Research Paper

Efficacy of Surfactant Combined with Peracetic Acid in Removing Listeria innocua from Fresh Apples

EWA PIETRYSIAK,1 JULIANNE M. KUMMER,1 INES HANRAHAN,2 AND GIRISH M. GANJYAL1*

1School of Food Science, Washington State University, Pullman, Washington 99164-6376; and 2Washington Tree Fruit Research Commission, 1719 Springwater Avenue, Wenatchee, Washington 98801, USA

MS 19-064: Received 11 February 2019/Accepted 15 July 2019/Published Online 23 October 2019

ABSTRACT

Large amounts of water used in the apple packing process, the presence of organic matter, and difficult-to-clean equipment are vectors for contaminating apples with foodborne pathogens, such as Listeria monocytogenes. There is a need to develop new cleaning methods for fresh apples and evaluate their antimicrobial efficacy. A series of surfactants of different chemical properties (cationic lauric arginate [LAE], anionic sodium dodecyl sulfate [SDS], and nonionic Tween 20 [T20]) alone and combined with peracetic acid (PAA) were evaluated for their efficiency in the removal of L. innocua, a surrogate of L. monocytogenes, from fresh apples. Whole fresh apples were inoculated with L. innocua (7 log CFU/mL) by a dipping method, dried, and subjected to wash treatments with selected cleaning solutions (water, PAA, PAA-LAE, PAA-SDS, and PAA-T20). The contact angle between the cleaning solutions and the surface of the apples was measured. The antimicrobial activity of the cleaning solutions and the efficacy of the cleaning treatment were evaluated by enumeration of L. innocua from treated apples and visualized by scanning electron microscopy. Stem bowl and calyx cavities of the apple harbored higher bacteria concentrations (~4.82 log CFU per apple), compared with the equatorial section (~2.66 log CFU per apple). Addition of 0.1% of selected surfactants led to a significant decrease in surface tension of cleaning solutions and allowed better spreading on the apple surface. Surfactants combined with PAA solution resulted in higher L. innocua reduction compared with PAA alone; however, their efficacy was limited. The most effective cleaning solution was PAA-T20, with reduction of approximately 2.2 log. Scanning electron microscopy imaging confirmed that most bacteria were harbored inside the surface irregularities. PAA alone and with surfactants caused damage and deformation of bacteria cells. Cleaning apples with PAA combined with surfactants may improve microbial safety in whole apples; however, the efficiency of the decontamination treatment can be reduced because of variations in apple morphology.

HIGHLIGHTS

• Combining surfactants with PAA decreased the population of L. innocua on apples.
• Treating apples with PAA-T20 reduced the load of L. innocua by 2.2 log.
• Stem bowl and calyx cavity are difficult-to-reach areas during the cleaning operation.
• Cleaning treatments were not effective in removing all L. innocua from apples.

Key words: Decontamination; Listeria innocua; Peracetic acid; Surfactants; Whole fresh apples

Apples are one of the most widely consumed fruits in the United States and worldwide. Fruits, especially fresh, are an important part of a healthy diet and are an excellent source of vitamins, minerals, and dietary fiber (23). However, fresh fruits such as apples and cantaloupes have been associated with multistate outbreaks of listeriosis, a severe invasive illness caused by Listeria monocytogenes (6).

In 2014 and 2015, an outbreak of L. monocytogenes in caramel apples led to the death of at least 3 people and the hospitalization of 35 people across 12 states in the United States. The apples were contaminated in one apple packing facility located in California. The presence of outbreak-related L. monocytogenes on the packing line was confirmed by environmental testing (5). In December 2017, L. monocytogenes was detected on apples in an apple packing facility in Michigan during routine final product testing, which led to the recall of sliced product from the affected lot (24).

The typical apple packing process is a fast-paced, multiple-step process in which fruits are first unloaded into a dump tank and then washed, sorted, labeled, and packaged before being shipped for retail sales (17). The large amount of water used in the apple packing process, the presence of organic matter, and difficult-to-clean equipment are vectors for contaminating apples with foodborne pathogens, such as L. monocytogenes. The apple packing process does not
include a kill step, such as heat treatment, that would inactivate or eliminate pathogens on the apples. The safety of the final product relies on good cleaning and sanitation practices and preventing fruit from contamination.

The apple cleaning process is challenging because of several factors, such as irregular shape, hydrophobic surface, and complex peel morphology (16). The surface of an apple, similar to the surface of many other plants, is covered with a hydrophobic layer of epicuticular waxes (13), which play a protective role and govern water-repelling properties. In addition, the surface of an apple peel is covered with different types of microstructures, such as microcracks and lenticel, that can serve as harborage sites for bacteria and protect bacteria from cleaning operations and disinfection (16). The hydrophobic characteristic of apple surface may limit the decontamination efficiency of water and aqueous solutions of commonly used sanitizers, preventing them from reaching the bacteria. Thus, there is a need to develop new cleaning methods for fresh apples and evaluate their antimicrobial efficacy.

A study on the removal of *Escherichia coli* and *Listeria innocua* from fresh lettuce leaves showed enhanced efficiency by adding surface active agents (surfactants) to the wash treatment (10). A surfactant is a chemical compound with the ability to alter surface or interfacial free energy (20). The use of a surfactant during the washing process helps reduce the surface tension between the liquid and the solid, which facilitates spreading of the washing solution over the surface of the produce and improves surface decontamination (18). This ability comes from an amphiphilic character of the surfactants. Amphiphilic molecules consist of polar and nonpolar groups, resulting in dual affinity (21). The polar group of surfactants disrupts cohesive forces between the water molecules and thereby decreases the surface tension and facilitates the spread of liquid on the nonpolar surface.

Based on its dissociation in water, a surfactant can be classified as cationic, anionic, and nonionic (21). Chemical composition determines the functional properties of surfactants and their ability to react with other components (8).

Peracetic acid (PAA) is a strong oxidizer widely used in the fresh produce industry. PAA presents good antimicrobial properties at low temperatures and at a range of operational pH values (15). Whereas chlorine reacts with organic matter, producing carcinogenic compounds such as chloramines and haloquinones, PAA does not (4). After PAA degradation, harmless residues (acetic acid and oxygen) readily dissolve in water and have no effect on the environment (9). In a water solution, PAA generates reactive oxygen species that damage the bacteria cell membrane and denature the cell enzymes and protein, leading to bacterial death (1).

A combination of surfactants with sanitizers should increase their antimicrobial potential by reducing surface tension, improving their spreadability on the apple surface, and facilitating detachment and deactivation of the pathogens. A series of selected surfactants of different chemical properties alone and combined with PAA were evaluated to examine their efficiency in removal of *L. innocua* from fresh apples. The cleaning treatments were designed to represent conditions in a typical apple packing process. Attachment of *L. innocua* to different apple sections was analyzed. The contact angle between the cleaning solutions and the surface of the apples was measured. The antimicrobial activity of the cleaning solutions and the efficacy of the cleaning treatment were evaluated and visualized by scanning electron microscopy (SEM).

**MATERIALS AND METHODS**

**Apples.** Gala apples, grown conventionally and harvested at commercial maturity, were provided by Borton Fruit Co. (Yakima, WA), Zirkle Fruit Co. (Yakima, WA), and Stemilt Growers (Wenatchee, WA). These apples were not commercially waxed. Apples were stored in a walk-in cooler (~4°C, regular atmosphere) at Washington State University (Pullman) for up to 2 weeks until being analyzed. All apples were of similar size and free of visible surface defects, mechanical injuries, and dirt.

**Inoculum preparation.** A nonpathogenic isolate of *L. innocua* ATCC 51742 was used as a surrogate strain to *L. monocytogenes*. The appropriateness of using *L. innocua* as a surrogate for *L. monocytogenes* has been previously reported (14, 19). The strain was stored at ~65°C in tryptic soy broth with 0.6% yeast extract (TSBYE; Difco, BD, Sparks, MD) and 25% glycerol.

Cultures were activated aerobically in 10 mL of sterile TSBYE in 50-mL sterile tubes (Fisher Scientific Co., Hanover Park, IL) at 35°C for 24 h, followed by a second activation using a 1:250 dilution of the first activation in 250 mL of TSBYE, and grown for 24 h at 35°C. Cultures were centrifuged at 5,000 × g for 10 min at 4°C (Eppendorf 5810, Hamburg, Germany), washed in 10 mL of sterile phosphate-buffered saline (PBS; pH 7.4) and resuspended in 50 mL of PBS. The concentration of inoculum was verified by 10-fold serial dilutions in PBS, plated on tryptic soy agar with 0.6% yeast extract (TSAYE; Difco, BD), and followed by a 48-h incubation at 35°C. The stock inoculum was diluted appropriately with water at room temperature to prepare an inoculum of the desired concentration.

**Apple inoculation.** Whole apples were pulled from refrigerated storage and placed overnight on a benchtop to equilibrate the apple temperature to the room temperature. Apples were inoculated with *L. innocua* by submerging 15 apples in 5 L of inoculum suspension (with an approximate concentration of 10⁷ CFU/mL) (22). Apples were carefully turned in the inoculum for 10 min to uniformly spread the inoculum and then air dried at room temperature for minimum of 1 h before wash treatment.

**Enumeration of *L. innocua* on different sections of the apple.** Apple samples (cubes that were approximately 3 by 3 by 1 cm) were cut using a sterilized knife from either core (calyx and stem bowl cavities) or equatorial sections (Fig. 1). Apple samples were placed in sterile stomacher bags with filters (Fisher Scientific), weighed, mixed with 25 mL of sterile PBS with 0.2% Tween 20 (T20) solution, and homogenized using a Stomacher 400 Lab blender (Steward Ltd., London, UK). Each sample was homogenized for 2 min at 300 rpm, allowed to rest for 1 min, and homogenized again for 2 min at 300 rpm. Homogenized suspensions were serially diluted in sterile PBS and spread plated with a turntable and glass rod on modified Oxford agar (Difco, BD). Plates were stored aerobically at 35°C for 48 h. The detection limit was approximately 2 log CFU per apple.
Preparation of cleaning solutions. Three types of surfactants were used: cationic lauric arginate (LAE; Cytoguard LA20, 20%, v/v; A&B Ingredients, Fairfield, NJ), anionic sodium dodecyl sulfate (SDS; Sigma, St. Louis, MO), and nonionic T20 (Sigma), alone and combined with PAA (Pace International, Wapato, WA). The cleaning treatments were prepared by adding LAE, SDS, and T20, respectively, to water to obtain solutions with a final surfactant concentration of 0.1% (w/w). For the solutions with PAA, the concentration of PAA was set at 80 ppm. PAA concentration was measured using a titration kit (LaMotte, Chestertown, MD).

Contact angle measurements. The contact angles of cleaning solutions, including water, 80 ppm of PAA, 0.1% LAE, 0.1% SDS, 0.1% T20, 0.1% LAE with 80 ppm of PAA, 0.1% SDS with 80 ppm of PAA, and 0.1% T20 with 80 ppm of PAA, were measured using the VCA Optima Video Contact Angle System (Ast Products, Inc., Billerica, MA). Apple samples (4 by 4 by 4 mm) were cut with a razor blade and placed on a microscope slide. Droplets (2 μL) of each wash solution were placed on the apple surfaces. The contact angles were recorded immediately. Nine measurements were performed for each treatment.

Antimicrobial activity of cleaning solutions. To examine the antimicrobial activity of the wash treatments, 0.2 mL of L. innocua stock solution was mixed with 9.8 mL of appropriate wash treatment (final concentration of 7 log CFU/mL), gently vortexed, and kept for 1 min at room temperature before plating on TSAYE plates. Plates were incubated at 35°C for 48 h for the microbial count.

Cleaning procedure. Applied treatments are summarized by Figure 2. Each time, three inoculated, untreated apples were subjected to microbial enumeration as a control.

Treatment 1. Following inoculation, apples were subjected to cleaning with one of the eight cleaning solutions (six apples for each treatment): (i) water, (ii) 0.1% LAE, (iii) 0.1% SDS, (iv) 0.1% T20, (v) 80 ppm of PAA, (vi) 0.1% LAE with 80 ppm of PAA, (vii) 0.1% SDS with 80 ppm of PAA, and (viii) 0.1% T20 with 80 ppm of PAA.

Apples were treated by spraying approximately 4.2 mL of cleaning solution on the surface of the apples using a plastic spray bottle and gently rubbing the apples with hands covered with gloves for 1 min. This was intended to simulate apple cleaning on the brush bed during the apple packing process. Half of the apples (three for each cleaning solution) were then sprayed with approximately 8.4 mL of water to rinse off detached bacterial cells. After cleaning treatment, the apples were left to dry under...
FIGURE 3. Contact angle of selected cleaning solutions on the apple surface. Representative images of PAA (A) and SDS (B) droplets on the apple peel surface used in the study. Mean ± standard deviation; n = 9. Bars labeled with different letters indicate a significant difference (P < 0.01).

The hood for a minimum of 1 h at room temperature. Experiments were repeated three times.

**Treatment 2.** Following inoculation, apples were subjected to cleaning with one of the five cleaning solutions (six apples for each treatment): (i) water, (ii) 80 ppm of PAA, (iii) 0.1% LAE with 80 ppm of PAA, (iv) 0.1% SDS with 80 ppm of PAA, and (v) 0.1% T20 with 80 ppm of PAA. Apples were treated by dipping in 250 mL of cleaning solution for 10 s followed by gently rubbing with hands covered with gloves for 1 min. Apples were then sprayed with approximately 8.4 mL of 80 ppm of PAA to rinse off and inactivate detached bacterial cells. After cleaning treatment, the apples were left to dry under the hood for a minimum 1 h at room temperature. Experiments were repeated three times.

**Enumeration of *L. innocua* after cleaning.** *L. innocua* was enumerated from the apples as described in “Enumeration of *L. innocua* on Different Sections of the Apple.” Only the apple core section (stem bowl and calyx cavities) was analyzed, because bacteria attach primarily in this section.

**SEM imaging.** Apple peel pieces (approximately 4 by 4 by 2 mm) were gently cut from the different apple sections with a razor blade. The cut pieces were fixed overnight (4°C) in 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.05 M PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid) buffer). The pieces were then washed with 0.05 M phosphate buffer (2 × 10 min) and water (1 × 10 min), fixed in a solution of 2% osmium tetroxide for 1 h at room temperature, rinsed twice with deionized water (2 × 10 min), and freeze-dried (Virtis, The Virtis Company, Inc., Gardiner, NY). Freeze-dried pieces were then mounted to aluminum stubs (specimen mount, Ted Pella, Inc., Redding, CA) using Pelco tabs (Ted Pella) and self-adhesive paper tracks. The stubs were gold coated on a Hummer sputter coater (Anatech, San Jose, CA) and observed with SEM (Quanta 200F, FEI Co., Hillsboro, OR) at an accelerating voltage of 20 kV. Samples were examined at a magnification range of ×200 to ×40,000. Representative images were recorded and analyzed.

**Statistical analysis.** Results were expressed as means with standard deviation. Data were analyzed using one-way analysis of variance. The Fisher least significant difference test was performed using Minitab 18 (Minitab Inc., State College, PA). *P* values of 0.01 or less were considered statistically significant.

**RESULTS AND DISCUSSION**

**Enumeration of *L. innocua* on different sections of an apple.** The core section of the apple surface (stem bowl and calyx cavity; Fig. 1) harbored significantly more cells of *L. innocua* than the equatorial section (Table 1). The core section of the apple harbored a relatively high amount of bacteria (~4.82 log CFU per apple), compared with the equatorial section (~2.66 log CFU per apple). Similar results were previously reported in studies on attachment of *E. coli* to the apple surface (3, 11, 12), where the stem bowl and calyx sections had the highest concentrations of bacteria. Thus, in this study, bacteria attachment and reduction were examined only in the stem bowl and calyx cavities of the apple.

We have previously shown that the stem bowl and calyx sections of apples are heavily covered with different types of microstructures, such as microcracks, lenticels, and trichomes, which serve as harbor sites for bacteria attached by physical entrapment (16). Microstructures may also protect bacteria from cleaning treatments by preventing the chemicals from reaching the harbored cells because of the surface tension. In addition, apples are characterized by an irregular shape and the presence of calyx and stem bowl cavities. The stem bowl cavity of a Gala apple can be 1 to 2 cm deep. The calyx cavity is not usually as deep as the stem bowl cavity; however, some apple varieties have open channels into the core region, allowing penetration of inoculum to the inside of the apple (3). The micro- and macrostructures of apples are hard to reach into during the typical industrial cleaning process. These important characteristics should be considered when developing new methods of fresh apple decontamination.

**Contact angle measurements.** The surface of an apple peel is covered with a natural layer of wax that governs hydrophobic character and water-repelling properties (13). The high surface tension of water prevents it from spreading on the apple surface. PAA, a commonly used sanitizer in the apple packing industry, at a concentration of 80 ppm (the

| Table 1. Attachment of *L. innocua* to different apple sections<sup>a</sup> |
|-----------------|-----------------|
| Apple section   | Log CFU/apple<sup>b</sup> |
| Core section    | 4.82 ± 0.71 A   |
| Equatorial section | 2.66 ± 0.62 B |

<sup>a</sup> The initial population of *L. innocua* used in this study was 6.94 ± 0.02 log CFU/mL.

<sup>b</sup> Means in this column not sharing the same letter are different (*P* < 0.01). Mean ± standard deviation; *n* = 15.
maximum allowed concentration for use in fresh produce cleaning) (7), did not change the surface tension of the water (Fig. 3). The addition of surfactants led to a significant decrease in the surface tension of cleaning solutions that was confirmed by contact angle measurements on the apple surface. The addition of 0.1% of selected surfactants allows better spreading of the cleaning solution on the surface of the apple and coverage of a larger surface with the same amount of liquid. SDS alone and combined with PAA exhibited the highest potential in reducing the surface tension of the solution. Lower surface tension and better spread of the cleaning solution on the apple surface may help reach microstructures present on the surface of the apple and lead to more effective decontamination.

Antimicrobial activity of cleaning solutions. To determine the antimicrobial potential of the selected cleaning solutions against L. innocua, the antimicrobial activity was examined (Fig. 4). The initial concentration of L. innocua was 7 log CFU/mL. All cleaning solutions containing PAA deactivated L. innocua after 1 min of contact time. PAA deactivates bacteria by generating reactive oxygen species, which damage cell membrane lipids and DNA, impair the enzymatic and transport systems by denaturation of proteins, and oxidize the sulphydryl bonds in cell membranes, thus increasing permeability and leading to bacterial death (1). Surfactants LAE and SDS also showed antimicrobial activity. LAE (0.1%) and SDS (0.1%) reduced the population of L. innocua by 6.9 log and 4.1 log, respectively. LAE, a compound derived from L-arginine, lauric acid, and ethanol, can damage the bacteria cell membrane and result in cell lysis (2). The biocidal effect of SDS is also based on its action on the cell membrane, and its antimicrobial potential was previously reported (25). T20 did not show antimicrobial activity alone, but with PAA, it was as effective as LAE and SDS. Combining selected surfactants with PAA dramatically increased their antimicrobial potential. Cleaning solutions that contained both surfactant and PAA showed high antimicrobial efficacy with short contact time (1 min). In addition, these cleaning solutions presented a lower contact angle when in contact with the apple surface compared with the PAA solution (Fig. 3). This may facilitate spreading of a cleaning solution on the apple surface and increase decontamination efficacy.

Enumeration of L. innocua after cleaning. All treatments used in this study were designed to simulate as much as possible the real conditions in an apple packing house. Commercial apple packing is a fast-paced process in which apples are first submerged in a dump tank and flume and then washed, predried, waxed, dried, sorted, and packed. The typical wash step during the apple packing process consists of rinsing apples with water and spraying cleaning solutions (which is optional). Apples are washed on a brush bed by the rotating movement of brushes. The time spent washing apples on a brush bed is limited (5 s to 2 min). Next, apples are rinsed with water or sanitizer solution and predried. Chemical composition, amount of cleaning solution, pH, and temperatures are specific to company practices (information gathered by us during survey visits in apple packing houses). The important factors that need to be considered when developing new methods of apple cleaning include the packing line setup and the limited time available for cleaning to be completed. Therefore, in this study, we evaluated two treatment settings (Fig. 5).

Treatment 1 consisted of cleaning apples with cleaning solutions, with or without water rinse. Cleaning apples with
water or with LAE, SDS, and T20 solutions did not significantly decrease the concentration of bacteria on the apple surface (Fig. 5). Application of PAA, with or without rinsing, reduced the concentration of *L. innocua* on the surface of the apples by 1.1 or 1.3 log, respectively. PAA-LAE (with and without rinsing) and PAA-T20 (with rinsing) were the most effective cleaning solutions used in this treatment. However, the maximum reduction observed was only up to 1.6 log, recorded after cleaning apples with PAA-T20.

Combining surfactants with PAA increased the efficacy of cleaning treatments. The use of surfactants decreased the surface tension and facilitated spreading of the solution on the apple surface, which might lead to an increased reduction of bacteria. Huang and Nitin (10) reported enhanced detachment of *L. innocua* from fresh lettuce leaves by use of surfactants when compared with cleaning with only water. Increased bacteria detachment was related to a decrease of the contact angle between the wash solutions and the surface of the leaves.

Our results showed that surfactants combined with sanitizer enhanced *L. innocua* reduction; however, their efficacy was limited, and intact bacteria cells were still detected on the apple surface.

Based on the results of Treatment 1, only solutions that were a combination of surfactant and sanitizer were used in Treatment 2. In this treatment, apples were first dipped into the cleaning solution, rubbed, and sprayed with sanitizer. Sanitizer spray was applied to inactivate the detached bacteria. Treatment 2 resulted in greater bacteria reduction compared with Treatment 1 (Fig. 6). Cleaning apples with water or PAA followed by PAA spray did not significantly reduce the concentration of bacteria. Probably, these cleaning solutions were not able to reach and detach or deactivate the bacteria cells. The addition of surfactant to solutions from other tests in this study.
PAA increased the bacterial reduction. All treatments containing surfactant (PAA-LAE, PAA-SDS, and PAA-T20) were more effective than water or PAA solution alone. Surfactants such as LAE, SDS, and T20 reduced the surface tension of water by adsorption at the liquid-solid interface, which helped reach bacteria harborage sites and increase bacteria reduction. However, applied treatment did not remove all bacteria. The maximum bacteria reduction, ~2.2 log, was recorded for samples treated with PAA-T20.

**SEM imaging.** SEM confirmed that applied treatments were not entirely effective in removing *L. innocua* from the surface of the apple peel. Bacteria cells were easy to locate in microcracks. *L. innocua* cells on untreated apples had a regular rod shape with a smooth surface (Fig. 7A).

In apples treated with cleaning solutions containing PAA alone or in combination with surfactants, *L. innocua* cells were deformed and exhibited a collapsed contour and damaged walls (Fig. 7B and 7C). Furthermore, in apples treated with PAA-LAE, bacteria clumping was observed (Fig. 7D). Nevertheless, not all bacteria cells were affected by cleaning treatments. A portion of the bacterial cells found in microcracks seemed to be unaffected (Fig. 7E and 7F), suggesting that the cleaning treatment did not reach the inside of all microcracks. The results of SEM analysis confirmed the protective role of microcracks and emphasizes the importance of considering apple morphology in removal of bacteria from the apple surface.

In summary, this study demonstrated the potential of use of PAA combined with surfactant in the apple cleaning process and emphasized the importance of apple morphology in bacteria attachment and decontamination. *L. innocua* attached primarily to the stem bowl and calyx cavities and further settled mainly in the surface microstructures, such as lenticels and microcracks. The use of a surfactant decreased the surface tension of the cleaning solutions, improved their spread on the hydrophobic surface of the apple, and increased bacterial reduction. However, part of the bacteria settled in hard-to-reach sites that were protected from cleaning operations and disinfection. Research is needed to optimize the cleaning treatment of apples during the commercial apple packing process. In such studies, additional surfactants at higher concentrations and in combination with other antimicrobial treatment should be evaluated. Cleaning treatments need to be designed to suit the conditions and equipment used in a typical apple industrial packing line.

**ACKNOWLEDGMENTS**

We thank the Franceschi Microscopy and Imaging Center at Washington State University for assistance in SEM imaging. We thank Dr. Amit Bandyopadhyay and Mr. Indranath Mitra from the School of Mechanical and Materials Engineering at Washington State University for their help in contact angle measurements and Dr. Stephanie Smith and Dr. Aleksandra Checinska Sielaff from the School of Food Science at Washington State University for their guidance in microbiological experiments. We thank Burton Fruit Co., Zirkle Fruit Co., and Stemilt Growers for providing the fruit used in this research. We also thank the Washington Tree Fruit Research Commission for providing the funding for conducting this research. This work was supported in part by the U.S. Department of Agriculture National Institute of Food and Agriculture, Hatch project, with accession 1016366.

**REFERENCES**


17. Predmore, A., and J. Li. 2011. Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combina-


