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Effectiveness of Broad-Spectrum Chemical Produce Sanitizers against Foodborne Pathogens as In Vitro Planktonic Cells and on the Surface of Whole Cantaloupes and Watermelons

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ABSTRACT

Over the past few years, foodborne disease outbreaks linked to enteric pathogens present on cantaloupe and watermelon surfaces have raised concerns in the melon industry. This research evaluated the effectiveness of commercially available produce sanitizers against selected foodborne pathogens, both in cell suspensions and on the outer rind surface of melons. The sanitizers (65 and 200 ppm of chlorine, 5 and 35% hydrogen peroxide, 5 and 50 ppm of liquid chlorine dioxide, various hydrogen peroxide–acid combinations, 0.78 and 2.5% organic acids, and 300 ppm of quaternary ammonium) were tested against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella,* and non-O157 Shiga toxin–producing *E. coli* (O26, O45, O103, O111, O121, and O145). The cell suspension study revealed the ability of all tested sanitizers to reduce all selected pathogens by 0.6 to 9.6 log CFU/ml in vitro. In the melon study, significant differences in pathogen reduction were observed between sanitizers but not between melon types. The most effective sanitizers were quaternary ammonium and hydrogen peroxide–acid combinations, with 1.0- to 2.2-log CFU/g and 1.3- to 2.8-log CFU/g reductions, respectively, for all pathogens. The other sanitizers were less effective in killing the pathogens, with reductions ranging from 0.0 to 2.8 log CFU/g depending on pathogen and sanitizer. This study provides guidance to the melon industry on the best produce sanitizers for use in implementing a broad-spectrum pathogen intervention strategy.

Key words: *Escherichia coli* O157:H7; Food safety; *Listeria monocytogenes*; Melons; Produce sanitizers; *Salmonella*

In the United States, consumption of cantaloupe and honeydew is approximately 11 lb per capita, and consumption of watermelons is approximately 16 lb per capita. In 2010, melons represented the third most consumed fruits in America *(1, 32)*. Melon popularity can be attributed to Americans' desire to eat a healthier diet, improvements in production and distribution methods, and new marketing campaigns for precut melon products and seedless varieties *(7)*. Over the past 15 years, the growing number of foodborne illness outbreaks associated with melon consumption has brought increased attention to melons as vehicles of human enteric pathogens.

Melons have been the source or vehicle of pathogen transmission in outbreaks involving *Salmonella, Escherichia coli,* and *Listeria* spp. *(4, 9, 10, 31)*. In 2011, the largest single-vehicle foodborne outbreak in the United States was linked to *Listeria monocytogenes* on cantaloupe grown and processed at one farm in Colorado, resulting in 146 illnesses and 30 deaths across 28 states *(9)*. In 2012, another multistate outbreak occurred as a result of *Salmonella* on cantaloupe grown in the Midwest and was later also traced to watermelon grown by the same farm *(10)*. These foodborne illness outbreaks attributed to melon products have raised concern from regulatory agencies (U.S. Food and Drug Administration) and resulted in mandatory requirements for melon producers regarding standards of producing, harvesting, and marketing their products through the Food Safety Modernization Act *(35)*.

Sources of melon contamination include the soil, irrigation water, manure used as fertilizer, wildlife, harvesting and processing equipment, wash water or packing ice, and human handlers during harvest, processing, or shipping *(6)*. Contact with pathogens is of high concern for cantaloupe, as the nature of the rind and stem scar tissue encourage the attachment and infiltration of microorganisms *(24, 31)*. Additionally, cracks or abrasions on the surface of watermelon, which contains a waxy outer cuticle, may allow for attachment and protection of pathogens *(8)*. This, combined with the relatively high pH values of cantaloupe and watermelon flesh (6.0 to 6.5 and 5.1 to 5.6, respectively), as well as high water and nutrient content, results in conditions conducive for pathogen survival and growth once the pathogens are transferred from the rind to flesh during processing *(14, 41)*.

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One method for reducing microbial contamination of melons is washing with chlorinated or hot water or a sanitizer to remove soil and reduce microbial contamination *(34)*. The use of these water interventions can provide additional assurance of the safety of the end produce. However, previous research indicated that these sanitizers have varying degrees of effectiveness against microbial contamination on a variety of produce, including melons. Its relatively low cost and ease of use contribute to the popularity of chlorine, the most commonly used produce sanitizer; however, chlorine treatments have exhibited inconsistent efficacy (1- to 8-log reductions on produce) *(5, 18, 22, 26, 30, 41)*.

Alternative sanitizers are available, and their antimicrobial efficacy has been explored both in aqueous solution and on the surface of produce. Chlorine dioxide resulted in 2- to 4-log reductions *(13, 19)*, while hydrogen peroxide and acid treatments have also reduced pathogen levels on produce by 1 to 6 log *(3, 16, 20, 27)*. The use of quaternary ammonium has shown potential for microbial reductions in aqueous systems, as well as on the surface of oranges, but it is currently not approved for direct use on food or in produce wash water *(11, 40)*.

These alternative sanitizers exhibit varying degrees of effectiveness against microbial contamination on a variety of produce, including melons, but their use has not been widely accepted by the melon industry due to the cost and inconvenience of testing concentrations. The objective of this study was to investigate the antimicrobial effectiveness of various broad-spectrum commercially available chemical produce sanitizers against foodborne pathogens in an aqueous model system (planktonic cells; in vitro) or on the outer rind surface of rough (cantaloupe) and smooth (watermelon) surface melons (in vivo). The in vitro study will provide evidence of microbial kill within the wash water itself, while the in vivo study will evaluate microbial kill on the melon surface. The combination of these objectives provides a holistic perspective of the effectiveness of commercially available produce sanitizers in reduction of foodborne pathogens. The study is a reflection of the produce industry's desires to know how effective different sanitizers are against foodborne pathogens.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. The *E. coli* O157:H7 inoculum consisted of three ATCC strains, 35150, 43895, and 43890. The non-O157 Shiga toxin–producing *E. coli* (STEC) inoculum consisted of six strains, O26:H11, O45:H2, O103:H2, O111:H2, O121:H19, and O145NM, all of which were obtained from the Michigan State University STEC Center Database. The *L. monocytogenes* inoculum for the cell suspension study contained strains FSL R2-499, FSL N1-277, and FSL O1-177, and the *Listeria innocua* inoculum for the melon study consisted of strains ATCC 33090 and DD680. The *Salmonella* inoculum consisted of *S. enterica* serovar Typhimurium (strains SA 3250 and ATCC 14028) and *S. enterica* serovar Enteritidis (strain ATCC 13076). The individual strains were grown in brain heart infusion broth (HiMedia, Mumbai, India) for two consecutive 24-h enrichments at 35°C. Following the enrichments, cells for the respective inocula were combined and harvested by centrifugation (10,000 × *g* for 10 min at 4°C; Sorvall Super T21, Thermo Fisher Scientific, Waltham, MA). The harvested cells were washed twice in 0.1% (wt/vol) peptone (HiMedia) and suspended in 30 ml of sterile deionized water to obtain 9.0 log CFU/ml (for the cell suspension study) or 3 liters of 0.1% (wt/vol) peptone to obtain 8.0 log CFU/ml (for the melon study). The viable count of each bacterial suspension was determined by surface plating on plate count agar (HiMedia). All cultures were obtained in a frozen state (–80°C) in glycerol-supplemented broth medium from the Iowa State University Microbial Food Safety Laboratory.

Melon preparation and inoculation. Melons (netted-type cantaloupe and personal-sized watermelon) were obtained from a local wholesaler or retailer, sourced from throughout the United States, within 3 days of harvest. No melons used in this study had previous antimicrobial treatment applications, and a randomly selected subset of melons was tested for the presence of foodborne pathogens (*Listeria* spp., *E. coli* O157:H7, non-O157 STEC, and *Salmonella*) prior to utilizing the melons in our study.

Melons were selected randomly and were visually checked prior to inoculation with pathogens to ensure the structural quality of the rinds. Prior to use, melons were washed under cool running tap water, lightly scrubbed (with a soft brush) to remove visible soil, and air dried at room temperature. Cantaloupes and watermelons were inoculated by submersion into one suspension (3 liters) of the respective bacteria, using manual rotation every minute for a total of 5 min with a gloved hand to cover all portions of the melon. Inoculated melons were air dried for 1 h on sterilized stainless steel racks in a biosafety hood at ambient temperature (22 ± 1°C) without the hood blower on to allow for bacterial attachment *(30)*.

Sanitizer preparation. Sanitizers were obtained or purchased from their respective suppliers and diluted in sterile deionized water to standardize water hardness according to the suppliers' instructions. Validation of concentrations throughout the experiment was conducted as recommended by the suppliers' recommendations (i.e., titration kits and test strips). Additionally, the pH of the solutions was checked and adjusted to ensure the accuracy of concentrations (Orion Star A211, –2 to 20 range, Thermo Scientific, South Burlington, VT). Additionally, due to organic matter buildup, solutions were routinely replaced following dipping of six melons to ensure consistency of sanitizer concentrations and activities throughout the experiment. These recommendations and steps were followed because the sanitizer solutions were at the limit or below the federal standard recommendations *(36)* with regard to their direct application in foods. Sanitizers, sources, and tested concentrations are provided in Table 1.

Bacterial analysis of cell suspension treated with sanitizers. For each sanitizer tested (Table 1), analysis of cell suspensions was conducted in triplicate, with duplicate samples taken for each analysis. Immediately following suspension of an inoculum in sterile deionized water, the inoculum was sampled, serially diluted (10-fold) in buffered peptone water (HiMedia), and plated on plate count agar to determine the initial concentration of bacteria. As a control, bacterial cell suspensions in sterile water to which no sanitizer was added were utilized. Appropriate amounts of the sanitizer treatments were applied directly to inoculum suspensions (100 ml), mixed by vortexing (Vortex, Fisher Scientific, Waltham, MA), and placed at 4°C for 5 min to mimic cold water application. Aliquots (1.0 ml) of control and treated cell suspensions were then serially diluted (10-fold) in buffered peptone water and plated on plate count agar for analysis of

TABLE 1. *Description of sanitizer categories, commercial names, sources, and concentrations utilized within the cell suspension and melon studies*

Chemical category[a]	Commercial name	Commercial source	Concn tested in cell suspension study		Concn tested in melon study
Chlorine (free)	Commercial bleach	Commercial bleach	65 ppm	200 ppm	200 ppm
Liquid chlorine dioxide	Chlorine dioxide aqueous solution	Birko	5 ppm	50 ppm	5 ppm
Hydrogen peroxide	Oxy-Tech	Eagle Enterprises LLC	5%	35%	5%
23% H_2O_2–5.3% PAA	SaniDate 5.0	BioSafe Systems LLC	80 ppm	200 ppm	Not tested[b]
18% H_2O_2–12% PAA	SaniDate 12.0	BioSafe Systems LLC	80 ppm	200 ppm	100 ppm
27% H_2O_2–2.0% PAA	StorOx 2.0	BioSafe Systems LLC	80 ppm	200 ppm	100 ppm
Quaternary ammonium chloride	Zep FS Amine Z	Zep		300 ppm	300 ppm
Organic acid–emulsifier	Pro-San	Microcide, Inc.		0.78%	0.78%
Lactic acid–citric acid	Veggiexide	Birko		2.5%	Not tested[b]
Acetic acid–PAA–H_2O_2	Birkoside MP-2	Birko		0.78%	0.78%

[a] H_2O_2, hydrogen peroxide; PAA, peroxyacetic acid.
[b] The sanitizer was not tested in melon study due to low efficacy in the cell suspension study.

bacterial survival. For counting the pathogen populations, the plates were incubated at 35°C for 24 to 48 h, depending on each organism's growth characteristics regarding the formation of visible colonies.

Sanitizer treatment of inoculated melons. For each sanitizer tested (Table 1), analysis was conducted in triplicate, with duplicate samples taken for each analysis. Each analysis set consisted of one noninoculated cantaloupe and one noninoculated watermelon that were tested to determine levels of background pathogens and three cantaloupes and watermelons that were each inoculated with the respective bacterial inoculum (*E. coli* O157:H7, non-O157 STEC, *L. innocua,* or *Salmonella*). Of the inoculated melons, one non–sanitizer-treated melon was selected for microbial analysis to determine the initial bacterial load and used as a control; the remaining two melons were selected for microbial analysis after sanitizer treatment. Pathogen-inoculated melons were treated with produce sanitizers by dipping them in 5 liters of prepared solutions in a sterile plastic tub at room temperature (22 ± 1°C) and using constant manual agitation for 2 min. A total of six inoculated melons were treated with each batch of diluted sanitizer to ensure that sanitizer concentrations were not reduced during the experiment. Following treatments, melons were rinsed with sterile municipal city water for 5 s (per sanitizer manufacturers' requirements, if applicable) and placed in a biosafety hood to air dry at room temperature for 1 h without the blower. Melons were then sampled by randomly removing approximately 60 rind plugs using a sterile stainless steel apple core borer (Mercer Cutlery, Deer Park, NY). The flesh was removed from the rind plugs using a sterilized knife and discarded. Rind samples were collected (25 g per melon sample) and homogenized with 225 ml of buffered peptone water using a sanitized commercial blender (Oster, Sunbeam Products, Boca Raton, FL) operating at the highest speed setting for 1 min. Melon homogenates were sampled by performing serial 10-fold dilutions in buffered peptone water and plating aliquots of selected dilutions of homogenate onto appropriate overlay selective agars *(42)*. Dey-Engley neutralizing broth was not utilized in this study because of its lack of inactivation in the selected sanitizers and antimicrobial effect on the targeted bacteria as observed in preliminary testing. Processing and plating onto selective agar was performed within 1 min after processing. The selective agars used in the present study included sorbitol MacConkey agar (Difco, BD, Sparks, MD) with tryptic soy agar (TSA; EMD Chemicals, Inc., Darmstadt, Germany) overlay (*E. coli* O157:H7), Possé agar *(23)* (non-O157 STEC), modified Oxford (MOX) agar (Difco, BD) with TSA overlay, and xylose lysine desoxycholate (XLD) agar (Difco, BD) with TSA overlay (*Salmonella*). The inoculated plates were incubated for 24 to 48 h at 35°C, depending on the organism's growth characteristics. Typical colonies were compared to those in the pure cultures. Presumptively positive isolates on agar plates displaying growth were confirmed by using appropriate agglutination kits (Oxoid Ltd., Basingstoke, UK).

Statistical analysis. Each study included three replicates with duplicate samples analyzed for each replication. For all studies, microbial counts were obtained and used to calculate means and standard deviations within the replicate analysis. Bacterial reduction was analyzed using PROC GLM (general linear model procedure) for mean separation of sanitizer treatment and least-square means for sanitizer and melon interactions. Analysis was performed using SAS 9.3 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Cell suspension study. In compliance with implementation of Food Safety Modernization Act standards *(35),* melon producers are encouraged to utilize practices based on Preventive Controls for Produce *(37)*. This process requires the identification of preventive control points and monitoring and documenting control. The washing step of produce is commonly identified as a preventive control within melon production. The wash water sanitizer concentration has critical limits and monitoring procedures established to ensure the antimicrobials will reduce pathogens within the water. The results from this cell suspension study can be utilized as validation data for the use of produce sanitizers. Rodgers et al. *(26)* showed that the cell suspension aqueous system study models optimal conditions for the establishment of a critical control point.

In the present study, produce sanitizers were compared at multiple concentrations and, when not significantly different ($P > 0.05$) in antibacterial effectiveness, the lower concentrations were selected for use in future work (Table 1). There were significant differences in bacterial reductions

TABLE 2. *Log reductions of pathogens in cell suspension following sanitizer treatments*

Sanitizer[a]	Concn	Mean log reduction (CFU/ml) ± SD of[b]: *E. coli* O157:H7	*Salmonella*	*L. monocytogenes*	Non-O157 STEC
Water (control), initial load		9.5 ± 0.1 A	8.7 ± 0.4 A	9.1 ± 0.3 A	9.2 ± 0.2 A
Chlorine	65 ppm	3.3 ± 0.5 EF	1.9 ± 0.5 E	3.0 ± 0.1 EFG	3.2 ± 0.9 E
	200 ppm	5.7 ± 0.3 D	4.1 ± 1.0 CDE	5.3 ± 0.2 CDE	4.9 ± 0.2 DE
Liquid chlorine dioxide	5 ppm	5.0 ± 0.5 DEF	5.3 ± 0.3 BCDE	5.2 ± 0.4 CDE	4.5 ± 0.4 DE
	50 ppm	9.5 ± 0.0 B	8.4 ± 0.6 BC	8.8 ± 0.1 B	9.3 ± 0.2 B
Hydrogen peroxide	5%	8.0 ± 0.8 BC	7.7 ± 1.0 BCD	2.6 ± 0.1 FG	7.5 ± 1.0 BC
	35%	9.5 ± 0.1 B	8.5 ± 0.7 BC	9.2 ± 0.1 B	9.3 ± 0.1 B
23% H_2O_2–5.3% PAA	80 ppm	4.9 ± 0.3 DEF	5.1 ± 0.3 BCDE	5.5 ± 0.4 CD	4.7 ± 0.3 DE
	200 ppm	6.6 ± 0.6 CD	6.2 ± 1.2 BCDE	5.2 ± 0.4 CDE	5.2 ± 0.4 CDE
18% H_2O_2–12% PAA	80 ppm	5.2 ± 0.4 DEF	6.8 ± 0.6 BCDE	4.9 ± 0.4 CDEF	5.4 ± 0.4 CDE
	200 ppm	9.6 ± 0.1 B	6.6 ± 0.8 BCDE	6.2 ± 0.5 CD	9.2 ± 0.2 B
27% H_2O_2–2.0% PAA	80 ppm	5.3 ± 0.1 DE	6.9 ± 1.2 BCD	4.8 ± 0.2 CDEF	5.0 ± 0.3 CDE
	200 ppm	9.6 ± 0.1 B	8.4 ± 0.8 BC	9.2 ± 0.3 B	9.2 ± 0.2 B
Quaternary ammonium chloride	300 ppm	6.1 ± 0.7 BD	4.5 ± 2.3 BCDE	7.2 ± 1.3 BC	6.3 ± 0.9 CD
Organic acid–emulsifier	0.78%	3.0 ± 0.7 F	3.8 ± 0.8 CDE	4.3 ± 0.8 CDE	3.9 ± 0.6 DE
Lactic acid–citric acid	2.50%	0.7 ± 0.1 G	3.0 ± 0.8 DE	1.8 ± 0.4 G	0.6 ± 0.1 F
Acetic acid–PAA–H_2O_2	0.78%	9.6 ± 0.1 B	9.1 ± 0.2 B	9.3 ± 0.1 B	9.4 ± 0.1 B

[a] Sanitizer treatments were applied for 5 min at 4°C. H_2O_2, hydrogen peroxide; PAA, peroxyacetic acid.
[b] Different letters indicate significant differences ($P < 0.05$) between log reductions within the same column.

observed between produce sanitizer treatments ($P < 0.05$, Table 2). The most effective categories of sanitizers were liquid chlorine dioxide (5 ppm) and peroxyacetic acid (PAA)–hydrogen peroxide (80 and 200 ppm), with 8- to 9-log CFU/ml reductions for *E. coli* O157:H7, *L. monocytogenes, Salmonella,* and non-O157 STEC. These results are supported by those of Rodgers et al. *(26),* who demonstrated greater than a 6-log pathogen reduction in a three-strain inoculum of *E. coli* O157:H7 and *L. monocytogenes* in an aqueous solution treated with peroxyacetic acid (80 ppm) and chlorine dioxide (3 to 5 ppm) for 20 to 70 s.

Hydrogen peroxide (5 and 35%) treatment also resulted in large reductions in pathogen populations (7 to 8 log CFU/ml) (Table 2). The only exception to this observation was the *L. monocytogenes* suspension treated with 5% hydrogen peroxide, which exhibited a greatly reduced log reduction (2.5 log CFU/ml). This finding is in contrast to the results reported for studies conducted by Robbins et al. *(25),* who observed a reduction of 6.0 log CFU/ml of *L. monocytogenes* Scott A cell suspension when the pathogen was treated with 3% hydrogen peroxide. These discrepancies can be attributed to variations between the study designs. Robbins et al. *(25)* conducted their study in a broth medium, utilizing a lower concentration of hydrogen peroxide but a longer contact time of 10 min and a much higher temperature of 20°C, which might have made the bacterial cells more susceptible to the action of a sanitizer.

Our results indicated that quaternary ammonium (300 ppm) caused a microbial reduction of 4 to 7 log CFU/ml (Table 2), with the greatest bacterial reduction in the *Listeria* cell suspension. These results are consistent with findings in a simulation study by Chaidez et al. *(11),* where they observed a 6-log reduction of *E. coli* and *Staphylococcus aureus* when treated with 200 mg/liter quaternary ammonium in different wash water conditions. A difference in survival rates between gram-positive and gram-negative bacteria when treated with quaternary ammonium was also observed. This difference in sanitizer efficacy can be attributed to the outer membrane structure of gram-negative cells, which leads to an increased resistance to cationic moieties in quaternary ammonium *(12).*

The organic acid combination product was the least effective sanitizer, with reductions in pathogen populations of up to 3 log CFU/ml or less depending on the foodborne pathogen (Table 2). The organic acid mixture tested included a combination of lactic and citric acids (2.50%), and our results are supported by the findings of Hawkins *(17),* who reported no effects of citric and other organic acids on *L. monocytogenes* or *E. coli* survival. Allende et al. *(2)* also showed no inhibitory effect of lactic acid-producing bacteria against *Listeria* in an aqueous solution at temperatures similar to those used in our study.

Melon inoculation study. The initial viable counts of bacteria attached to surfaces of melons were 4.2, 3.8, 4.5, and 3.3 log CFU/g for *E. coli* O157:H7, *Salmonella, Listeria* spp., and non-O157 STEC, respectively. The reductions in viable bacterial counts varied from 0 to 2.8 log CFU/g depending on the sanitizer type and pathogen (Table 3). However, significant differences ($P < 0.05$) were observed between sanitizer-treated melons and the untreated control, as well as between the sanitizers and water and chlorine treatments. Because there were no statistically significant interactions between melon types (cantaloupe versus watermelon), the analysis reported all melon data collectively.

One notable observation was the variation in surface types between the cantaloupes and watermelons. The cantaloupes specifically had varying degrees and depths of netting and often were smooth with minor netting. Even

TABLE 3. *Log reduction of pathogens on melon surfaces following sanitizer treatments*

Sanitizer	Concn	Mean log reduction (CFU/ml) ± SD of[b]: *E. coli* O157:H7	*Salmonella*	Listeria spp.	Non-O157 STEC
Control (no treatment), initial load		4.2 ± 0.2 A	3.8 ± 0.3 A	4.5 ± 0.2 A	3.3 ± 0.4 A
Water		0.7 ± 0.5 D	−0.5 ± 0.4 D	1.2 ± 0.5 B	−0.1 ± 0.5 D
Chlorine	200 ppm	0.6 ± 0.5 D	1.2 ± 0.4 C	1.9 ± 0.5 B	0.6 ± 0.6 CD
Liquid chlorine dioxide	5 ppm	1.6 ± 0.5 BC	2.1 ± 0.4 BC	0.8 ± 0.5 B	1.6 ± 0.6 BC
Hydrogen peroxide	5%	1.4 ± 0.5 BC	1.5 ± 0.6 BC	2.4 ± 0.5 B	1.8 ± 0.7 BC
18% H_2O_2–12% PAA	100 ppm	2.3 ± 0.5 BC	2.0 ± 0.5 BC	1.8 ± 0.5 B	1.6 ± 0.8 BC
27% H_2O_2–2.0% PAA	100 ppm	0.9 ± 0.5 CD	2.6 ± 0.5 B	1.5 ± 0.5 B	0.9 ± 0.7 BCD
Quaternary ammonium chloride	300 ppm	2.4 ± 0.5 B	2.3 ± 0.4 B	0.8 ± 0.5 B	2.1 ± 0.5 B
Organic acid–emulsifier	0.78%	0.8 ± 0.5 D	2.8 ± 0.5 B	1.2 ± 0.5 B	1.5 ± 0.6 BC
Acetic acid–PAA–H_2O_2	0.78%	2.3 ± 0.5 BC	2.8 ± 0.4 B	1.2 ± 0.5 B	0.3 ± 0.5 CD

[a] Sanitizer treatments were applied for 2 min. H_2O_2, hydrogen peroxide; PAA, peroxyacetic acid.
[b] Different letters indicate significant differences ($P < 0.05$) between log reductions within the same column.

with these differences in netting, our study did not show a significant difference between melon types, which is not consistent with previous research *(3, 5, 13, 26, 38)*.

Quaternary ammonium (300 ppm) resulted in 2-log or greater reductions of pathogenic *E. coli* and *Salmonella* but less than a 1-log reduction of *Listeria*. These results were not consistent with the results shown by Chaidez et al. *(11),* who noted a 6-log reduction of gram-positive pathogens. This may be attributed to the ability of *Listeria* to attach to the surface of the melons through interactions with the melon's surface structures *(24)* and to display an increased resistance to sanitizers as a result of cell attachment *(21, 28)*. The use of quaternary ammonium has been limited in studies on fresh produce because it is not approved for direct food contact, though it has been successfully utilized and accepted to reduce microbial contamination on whole produce that is peeled prior to consumption *(33, 40)*. The reduction in gram-negative bacterial populations observed in the present study could be used as evidence of the potential for use of quaternary ammonium in the melon industry to control some pathogens of concern, considering that the rinds of melons are not eaten.

When concentrations of sanitizers were compared, hydrogen peroxide and PAA treatments were tested at 2 and 12%. The higher concentration of PAA (12%) resulted in 2-log or greater reductions of *E. coli* O157:H7 and *Salmonella* and slightly lower, less than 1-log reductions, of *Listeria* and non-O157 STEC populations. An additional sanitizer containing hydrogen peroxide, PAA, and acetic acid (0.78%) produced similar results for bacterial reductions, though non-O157 STEC exhibited very little reduction in the presence of this sanitizer. These results are consistent with the results reported by Wang et al. *(38),* who noted approximately 1.5-log reductions of *E. coli* O157:H7 on apples and cantaloupes treated with PAA at 80 ppm. However, Rodgers et al. *(26)* reported much greater reductions (~4.4 log) of *E. coli* O157:H7 and *L. monocytogenes* on a variety of produce (cantaloupe melons, apples, and strawberries) with the use of PAA at 80 ppm. Some of these differences may be attributed to the strains of *E. coli* O157:H7 and *L. monocytogenes* used in the methods.

Chlorine dioxide has much more oxidative power than chlorine *(33),* which resulted in greater reductions of bacteria under the present experimental conditions. Liquid chlorine dioxide (5 ppm) resulted in 1.5- to 2-log reductions of *E. coli* O157:H7 and *Salmonella,* but less than a 1-log reduction was achieved for *Listeria*. These results were similar to those of previous work with chlorine dioxide, which showed reductions of *E. coli, Listeria,* and *Salmonella* on cantaloupes by 2 to 3 log at 0.5 to 5 mg/liter for up to 10 min of exposure *(19)* and of total bacterial populations by 3 to 4 log on apples, guavas, lettuce, and cucumbers *(13)*. Chlorine resulted in less than a 1-log reduction of *E. coli* O157:H7 and less than 2-log reductions of *Listeria* and *Salmonella* in our study. These results were consistent with those of many previous studies with regard to bacterial reductions on melons *(13, 15, 19, 29, 39)*.

Hydrogen peroxide (5%) resulted in 1.5- to 2.5-log reductions of all bacteria, which was consistent with the cantaloupe study results reported by Sapers et al. *(27)* and Ukuku and Fett *(29)*. However, Sapers et al. *(27)* indicated that the addition of detergents could improve the efficacy of hydrogen peroxide and that the addition of acids (acetic acid and PAA) can greatly improve this treatment for most pathogens.

When considering both melon types and all pathogens tested, analysis showed that the five sanitizer treatments were consistently more effective at reducing pathogen loads than the baseline water-and-chlorine treatment. These sanitizers included 18% H_2O_2–12% PAA (100 ppm, 1.6- to 2.3-log reductions depending on the pathogen), quaternary ammonium chloride (300 ppm, 0.8- to 2.4-log reductions depending on the pathogen), acetic acid–PAA–H_2O_2 (0.78%, 0.3- to 2.8-log reductions depending on the pathogen), liquid chlorine dioxide (5 ppm, 0.8- to 2.1-log reductions depending on the pathogen), and hydrogen peroxide (5%, 1.4- to 2.4-log reductions depending on the pathogen). It should be noted that for individual melon types (cantaloupe and watermelon), some sanitizers produced

greater than 3-log reductions of selected bacteria (data not shown); however, considering the overall melon analysis, no sanitizer treatment resulted in greater than a 2.8-log reduction of any pathogen studied. This should be a consideration for future studies concerning produce sanitizers and alternative treatments for a combined hurdle effect in pathogen reduction on whole melons. Additionally, this study optimized the conditions for bacterial contact and attachment to melon surfaces, starting with an 8-log CFU/ml inoculum but resulting in 4.5-log or less attachment to melon surfaces for all tested pathogens. This low pathogen attachment under ideal conditions should be considered when analyzing the effectiveness of sanitizers to reduce pathogen loads in less than ideal environmental conditions for bacterial presence and attachment.

The results of this study will be disseminated to melon producers and processors to highlight the benefit to food safety through prevention of microbial contaminants in the melon industry. It will also be important to discuss the willingness of industry members to utilize these sanitizer treatments in their facilities, based on chemical makeup, benefits, and cost of use. Further work should be conducted to determine the optimal use of these sanitizers to improve efficacy and the possibility of combined treatments to utilize hurdle technologies for greater microbial reductions. The results of this study will be further used to determine the role that these sanitizers may serve in preserving the quality and shelf life of melons through improvement of melon surface and flesh integrity, as well as yeast and mold reduction.

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