Investigation of handling practices for fresh produce and the efficacy of commercially available produce washes on removal of pathogens and natural microflora on whole cantaloupe surfaces

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A B S T R A C T

The objectives of our study were to collect descriptive data of handling practices for fresh produce and to evaluate the efficacy of commercially available washes for reducing pathogens and natural microflora on whole cantaloupes. A total of 51 people answered a produce handling survey. Information from the survey was used to develop two experiments. In one experiment, cantaloupes were washed with water (control), 9% vinegar solution, or a commercial antimicrobial for fruit and vegetables (CAFVT) for 2 min by using a continuous water motion system. Cantaloupe surfaces were tested on day 0 for initial aerobic plate counts (APC); then wedges or cubes in refrigeration storage were tested on days 1, 3, and 6 for APC. In a second experiment, Salmonella spp. (8.54 log10 CFU/ml) or Listeria monocytogenes (8.52 log10 CFU/ml) inoculated cantaloupes were washed with cold tap water (control) or a commercial produce wash (CPW) for 30, 60, or 120 s. APC populations for surfaces of untreated and cantaloupes treated with water, 9% vinegar solution, or CAFVT were 3.88, 3.39, 3.01, and 2.98 log10 CFU/cm², respectively. Cubes from treated cantaloupes reached populations between 6.2 and 8 log10 CFU/g on day 6 of storage, while populations of wedges from treated cantaloupes reached populations between 5.2 and 7.6 log10 CFU/g on day 6 of storage. The CPW was capable of reducing ca. 1.26 and 1.12 log10 CFU/cm² of Salmonella spp. and L. monocytogenes populations, respectively, on the surface of cantaloupes. Pathogenic populations for residual wash water were reduced below the detection limit of 1.95 log10 CFU/ml.

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1. Introduction

Changes in life style and the awareness of health benefits have markedly increased the demand and consumption of fruits and vegetables (Bruhn, 2009). However, along with this increase in consumption, produce-related outbreaks and illnesses have been recognized worldwide (Lynch, Tauxe, & Hedberg, 2009).

In the United States (U.S.), melons, especially cantaloupes, have been associated with foodborne illness outbreaks linked to Salmonella serovars and Listeria monocytogenes. In 2012, a multistate outbreak of salmonellosis resulted in a total of 261 ill persons with outbreak strains of S. Typhimurium (228) and S. Newport (33), with 3 deaths reported in the state of Kentucky (CDC, 2012). During 2011, multistate outbreaks of listeriosis and salmonellosis (S. Panama) were linked to cantaloupe consumption. The listeriosis outbreak involved 147 ill persons, 33 deaths, and 1 miscarriage in 28 states, while salmonellosis outbreaks resulted in only 20 ill persons (CDC, 2011a, 2011b). Moreover, in 2008 another salmonellosis outbreak (S. Litchfield) involved 51 ill persons in 16 states in the U.S. and 9 illnesses in Canada, no deaths were reported (CDC, 2008).

Fresh produce, including cantaloupes, can become contaminated with pathogenic microorganisms at any point along the farm-to-table food chain (e.g. production, harvesting, packing, processing, and foodservice handling). Washing plays an important role on fresh produce quality and safety. Washing procedures are used mainly to remove soil, chemical residues (i.e. pesticides), and other debris from the surface of produce. Washing procedures and sanitizing agents are of concern because inadequate handling can result in produce damage, cross-contamination, and chemical and/
or microbial contaminant internalization (Pao, Long, Kim, & Kelsey, 2012; Park, Gray, Oh, Kronenberg, & Kang, 2008). Methods to reduce microbial contamination on the surface of produce usually involve the use of sanitizers and mechanical action.

Studies exploring the efficacy of various washing treatments including antimicrobial chemicals such as hydrogen peroxide (Ukuku, 2006), peracetic acid (Rocha-Bastos, Ferreira-Soares, Andrade, Arruda, & Alves, 2005), nisin and its combination with EDTA, sodium lactate, and potassium sorbate (Ukuku & Fett, 2004), plant antimicrobial extracts (Upadhyay, Upadhyay, Mooyottu, & Kollanoor-Johny, 2014), and hot water surface pasteurization (Fan, Annois, Beaulieu, & Sites, 2008; Ukuku, 2006) in reducing pathogenic microorganisms on cantaloupes have yielded mixed results. However, washing treatments applied by immersion in the washing solutions with or without physical action (e.g., scrubbing or agitation) reduced attachment of pathogenic microorganisms on cantaloupes surface by 2–4.9 log_{10} CFU units. It is important to note that as the time interval between contamination and washing treatment application increases, washing treatment efficacy decreases (Gil, Selma, Lopez-Galvez, & Allende, 2009; Sapers, 2001).

The incidence of food-related outbreaks in school settings and the effort to improve availability of fruits and vegetables in meals offered to young children prompted the need to develop interventions and prevention strategies for handling produce at the school level. Reported foodborne disease outbreaks in schools have been analyzed to identify etiology, mode of transmission, number of affected children, morbidity and mortality, and strategies of prevention (Daniels et al., 2002; Lee & Greig, 2010; Venuto, Halbrook, Hinners, Lange, & Mickelson, 2010). Because limited information is known about fruit and vegetable handling practices in schools, and such information is imperative for the development and implementation of produce intervention strategies, there is a need to conduct research to examine produce-handling practices in school foodservice facilities. Therefore, the objectives of this study were to collect descriptive data of handling practices for fresh produce used in foodservice facilities and to evaluate the efficacy of commercial washes for reducing pathogens (Salmonella spp. and L. monocytogenes) and natural microflora on whole cantaloupes.

2. Materials and methods

The study consisted of three phases; a survey (phase I) of school foodservice employees that was conducted between June and July 2014. The survey consisted of 23 questions, exploring four different variables related to produce washing: (1) identification of personnel responsibilities; (2) equipment in facilities for washing produce; (3) produce washing practices; and (4) produce storage practices. Based on results obtained from phase I, two experiments (phases II and III) were conducted to evaluate the efficacy of commercial washes for reducing natural microflora and pathogens on whole cantaloupes, respectively.

2.1. Investigation of handling practices for fresh produce (phase I)

2.1.1. Questionnaire

For the development of the questionnaire, employees (n = 2) of a large foodservice facility were interviewed to gather information for the procedures used to wash, prepare, and store produce (e.g., lettuce, tomatoes, and cantaloupes), then employees were asked to demonstrate practices and observational data collected. Utilizing this information, a draft of the questionnaire was prepared and then reviewed by researchers at the Center of Excellence for Food Safety Research in Child Nutrition Programs at Kansas State University, Manhattan, Kans., who work closely with school foodservice personnel. Appropriate modifications included changing the order of questions, organizing questions by categories and providing questions with multiple responses to decrease the overall response time. For the final questionnaire, a total of 23 questions were developed to assess food safety practices of interest and were organized in four main categories: (1) identification of personnel responsibilities; (2) equipment in facilities for washing produce; (3) produce washing practices; and (4) produce storage practices.

2.1.2. Sample selection and data collection

Through the collaboration of the Food Science Institute and the Center of Excellence for Food Safety Research in Child Nutrition Programs at Kansas State University and the Institute of Child Nutrition at the University of Mississippi, Oxford, Miss. Questionnaires were provided to a total of 52 foodservice employees in the states of Kansas (18 attendees) and Virginia (34 attendees) attending state workshops. Participation of the employees was voluntary and anonymous. Data were collected from June to July 2014.

2.1.3. Data analysis and further research

Descriptive statistics were used to assess participants’ responses. Microsoft Excel (Excel:mac 2011 version 14.4.6) was used to arrange data, obtain frequencies, calculate medians and percentages, and to depict results in graphs and tables.

Results of Phase I provided information regarding the processing to which produce is subjected before it is served in school cafeterias. For example, the questionnaire results identified that 10% of the foodservice facilities, under the study, used antimicrobial products to wash produce (e.g., FIT, Eat Cleaner) while 53% used tap water. It was also identified that approximately 20% of respondents kept fresh-cut produce (e.g., prepared shredded lettuce, sliced tomatoes, and cubed or wedged cantaloupes) in refrigeration storage for 1 day while approximately 6% kept the prepared produce for 3 days. Produce was usually stored in plastic containers with lids. Remaining results from the survey are further discussed in the results and discussion section of this manuscript.

Produce, such as cantaloupes, have been identified as the food vehicle for salmonellosis and listeriosis outbreaks (CDC, 2015). These outbreaks have stressed the need to investigate disinfectant agents for their effectiveness in reducing populations of microorganisms in produce. Thus, the information obtained in phase I was used to develop experiments defined in phase II and phase III with the purpose of determining efficacy of washing techniques for reducing pathogens (Salmonella spp. and L. monocytogenes) and natural microflora on whole cantaloupe surfaces.

2.2. Efficacy of washing treatments on natural microflora of whole cantaloupes (phase II)

2.2.1. Experimental design

Per discussion with the produce manager at a retail store in Manhattan, Kans., cantaloupes from the same provider and production lot were purchased from this local retail store. Each item was inspected to ensure absence of bruises or lacerations on the surface of the produce. Cantaloupes were washed separately with water (control), a solution of vinegar, and a commercial antimicrobial fruit and vegetable treatment (CAVFT). A whole unwashed and untreated cantaloupe was used to determine initial microflora load. After washing, each cantaloupe was manually cut in half. One half was cut into four visually equal-sized wedges (slices) with the rind intact and the remaining half was cut into cubes with rinds removed carefully. Then cantaloupes were stored in plastic containers with lids at 4 ± 1°C for 6 days. Treated and untreated cantaloupes were tested after washing treatment on day 0 and on...
day 1, 3, and 6 of storage. Two replications were conducted, and samples of each treatment were analyzed in duplicate. The average was used for statistical analysis.

2.2.2. Washing, cutting, culling, and storage procedures

Cantaloupes (Cucumis melo var. reticulatus, weighing 1552.26 ± 262.54 g) were washed separately with tap water (pH = 9.7; free chlorine = 2.78 ppm), which was used as control. The higher pH of water was verified through the City of Manhattan’s Water Division. Treatments included a 9% vinegar solution containing 0.45% acetic acid (pH = 3.02) which was prepared by mixing 12 L of distilled white vinegar (5% acetic acid; The Kroger Co., Cincinnati, Ohio) with 120 L of tap water, and a commercial antimicrobial fruit and vegetable treatment (CAFVT; pH = 2.82; containing lactic acid (1061±1391 ppm)), sodium hydrogensulfate, dodecylbenzenesulfonic acid (76–111 ppm); Ecolab, St. Paul, Minn.) for 120 s by using a continuous water motion system (Model 50/SP66L2B1; Produce Soak by Power Soak Systems, Kansas City, Mo.). Temperature of water used to prepare washing treatments was 18 ± 1 °C.

The continuous water motion washing system consisted of a stainless steel wash tank with two bays (ca. 150 L), a stainless steel self-draining parallel flow pump, a pump motor connected to the wash tank, water inlet holes which run full length of the back wall of the wash tank, and six low profile wash jets (each bay with low profile jets; average flow rate ca. 10 gpm per jet) located above wash pump inlet holes. During the washing operation, the pump located on a side of the wash tank was fed with water in a first direction via a pump inlet connected to an intake port passing through the right side wall of the wash tank, and then water was impelled out from the pump in a second direction substantially parallel to the first direction via a pump outlet connected to an outlet chamber and wash jets (US Patent No. 2002335694).

After application of washing treatments, the surfaces of cantaloupes were tested for microbial enumeration (day 0). To investigate the effect of washing treatment over storage time, each cantaloupe was manually cut in half and its seeds removed using a sterile stainless steel knife. One half was cut into four visually equal-sized wedges (ca. 16 × 5 cm) with the rind intact. The remaining half was further cut into cubes (ca. 2 × 2 cm) with rinds removed carefully. Cantaloupes were stored in plastic containers (Snapware®, Mira Loma, Calif.) at 4 ± 1 °C for a total of 6 days and samples of wedged and cubed cantaloupes were separated and tested on days 0, 1, 3, and 6.

2.2.3. Sampling and enumeration procedures

Populations of cantaloupe native microflora on treated and untreated cantaloupes were determined. For day 0 sampling only, a sterilized stainless steel cork-borer (ø = 3.8 cm) was used to randomly mark a total of five rind plugs per cantaloupe, then the rind plugs were removed with a sterile scalpel. The procedure to remove the plugs consisted of cutting around the core-borer mark and excising the circular area of rind tissue to a depth of 1 ± 0.5 mm, resulting in a composite sample (56.7 cm2). The composite sample was placed in a sterile filtered stomacher bag (177 mm × 305 mm; Fisher Scientific, Pittsburgh, Pa.) in which 50 ml of sterile 0.1% peptone water (Difco; Franklin Lakes, N.J.) was added and then stomached on medium speed for 1 min (Seward 400 Stomacher, Seward Limited; Worthing, Great Britain) and subsequently serially diluted by using 9 ml of 0.1% peptone water blanks. For all other sampling days (1, 3, and 6), pieces of cubed and wedged cantaloupe from each washing treatment were selected and cut with a sterile stainless steel knife to obtain 30 ± 0.3 g samples.

When preparing wedged cantaloupe samples, it was ensured that each sample (30 ± 0.3 g) included the rind portion attached to the fruit flesh. Then samples were transferred to a sterile stomacher bag in which 300 ml of sterile 0.1% peptone water was added and then stomached on medium speed for 1 min and subsequently serially diluted by using 9 ml of 0.1% peptone water blanks. All samples were surface plated (0.25 ml aliquots in quadruplicate or 0.1 ml aliquots in duplicates) onto tryptic soy agar (Difco; Flanklin Lakes, N.J.) and incubated at 37 °C for 18–24 h for total aerobic plate counts (APC).

2.2.4. Statistical analysis

Microbial data (CFU/g) were analyzed after log transformation. The experiment followed a randomized complete block (replication as block factor) with a split–split plot design. Data was analyzed using the PROC GLIMMIX procedures with NOBOUND option of SAS version 9.4 (SAS Institute, Cary, N.C.). Washing treatments (washed whole cantaloupes) were considered as whole plot factors, and microbial populations over time were considered as subplot factors, and shape type (wedged or cubed cantaloupe pieces) and microbial populations over time were considered as subplot factor and sub-subplot factors, respectively. Appropriate interactions were tested first at a significant level of 0.05, followed by test of main effects. The SLICEDIFF option was used to explore the differences in the level of one effect inside the levels of other effect. Then, appropriate corresponding least squares means were determined and pairwise comparisons were conducted using Bonferroni’s adjustment.

2.3. Effectiveness of a commercially available fruit and vegetables wash for reducing pathogens on whole cantaloupes (phase III)

2.3.1. Experimental design

For phase III trials, whole cantaloupes were inoculated with either a five-strain cocktail of Salmonella spp. or a three-strain cocktail of L. monocytogenes. Cantaloupes were washed separately with tap water (as control) and a commercial produce wash (CPW) at various exposure times (30, 60, or 120 s). L. monocytogenes inoculated cantaloupes were treated with the commercial produce wash and tap water for only 120 s exposure time. The trials were replicated five times for Salmonella spp. inoculated cantaloupes and three times for L. monocytogenes inoculated cantaloupes.

2.3.2. Bacterial strains

Mixtures of each pathogen isolated from different sources were used as inocula. Salmonella spp. strains used in the study included RM 3363 (serovar Poona), a human isolate associated with a cantaloupe outbreak in 2002; RM 6832 (serovar Newport), isolated from cantaloupe at a food distribution center as part of large survey; RM 2247 (serovar Baildon), a clinical isolate associated with outbreak due to tomatoes from Florida; RM 6825 (serovar Gaminara), isolated from tomato at a food distribution center as part of large survey; and ATCC 13311 (Salmonella Typhimurium, Manassas, Va.), a human feces isolate. L. monocytogenes strains included RM 3818 (serotype 4b), isolated from mint; ATCC 19115 (serotype 4b, Manassas, Va.), a human isolate; ATCC 19118 (serotype 4e, Manassas, Va.), a chicken isolate; and SLR-2249 (laboratory strain with the ActA gene removed, St. Cloud, Minn.). All RM strains were kindly provided by Dr. Robert Mandrell (USDA ARS, Albany, Calif.).

2.3.3. Inoculum preparation

For inocula preparation, one loopful of each culture strain was used to inoculate 9 ml of tryptic soy broth (TSB; Difco; Flanklin Lakes, N.J.) and incubated at 37 °C for 24 h. A final transfer of 0.5 ml was made into 30 ml of TSB, which was incubated at 37 °C for 18–24 h. Cells of each strain were collected by centrifugation (4960 × g for 15 min; JA-17 rotor, Model J2-21 M/E; Beckman Coulter, Inc., Pasadena, Calif.) at 4 °C. The cell pellets were then
resuspended in 30 ml of sterile 0.1% peptone water (Difco; Franklin Lakes, N.J.), and transferred into a small plastic vial equipped with an atomizer to form a mixed strain cocktail. The same procedures were used for the preparation of *S.* spp. and *L. monocytogenes* cocktail inoculums. The cell density of *S.* spp. and *L. monocytogenes* cocktail inoculums was 8.54 and 8.52 log10 CFU/ml, respectively, as determined by plating serial dilutions onto xylose-lysine deoxycholate (XLD; Difco; Franklin Lakes, N.J.) for *Salmonella* spp. or modified Oxford medium (MOX; Difco; Franklin Lakes, N.J.) for *L. monocytogenes*, with incubation at 37 °C for 24 h. The inoculum was maintained at 22 ± 2 °C and applied to produce within 1 h of preparation.

### 2.3.4. Procedure of inoculation

Per discussion with the produce manager at a retail store in Manhattan, Kans, and purchaser from K-State Dining Services, cantaloupes from the same provider and production lot were obtained from the K-State Dining Services and local retail stores in Manhattan, Kans. Cantaloupes were stored at 4 °C for no more than 24 h prior to inoculation; before inoculation, samples were tempered at room temperature (22 ± 2 °C) for approximately 2 h.

Inside a biosafety cabinet, a fine mist of the inoculum (ca. 8–10 ml per eight or ten full sprays, respectively) was sprayed onto the cantaloupe’s surface using a plastic bottle with an atomizer (8 oz, high-density polyethylene (HDPE), The Bottle Crew, West Bloomfield, Mich.). To assure for complete inoculum coverage, cantaloupes were rotated by using a glove-covered hand. After inoculation, cantaloupes were allowed to dry inside the biosafety cabinet for 1 h to permit cell attachment. The same procedure was repeated for all cantaloupes inoculated either with *Salmonella* spp. or *L. monocytogenes*.

### 2.3.5. Washing procedures

Cantaloupes (C. melo var. reticulatus, weighing 1552.26 ± 262.54 g) inoculated with *Salmonella* spp. as described above were washed separately with the commercial produce wash (citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract; HealthPro Brands, Cincinnati, Ohio; pH = 3.6) or cold tap water (pH = 9.4; free chlorine = 2.78 ppm; 20 ± 2 °C; chloride = 50 ppm) for three different exposure times (30, 60, and 120 s). Cantaloupes inoculated with *L. monocytogenes* were washed with the commercial produce wash and cold tap water (20 ± 2 °C) for 120 s. The commercial fruit and vegetables wash treatment was prepared by mixing the produce wash product in powder form (containing citric acid, sodium lauryl sulfate, sodium carbonate, magnesium carbonate, and grapefruit oil extract; 28 g) with 8 L of cold tap water according to the manufacturer’s directions (HealthPro Brands, Cincinnati, Ohio).

Washing treatments were applied by submerging the cantaloupes under the surface of the wash solutions and stirring with a sterile L spreader to ensure for complete coverage and contact of cantaloupe’s surface with wash solution. A metal colander disinfected with 70% ethanol (Ethanol 200 proof, Decon Laboratories, INC., King of Prussia, Pa.) was used to hold cantaloupes during washing. After the treatment application, cantaloupes were rinsed with tap water (1 L per unit) and then allowed to dry for 30 min before sampling.

### 2.3.6. Sampling and enumeration procedures

A sterilized stainless steel cork-borer (a = 3.8 cm) was used to randomly mark a total of five ring plugs per cantaloupe, then ring plugs were removed with a sterile scalpel. The procedure to remove the plugs consisted in cutting around the core-borer mark and excising the circular area of rind tissue to a depth of 1 ± 0.5 mm, resulting in a composite sample (56.7 cm^3^).

The composite sample was placed in a sterile filtered stomacher bag (177 mm × 305 mm; Fisher Scientific, Pittsburgh, Pa.) and 30 ml (*Salmonella* spp. samples) or 50 ml (*L. monocytogenes* samples) of sterile 0.1% peptone water was added to the bags that were then stomached on medium speed for 1 min (Seward 400 Stomacher, Seward Limited; Worthing, Great Britain). Samples were serially diluted by using 9 ml of 0.1% peptone water, and then surface plated (0.1 ml) onto XLD media for *Salmonella* spp. recovery or MOX media for *L. monocytogenes* recovery. Additionally, non-inoculated cantaloupes were sampled, using the same procedure, for standard aerobic plate counts. Samples were serially diluted and plated onto tryptic soy agar and incubated at 36 °C for 24 h to estimate aerobic plate counts.

### 2.3.7. Statistical analysis

For phase III trials, a randomized complete block design (RCBD), with replication as block factor was used to test the effects of washing treatments in combination with exposure time on reducing *Salmonella* spp. populations and a generalized RCBD with repetition day as block factor was used to test the effects of washing treatments on reducing *L. monocytogenes* populations. Data sets were analyzed using PROC MIXED in SAS version 9.4 (SAS institute, Cary, N.C.) with washing treatment and exposure time being treated as fixed effects. When pertinent, two-way interactions were tested first at a significant level of 0.05, followed by a test of main effects. The appropriate corresponding least squares means were determined and pairwise comparisons were conducted using Fisher’s protected LSD. Mean log10 reductions and associated standard errors were estimated from contrasts of the treatment combination minus the inoculated samples at each trial.

### 3. Results and discussion

#### 3.1. Investigation of handling practices for fresh produce (phase I)

##### 3.1.1. Foodservice personnel identification

A total of 51 people responded to the survey: 61% (31) were school cafeteria employees, 8% (4) worked for a State Agency, and 2% (1) responded to USDA personnel. Additionally, 29% (15) of the respondents answered the questionnaire based on observed practices in foodservice facilities as food safety consultants, School Food Authority (SFA), board of education member, school district office-clerk, hospital foodservice, or private school foodservice.

##### 3.1.2. Type of produce

Respondents were asked to indicate all types of fruits and vegetables used in school facilities. Ninety-two percent (47 of 51) of respondents indicated using whole fresh vegetables in their facilities, 82% (42) reported using pre-prepared vegetables, only 10% (5) reported to use fruits and vegetables in other forms (e.g., canned, bulk packaged), 6% (3) reported not using vegetables in their facilities, and 6% (3) did not respond to this question.

##### 3.1.3. Produce washing, preparation, and storage

Fifty-three percent of respondents (27) reported using cold tap water to wash fresh fruit and vegetables, 31% (16) indicated using a washing sink with or without antimicrobial solutions, 10% (5) indicated using antimicrobial products, 2% (1) indicated using other methods, and 4% (2) did not respond to this question. Among the 10% of respondents that indicated using an antimicrobial solution, it was specified that they used antimicrobials available in the market such as FIT fruit and vegetable wash, Eat Cleaner fruit and vegetable wash, and a generic fruit and vegetable wash.

Questions regarding storage practices after washing of produce showed that 49% (25) of the respondents stored whole fruits and vegetables for next day preparation and consumption. Respondents...
specified whole fruits and vegetables washed and stored for next day preparation or consumption included: carrots (80%), tomatoes (72%), cantaloupes (64%), romaine lettuce (60%), green leaf lettuce (52%), and others (48%: apples, oranges, bananas, kiwi, grapes, berries, stone fruit, pears, and plums). Forty-three percent (22) reported that their facility does not store whole fruits and vegetables for next day consumption after washing, and 8% (4) did not respond to this question.

Unwashed whole fruits and vegetables were stored for different time periods, 43% (22) of respondents indicated storing unwashed whole fruits and vegetables for up to 7 days, 22% (11) of respondents indicated storing fruits and vegetables for 1 day (or overnight for use the following day), 10% (5) for 6 days, 8% (4) for 3 days, 8% (4) for 2 days, 8% (2) indicated this did not apply to their facility, and 2% (1) did not respond to this question.

Respondents were also asked to identify the type of containers used in their facilities to store fruits and vegetables. Seventy-three percent of respondents (37) indicated they stored fruits and vegetables in plastic containers with lids, 14% (7) used baking sheets covered with plastic bun bags, 6% (3) used baking sheets with racks and covered with plastic bun bags, and 27% (14 of 51) of the respondents reported other means to store fruits and vegetables such as either 4 or 6” steam table pans with or without clear plastic wrap, plastic containers without lids, plastic bags, fruit bowls, and boxes or original packaging.

The fruits and vegetables of main focus for our survey were cantaloupes, green leaf lettuce, and tomatoes due to their association with foodborne outbreaks in the past (Ackers et al., 1998; Behravesh et al., 2012; CDC, 2011a, 2011b; Taylor et al., 2010; Walsh, Bennett, Mahovic, & Gould, 2014). Seventy-eight percent (52%) of respondents indicated they stored whole cantaloupes, green leaf lettuce, and tomatoes for next day consumption after washing, and 8% (48%) included somewhat trained, and not trained, in which the anchors “very well trained” and “not trained” corresponded to highest and lowest level, respectively, of training on the scale. Thirty-nine percent (20) of respondents indicated having a well trained staff, 37% (19) indicated staff was adequately trained, 10% (5) indicated having a very well trained staff, 8% (4) responded that staff were somewhat trained, 2% (1) indicated that staff were not trained, and 4% (2) did not respond to the question. When analyzing responses by state, 43% of respondents from the state of Kansas indicated their staff or personnel was “adequately trained,” while 41% of respondents from

3.1.4. Facility personnel and equipment

Respondents were asked to rate staff training with regards to washing and preparing fruits and vegetables and were provided with this scale: very well trained, well trained, adequately trained, somewhat trained, and not trained, in which the anchors “very well trained” and “not trained” corresponded to highest and lowest level, respectively, of training on the scale. Thirty-nine percent (20) of respondents indicated having a well trained staff, 37% (19) indicated staff was adequately trained, 10% (5) indicated having a very well trained staff, 8% (4) responded that staff were somewhat trained, 2% (1) indicated that staff were not trained, and 4% (2) did not respond to the question. When analyzing responses by state, 43% of respondents from Rhode Island indicated their staff or personnel was “adequately trained,” while 41% of respondents from

### Table 1

<table>
<thead>
<tr>
<th>Type of Fruits and Vegetables Prepared in Foodservice Facilities and Different Storage Type*</th>
<th>Cubed cantaloupe</th>
<th>Cantaloupe wedges with rind</th>
<th>Cantaloupe wedges without rind</th>
<th>Sliced tomatoes</th>
<th>Diced tomatoes</th>
<th>Shredded lettuce</th>
<th>Leaf for sandwich</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What fruits and vegetables do you prepare in your facility?</strong></td>
<td>26(51)</td>
<td>18(33)</td>
<td>11(22)</td>
<td>40(78)</td>
<td>30(59)</td>
<td>26(51)</td>
<td>40(78)</td>
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<tr>
<td>PCWLB</td>
<td>(29/57)</td>
<td>16(31)</td>
<td>18(35)</td>
<td>35(69)</td>
<td>30(59)</td>
<td>25(49)</td>
<td>31(61)</td>
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<tr>
<td>BSCPB</td>
<td>1(2)</td>
<td>2(4)</td>
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<td>1(2)</td>
<td>0</td>
<td>1(2)</td>
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<tr>
<td>BSRCPB</td>
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<td>0</td>
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<td>1(2)</td>
<td>1(2)</td>
<td>4(8)</td>
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<td>5(10)</td>
<td>7(14)</td>
<td>15(29)</td>
<td>9(18)</td>
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<td>26(51)</td>
<td>24(47)</td>
<td>8(16)</td>
<td>13(25)</td>
<td>9(18)</td>
<td>7(14)</td>
</tr>
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<td><strong>How does your facility store prepared fresh fruits and vegetables?</strong></td>
<td></td>
<td></td>
<td></td>
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<td>1 day</td>
<td>20(39)</td>
<td>16(31)</td>
<td>16(31)</td>
<td>22(43)</td>
<td>–</td>
<td>19(37)</td>
<td>31(61)</td>
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<tr>
<td>2 day</td>
<td>6(12)</td>
<td>3(6)</td>
<td>3(6)</td>
<td>10(20)</td>
<td>–</td>
<td>7(14)</td>
<td>4(8)</td>
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<td>5(10)</td>
<td>5(10)</td>
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<td>10(20)</td>
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<td>1(2)</td>
<td>1(2)</td>
<td>1(2)</td>
<td>0</td>
<td>2(4)</td>
<td></td>
</tr>
<tr>
<td>Up to 7 days</td>
<td>1(2)</td>
<td>1(2)</td>
<td>2(4)</td>
<td>2(4)</td>
<td>–</td>
<td>1(2)</td>
<td>2(4)</td>
</tr>
<tr>
<td>N/A</td>
<td>15(29)</td>
<td>22(43)</td>
<td>21(41)</td>
<td>9(18)</td>
<td>–</td>
<td>11(22)</td>
<td>6(12)</td>
</tr>
</tbody>
</table>

*Response of participant N and (%)

* PCWL – Plastic containers with lids; BSCPB – Baking sheets covered with plastic bun bags; BSRCPB – Baking sheets with racks and covered with plastic bun bags; Other included – 4 or 6” steam table pans with or without clear plastic wrap, plastic container without lids, plastic bags, fruit bowls, and boxes or original packaging; NA – not apply.

Calculated from the information provided in the text.
the state of Virginia indicated personnel was “well trained” (Fig. 1).

Additionally, respondents were asked if their facilities possess adequate equipment dedicated to washing and preparing fresh fruits and vegetables. Sixty-five percent of respondents (33) indicated possessing adequate equipment and 29% of the respondents (15) indicated lacking adequate equipment to wash and prepare fresh fruits and vegetables. Among the 29% of respondents indicating lacking of equipment, 80% of these respondents indicated lacking sinks, 53% indicated lacking countertop space, 47% indicated lacking refrigerators, 27% indicated lacking cutting boards, 13% indicated lacking knives, and 27% indicated lacking other equipment (e.g., stationers and salad spinners). When asked specifically about refrigerator capacity and space to accommodate fresh fruits and vegetables, 65% of respondents (33) indicated having enough refrigerator capacity and space to accommodate fresh fruits and vegetables, 25% (13) indicated lacking refrigerator capacity and space to accommodate fruits and vegetables, and 10% (5) did not respond to the question.

Respondents were asked to freely comment about their equipment needs and problems. Respondents noted a lack of equipment to perform their job and limited kitchen space. In addition, some mentioned that they have old equipment and very old facilities, serving more students than the facility was designed and built to serve. Some respondents mentioned having three-compartment sinks; however, they lack a sink designated for fruit and vegetable washing and preparation. In addition, one participant noted that their facility has equipment for refrigeration, but the equipment is obsolete and needs to be replaced. One concern was the layout of the kitchen meeting current food safety recommendations.

The implementation of safety programs based on HACCP principles, strict standards, constant training, and personnel supervision are key factors that could help to reduce the risk of contamination at any level of school meal preparation. Therefore, observational research should be conducted in school settings in order to verify adherence to good manufacturing practices during preparation of school meals, and to evaluate improvement of produce safety handling. Other research efforts should focus on practical interventions to reduce potential cross-contamination in school facilities during preparation and handling, along with prevention efforts on improving personnel training and skills to prepare fruits and vegetables (Daniels et al., 2002; Lee & Greig, 2010).

### 3.2. Efficacy of washing treatments on native microflora of whole cantaloupes (phase II)

Aerobic plate count (APC) populations of untreated (control) cantaloupe surfaces averaged 3.88 log_{10} CFU/cm². Aerobic plate counts of cantaloupe rinds varied after washing treatments. Washing with tap water showed populations of 3.39 log_{10} CFU/cm², whereas populations after washing with the CAFVT and 9% vinegar solution were 2.98 log_{10} CFU/cm² and 3.01 log_{10} CFU/cm², respectively.

Aerobic bacteria populations transferred from cantaloupe rind surfaces to fresh-cut pieces (wedges or cubes) were determined immediately after preparation on day 0 and then sampled on day 1, 3, and 6 of storage. Cubes from untreated (control) cantaloupes showed populations of 2.80 and 3.43 log_{10} CFU/g on day 0 and day 1, respectively, and populations increased significantly on day 3 and day 6, reaching 7.19 and 8.50 log_{10} CFU/g, respectively. Aerobic plate count populations of cubed and wedged cantaloupes from whole washed and unwashed (untreated) cantaloupes were significantly different over time (P < at 0.05/16 = 0.0031; Table 2). Populations of cubes from cantaloupes washed with 9% vinegar solution and CAFVT ranged from 1.01 to 3.30 log_{10} CFU/g on day 0 to day 3. However, by day 6 populations increased significantly, reaching approximately 6.3 and 8.07 log_{10} CFU/g, respectively. Cubes from cantaloupes washed with tap water showed populations >2.3 log_{10} CFU/g on day 0 and day 1, and reached populations >4.6 log_{10} CFU/g on day 3. Although populations increased up to 6.62 log_{10} CFU/g on day 6, these counts were statistically similar to day 3 counts.

Wedges from untreated (control) and CAFVT washed cantaloupes showed populations over 5.6 log_{10} CFU/g on day 3 and approximately 8 log_{10} CFU/g on day 6. Wedges from cantaloupes washed with 9% vinegar solution showed populations between 1.39 and 2.97 log_{10} CFU/g on day 0–3, however populations increased continuously and reached 5.20 log_{10} CFU/g on day 6. Populations of wedges from cantaloupes washed with water ranged between 2.99 and 4.86 log_{10} CFU/g on day 0–3, and increased up to 7.40 log_{10} CFU/g on day 6.

Significant differences of APC populations among washing treatments were observed only for cubed cantaloupes on day 3 and wedged cantaloupes on day 1 and day 3 (P < at 0.05/16 = 0.0031; Table 2). On day 1 sampling, wedges from cantaloupes washed with 9% vinegar solution showed the lowest population with 1.39 log_{10} CFU/g, while wedges from cantaloupes washed with water showed the highest population, 4.86 log_{10} CFU/g. On day 3 sampling, cubes from cantaloupes washed with 9% vinegar solution and CAFVT showed the lowest APC populations with 3.30 and 2.47 log_{10} CFU/g, respectively, while cubes from untreated (control) cantaloupes showed the highest population with 7.19 log_{10} CFU/g.

Similarly, wedges from cantaloupes washed with 9% vinegar solution showed the lowest population with 2.04 log_{10} CFU/g. However, wedges from cantaloupes washed with CAFVT along with wedges from the untreated (control) showed the highest populations with approximately 5.6 log_{10} CFU/g. Interestingly, on day 0, wedges from CAFVT-washed cantaloupes showed lower APC populations by ≥0.96 log when compared to APC populations from wedges obtained from untreated and tap water washed cantaloupes, whereas cubes from 9% vinegar solution and CAFVT-washed cantaloupes showed lower APC populations by ≥1.1 log when compared to APC populations from cubes obtained from untreated and tap water washed cantaloupes. This indicates that washing cantaloupes with 9% vinegar solution and CAFVT reduced naturally occurring microflora on the surface of cantaloupe, which may have helped reduce the probability of transferring microorganisms from the rind to the flesh during cutting or transformation from whole...
cantaloupe to cubes.

It is worth noting that fresh-cut melons prepared at home kitchens have a suggested 7-day shelf life at 5 °C (CDC, 2013). However, shelf life for fresh-cut fruits for catering and foodservice is only 1–2 days (Barth, Hankinson, Zhuang, & Breidt, 2009).

Similarly, our results indicated that storage of fresh-cut (wedged and cubed) cantaloupes at refrigeration temperatures (4 ± 1 °C) should not exceed 3 days of storage since aerobic plate count populations reached ≥ 5.2 log10 CFU/g on day 6, even though washing treatments were applied prior to preparation. Moreover, it is important to keep in mind that the risk of recontamination can be amplified by further processing steps due to poor employee hygiene or improper handling with poorly sanitized utensils, equipment, or surfaces.

The reduced efficacy of washing treatments on fresh-cut pieces (wedges, slices, and cubes) of cantaloupes over storage time may be due to strong attachment of microorganisms (influenced by cantaloupe surface morphology), and the formation of biofilms enhanced by the availability of nutrients in cantaloupe juices after the fruit was cut (Nguyen-the & Carlin, 1994; Ukuku, Bari, Kawamoto, & Ishihki, 2005; Ukuku & Fett, 2002b).

3.3. Effectiveness of a commercially available fruit and vegetable wash for reducing pathogens (Salmonella spp. and L. monocytogenes) on whole cantaloupes (phase III)

Non-inoculated cantaloupes sampled for standard aerobic plate counts during Salmonella spp. and L. monocytogenes trials had total aerobic plate count populations of 4.70 log10 CFU/cm² and 4.80 log10 CFU/cm², respectively. No two-way interaction effect was observed (washing treatment × exposure time) on reducing Salmonella spp. populations on cantaloupe surfaces (Table 3). However, Salmonella spp. populations were affected (P < 0.05) by the commercial produce wash and exposure time (Table 3). The average Salmonella spp. population on the surface of cantaloupes after washing with tap water and the commercial produce wash solution were 5.50 and 4.87 log10 CFU/cm², respectively (Table 4). With respect to exposure time, pooled data across washing treatments showed that 60 and 120 s exposure times achieved the lowest Salmonella spp. population recovery after washing procedures (Table 4), while exposure time of 30 s showed the highest recovery of Salmonella spp. populations after washing. Sampling of residual wash treatment water resulted in recovery of 4.30 log10 CFU/ml of Salmonella spp. populations from the cold tap water and populations below the detection limit of 1.95 log10 CFU/ml for the commercial produce wash (Table 5).

The inoculated populations of Salmonella spp. on the surface of cantaloupes (n = 6) that were not washed averaged 6.13 log10 CFU/cm². Compared to the inoculated samples recovery, a reduction of 0.62 log10 CFU/cm² was observed on the rind of cantaloupes washed with tap water while reductions of 1.26 log10 CFU/cm² (P < 0.05) were observed on the rind of cantaloupes washed with the commercial produce wash. This difference in log reductions may be due to a difference of pH in washing treatments; pH measurements of washing treatments indicated pH values of ca. 3 for the commercial produce wash and ca. 9 for tap water. Various researchers have reported that the antimicrobial action of organic acids is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by dissociation of hydrogen ions from acid (FDA, 2013; Rico, Martin-Diana, Barat, & Ryan, 2007; Parish et al., 2003).

Exposure time for L. monocytogenes inoculated cantaloupes to washing treatments was 120 s. This decision was based on the results obtained from the Salmonella spp. trial where application of washing for 120 s showed the lowest Salmonella spp. population recovery (Table 4). Similarly to Salmonella spp., in these set of trials, the commercial produce wash had a significant effect (P < 0.05) on L. monocytogenes populations after washing procedures (Table 4). Application of the commercial produce wash for 120 s achieved 1.12 log10 CFU/cm² reduction of L. monocytogenes population on cantaloupe rind. However, a reduction of 0.63 log10 CFU/cm² was achieved by washing with cold tap water for 120 s.

Moreover, sampling of residual water after treatment indicated that L. monocytogenes-inoculated cantaloupes transferred the pathogenic load to regular tap water by 4.47 log10 CFU/ml while recovery of microorganisms in the commercial produce wash water after treatment was below the detection limit of 1.95 log10 CFU/ml (Table 5).

Parnell, Harris, and Suslow (2005) reported Salmonella Typhimurium populations log reductions of 0.7 and 1.8 log10 CFU/melon on cantaloupes washed by immersion for 60 s with water and 200 ppm total chlorine, respectively. Fishburn, Tang, and Frank (2012) evaluated the efficacy of five home-used washing technologies (diluted chlorine bleach, electrolyzed oxidizing water, ozone, veggie wash or running tap water) in reducing L. monocytogenes populations on cantaloupes that were washed by submersion for 2 min. Electrolyzed oxidizing water, ozone, veggie wash, and running tap water showed 0.55 log reductions of L. monocytogenes, respectively.

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-values of the main effects and interaction effects for recovered Salmonella spp. and L. monocytogenes after application of washing treatments.</td>
</tr>
<tr>
<td>Effect*</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Wash treatment</td>
</tr>
<tr>
<td>Exposure time</td>
</tr>
<tr>
<td>Wash treatment × Exposure time</td>
</tr>
</tbody>
</table>

Table 2

Aerobic plate count populations (APC; log10 CFU/g) on fresh-cut cantaloupe prepared from washed whole cantaloupes after storage at 4 ± 1 °C for up to 6 days (n = 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surface Cantaloupe cubes (2 × 2 cm)</th>
<th>Cantaloupe wedges (16 × 5 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D1</td>
</tr>
<tr>
<td>Untreated</td>
<td>3.88</td>
<td>2.90bs*</td>
</tr>
<tr>
<td>Tap water</td>
<td>3.39</td>
<td>2.56bs</td>
</tr>
<tr>
<td>95 vinegar solution</td>
<td>3.01</td>
<td>1.07bs</td>
</tr>
<tr>
<td>CAFVT</td>
<td>2.98</td>
<td>1.39bs</td>
</tr>
</tbody>
</table>

D = Day; CAFVT = commercial antimicrobial fruit and vegetable treatment.

* Means with different superscripts within a row are significantly different at Bonferroni P = 0.05/96 = 0.00052.

** Standard error (SE) = 0.5905; D = day of storage.
applying the FIT prototype wash. Harris et al. (2001) found that Salmonella log10 CFU/cm2, which are similar results to those reported by Park et al., 2008). Material; Park et al., 2008). not affected regardless of water quality (presence of organic material; Park et al., 2008). potato tubers. Additionally, effectiveness of the produce wash was whereas diluted chlorine bleach achieved 1.43 log reduction.

Various researchers have studied the efficacy of a produce wash (FIT) on tomatoes (Beuchat, Harris, Ward, & Kajs, 2001; Harris, Beuchat, Kajs, Ward, & Taylor, 2001), strawberries (Lukasik et al., 2003), and potatoes (Park et al., 2008). Beuchat et al. (2001) reported Salmonella reductions of >6.83 log10 in tomatoes when applying the FIT prototype wash. Harris et al. (2001) found that application of the FIT produce wash resulted in Salmonella reductions in tomatoes greater than those achieved with sterile water and Dey and Engley (D/E) broth. Washing strawberries by immersion for 2 min with the FIT produce wash achieved 2 log reductions of E. coli O157:H7 and Salmonella Montevideo on the surface of strawberries (Lukasik et al., 2003). In another study, flume water enhanced with the FIT produce wash resulted in reductions of enteric pathogens between 1.4 and 1.8 log10 CFU/g on surfaces of potato tubers. Additionally, effectiveness of the produce wash was not affected regardless of water quality (presence of organic material; Park et al., 2008).

In our study, the application of the commercial produce wash achieved Salmonella spp. and L. monocytogenes reductions of >1 log10 CFU/cm2, which are similar results to those reported by Lukasik et al. (2003) and Park et al. (2008). Log reduction results reported by Beuchat et al. (2001) and Harris et al. (2001) were significantly higher than our findings. Therefore, it is important to note that methods used for application of produce wash and recovery of microorganisms by these researchers were different than those used in the current study. Moreover, the current study focused on methods that are used in foodservice operations and not necessarily methods used in the laboratory settings. Although the commercial washing treatment was capable of achieving >1 log reduction of Salmonella spp. and L. monocytogenes populations on cantaloupe rind, these reductions are insufficient to assure microbial safety of cantaloupes. Minimal reduction of pathogenic microorganism populations could be attributed mainly to the characteristics of a cantaloupe’s surface, which is a complex meshwork of tissue that provides binding sites that are difficult to reach with sanitizers (Ukuku & Fett, 2002a,b; Wang, Feng, Luo, & Zhang, 2007). However, the commercial produce wash showed significant potential to maintain adequate microbial water quality and reduced the risk of cross-contamination when new produce is introduced to the washing sink or tank.

4. Conclusions and implications

In the survey responses, a small percentage of respondents (10%) indicated they use antimicrobial washes for washing produce. Prepared fruits and vegetables of interest (green leaf lettuce, tomato, and cantaloupes) were stored under refrigerated conditions overnight or as long as 7 days. Challenges faced for school foodservice personnel included limitations in existing kitchen equipment and infrastructure, training, and skills of personnel to wash and prepare fruits and vegetables. While schools are the only foodservice environment required to have a food safety program based on HACCP-principles ensuring that directors, managers, and employees fully understand the importance of a properly maintained and managed food safety program will help to prevent food safety hazards that arise during food preparation (receiving, storing, preparing, cooking, cooling, reheating, holding, assembling, packaging, transporting) and service are adequately controlled. School foodservice managers should be encouraged to reinforce preexisting food safety knowledge through training courses and certifications, and should emphasize proper food safety practices or behaviors in order to create a culture of food safety and reduce the risk of foodborne illnesses outbreaks.

Washing cantaloupes with 9% vinegar solution, CAFVT, or CPW reduced natural microflora or pathogenic populations on the surface of cantaloupes by approximately 1 log. However, it is important to note that the approximate infectious dose of pathogenic microorganisms, such as L. monocytogenes, is estimated to be as low as one cell in immunocompromised individuals, and these washing treatments might not be able to ensure cantaloupe safety if pathogenic populations >1 log are present on the surface or internalized in the produce. Therefore, the use of disinfectants such as 9% vinegar solution, CAFVT, and CPW would assist mainly to maintain process/wash water free of microbial contaminants and reduce the risk of cross-contamination when new produce is introduced to the washing sink or tank.
Acknowledgments

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References


