

Efficacy of Home Washing Methods in Controlling Surface Microbial Contamination on Fresh Produce

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ABSTRACT

Much effort has been focused on sanitation of fresh produce at the commercial level; however, few options are available to the consumer. The purpose of this study was to determine the efficacy of different cleaning methods in reducing bacterial contamination on fresh produce in a home setting. Lettuce, broccoli, apples, and tomatoes were inoculated with *Listeria innocua* and then subjected to combinations of the following cleaning procedures: (i) soak for 2 min in tap water, Veggie Wash solution, 5% vinegar solution, or 13% lemon solution and (ii) rinse under running tap water, rinse and rub under running tap water, brush under running tap water, or wipe with wet/dry paper towel. Presoaking in water before rinsing significantly reduced bacteria in apples, tomatoes, and lettuce, but not in broccoli. Wiping apples and tomatoes with wet or dry paper towel showed lower bacterial reductions compared with soaking and rinsing procedures. Blossom ends of apples were more contaminated than the surface after soaking and rinsing; similar results were observed between flower section and stem of broccoli. Reductions of *L. innocua* in both tomatoes and apples (2.01 to 2.89 log CFU/g) were more than in lettuce and broccoli (1.41 to 1.88 log CFU/g) when subjected to same washing procedures. Reductions of surface contamination of lettuce after soaking in lemon or vinegar solutions were not significantly different ($P > 0.05$) from lettuce soaking in cold tap water. Therefore, educators and extension workers might consider it appropriate to instruct consumers to rub or brush fresh produce under cold running tap water before consumption.

Over the past decade, there has been an increase in per capita consumption of fresh fruits and vegetables (4) because of an increased awareness in a healthy diet. Fruits and vegetables are generally considered safe to eat (16); however, they have been linked to several outbreaks of foodborne illness (22). Pathogenic microorganisms, such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, have been associated with fresh produce over the past two decades. Direct or indirect pathogen contamination of fresh produce can occur at many points in the production chain during growth and processing (5, 10), thus presenting a food safety challenge to consumers.

Listeria monocytogenes is a common contaminant in raw milk, uncooked seafood, and fresh produce. Pregnant women, alcoholics, young children, elderly and immunocompromised individuals are most susceptible to infection by this human pathogen. It causes 1,700 cases of listeriosis and 450 deaths annually in the U.S. (11) and has been associated with several produce recalls (9, 14), including red bell peppers, romaine lettuce, sprouts, and apple slices within the past 3 years. Presence of this pathogen has also been reported in potatoes, radishes, cabbage, cucumbers, and mushrooms obtained from the market (12). According to a previous study (20), *L. monocytogenes* has the ability to survive and grow on head lettuce when stored at 5 and 12°C for 7 and 14 days. Although complete elimination of

pathogenic microorganisms from fresh produce is not currently achievable, a reduction of potential contamination is desirable (19).

Consumers generally need more practical information on how to reduce bacterial contamination on fresh produce. Because *L. monocytogenes* is a natural contaminant of raw materials, it is of great importance to investigate cleaning methods that can control its contamination. Therefore, the goal of this study was to investigate effectiveness of cleaning methods that can be used by consumers in a home setting to reduce microbial contamination on fresh produce.

MATERIALS AND METHODS

Bacterial strain and inoculation preparation. The bacterial strain used in this study was *Listeria innocua* (ATCC, 33090), which was used as a surrogate for *L. monocytogenes* in previous studies (1, 17). The bacteria were stored at -80°C in 15% glycerol stocks. Before each experiment, stocks were streaked onto tryptic soy agar (TSA; Difco, Sparks, Md.) containing 0.6% yeast and incubated at 37°C for 48 h. Bacterial subcultures were prepared by two successive transfers of cells in tryptic soy broth (TSB; Difco) supplemented with 0.6% yeast extract after incubation at 37°C for 24 h. The final broth culture was grown at 37°C for 18 h in TSB and diluted 10-fold with 0.1% peptone water to yield a final inoculum of approximately 10⁸ CFU/ml. The inoculum was applied to the selected fresh produce within 1 h of preparation. Concentration of inoculum was determined by surface plating of serially diluted culture onto *Listeria* selective agar (Oxford formulation) with supplement (SR020E; Oxoid, Basingstoke, UK). Plates were incubated at 37°C for 48 h before the colonies were counted.

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TABLE 1. Reduction of *Listeria innocua* contamination on the surface of lettuce after cleaning^a

	<i>L. innocua</i> reduction (log CFU/g)						
	W2/15	W2/15 × 2	W30/15	W0/15	L2/15	V2/15	VW2/15
Trial 1 ^b	1.85 (0.38)	1.96 (0.36)	1.83 (0.12)	1.45 (0.17)	1.78 (0.08)	1.77 (0.19)	1.92 (0.15)
Trial 2	1.74 (0.17)	1.76 (0.13)	1.85 (0.04)	1.37 (0.15)	1.66 (0.29)	1.98 (0.18)	1.54 (0.05)
Average ^c	1.79 B (0.27)	1.86 B (0.27)	1.84 B (0.08)	1.41 A (0.15)	1.72 B (0.20)	1.88 B (0.20)	1.73 B (0.23)

^a W2/15, soak 2 min in water and rinse 15 s; W2/15 × 2, soak 2 min in water and rinse 15 s (twice); W30/15, soak 30 min in water and rinse 15 s; W0/15, rinse 15 s; L2/15, soak 2 min in lemon solution and rinse 15 s; V2/15, soak 2 min in vinegar and rinse 15 s; VW2/15, soak 2 min in Veggie Wash and rinse 15 s.

^b Two trials were performed on different days using the sample purchased from the same store the day before the experiment. Initial inoculation levels were 7.66 ± 0.01 log CFU/g. Values presented are mean log reductions with the standard deviation in parentheses.

^c Same treatments from two trials were averaged for comparison among treatments. Means with different letters are significantly different ($P < 0.05$).

Procedure for inoculating fresh produce. Lettuce, tomatoes, apples, and broccoli were used as models to represent fresh produce of different surface characteristics. Samples of produce were purchased from a local grocery store on the day before the experiment and stored in their original boxes at 4°C. Fresh produce that was bruised or had cracks was not used in the experiment. Background tests were performed on tap water and uninoculated produce to confirm the absence of *Listeria* spp. Apples were the only produce pretreated in warm water (45°C) for 2 min before inoculation with *L. innocua* to remove wax coating that might interfere with bacteria attachment to the fruit. The fresh produce was individually submerged in 3 liters of bacterial inoculum (approximately 10^8 CFU/ml) and agitated by stirring with a sterilized stainless steel spoon for 3 min. The inoculated produce was air dried for 10 min in a biosafety cabinet before it was subjected to the selected treatments. The positive controls (no cleaning after inoculation) for each produce were analyzed to determine the baseline contamination level of *L. innocua*.

Produce cleaning procedure. Inoculated samples were subjected to combinations of the following cleaning procedures: (i) soak for 2 min in tap water (room temperature or warm at 40°C), Veggie Wash solution (2.0 oz/gal of water, Beaumont Products Inc., Kennesaw, Ga.), 5% vinegar solution, or 13% lemon solution and (ii) rinse with cold tap water (15 s), rinse and rub with running tap water (15 s), brush under running tap water (15 s), or wipe with wet/dry paper towel (15 s). During soaking, the produce was rotated or turned to ensure full surface coverage by treatment. The intensity of each procedure was uniformly maintained to reduce variability within treatments. The efficacy of soaking in tap water followed by a rinse step was also tested for stem and blossom ends of apples (inaccessible areas) and for flower and stem sections of broccoli. Each kind of produce had three replicates of each treatment per experiment, and each experiment was performed two times.

Microbiological analysis. After various cleaning procedures, samples were placed in stomacher bags and diluted 1:10 (wt/vol) with 0.1% peptone water. Samples of lettuce and broccoli were pummeled in a Stomacher 400 Circulator (Seward Limited, London, UK) at normal speed setting for 2 min. For apples and tomatoes, *L. innocua* was detached from the surface by hand rubbing for 2 min in 0.1% peptone water. A sterile stainless steel cork bore was used to separate the stem and blossom ends of

apples to further determine the efficacy of washing methods to the inaccessible areas. A sterile knife was used to cut the broccoli flower and stem sections to separately evaluate *L. innocua* contamination in each portion. Populations of *L. innocua* on treated samples were determined by plating 0.1 ml of serially diluted homogenized sample on *Listeria* selective agar (Oxoid). Three replicates of each sample were analyzed, and each replicate consisted of a minimum of three plates with serial dilutions. Plates were incubated for 48 h at 37°C before the colonies were counted. *L. innocua* colonies were confirmed with CAMP Test and Micro-ID *Listeria*.

Statistical analysis. Microbiological counts (CFU per gram) of samples and the data were transformed to log reduction before statistical analysis. Log reductions for each treatment were compared for statistical significance by the GLM procedure with SPSS-PC (SPSS Inc., Chicago, Ill.). Differences of means for treatments were separated by least significant difference at $P < 0.05$.

RESULTS

Viable *L. innocua* recovered after cleaning was entirely attributed to the inoculation. There was no trace of *Listeria* spp. detected in the tap water and uninoculated samples used in the experiments. Viable *L. innocua* reductions obtained after cleaning were relative to populations on inoculated produce (positive control). The inoculation level used in the experiment was higher than natural contamination to allow valid observation of bacterial reductions after different cleaning methods.

Lettuce. Rinsing lettuce leaves under cold running tap water for 15 s without prior soaking showed the lowest significant ($P < 0.05$) bacterial reduction of 1.41 log CFU/g among all other treatments (Table 1). Reductions of *L. innocua* populations ranged from 1.72 to 1.88 log CFU/g after soaking lettuce leaves in 13% lemon, 5% vinegar, Veggie Wash solution, or tap water (room temperature or warm at 40°C) for 2 min, followed by a 15-s rinse under running tap water. Soaking lettuce leaves in lemon and vinegar solutions showed no difference ($P > 0.05$) in bacterial reductions from soaking in cold tap water for 2 min. There-

TABLE 2. Reduction of *Listeria innocua* contamination on the surface of broccoli after cleaning^a

	<i>L. innocua</i> reduction (log CFU/g)			
	W2/15	W0/15	WW2/15	VW2/15
Trial 1 ^b	1.53 (0.21)	1.30 (0.20)	1.47 (0.06)	1.50 (0.10)
Trial 2	1.67 (0.06)	1.53 (0.12)	1.77 (0.21)	1.50 (0.17)
Average ^c	1.60 A (0.15)	1.41 A (0.19)	1.62 A (0.21)	1.50 A (0.13)

^a W2/15, soak 2 min in water and rinse 15 s; W0/15, rinse 15 s; WW2/15, soak in warm water for 2 min and rinse 15 s; VW2/15, soak 2 min in Veggie Wash and rinse 15 s.

^b Two trials were performed on different days using the sample purchased from the same store the day before the experiment. Initial inoculation levels were 7.60 ± 0.28 log CFU/g. Values presented are mean log reductions with the standard deviation in parentheses.

^c Same treatments from two trials were averaged for comparison among treatments. Means with different letters are significantly different ($P < 0.05$).

fore, lemon and vinegar solutions were not used in the later experiments for other produce. Soaking for 30 min in tap water was considered too long to be practical and was not significantly different ($P > 0.05$) from 2 min of soaking in tap water. Mean bacterial reductions in all treatments were not significantly different for trials 1 and 2, except in Veggie Wash treatments. The discrepancy observed between Veggie Wash treatments could be from a minor variation in lettuce surface structure and rinse procedures.

Broccoli. Mean bacterial reductions obtained from trials 1 and 2 among all treatments were not different ($P > 0.05$). Mean reduction of *L. innocua* after rinsing and soaking broccoli in either cold tap water, warm tap water (40°C), or Veggie Wash solution for 2 min, followed by a 15-s rinse under cold running tap water were in the range of 1.50 to 1.61 log CFU/g and were not different ($P > 0.05$) from each other (Table 2). Similar to the observations in lettuce,

soaking broccoli in either cold water, warm water, or Veggie Wash solution before rinsing improved the reduction compared with rinsing only (1.41 log CFU/g), although the differences were not significant. A separate analysis of flower and stem sections indicated a higher contamination level on the flower (7.47 log CFU/g) than on the stem portion (6.93 log CFU/g). The larger surface area of the flower section could contribute to the higher bacterial population. Nevertheless, the flower showed greater reduction (1.49 log CFU/g) than the stem portion (0.46 log CFU/g) after soaking in water for 2 min followed by a rinse step.

Apples. A 2-min soak in either tap water or Veggie Wash solution followed by rinsing (15 s) under running tap water resulted in reductions of 2.32 and 2.28 log CFU/g, respectively, which showed no significant differences ($P > 0.05$) when compared with rubbing (2.11 log CFU/g) or brushing (2.03 log CFU/g) under running tap water without a prior soaking step (Table 3). However soaking before rinsing (2.32 log CFU/g) significantly improved bacteria reduction compared with rinsing alone (2.01 log CFU/g). Wiping apples with dry or wet paper towel had the lowest significant ($P < 0.05$) reductions of 0.66 and 0.96 log CFU/g, respectively, among all treatments. Further investigation revealed that the stem and blossom ends had higher residual contamination of *L. innocua* (3.70 log CFU/g) than the surface (1.18 log CFU/g) after soaking in water followed by rinsing. The uneven surfaces characterized by stem and blossom ends could result in accumulation of more bacteria. There was no significant difference observed in trials 1 and 2 for all treatments, except the mean reductions for rub and rinse (RW0/15). Although care had been taken to standardize the cleaning procedures, minor variations in movement of rubbing and rinsing, as well as changes in water flow rate, could contribute to the observed differences.

Tomatoes. Veggie Wash solution had a reduction of 2.89 log CFU/g and was significantly higher ($P < 0.05$) than all other cleaning methods (Table 4). Rinsing after soaking and rinsing with rubbing without presoaking resulted in bacterial reductions of 2.53 and 2.36 log CFU/g, respectively; the difference was not significant ($P > 0.05$)

TABLE 3. Reduction of *Listeria innocua* contamination on the surface of apples after cleaning^a

	<i>L. innocua</i> reduction (log CFU/g)						
	W2/15	RW0/15	W0/15	BW0/15	VW2/15	DT	WT
Trial 1 ^b	2.16 (0.14)	2.34 (0.21)	2.09 (0.01)	1.99 (0.31)	2.40 (0.03)	0.56 (0.12)	0.82 (0.27)
Trial 2	2.48 (0.22)	1.88 (0.19)	1.94 (0.14)	2.06 (0.05)	2.16 (0.17)	0.76 (0.12)	1.09 (0.39)
Average ^c	2.32 D (0.24)	2.11 CD (0.31)	2.01 C (0.13)	2.03 CD (0.20)	2.28 CD (0.17)	0.66 A (0.15)	0.96 B (0.33)

^a W2/15, soak 2 min in water and rinse 15 s; RW0/15, rub while rinsing 15 s; W0/15, rinse 15 s; BW0/15, brush while rinsing 15 s; VW2/15, soak 2 min in Veggie Wash and rinse 15 s; DT, wipe with dry paper towel 15 s; WT, wipe with wet paper towel 15 s.

^b Two trials were performed on different days using the sample purchased from the same store the day before the experiment. Initial inoculation levels were 5.35 ± 0.35 log CFU/g. Values presented are mean log reductions with the standard deviation in parentheses.

^c Same treatments from two trials were averaged for comparison among treatments. Means with different letters are significantly different ($P < 0.05$).

TABLE 4. Reduction of *Listeria innocua* contamination on the surface of tomatoes after cleaning^a

	<i>L. innocua</i> reduction (log CFU/g)					
	W2/15	RW0/15	W0/15	VW2/15	DT	WT
Trial 1 ^b	2.51 (0.19)	2.56 (0.18)	2.37 (0.19)	2.98 (0.37)	1.89 (0.05)	2.00 (0.37)
Trial 2	2.54 (0.28)	2.16 (0.27)	1.82 (0.12)	2.80 (0.11)	1.81 (0.13)	1.88 (0.17)
Average ^c	2.53 B (0.28)	2.36 B (0.29)	2.10 A (0.33)	2.89 C (0.26)	1.85 A (0.10)	1.94 A (0.27)

^a W2/15, soak 2 min in water and rinse 15 s; RW0/15, rub while rinsing 15 s; W0/15, rinse 15 s; VW2/15, soak 2 min in Veggie Wash and rinse 15 s; DT, wipe with dry paper towel 15 s; WT, wipe with wet paper towel 15 s.

^b Two trials were performed on different days using the sample purchased from the same store the day before the experiment. Initial inoculation levels were 5.25 ± 0.21 log CFU/g. Values presented are mean log reductions with the standard deviation in parentheses.

^c Same treatments from two trials were averaged for comparison among treatments. Means with different letters are significantly different ($P < 0.05$).

between these two cleaning procedures. Rinsing (15 s) under running tap water without prior soaking had a significantly lower bacterial reduction of 2.10 log CFU/g than rinsing with rubbing without presoaking (2.36 log CFU/g) or rinsing after soaking (2.53 log CFU/g). These results indicate that rubbing and soaking improved bacterial reduction. Wiping with a wet or dry paper towel without soaking or rinsing had significantly low bacterial reductions of 1.94 and 1.85 log CFU/g, respectively. Generally, in both trials, bacterial reductions in each cleaning procedure was not significantly different ($P > 0.05$), except in rinsing under cold tap water. The variation observed in rinsing could be a result of minor differences in water flow rates.

DISCUSSION

In our experiments, with the exception of broccoli, rinsing the fresh produce under cold running tap water without prior soaking resulted in a lower reduction compared with rinsing with a presoaking step. These results suggest that soaking followed by rinsing in tap water yields more bacterial reductions. However, soaking for 30 min was considered too long to be practical and was not significantly different ($P > 0.05$) from a 2-min soaking. Veggie Wash had a significantly greater effect in reducing *L. innocua* in tomatoes, but not in apples, broccoli, and lettuce. Reductions of *L. innocua* in lettuce after soaking in 13% lemon, 5% vinegar, or Veggie Wash solutions were not statistically significant ($P > 0.05$) from lettuce soaked in cold tap water. Therefore, it is more cost effective to soak and clean fresh produce with cold tap water than with other homemade or commercial cleaning solutions. It should be noted that the model system used in this study was designed to evaluate the effectiveness of cleaning methods after a short period of surface contamination on fresh produce. However, during extended cold storage, *Listeria* can enter physiological states or associate with native microflora that make them more resistant to removal from produce surfaces.

Listeria innocua population reductions in both tomatoes and apples were greater than in lettuce and broccoli when subjected to the same treatment. The difference in *L.*

innocua reduction was probably due to the different morphological characteristics of tomatoes and apples. The smooth nature of apple and tomato surfaces and the coating materials applied during processing might have contributed to loose attachment of bacteria, which was easily washed off when subjected to cleaning procedures. Grooves on lettuce and broccoli provided more surface area for bacteria attachment and protected the bacteria from (made them less accessible to) sanitizing solutions. Efficacy of sanitizing agents as influenced by the morphology of the produce has been reported (18).

Our results indicated that stem and blossom ends of apples were more contaminated than the surface after soaking and rinsing. A previous study (2) suggested that the stem and calyx areas of apples provide sites where bacteria pool, attach, and form biofilms, which result in bacterial protection against sanitizers. Stems and blossom ends are known to resist cleaning and are difficult to access by rubbing (7, 15), which becomes a potential food safety hazard to the consumer. Transfer of *Salmonella montevideo* by cutting through contaminated tomato stem ends to the center and bottom parts has been reported (16). *Salmonella* has also been demonstrated to transfer from contaminated rind to the edible portion of cantaloupe (21). The transfer of pathogens from contaminated stem scar or blossom to the edible parts during the preparation of fresh fruits poses a health risk. Therefore, consumers should discard stem and blossom ends of fresh produce after washing under cold tap water to avoid transfer of contaminated pathogen to the edible parts. Parnell and Harris (19) reported that wetting, rubbing, rinsing, and drying methods are simple methods that reduced surface microorganisms on intact apples. Our results indicated that wiping apples with a wet or dry paper towel had the lowest population reductions compared with other treatments. Friction involved in wiping with wet/dry paper towel was not effective to reduce bacteria on apples and tomatoes. Therefore, wiping fresh produce with wet or dry paper towel without soaking and rinsing is not recommended.

Chlorinated water at 50 to 200 ppm active chlorine is currently being used at commercial levels to reduce micro-

bial contamination; however, the reduction is less than 2 log CFU/g on fruits and vegetables (3, 6, 8). Application of gaseous sanitizers on vegetables and fruits has also been reported to result in less than a 2.00-log bacterial reduction (13). Wei et al. (25) reported that washing tomatoes contaminated with low doses of *S. montevideo* with tap water was effective in removing the bacteria. In our study, rinsing lettuce, broccoli, apples, and tomatoes under cold running tap water had a *L. innocua* reduction within the range of 1.41 to 2.10 log CFU/g. The reduction range of water-based treatments in our study was generally comparable with reductions when chlorinated water, NaOCl, Tween 80, acetic acid, and 1% hydrogen peroxide were used on other foodborne pathogens (6, 26). On the basis of the model presented, vinegar and lemon solutions were not significantly different from cold tap water in reducing *L. innocua*. Wiping apples and tomatoes with wet or dry paper towel showed lower bacterial reductions compared with soaking and rinsing procedures. Extension efforts of U.S. Department of Agriculture and Food and Drug Administration does not recommend the use of commercial sprays or washes; washing produce with tap water is usually adequate (23, 24).

Results from this study suggest that washing produce under cold running tap water with rubbing and brushing, where applicable, has a potential to reduce surface bacterial contamination. Therefore, education and extension personnel might consider it appropriate to instruct consumers to rub or brush fresh fruits and vegetables under cold running tap water before consumption.

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