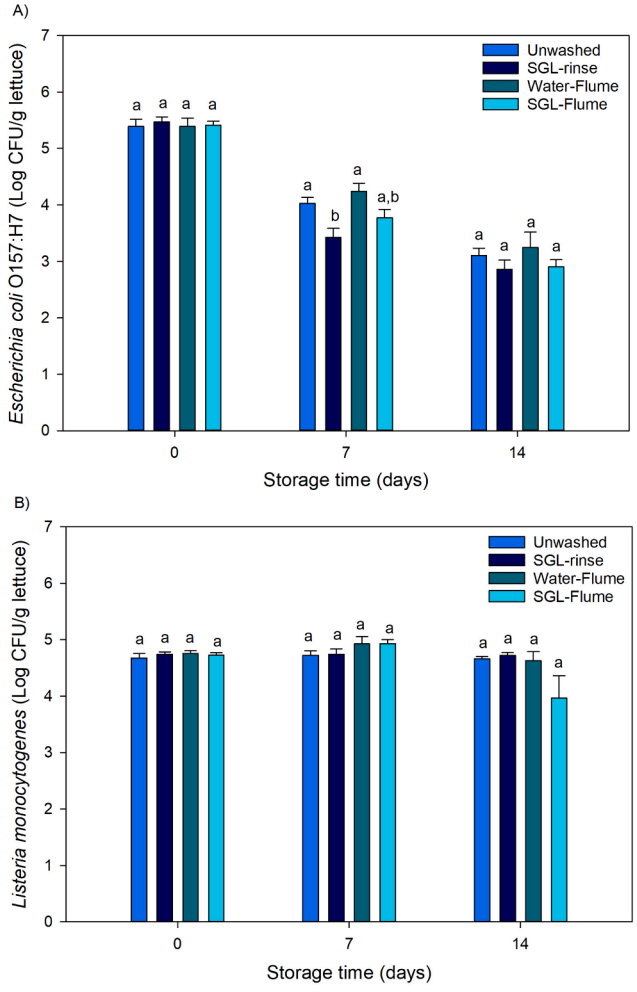

Fig. 4. Effect of treatments on A) *E. coli* O157:H7 and B) *L. monocytogenes* populations on washed fresh-cut lettuce samples immediately after inoculation (day 0), and after storage for 7 and 14 days at 5 °C. Different letters indicate significant differences between treatments within each storage day at the $\alpha = 0.05$ probability level.

3.2.3. Effects of SGL on product quality and shelf life

3.2.3.1. Packaging conditions and gas composition. The variability of headspace gas composition at 7- and 14-day evaluation times for iceberg lettuce, washed and unwashed, packaged using the same OTR bags, is shown in Fig. 5A and 5B. Concentrations of O_2 and CO_2 in the package headspace on each evaluation day were dependent on the treatment conditions. Following 14 days of storage, the O_2 concentration of SGL-flume washed samples were the highest among all samples. No significant differences among treatments were reported for the O_2 concentration over the storage time ($p > 0.05$). Even though the CO_2 concentration increased significantly during storage for all treatments ($p < 0.05$), this increase was particularly steep for SGL-only treated samples. However, the CO_2 concentration of SGL-flume and flume washed samples increased more gradually than in other treatments. No significant differences in CO_2 concentration between SGL-flume and flume washed samples were reported over the storage time. Use of SGL in conjunction with a chlorinated flume wash, therefore, does not appear to have any detrimental effect on package atmosphere.

3.2.3.2. Loss of tissue integrity. Loss of tissue integrity leads to an increase in ion leakage which is assessed by measuring the electrical conductivity of the solution produced by soaking the tissue samples in water (Luo, 2007). In this study, washed and unwashed lettuce samples exhibited an increase in the percentage of electrolyte leakage (EL) over storage time from 7 to 14 days. At 14 days storage, unwashed samples (Fig. 6A) and samples that were treated by SGL-rinse or SGL-only exhibited similar percentage of EL. The SGL-flume and Flume washed samples resulted in significantly lower loss of tissue integrity ($p < 0.05$) than unwashed, SGL-rinse or SGL-only treated samples as indicated by the lower EL measurements of 4.1% and 7.6%, for SGL-flume and flume-only samples, respectively. No significant difference ($p > 0.05$) was observed in the percentage of EL for SGL-flume and flume only samples. The high EL for unwashed samples clearly indicates that washing samples is necessary to remove exudates and prevent degradation caused by excess microbial growth. The high EL for SGL-only samples indicates that SGL treatment alone causes considerable tissue damage when allowed to remain on samples. The only slightly lower EL for SGL-rinse samples indicates that the rinse treatment was inadequate to remove SGL residues, although it is an improvement over SGL-only. The relatively low EL for flume washed samples indicates that this wash treatment is effective in removing exudates and microbes sufficient to maintain tissue integrity without causing significant damage. However, the application of SGL prior to flume washing samples resulted in the lowest EL, indicating that this treatment reduced the microbial rebound

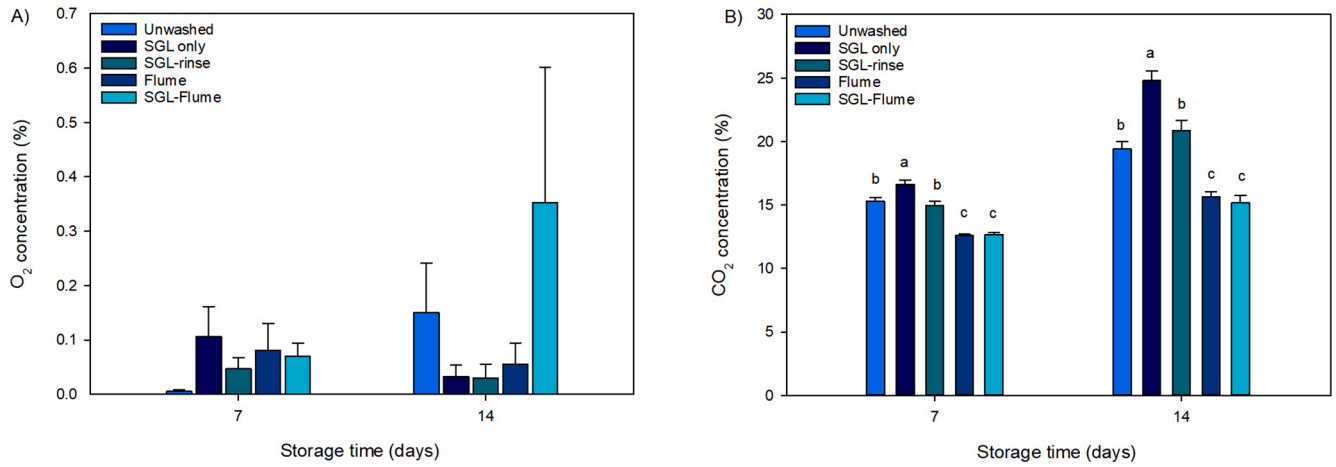

Fig. 5. Effect of treatments on changes of headspace composition (A) O_2 and (B) CO_2 of fresh-cut lettuce samples stored at 5 °C. Different letters indicate significant differences between treatments within each storage day at the $\alpha = 0.05$ significance level.

observed in samples washed with chlorine alone. This result also suggests that flume washing successfully removed the SGL residues that could cause tissue damage, which is a critical finding from a quality perspective. These results indicate that SGL-flume washing treatment may be more beneficial in minimizing loss of tissue integrity than the other investigated treatments.

3.2.3.3. Off-odor. Off-odor scores were significantly higher for all samples on day 14 than on day 7 ($p < 0.05$) (Fig. 6B). On day 7, off-odor scores of SGL-only washed samples were significantly higher than those of all treatments except for SGL-rinse. Flume and SGL-flume treated samples were not significantly different ($p > 0.05$) as they presented scores of 15 and 17, respectively. After 14 days of storage, SGL-only samples scored the highest among all treatments for off-odor (above moderate off-odor). In contrast, the SGL-flume and flume washed samples exhibited the lowest off-odor score (slight off-odor). Since off-odor is a sign of tissue degradation, these results are in agreement with our results for electrolyte leakage. Consequently, SGL can be applied to the lettuce samples without causing tissue damage and exacerbating off-odor, if it is followed by flume washing.

3.2.3.4. Overall visual quality. After storage at 5 °C for seven days, visual quality of the unwashed, flume, SGL-flume and SGL-rinse samples were not significantly different ($p > 0.05$); average attribute ratings ranged from 55 to 63. SGL-rinse and SGL-only received the lowest scores with mean scores of 55 (neither like or dislike) and 45 (dislike slightly), respectively. As the storage time increased, so did the differences between the quality of unwashed, and flume or SGL-flume washed lettuce samples (Fig. 6C). The unwashed sample mean score showed the largest decline in quality (29%), followed by SGL-only (26%), SGL-rinse (18%), SGL-flume (13%), and flume (9%). The SGL-flume and flume samples did not differ significantly in their quality ratings over the 14 days of storage. This finding indicates that, for these treatments, the iceberg lettuce samples maintained acceptable quality (like slightly or better) during the two weeks of storage.

4. Discussion

The main purpose of this study was to examine the potential of a combination of silver dihydrogen citrate, glycerin, and lactic acid (SGL solution) to reduce the *E. coli* population immediately after washing and during cold storage of fresh-cut lettuce beyond that achievable with chlorine alone. This study demonstrated the efficacy of this novel formulation's anti-microbial activity on foodborne pathogens in laboratory scale experiments and confirmed its application using non-pathogenic *E. coli* strains on fresh-cut lettuce processed on a pilot-plant scale. Despite recognizing the differences between different types of lettuce, the advantage of including both iceberg and romaine lettuce in this study is that these are the main ingredients used by the fresh-cut industry and are often associated with outbreaks and recalls (CDC, 2018, 2019; FDA, 2022). The data for different lettuce varieties (e.g., romaine or iceberg lettuce) shows that the SGL-flume treatment has general application for lettuce.

Washing, a crucial step in the processing of fresh-cut lettuce, is designed to remove dirt, decrease microbial load, and promote pathogen inactivation. Chlorine has been used by the fresh-cut industry for over 40 years as one of the most effective sanitizers (Gil et al., 2009). Many studies have examined the use of chlorine at concentrations ranging from 1 to 100 mg/l for washing leafy greens (Luo et al., 2012). The importance of maintaining at least 10 mg/l free chlorine (as hypochlorous acid, HOCL) in the wash water has been demonstrated in pilot plant studies to reduce bacterial survival and prevent cross-contamination (Luo et al., 2012, 2018). Chlorine efficacy is often correlated with chlorine concentration, pH, and exposure time during washing (Zhou et al., 2015). In this study, the minimal 15 mg/l of free chlorine used in the flume system was insufficient to fully disinfect the inoculated fresh-cut lettuce prior to packaging and refrigerated storage. Despite this result, it was clear that washing lettuce resulted in lower culturable populations of *E. coli* than those found on unwashed samples (Fig. 3). In addition, only when the SGL solution was sprayed on the samples and then washed in the flume system (Stage 2 studies) did the log reduction exceed 1 log CFU/g. Admittedly, such bacterial reductions would be considered sufficient only when the initial counts are not excessively high. Possibly, the initial inoculum concentration used in this study was excessively high and did not represent a realistic contamination level, or the exposure time to the sanitizers was insufficient. Such explanation suggests the need for more pilot plant-based trials to optimize the effectiveness of the washing process. It is important to note, however, that the effectiveness of sanitizers for microbial reduction is generally lower in commercial-scale or pilot-plant operations than in laboratory-scale studies. This is because, in the latter, the operational parameters are more controllable. Consequently, the laboratory tests (Stage 1) were able to achieve higher inactivation (3.15 log reduction) of *E. coli* O157:H7 and (2.94 log reduction) *L. monocytogenes* immediately after washing. A quantitative microbial risk assessment model developed by Pang et al. (2017) may be useful to understand how different intervention technologies are effective in reducing the risk of illness associated with the consumption of lettuce contaminated with *E. coli* O157:H7. Using data from several studies on the effect of reducing *E. coli* O157:H7 in lettuce through washing in chlorinated water, the authors

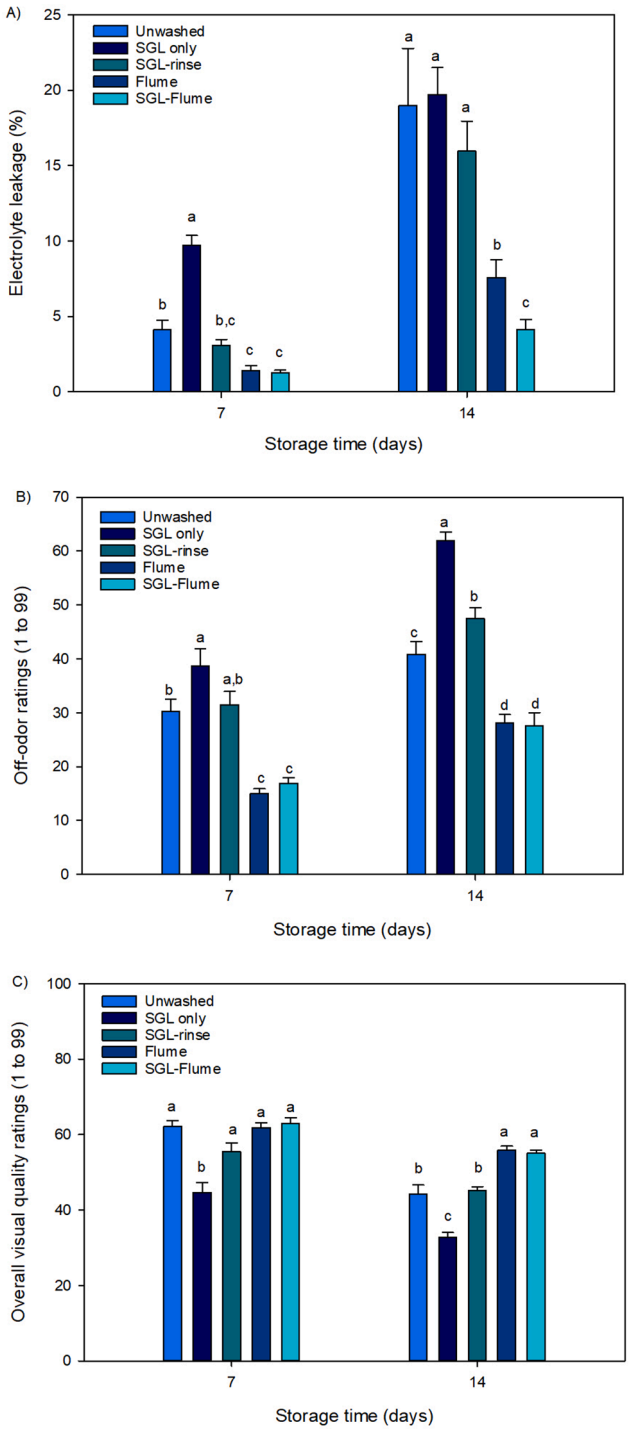

Fig. 6. Effect of treatments on changes in electrolyte leakage (A), off-odor (B) and overall visual quality (C) of fresh-cut lettuce samples stored at 5 °C. Different letters indicate significant differences between treatments within each storage day at the $\alpha = 0.05$ significance level.

estimated that the number of illness cases was reduced by 92% (Pang et al., 2017). The effectiveness in reducing the risk of foodborne illness from using SGL in combination with chlorinated water to wash lettuce needs to be explored in future risk assessments.

In the Stage 2 pilot-plant trials, the highest log reduction observed for SGL-flume washed samples suggests that the currently established methods that use free chlorine could benefit from the pre-application of SGL solution to enhance sanitizer efficacy. The synergistic effects of combining sanitizers with other compounds like silver ions have been previously reported for drinking water applications, for example (Pedahzur et al., 1995, 1997). Pedahzur et al. (1995) found that combining silver ions with hydrogen peroxide can be up to 30 times more potent for the inactivation of *E. coli* than either component alone, and that the synergistic combination had great potential for drinking water disinfection (Pedahzur et al., 1997). The authors observed that at low hydrogen peroxide (H_2O_2) concentrations, *E. coli* inactivation was proportional to the concentration of both silver and H_2O_2, whereas the efficacy of the treatment was less dependent on silver concentration when higher H_2O_2 concentrations were applied. They reported over 3-log reduction for certain concentration ranges, owing to the synergistic effect of the two compounds during a laboratory scale application.

NASA had proposed ionic silver as an antimicrobial additive for purifying drinking water using silver chloride and silver bromide-based filter cartridges in early 1974 to replace iodine which could pose a risk to the health of the crew over long term missions. Additional research into potable spacecraft water systems by another group of NASA scientists using silver fluoride indicated that ionic silver is an effective biocide against many bacteria that were previously recovered from spacecraft drinking water (Birmele et al., 2011). In this context, seeking to improve the knowledge of silver biocides, the advantages and disadvantages of switching to a silver disinfection system for spacecraft water were recently discussed by NASA scientists (Li & Calle, 2018).

Silver ions have been shown to possess strong antimicrobial properties in pharmaceutical and biomedical applications. The antimicrobial activity of silver nanoparticles is related as they release silver ions over time (Zhang et al., 2011). Silver chloride, for example, in the form of nanoparticles showed bactericidal effects against bacteria common in infections such as *S. aureus* and *E. coli* (Trinh et al., 2015) and in the treatment of wounds (Kang et al., 2016). In the latter case, the slow release of silver ions is necessary for a continuous bactericidal concentration of Ag ions in the wound (Kang et al., 2016). Additional activities of silver nanoparticles and their various applications can be found elsewhere (Sofi et al., 2022).

In addition to nanoparticles, silver ions can also be stabilized in the presence of citric acid, as is the case with the patented molecule silver dihydrogen citrate (SDC). SDC is potentially an environmentally friendly alternative to “harsh” disinfectants and has proven to be effective against surfaces contaminated with human norovirus (Buckley et al., 2018; Manuel et al., 2016) and bacteria (Masuku et al., 2012, 2014). The silver ion present in this aqueous disinfectant is weakly bound to a citrate ion. After this complex is formed, it provides a stabilized form of silver ion in an organic acid (citric acid). The bioavailability of silver ions allows the SDC complex to effectively kill microorganisms (Generali et al., 2020).

The ability of the SGL solution to accelerate the death of *E. coli* in fresh-cut lettuce during cold storage can be attributed to the antibacterial effect of silver ions and lactic acid acting in sequential combination with chlorine in the flume system. Although SGL-only and SGL-rinse exhibited slow long-term bactericidal effect, SGL when not thoroughly rinsed off also caused tissue damage under these conditions. Tissue damage in turn provided exudates which could support microbial growth. This provides a reasonably good explanation for the lower efficacy of SGL-only and SGL-rinse treatments to reduce *E. coli* on lettuce samples compared to SGL-flume treatment. In the SGL-flume treatments, lettuce is subjected to vigorous rinsing, and no SGL-associated tissue damage, and hence a less supportive environment for microbes. Furthermore, additional inactivation with both longer SGL exposure and chlorinated water contact times for the samples conveyed to and washed in the flume tank, than those sprayed on the conveyor with the overhead water rinse, leads to more permanent damage to bacterial cells.

While it would be interesting to quantify the relative presence of any residue of SGL or silver ion in the final washed lettuce, the lack of these analyses does not undermine the findings from this study or its general application. Our purpose was not to track the specific concentration of silver ions on leaf surfaces, but to measure changes in the concentrations

of *E. coli* and *L. monocytogenes* to determine the effectiveness of SGL on both laboratory and pilot-plant scales. In addition, the FDA has determined that the solution of silver dihydrogen citrate manufactured by Pure Bioscience, Inc. can be used as an antimicrobial solution applied by spray or dip on fruits and vegetables and will not have a significant adverse impact on the enviroment or human health (FDA, 2015). Concerns about silver and silver nanoparticle safety, environmental toxicity and the risks to human health have been extensively discussed in literature reviews (Maillard & Hartemann, 2012; Mijnendonckx et al., 2013).

The results of post-washing inoculation indicated that any residue of the SGL solution that might remain did not affect the reduction of *E. coli* O157:H7 because the same trends were observed for all samples. Furthermore, after 14 days of storage, there were no statistically significant differences between *E. coli* O157:H7 populations on washed and unwashed samples (Fig. 4A). In addition, the *L. monocytogenes* populations on washed and unwashed samples were not statistically different throughout the storage period (Fig. 4B). The *L. monocytogenes* populations were maintained at their initial inoculation level of 10^5 CFU/g for the duration of storage. We hypothesized that if the SGL solution had been present as a residue, a significant decline in the *L. monocytogenes* population over the storage period would be detected, but that did not occur. It is possible that residue could have been bound to the plant tissue and therefore unavailable to exhibit antimicrobial activity. However, this is unlikely because no adverse quality effects on the plant tissue after flume washing were detected as they were for the SGL-only and SGL-rinse treatments. In conclusion, results from inoculating the samples with foodborne pathogens after washing indicated that if any residue remained after treatment with SGL solution followed by flume washing, it had no bactericidal effect on pathogens introduced after the treatment. The lack of difference in quality between SGL-flume and flume washed samples provided further evidence that any remaining residues were negligible, as they did not impact electrolyte leakage, off-odor or visual quality of lettuce leaves. In contrast, when SGL was not followed by a wash (i.e., SGL-only samples), there was greater damage to lettuce tissue (Fig. 6A), greater off-odor (Fig. 6B) and lower visual quality (Fig. 6C). Therefore, flume wash after SGL treatment removed most SGL residues, and the combined treatment maintained quality and shelf-life at least as effectively as chlorinated flume wash alone.

Moreover, the sequential treatment of SGL and chlorinated flume wash did seem to have a stronger bactericidal effect on the tested pathogens than did chlorine alone, since pathogen populations did not rebound after this treatment as they did after chlorine only treatment. The unique long-term potency of silver ions on *E. coli*, which is not seen in common sanitizers, has been previously reported by Chamakura et al. (2011). Fluorescent microscopic analyses conducted by those authors indicated that *E. coli* cells treated with silver nanoparticles sustained cell wall and membrane damage resulting in cell death. According to the authors, no silver was detected inside the *E. coli* cells (Feng et al., 2000). Chamakura et al. (2011) inferred that the inactivation of *E. coli* occurred mainly due to damage to the cell wall and membrane with the result of an increase in membrane permeability that led to the 'permeation' of intracellular materials and the leakage of ions such as potassium.

A similar explanation for the *E. coli* inactivation by silver ions was reported by Pedahzur et al. (1997). Feng et al. (2000) reported that silver ions caused serious damage to the microbial cell wall, shrinking or detaching of the cytoplasmic membrane from the cell wall, and damage to microbial DNA, resulting in inability of the cells to replicate. All these phenomena were reported to cause microbial death (Feng et al., 2000).

The antimicrobial action of lactic acid, as explained by Alakomi et al. (2000), is largely attributed to its ability to penetrate the cytoplasmic membrane, resulting in reduced intracellular pH and rupture of the outer membrane. In another study, citric and lactic acids achieved similar reduction of *E. coli* populations as chlorine did (~2.0 log CFU/g reduction). However, lactic acid was the most effective of these treatments against *L. monocytogenes* achieving a 1.5 log CFU/g reduction Akbas and Ölmez (2007). More recently, Chhetri et al. (2020) compared the effect of lactic acid and chlorine on the viability of *E. coli* O157:H7 on spinach leaves and reported that although 100 mg/L chlorine had a greater initial impact than 0.5% lactic acid, the lactic treatment eventually surpassed chlorine in bactericidal efficacy. Within 48 h, 0.5% lactic acid treatment resulted in > 4 log reduction in viable *E. coli* O157:H7 cells and a greater number of dead than live cells.

In summary, both silver ions and lactic acid are known to cause damage to cell membranes, but lactic acid is able to penetrate the cell and acidify its interior (Alakomi et al., 2000), while silver ions cause vital ions to leak out of the cell (Chamakura et al., 2011). Consequently, lactic acid may accelerate the process of cell death initiated by the silver ions. Whatever the mechanism of antimicrobial action, our concern as food safety scientists is to develop an approach that can kill pathogenic bacteria in food. This study used a solution containing silver ions and lactic acid which demonstrated effective antimicrobial activity. An in-depth discussion of the mechanism of action of silver and lactic acid as antimicrobials, or on how the concentration of silver ions would impact human health and the environment are beyond the scope of this study. Our purpose is to explain in which circumstances the SGL solution will be useful in promoting the food safety of freshly cut lettuce. Future studies of the silver ion's modes of action could expedite the development of novel applications for the sanitation of fresh-cut lettuce, with the objective of reducing the burden of food-related outbreaks for the public health system.

The impact of SGL on quality and shelf life were compared to that from traditional processing. Processing operations such as cutting, washing, drying, and packaging, involved in the processing of fresh-cut lettuce, cause damage to tissues, increasing respiration rate, and leading to rapid deterioration in quality and reduced product shelf life (Martínez-Sánchez et al., 2011; Varoquaux & Wiley, 1994). Thus, to assess the usefulness of a new sanitizer or process aid, it is extremely important to evaluate how the quality and sensory parameters are affected by it, either in comparison with the traditional approach or in conjunction with the traditional approach.

The package headspace gas composition is largely influenced by the respiration rate of the product gas transmission of the package films (Pereira et al., 2019). Inappropriate control of package gas concentration leads to off-odor. As reported by other studies, (Luo, 2007; Nguyen-the & Carlin, 1994; Tudela et al., 2013) a strong relationship between discernable off-odor and CO_2 concentration was also observed in the present research. In contrast, an inverse relationship between the intensity of off-odors and the concentration of O_2 was apparent, which is comparable to the results reported by Kim et al. (2005b). Atmospheres containing low O_2 and high CO_2 gas concentrations probably favor the growth of lactic acid bacteria (Luo, 2007; Nguyen-the & Carlin, 1994). Consequently, as the O_2 concentration in the packages is depleted, anaerobic fermentation by lactic acid bacteria occurs and thus leads to the accumulation of fermentative volatiles such as ethanol and acetaldehyde, which are correlated with the development of off-odor (Luo, 2007; Tudela et al., 2013). Off-odors associated with low O_2 in combination with high CO_2 developed in unwashed, SGL only, and SGL-rinsed samples. However, SGL-flume and flume washed samples received lower off-odor scores than the other treatments, although similar to each other. These treatments were also similar with respect to lower CO_2 concentrations, which indicate superior tissue integrity of these samples along with the apparent steady-state CO_2 concentrations that were measured for SGL-flume samples. Therefore, the combination of the SGL solution with flume washing may be close to the optimum for inhibiting production of ethanol and acetaldehyde and, consequently, maintaining low off-odor.

During processing of fresh-cut product, lettuce tissue can be injured, which results in an opportunity for microbial growth with consequent negative impacts on product quality (Luo, 2007). Electrolyte leakage indicates cell membrane injury (Hong et al., 2000). Although the exact mechanism for electrolyte leakage is unknown (Kim et al., 2005a),

increases in electrical conductivity have been consistently reported to reflect the influx of electrolytes from ruptured cells and consequent loss of tissue integrity (Jiang et al, 2001; King & Ludford, 1983; Koukounaras et al., 2019; Luo et al., 2004). The increased electrolyte leakage observed in unwashed samples, SGL-only and SGL-rinse treated samples coincides with significant decline in visual quality observed in these samples. Nevertheless, the same processes did not have a significantly adverse impact on SGL-flume treated samples, nor the associated amount of mechanical injury as evidenced by the stability of their electrical conductivity throughout storage. The reduced and stable electrolyte leakage from these lettuce samples during storage may also indicate that the membrane damage recovery process was more active for these samples than for samples subjected to other treatments.

Since produce quality assessment is primarily based on visual appearance, lettuce samples were evaluated for visual quality on days 7 and 14 of storage at 5 °C. The 14-day storage duration was chosen because it is the common shelf life of products washed in flume systems in the United States (Bornhorst et al., 2018). The storage temperature was selected based on current FDA guidelines for packaged ready-to-eat leafy greens that require temperature control at 5 °C or less to minimize pathogen proliferation throughout the supply chain (FDA, 2018). Additional information on the impact of temperature on the quality of packaged leafy greens during retail storage is described by De Frias et al. (2015).

The visual quality attributes of appearance, freshness, and color are factors that significantly affect the consumer acceptability of fresh-cut products. Our results are aligned with those of Baur et al. (2004) in which unwashed samples resulted in lower quality scores than that of washed samples. For example, SGL-flume and flume-only washed samples retained 87% and 91% of overall quality, respectively, whereas unwashed samples retained 71%. In other words, unwashed samples clearly demonstrate that washing after cutting is a crucial processing step in retaining the quality of iceberg lettuce (Baur et al., 2004). The overall visual quality of SGL-flume and flume washed samples was not significantly different; and this can be explained by the way the flume system operates. In well-adjusted jacuzzi flume tanks, operating with optimal process control, fresh-cut lettuce is exposed to sanitizing solution, while no mechanical damage is detected. This finding is reflected in low values of electrical conductivity for SGL-flume and flume washed samples indicating effective produce washing and good product quality (Hussain Dar et al., 2020). As a result, the findings of this study indicate that the application of SGL solution does not detract from the quality of lettuce washed in conventional flume systems.

5. Conclusion

The present work evaluated an innovative formula consisting of silver dihydrogen citrate, glycerin, and lactic acid bacteria (SGL) for the processing of fresh-cut lettuce. The application of the SGL solution as a prewash step in combination with the traditional flume washing system achieves improved inactivation of bacterial cells immediately after washing and potentially accelerates bacterial death during cold storage. The laboratory scale experiments showed greater than 3.15 log reduction of *E. coli* O157:H7 and 2.94 log reduction of *L. monocytogenes* directly after washing for samples that were initially rinsed with SGL solution. For *E. coli* O157:H7, a significant difference of 2.05 logs between SGL treatment and the control was maintained until day 7. In the pilot-plant scale study, results show that the ability to accelerate the death of generic *E. coli* was observed only for lettuce samples sprayed with SGL during cutting followed by flume washing (SGL-flume), but was not observed for samples that were only flume washed. At the end of the shelf life, the generic *E. coli* populations in SGL-flume washed samples were reduced by 1.48 log CFU/g, whereas such populations were reduced by only 0.70 log CFU/g in the samples that were only flume washed. Post-wash inoculation of SGL-flume washed lettuce samples showed neither any significant declines in pathogen populations nor declines in quality or shelf life of lettuce samples. Therefore, the SGL solution shows promise as a novel formulation for improving the efficacy of the leafy greens washing process to inactivate bacterial pathogens without compromising quality. The results can assist leafy green processors in determining whether to adopt the evaluated process aid to improve product safety outcomes. Evaluation of the cost-effectiveness of the evaluated process aid, and information regarding its proper use remain to be addressed in future work.

CRediT authorship contribution statement

Gabriella Mendes-Oliveira: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Yaguang Luo:** Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Bin Zhou:** Methodology. **Ganyu Gu:** Methodology. **Zi Teng:** Methodology. **Samantha Bolten:** Methodology, Writing – review & editing. **Eunhee Park:** Methodology, Writing – review & editing. **Daniel Pearlstein:** Methodology. **Ellen R. Turner:** Methodology, Writing – review & editing. **Patricia D. Millner:** Writing – review & editing. **Xiangwu Nou:** Conceptualization, Methodology, Formal analysis, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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