applied microbiology

MDPI

Article

Encapsulated Plant-Derived Antimicrobial Reduces Enteric Bacterial Pathogens on Melon Surfaces during Differing Contamination and Sanitization Treatment Scenarios

Keila L. Perez-Lewis [1], Yagmur Yegin [1], Jun Kyun Oh [2], Alejandro Castillo [1], Luis Cisneros-Zevallos [3], Chris R. Kerth [4], Ethan Scholar [2] and Thomas M. Taylor [4,*]

1 Department of Food Science and Technology, Texas A&M AgriLife Research, College Station, TX 77843, USA; kperezlewis@gmail.com (K.L.P.-L.); yyegin@mit.edu (Y.Y.); a-castillo@tamu.edu (A.C.)
2 Department of Chemical Engineering, Texas A&M University, College Station, TX 77843, USA; junkyunoh@dankook.ac.kr (J.K.O.); ethanscholar@tamu.edu (E.S.)
3 Department of Horticultural Sciences, Texas A&M AgriLife Research, College Station, TX 77843, USA; lcisnero@tamu.edu
4 Department of Animal Science, Texas A&M AgriLife Research, College Station, TX 77843, USA; c-kerth@tamu.edu
* Correspondence: matt_taylor@tamu.edu; Tel.: +1-979-862-7678

Citation: Perez-Lewis, K.L.; Yegin, Y.; Oh, J.K.; Castillo, A.; Cisneros-Zevallos, L.; Kerth, C.R.; Scholar, E.; Taylor, T.M. Encapsulated Plant-Derived Antimicrobial Reduces Enteric Bacterial Pathogens on Melon Surfaces during Differing Contamination and Sanitization Treatment Scenarios. *Appl. Microbiol.* **2021**, *1*, 460–470. https://doi.org/10.3390/applmicrobiol1030030

Academic Editors: Yiannis Kourkoutas, Eugenia Bezirtzoglou, Christina Tsigalou and Gregoria Mitropoulou

Received: 7 September 2021
Accepted: 8 October 2021
Published: 12 October 2021

Abstract: This study aimed to quantify survival in *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium isolates on melon rind surface samples achieved by sanitizer treatment under three differing melon contamination and sanitization scenarios. Sanitizing treatments consisted of the plant-derived antimicrobial (PDA) essential oil component (EOC) geraniol (0.5 wt.%) entrapped in the polymeric surfactant Pluronic F-127 (GNP), 0.5 wt.% unencapsulated geraniol (UG), 200 mg/L hypochlorous acid at pH 7.0 (HOCl), and a sterile distilled water wash (CON). The experimental contamination and sanitization scenarios tested were: (1) pathogen inoculation preceded by treatment; (2) the pathogen was inoculated onto samples twice with sanitizing treatment applied in between inoculation events; or (3) pathogen inoculation followed by sanitizing treatment. Reductions in the numbers of surviving pathogens were dependent on the sanitizing treatment, the storage period, or the interaction of these effects. GNP treatment provided the greatest reductions in surviving pathogen counts on melon rinds, but these did not regularly statistically differ from those achieved by HOCl or UG treatment. GNP treatment provided the best pathogen control under differing conditions of pre- and/or post-harvest cross-contamination and can be applied to reduce the risk of pathogen transmission on melon rinds.

Keywords: antimicrobial nanoparticles; melons; enteric pathogens; produce sanitizers; cross-contamination; post-harvest contamination; geraniol; micro-encapsulation

1. Introduction

The consumption of fresh and minimally processed fruits and vegetables has been recommended to supply a variety of essential nutrients and health protection benefits [1,2]. Nevertheless, their safe consumption is challenged by unintentional contamination and subsequent functioning as vehicles of human pathogens, causing outbreaks of foodborne illness. In the United States, the U.S. Centers for Disease Control and Prevention (CDC) reported that, in 2017 in the U.S., *Salmonella enterica* was the most frequently identified confirmed bacterial etiological foodborne disease agent, causing more cases of illness than any other foodborne microbial pathogen except the Human Noroviruses. Additionally, *Salmonella* was linked to more cases of U.S. foodborne disease-related hospitalizations than all other CDC-evaluated microbial pathogens [3]. The Shiga-toxigenic *Escherichia coli* (STEC) were ranked the second most frequent cause of foodborne disease-related hospitalizations overall [3]. Recently, the European Food Safety Authority (EFSA) reported a multi-national

outbreak of *Salmonella* Braenderup resulting in 348 identified cases; imported melons were suspected as the transmission vehicle in the outbreak [4]. In the years since 2017, a number of high-impact multistate disease outbreaks have occurred in the U.S. involving the transmission of these enteric pathogens to consumers of fresh and minimally processed fruits and vegetables, resulting in approximately 2185 cases of human illness, 521 disease-related hospitalizations, and at least five fatalities [5]. These outbreaks, and resulting incidents of illness, hospitalization, and death, result in substantial financial and quality of life losses, as well as recalls that reduce food availability for consumption [6,7].

The U.S. FDA Food Safety Modernization Act (FSMA) and its implementing final rule for preserving the safety of fresh fruits and vegetables mandates that food crop producers assess and take steps to reduce food safety hazard risks throughout fruit and/or vegetable production and post-harvest handling/packing [8]. One strategy to reduce the spread of microbiological food safety hazards, including human pathogens, is the application of sanitizers in post-harvest washing waters to reduce cross-contamination risk and reduce microbial hazard numbers on contaminated crop surfaces. Post-harvest fruit and vegetable sanitizing treatments have been extensively studied and reported in the scientific and industry literature. Ramos et al. [9] reviewed the efficacy of several chemical and physical sanitizing interventions for fruit and vegetable safety protection. They concluded that while chlorine-based sanitizers (e.g., HOCl, ClO_2) are effective for reducing pathogen counts on various commodity surfaces and maintaining the sanitary condition of washing waters, they are easily counteracted by excess organic load or may require unsuitably high concentrations for multi-log cycle reductions on fruit and vegetable surfaces. Others reached similar conclusions regarding the utility of chlorous sanitizers, as well as other disinfectants, citing high reactivity, consumer dislike of treated produce, and other reasons why novel sanitizing interventions for produce safety protection are necessary [10]. One such strategy is the application of a biopreservative hurdle, such as a plant-derived antimicrobial (PDA) to decontaminate a fruit or vegetable commodity. These sanitizers are natural and decompose into innocuous substances, exerting a multifaceted antimicrobial mechanism against produce-contaminating bacterial organisms [11,12]. Nevertheless, recently published reviews have concluded the utility of some classes of PDAs is limited by their inherent hydrophobicity and volatility, suggesting their incorporation into edible or active packaging films can enhance their delivery to pathogens contaminating fruit or vegetable surfaces [13,14].

As an alternative to PDA/essential oil component (EOC)-impregnated films, our research group has explored the use of emulsion encapsulation for effectively delivering PDA to pathogens contaminating the surface of leafy vegetables [15,16], as well as smooth-skinned fruits such as tomato [17]. A consistent observation in these research studies was that EOC emulsion encapsulation yielded equivalent or better reductions in *Salmonella* and/or *E. coli* O157:H7 numbers versus hypochlorous acid (HOCl) at 200 mg/L. In some cases, encapsulation of the EOC resulted in pathogen loads being reduced to non-detectable counts versus washing with sterile water, use of a surfactant, or HOCl [16,17]. Yegin et al. [18] demonstrated entrapment of the rose oil EOC geraniol into polymeric nanoparticles enhanced mass transport to the outer membrane leaflet of *E. coli* O157:H7 cells. This benefit of EOC encapsulation has been recently reviewed with respect to the development of useful antimicrobial technologies for food and pharmaceutical applications [19]. Additionally, our group reported geraniol-loaded nanoparticles outperformed unencapsulated geraniol and 200 mg/L HOCl on spinach surfaces, reducing *E. coli* O157:H7 and *S.* Typhimurium to non-detectable numbers (<0.5 $\log_{10}$ CFU/cm^2) when applied multiple days prior to pathogen application. Similar effects were observed when *E. coli* O157:H7- or *S.* Typhimurium-inoculated spinach was sanitizer-treated and then re-inoculated 3 days later, simulating pathogen contamination of the food crop during pre- and post-harvest operations [20]. These findings data indicated encapsulated geraniol afforded longer-lasting food safety protection versus other conventional chemical sanitizing treatments, even under conditions of gross failures in good agricultural practices (GAPs).

The primary objective of this study was to compare the numbers of surviving *S*. Typhimurium and *E. coli* O157:H7 inoculated on muskmelon rind surfaces following differing sanitizing treatments under conditions of simulated pre-harvest and/or post-harvest contamination and subsequent refrigerated storage. The null hypothesis for the study for each of the differing experimental contamination and sanitization scenarios (hereafter scenarios) was that pathogen survivor counts achieved by sanitizing treatment would not differ from one another.

2. Materials and Methods

2.1. Melon Preparation for Microbial Inoculation and Testing

Non-waxed Texas-grown muskmelons were purchased from a College Station, TX-based wholesaler and transported to the Food Microbiology Laboratory (Department of Animal Science, Texas A&M University, College Station, TX, USA). Upon arrival at the Food Microbiology Lab, melons were washed in sterile distilled water and placed on sterile racks to dry over 1 h at ambient condition. After drying, flame-sterilized stainless steel borer (Brazos Valley Welding, Bryan, TX, USA) and forceps were used to excise 10 cm^2 melon rind sample discs (2 mm approximate depth), and three excised discs were composited together into sterile plastic dishes (total surface area 30 cm^2 per sample).

2.2. Microorganisms for Sample Inoculation and Sample Inoculation Processes

Melon samples were inoculated with a mixture of *S. enterica* serovar Typhimurium ATCC 700720 and *E. coli* O157:H7 ATCC 700728, both naturally resistant to 100 µg/mL rifampicin. Stock cultures of these microorganisms were revived and prepared with methods previously described [20]. Each microorganism was extracted from frozen storage, passed twice in tryptic soy broth (TSB; Becton, Dickinson and Co., Sparks, MD, USA) incubating at 35 °C for 24 h, and then washed twice by centrifugation (15 min, 2191× *g*, 25 °C) and resuspended in 0.1% (*w*/*v*) peptone diluent (Becton, Dickinson and Co.) prior to mixing to prepare the inoculum. The inoculum fluid containing both organisms was prepared to a target of 8.0 $\log_{10}$ CFU/mL; samples were then spot-inoculated by application of ten 10-µL spots onto the sample surface, distributing a total 0.1 mL amongst the three discs comprising a sample. Inoculated samples were covered and then held at ambient conditions in a biological safety cabinet for 1.0 h to facilitate microbial attachment to melon rind sample surfaces.

2.3. Sample Inoculation and Treatment Scenarios

Three scenarios were designed and completed to determine the differing impacts of differences in the sequence of contamination(s) and sanitizing treatment on pathogen survival/reduction. Scenario 1 simulated events wherein the bacterial pathogen contaminates the produce item pre-harvest, most likely through fecal residue contacting the produce item (e.g., direct feces contact on fruit surface, feces-contaminated overhead irrigation water droplet contact). The item is harvested and then sanitized during post-harvest handling prior to refrigerated packing, distribution, and retail. Scenario 2 simulated produce item contamination events occurring both pre- and post-harvest, with sanitization treatment applied between the contamination events. Finally, scenario 3 replicated events where harvested melons encounter cross-contamination following sanitizing treatment application. Scenarios 2 and 3 assumed a breakdown in post-harvest sanitary handling of produce items, while scenarios 1 and 2 assumed pre-harvest contamination of produce occurring.

2.4. Sanitizing Treatment Preparation and Application to Sample Surfaces

Geraniol-loaded encapsulates/nanoparticles (GNPs), as well as other sanitizing treatments, were formulated and prepared as already previously described [17,19]. GNPs were formulated to contain 0.5 wt.% geraniol (CAS #106-24-1; TCI America, Portland, OR, USA; >98%) in the sanitizing fluid. Unencapsulated geraniol (UG) was prepared in sterile distilled water to 0.5 wt.%, as well as 200 mg/L hypochlorous acid (HOCl; pH 7.0 ± 0.1), and,

finally, a control treatment consisting of sterile tepid distilled water rinsing (CON). Sanitizing treatments were applied to inoculated samples or non-inoculated samples (depending on which treatment scenario was being tested) by immersing the sample tissue in 20 mL of sanitizing fluid for 2 min. Following sanitizing treatment, sample discs were aseptically removed, placed on sanitary paper towel for 15 min to drip off any residual treatment fluid, and placed into a new sterile plastic dish for subsequent refrigerated storage, or were prepared for immediate microbiological analysis. Samples assigned to refrigerated storage were covered with an oxygen-permeable low-density polyethylene film, and stored at 5 ± 1 °C for either 3, 5, 7, or up to 10 days. For samples that were subjected to scenario 2, all samples were removed at day 3 of refrigerated storage and re-inoculated with a mixture of organisms prepared to a target of 7.0 $\log_{10}$ CFU/mL per previous assessment of post-harvest contamination in commercial fruit and vegetable packinghouses [20,21]. Re-inoculated samples were then either returned for further refrigerated storage or prepared for microbiological analysis. Samples tested within melon contamination and sanitization scenario 3 were subjected to sanitizing treatment without being first inoculated and were then placed at refrigeration (Frigidaire Corp., Charlotte, NC, USA). After 3 days of refrigerated storage, all samples were removed from refrigerated storage, inoculated with cocktailed pathogens (inoculum fluid 8.0 $\log_{10}$ CFU/mL), and then either returned for further refrigerated storage or prepared for microbiological analysis.

2.5. Microbiological Analysis of Samples

All samples were prepared for microbiological testing by placing sample tissue discs into a sterile stomacher bag containing 99 mL 0.1% (*w*/*v*) peptone diluent (Becton, Dickinson and Co.) and by pulverizing samples for 60 sec prior to preparation of serial dilutions in 0.1% peptone diluent. Serial dilutions were spread onto the surfaces of lactose-sulfite-phenol red-rifampicin (LSPR) agar, supplemented with 100.0 mg/L rifampicin (Sigma-Aldrich Co., St. Louis, MO, USA), according to the formula of Castillo et al. [22]. *E. coli* O157:H7 and *S.* Typhimurium colonies were differentially counted on LSPR agar surfaces following 24–36 h incubation at 36 ± 1 °C, where *E. coli* colonies displayed lactose fermentation, while *S.* Typhimurium exhibited sulfite reduction (blackening of colonies) without lactose fermentation.

2.6. Experimental Design and Data Analysis

Sanitizing treatment experiments on melon samples were completed using a factorial arrangement of a completely randomized design, with assignment of samples to treatment conditions and storage period for each experimental scenario. All experimental trials were replicated three times in identical fashion ($N = 3$). All microbiological data were recorded and $\log_{10}$-transformed prior to statistical analysis. Two-way ANOVA was followed by Tukey's Honestly Significant Differences (HSD) multiple comparisons test, performed using GraphPad Prism version 9.2.0 for macOS Big Sur, v.11.4 (GraphPad Software, San Diego, CA, USA, www.graphpad.com, accessed on 11 October 2021). Data were analyzed testing the main effects of sanitizing treatment (GNP, UG, HOCl, CON), storage period (0, 3, 5, 7, 10 days), and their interaction for each of the three experimental scenarios. Microbiological data gathered for *Salmonella* were not compared to like data for *E. coli* O157:H7. Finally, the interaction of sanitizing treatment by melon treatment scenario was tested in order to discern which sanitizing treatment yielded the greatest apparent antimicrobial activity (observed as reductions in numbers of surviving pathogen cells) across the differing contamination scenarios.

3. Results

3.1. Scenario 1: Sanitizing Treatments to Reduce Pathogens Contaminating Melons Pre-Treatment

The mean numbers of *E. coli* O157:H7 and *S.* Typhimurium cells in inoculum fluid applied to melons prior to sanitizing treatment were 7.57 ± 0.08 and 7.61 ± 0.13 $\log_{10}$ CFU/mL, respectively, and did not statistically differ from one another ($p > 0.05$). Figure 1A,B presents

mean survivor counts of *S.* Typhimurium and *E. coli* O157:H7 inoculated onto melon rinds by storage days, respectively. Amongst sanitizing treatments, GNP treatment produced the greatest decline in numbers of both microorganisms, 2.97 and 3.67 $\log_{10}$ CFU/cm^2 in *Salmonella* and *E. coli* O157:H7, respectively, over 10 days' refrigerated storage post treatment. HOCl (200 mg/L) treatment produced the second greatest reductions in microbe loads, though mean numbers of surviving pathogen cells did not statistically differ at day 10 for GNP and HOCl-treated *E. coli* O157:H7 counts (Figure 1B). Surviving pathogen counts on GNP-treated melon samples were >1.0 $\log_{10}$-cycle less than those on HOCl-treated melons, indicating a >90% greater reduction by GNP application over HOCl, a widely used fruit and vegetable post-harvest water sanitizer. UG and CON treatments produced moderate reductions, <1.0 $\log_{10}$-cycle on melon sample surfaces, in many instances producing non-statistical differences in survivor counts (Figure 1).

3.2. Scenario 2: Efficacy of Sanitizing Treatments Applied to Reduce Pathogens Contaminating Melons Prior to and Following Treatment

Scenario 2 was designed to simulate conditions of fruit production, harvest, and post-harvest handling where pathogen contamination occurs both during pre-harvest production and following post-harvest washing. Since experimental samples for all three experimental scenarios were prepared and initiated on the same days for each of the three replicates, inoculation fluids prepared for samples for scenario 1 were also used for scenario 2 samples, and they had the same mean counts of each organism at day 0 inoculation. Following three days of refrigerated incubation, all samples were removed and inoculated a second time with a blend of microbes. Means of *S.* Typhimurium and *E. coli* O157:H7 inoculated onto samples at day 3 were 6.55 ± 0.11 and 6.48 ± 0.04 $\log_{10}$ CFU/mL. Sanitizing treatment exerted a significant effect on resulting microbial loads on melons but without interaction of the treatment with the storage period (Figure 2). The second inoculation event after three days of refrigerated storage likely negated reductions that would otherwise have been observed, although GNP treatment still yielded significant reductions in microbe numbers (0.99 and 0.58 $\log_{10}$-cycles for *S.* Typhimurium and *E. coli* O157:H7, respectively) (Figure 2). Nonetheless, HOCl and GNP treatment produced non-differing survivor counts, nor were statistically significant differences observed to exist between HOCl and UG counts for each organism on melons.

3.3. Scenario 3: Efficacy of Sanitizing Treatments to Reduce Pathogens Contaminating Melons Post-Treatment

Scenario 3 assumed a potential scenario where melons were harvested without prior pathogen contamination, with pathogen contamination occurring following post-harvest sanitizer application (e.g., during bulk packing or chilled distribution). Inoculation fluids for melons used during scenarios 1 and 2 were also used for scenario 3-assigned samples, so mean numbers of organisms for samples at day 3 of the experimental timeline were the same. Surprisingly, unlike data gathered for the other scenarios, the storage period was the only significant main effect for both microbes ($P < 0.0001$). Neither sanitizing treatment nor the interaction of main effects exerted significant effects against pathogens on melon sample surfaces (Figure 3). Pathogen counts declined over 10 days of refrigerated storage following sanitizing treatment on day 0 and inoculation on day 3, with cumulative reductions of 1.4–1.5 $\log_{10}$ CFU/cm^2 for *Salmonella* and *E. coli* O157:H7.

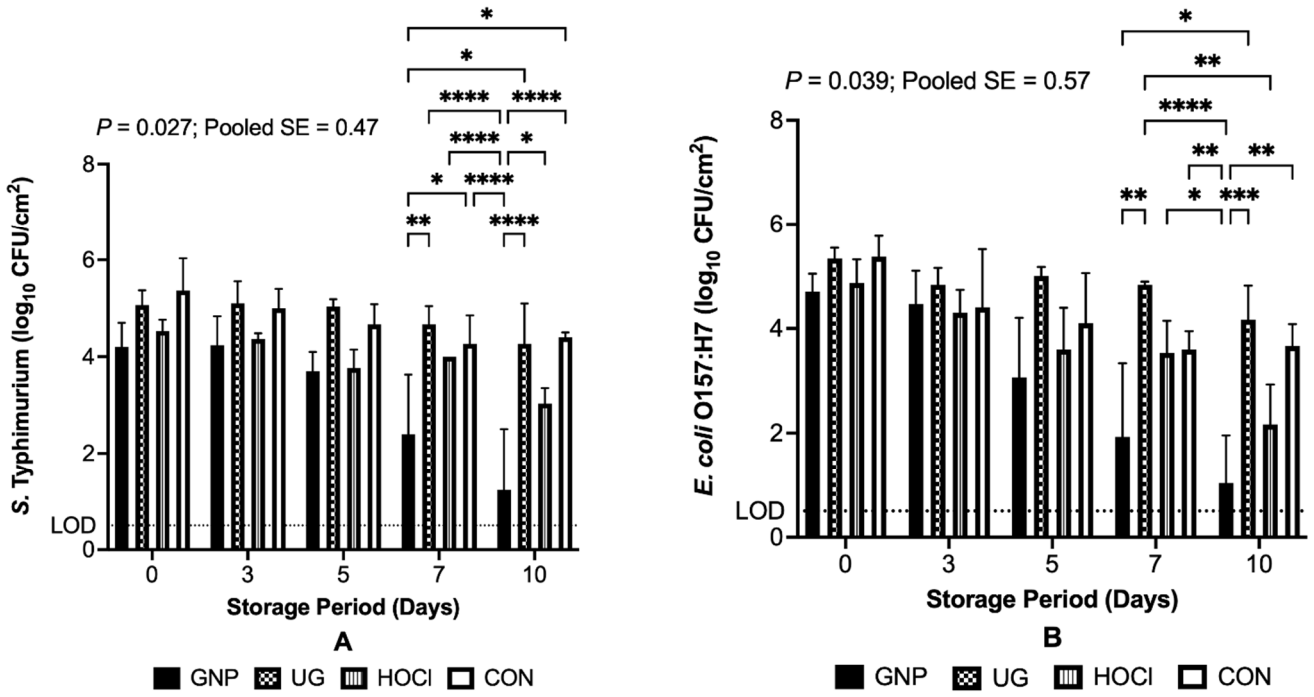

Figure 1. *Salmonella* Typhimurium (**A**) and *Escherichia coli* O157:H7 (**B**) on melons following sanitizer treatment when contamination precedes sanitizing treatment (scenario 1). Bars depict means of three identical replicates ($N = 3$); error bars depict one standard deviation. GNP: 0.5 wt.% geraniol-loaded nanoparticles; UG: unencapsulated 0.5 wt.% geraniol; HOCl: 200 mg/L hypochlorous acid, pH 7.0; CON: sterile distilled water. LOD: assay limit of detection. Individual statistically different means are connected by overhead brackets; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$ by Tukey's HSD means separation process. Pooled SE: pooled standard error.

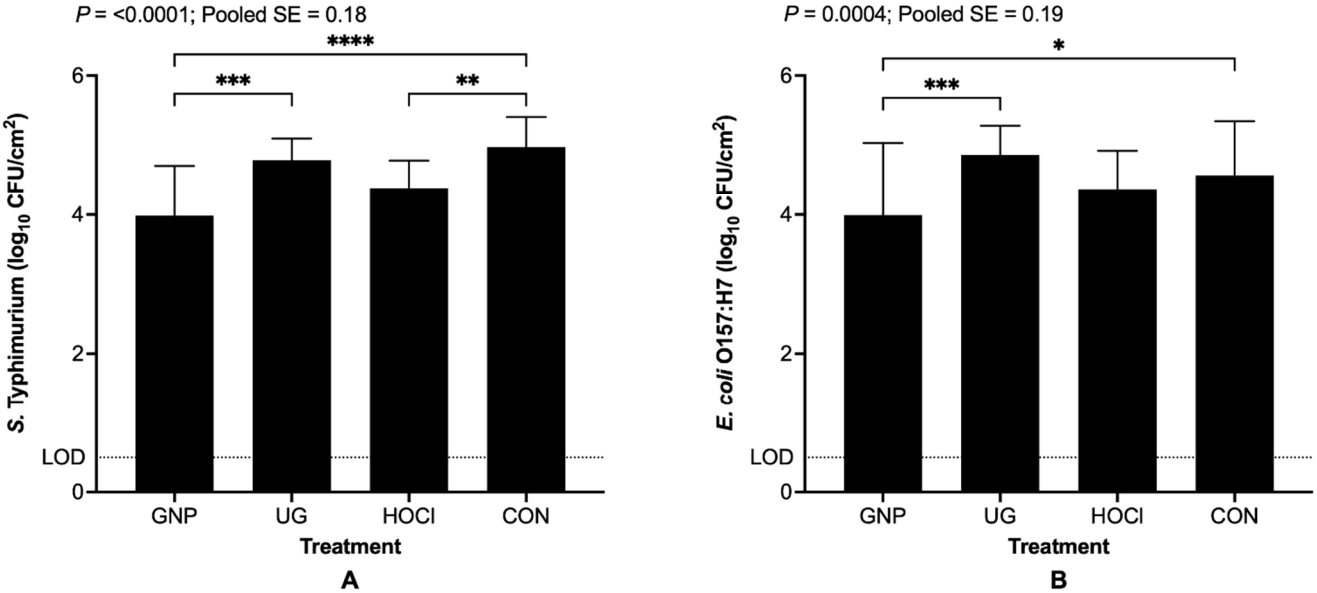

Figure 2. *Salmonella* Typhimurium (**A**) and *Escherichia coli* O157:H7 (**B**) on melons following sanitizing treatment when inoculation occurs both prior to sanitizing treatment and again after 3 days at 5 °C (scenario 2). Bars depict means of three identical replicates ($N = 3$); error bars depict one standard deviation. GNP: 0.5 wt.% geraniol-loaded nanoparticles; UG: unencapsulated 0.5 wt.% geraniol; HOCl: 200 mg/L hypochlorous acid, pH 7.0; CON: sterile distilled water. LOD: assay limit of detection. Individual statistically different means are connected by overhead brackets; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$ by Tukey's HSD means separation process. Pooled SE: pooled standard error.

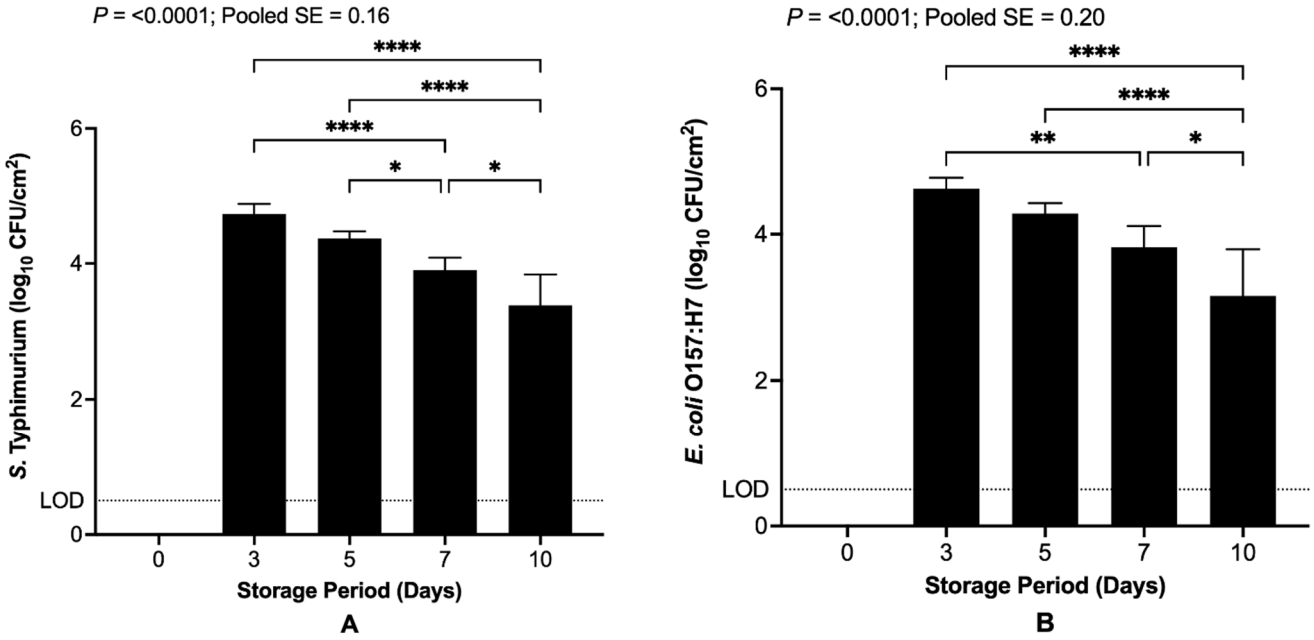

Figure 3. *Salmonella* Typhimurium (**A**) and *Escherichia coli* O157:H7 (**B**) on melons during 10 days' storage at 5 °C when inoculation follows sanitizing treatment and 3 days' refrigerated storage (scenario 3). Bars depict means of three identical replicates ($N = 3$); error bars depict one standard deviation. Sanitizing treatments consisted of 0.5 wt.% geraniol-loaded nanoparticles, unencapsulated 0.5 wt.% geraniol, 200.0 mg/L hypochlorous acid (pH 7.0), and sterile distilled water (control). LOD: assay limit of detection. Individual statistically different means are connected by overhead brackets; *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$ by Tukey's HSD means separation process. Pooled SE: pooled standard error.

3.4. The Interaction of Sanitizing Treatment x Treatment Scenario

Tables 1 and 2 present the survival of *S*. Typhimurium and *E. coli* O157:H7 on melons as a function of the interaction of sanitizing treatment (GNP, UG, HOCl, CON) by treatment scenario (1, 2, 3). Surprisingly, for both organisms, scenario 3 mean counts of surviving pathogen cells were generally lower across all treatments, including the CON, than for other scenarios 1 or 2. This potentially resulted from the day 0 samples being left intentionally uninoculated on scenario 3 trials versus 1 and 2, leading to overall lower mean survivor counts following statistical analysis. As was demonstrated in Figures 1–3, GNP treatment yielded lower numerical means than all other treatments for each experimental scenario, though GNP-specific means were not typically statistically lower than those obtained for HOCl or UG treatments ($p > 0.05$) (Tables 1 and 2).

Table 1. *Salmonella* Typhimurium survival ($\log_{10}$ CFU/cm^2) on melon sample surfaces by the interaction of sanitizing treatment × experimental contamination and sanitization scenario.

Sanitizing Treatment [1]	Scenario 1 [2]	Scenario 2	Scenario 3
GNP	3.15 E [3]	3.99 BC	3.17 E
UG	4.85 A	4.79 A	3.45 CDE
HOCl	3.94 BCD	4.39 AB	3.52 CDE
CON	4.74 A	4.98 A	3.36 DE

[1] GNP: 0.5 wt.% geraniol in 0.5% Pluronic F-127 nanoparticle; UG: 0.5 wt.% unencapsulated geraniol; HOCl: 200 mg/L hypochlorous acid, pH 7.0; CON: sterile distilled water wash. Treatments were applied for 2 min by immersion followed by draining of treatment fluids and sample placement in sterile covered dishes. [2] Treatment scenarios were designed to inoculate the organism prior to sanitizing treatment (1), inoculate prior to treatment, and again after 3 days' storage at 5 °C (2), or inoculate after sanitizing treatment and 3 days' storage at 5 °C (3). [3] Values depict means from three independent replicates ($N = 3$); means not sharing letters (A, B, C, . . .) differ by 2-way analysis of variance and Tukey's Honestly Significant Differences (HSD) test at $p = 0.05$. $p < 0.0001$; Pooled Standard Error = 0.13.

Table 2. *Escherichia coli* O157:H7 survival (log_{10} CFU/cm^2) on melon sample surfaces by the interaction of sanitizing treatment × experimental contamination and sanitization scenario.

Sanitizing Treatment [1]	Scenario 1 [2]	Scenario 2	Scenario 3
GNP	3.04 E [3]	3.99 BCD	3.01 E
UG	4.77 A	4.87 A	3.49 DE
HOCl	3.69 CDE	4.37 ABC	3.45 DE
CON	4.23 ABC	4.57 AB	3.17 E

[1] GNP: 0.5 wt.% geraniol in 0.5% Pluronic F-127 nanoparticle; UG: 0.5 wt.% unencapsulated geraniol; HOCl: 200 mg/L hypochlorous acid, pH 7.0; CON: sterile distilled water wash. Treatments were applied for 2 min by immersion followed by draining of treatment fluids and sample placement in sterile covered dishes. [2] Treatment scenarios were designed to inoculate the organism prior to sanitizing treatment (1), inoculate prior to treatment, and again after 3 days' storage at 5 °C (2), or inoculate after sanitizing treatment and 3 days' storage at 5 °C (3). [3] Values depict means from three independent replicates ($N = 3$); means not sharing letters (A, B, C, . . .) differ by 2-way analysis of variance and Tukey's Honestly Significant Differences (HSD) test at $p = 0.05$. $p = 0.0006$; Pooled Standard Error = 0.15.

4. Discussion

In the current study, geraniol (0.5 wt.%)-loaded nanoparticles, unencapsulated geraniol (0.5 wt.%), 200 mg/L HOCl (pH 7.0), and sterile distilled water were used to sanitize *S.* Typhimurium- and *E. coli* O157:H7-inoculated melon rind surfaces. The three experimental melon contamination scenarios were designed to simulate events potentially occurring wherein pre-harvest and/or post-harvest fruit contamination by pathogens might occur. We were interested in observing whether PDA encapsulation would result in a longer-lasting selective pressure against microorganisms versus other sanitizing treatments, thereby indicating the utility of encapsulation to provide improved fruit microbiological safety protection versus other antimicrobial/sanitizing treatments reported in the literature.

With respect to scenario 1 samples, results here differ from those we previously reported on identically completed experiments handling spinach leaves [16]. Melons retained 0.2–1.2 log_{10} CFU/cm^2 higher numbers of both organisms on CON samples at day 0 versus spinach samples, though sample inoculation methods used in the current study were applied in the same fashion as for spinach samples previously utilized [16]. The interaction of main effects of sanitizing treatment and storage period was determined to occur for scenario 1 melon samples, influencing the resulting counts of pathogens over 10 days' refrigerated storage following treatment (Figure 1). Application of unencapsulated geraniol (UG) in the current study performed similarly compared to previous research with respect to antimicrobial activity on melon surfaces [23]. With respect to differences observed in reductions in pathogen counts for melons versus spinach from our previous study, previous research has suggested the observed results stem from multiple factors. Annous et al. [24] suggested cantaloupe rinds and netting afford bacterial organisms the opportunity for strong attachment and subsequent biofilm formation. *Salmonella* cells inoculated onto melon rinds for 2 h at 20 °C demonstrated signs of adhesion and attachment via SEM, likely similar to the current study due to the 1 h of pathogen attachment allowed prior to treatment with differing sanitizing treatment (Section 2.2). Guzel et al. [25] reported Listeria innocua cells inoculated onto Romaine lettuce were reduced by at least 0.5 log10 CFU/g more than when on cantaloupe rind surfaces by application of pH 7.0 HOCl (200.0 ppm), which suggested a function of differing contact angle characteristic between the two commodities. It is also known that surface chemistry and topography of substrates can strongly influence bacterial adhesion [26,27]. The differences in chemical and morphological characteristics of spinach and melon can lead to different number of adherent bacteria for a given inoculation concentration. Supplemental Table S1 confirms this suggestion, demonstrating lower contact angle values (i.e., decreased wettability) for cantaloupe rinds as compared to spinach leaf surface. Additionally, Supplemental Figure S1A demonstrates cantaloupe surface structure, indicating depressions between as compared to spinach cells being raised, supporting the suggestion of produce item surface architecture interacting with the contact angle to effect surface wetting and distribution of hydrophilic and amphiphilic sanitizers to attached bacterial cells. The ability of nanoparticle suspension to reach such crevices

and attached bacteria in these locations is also dependent on the surface morphology and whether the wetting is following the Cassie state or the Wenzel state [26,28]. Overall, the interplay among surface texture and surface chemistry (energy) will not only control the bacterial contamination/attachment but also nanoparticulate deposition.

Scenario 2 experiments sought to simulate multiple pathogen contamination events occurring for fresh fruits, during pre- and then post-harvest handling, with the application of the sanitization treatment occurring between the two contamination events. For melons, the main effect of treatment was highly significant with respect to pathogen survival, though an interaction of sanitizing treatment and storage period was not (Figure 2). As was the case from experiments described for scenario 1, reductions in microbial counts were low, not exceeding 0.99 $\log_{10}$ CFU/cm^2. GNP treatment achieved the greatest numerical reductions in the numbers of both organisms, though survivor numbers did not statistically differ from those obtained from HOCl samples (Figure 2). The reductions observed in the current study for GNP treatment were significantly less than those we recently reported on spinach [20]. A lack of more impressive reductions in microbial counts may be at least partially explained by the second inoculation/contamination event, where the numbers of the organisms were refreshed on sample surfaces. However, the lack of an interaction of main effects of treatment by storage period was surprising, given past findings and the expectation that dual sequential inoculations would result in sanitization treatment-induced pathogen reductions being negated by the second inoculation event. This outcome may have resulted from the differences in melon rind versus spinach leaf surface physico-chemistry and topography, lending itself to more substantial reductions in the latter [26,29]. Past research into the application of sanitizing chemical treatments has not assessed the potential for edible produce commodities to be repeatedly contaminated pre- and/or post-harvest, as this would represent a gross loss of produce safety hazard control. Nonetheless, the identification of sanitizing treatments effective to provide food safety hazard risk reduction and/or hazard control under such circumstances would represent progress in the fight against produce-borne microbial disease for fruit and vegetable consumers.

Scenario 3 experiments detail the results of testing when the sanitizing treatment preceded pathogen inoculation/contamination. As with scenario 2 experiments, research had not evaluated such events directly with the exception of Perez-Lewis et al. [16]. Others have evaluated the impacts of sanitizing treatments in fruit or vegetable washing systems on inoculated items and subsequent control against cross-contamination of pathogens onto non-inoculated produce items. López-Gálvez et al. [30] reported no recovery of *E. coli* on non-inoculated lettuce from washing waters recovered from *E. coli* inoculated onto lettuce infused with 40.0 mg/L HOCl. Murray et al. [31] likewise reported low-level reduction of 1.8 $\log_{10}$-cycles in *E. coli* O157:H7 recovered from chlorinated (2.0 ppm) spent commercial wash water onto shredded lettuce. This reduction is similar to that obtained here for scenario 3, though direct comparison is not feasible given differences in HOCl content as well as the use of lettuce versus melon rind. In the current study, for melons, only the storage period exerted a statistical influence on the resulting microbial counts for both pathogens ($p \leq 0.0001$) (Figure 3); a similar effect was previously observed for experiments on spinach samples inoculated prior to pathogen application [16].

5. Conclusions

Cantaloupes and muskmelons have acted as human microbial pathogen transmission vehicles repeatedly in the U.S. in the past, as well as in Europe. Sanitization of melon surfaces by chemical and/or physical methods is nonetheless a useful practice for reducing the risk of foodborne microbial disease by reducing the spread of contaminating microbes during post-harvest handling of whole and/or sliced melons. Results from the current study on pathogen reduction assessment indicate that emulsion encapsulation of the PDA geraniol yielded the greatest reductions versus other sanitizing treatments, regardless of which melon contamination and treatment scenario was tested (Tables 1 and 2). Emulsion of the EOC geraniol, we submit, enhanced the observed antimicrobial activity for the GNP

treatment through facilitating a longer-lasting selective pressure against pathogen cells via slow nanoparticle breakdown during refrigerated storage [18,27]. Melon microbiological safety improvement from the use of chemical sanitizing treatments, particularly those incorporating emulsified PDA, can assist in protecting U.S. produce safety more effectively during post-treatment refrigerated storage by longer selective pressure against chlorine or other sanitizing chemical interventions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/applmicrobiol1030030/s1, Figure S1: Laser confocal microscopy images of cantaloupe melon rind (A) and spinach leaf (B) surfaces not subjected to pathogen inoculation or sanitization, Table S1: Water contact angle (θ) for cantaloupe and spinach surfaces.

Author Contributions: Conceptualization, L.C.-Z., A.C., E.S. and T.M.T.; methodology, L.C.-Z., E.S. and T.M.T.; formal analysis, K.L.P.-L., Y.Y. and J.K.O.; data curation, K.L.P.-L. and T.M.T.; writing—original draft preparation, T.M.T.; writing—review and editing, K.L.P.-L., Y.Y., A.C., L.C.-Z., C.R.K. and E.S.; supervision, T.M.T.; funding acquisition, A.C., L.C.-Z., E.S. and T.M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by Agriculture and Food Research Initiative (grant no. 2011-67017-30028) from the USDA National Institute of Food and Agriculture. Authors K.L.P.-L. and Y.Y. received graduate assistantship support from Texas A&M University.

Data Availability Statement: Recorded and $\log_{10}$-transformed microbiological data, coded by experimental treatment, pathogen, scenario, replicate, and storage period, were deposited with the Texas Data Repository and available at http://data.tdl.org/, at https://doi.org/10.18738/T8/Z7PTTY (accessed on 11 October 2021).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. U.S. Department of Agriculture; U.S. Department of Healtha and Human Services. Dietary Guidelines for Americans, 2020–2025, 9th ed. Available online: www.dietaryguidelines.gov (accessed on 7 September 2021).
2. World Health Organization. Healthy Diet. Available online: https://www.who.int/initiatives/behealthy/healthy-diet (accessed on 3 August 2021).
3. Centers for Disease Control and Prevention. Surveillance for Foodborne Disease Outbreaks, United States. Annual Report. 2017. Available online: https://www.cdc.gov/fdoss/pdf/2017_FoodBorneOutbreaks_508.pdf (accessed on 15 July 2021).
4. European Centre for Disease Prevention and Control (ECDC); European Food Safety Authority (EFSA). Multi-Country Outbreak of *Salmonella* Braenderup ST22, Presumed to Be Linked to Imported Melons. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/en-6807 (accessed on 7 September 2021).
5. U.S. Centers for Disease Control and Prevention. List of Selected Multistate Foodborne Outbreak Investigations. Available online: https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html (accessed on 1 June 2020).
6. Batz, M.B.; Hoffmann, S.; Morris, J.G., Jr. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot.* **2012**, *75*, 1278–1291. [CrossRef]
7. USDA-ERS. Cost Estimates of Foodborne Illnesses. Available online: https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses/ (accessed on 24 May 2021).
8. FDA. Title 21, U.S. Code of Federal Regulations, §112: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Available online: https://www.ecfr.gov/cgi-bin/text-idx?SID=6065be805bbe8488f8cf57bf0299dfd9&mc=true&node=pt21.2.112&rgn=div5 (accessed on 23 March 2020).
9. Ramos, B.; Miller, F.A.; Brandão, T.R.S.; Teixeira, P.; Silva, C.L.M. Fresh fruits and vegetables-an overview on applied methodologies to improve its quality and safety. *Innov. Food Sci. Emerg. Technol.* **2013**, *20*, 1–15. [CrossRef]
10. Luna-Guevara, J.J.; Arenas-Hernandez, M.M.P.; Martinez de la Peña, C.; Silva, J.L.; Luna-Guevara, M.L. The role of pathogenic *E. coli* in fresh vegetables: Behavior, contamination factors, and preventive measures. *Int. J. Microbiol.* **2019**, *2019*, 2894328. [CrossRef] [PubMed]
11. Nowotarska, S.W.; Nowotarski, K.J.; Friedman, M.; Situ, C. Effect of structure on the interactions between five natural antimicrobial compounds and phospholipids of bacterial cell membrane on model monolayers. *Molecules* **2014**, *19*, 7497–7515. [CrossRef] [PubMed]
12. Wu, D.; Lu, J.; Zhong, S.; Schwarz, P.; Chen, B.; Rao, J. Influence of nonionic and ionic surfactants on the antifungal and mycotoxin inhibitory efficacy of cinnamon oil nanoemulsions. *Foods Funct.* **2019**, *10*, 2817–2827. [CrossRef] [PubMed]

13. Deng, L.-Z.; Mujumdar, A.S.; Pan, Z.; Vidyarthi, S.K.; Xu, J.; Zielinska, M.; Xiao, H.-W. Emerging chemical and physical disinfection technologies of fruit and vegetables: A comprehensive review. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2481–2508. [CrossRef]
14. de Corato, U. Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: A comprehensive critical review from the traditional technologies into the most promising advancements. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 940–975. [CrossRef] [PubMed]
15. Ruengvisesh, S.; Kerth, C.R.; Taylor, T.M. Inhibition of *Escherichia coli* O157:H7 and *Salmonella enterica* isolates on spinach leaf surfaces using eugenol-loaded surfactant micelles. *Foods* **2019**, *8*, 575. [CrossRef] [PubMed]
16. Perez-Lewis, K.L.; Yegin, Y.; Cisneros-Zevallos, L.; Castillo, A.; Kerth, C.R.; Akbulut, M.; Taylor, T.M. Geraniol-loaded polymeric nanoparticles inhibit enteric pathogens on spinach during posttreatment refrigerated and temperature abuse storage. *Front. Sustain. Food Syst.* **2018**, *2*, 16. [CrossRef]
17. Ruengvisesh, S.; Oh, J.K.; Kerth, C.R.; Akbulut, M.; Taylor, T.M. Inhibition of bacterial human pathogens on tomato skin surfaces using eugenol-loaded surfactant micelles during refrigerated and abuse storage. *J. Food Saf.* **2019**, *39*, e12598. [CrossRef]
18. Yegin, Y.; Perez-Lewis, K.L.; Zhang, M.; Akbulut, M.; Taylor, T.M. Development and characterization of geraniol-loaded polymeric nanoparticles with antimicrobial activity against foodborne bacterial pathogens. *J. Food Eng.* **2016**, *170*, 64–71. [CrossRef]
19. Lammari, N.; Louaer, O.; Meniai, A.H.; Elaissari, A. Encapsulation of essential oils via nanoprecipitation process: Overview, progress, challenges and prospects. *Pharmaceutics* **2020**, *12*, 431. [CrossRef]
20. Yegin, Y.; Perez-Lewis, K.L.; Liu, S.; Kerth, C.R.; Cisneros-Zevallos, L.; Castillo, A.; Akbulut, M.; Taylor, T.M. Antimicrobial-loaded polymeric micelles inhibit enteric bacterial pathogens on spinach leaf surfaces during multiple simulated pathogen contamination events. *Front. Sustain. Food Syst.* **2021**, *5*, 646980. [CrossRef]
21. Harris, L.J.; Farber, J.N.; Beuchat, L.R.; Parish, M.E.; Suslow, T.V.; Garrett, E.H.; Busta, F.F. Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comp. Rev. Food Sci. Food Saf.* **2003**, *2*, 78–141. [CrossRef]
22. Castillo, A.; Lucia, L.M.; Goodson, K.J.; Savell, J.W.; Acuff, G.R. Use of hot water for beef carcass decontamination. *J. Food Prot.* **1998**, *61*, 19–25. [CrossRef] [PubMed]
23. Zhang, Y.; Ma, Q.; Critzer, F.; Davidson, P.M.; Zhong, Q. Organic thyme oil emulsion as an alternative washing solution to enhance the microbial safety of organic cantaloupes. *Food Control* **2016**, *67*, 31–38. [CrossRef]
24. Annous, B.A.; Solomon, E.B.; Cooke, P.H.; Burke, A. Biofilm formation by *Salmonella* spp. on cantaloupe melons. *J. Food Saf.* **2005**, *25*, 276–287. [CrossRef]
25. Guzel, M.; Moreira, R.G.; Omac, B.; Castell-Perez, E. Quantifying the effectiveness of washing treatments on the microbial quality of fresh-cut romaine lettuce and cantaloupe. *LWT Food Sci. Technol.* **2017**, *86*, 270–276. [CrossRef]
26. Oh, J.K.; Yegin, Y.; Yang, F.; Zhang, M.; Li, J.; Huang, S.; Verkhoturov, S.V.; Schweikert, E.A.; Perez-Lewis, K.; Scholar, E.A.; et al. The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. *Sci. Rep.* **2018**, *8*, 17247. [CrossRef]
27. DeFlorio, W.; Liu, S.; White, A.R.; Taylor, T.M.; Cisneros-Zevallos, L.; Min, Y.; Scholar, E.M.A. Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Comp. Rev. Food Sci. Food Saf.* **2021**, *20*, 3093–3134. [CrossRef]
28. Zhang, M.; Oh, J.K.; Huang, S.-Y.; Lin, Y.-R.; Liu, Y.; Mannan, M.S.; Cisneros-Zevallos, L.; Akbulut, M. Priming with nano-aerosolized water and sequential dip-washing with hydrogen peroxide: An efficient sanitization method to inactivate *Salmonella* Typhimurium LT2 on spinach. *J. Food Eng.* **2015**, *161*, 8–15. [CrossRef]
29. Wang, H.; Feng, H.; Luo, Y.; Zhang, A. Produce surface characteristics affect product quality and safety. *Acta Hort.* **2007**, *746*, 131–138. [CrossRef]
30. López-Gálvez, F.; Allende, A.; Selma, M.V.; Gil, M.I. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *Int. J. Food Microbiol.* **2009**, *133*, 167–171. [CrossRef]
31. Murray, K.; Aldossari, H.; Wu, F.; Warriner, K. Dynamic changes in free-chlorine levels within a commercial post-harvest wash and prevention of cross-contamination between shredded lettuce batches. *Food Control* **2018**, *85*, 127–134. [CrossRef]