

Efficacy of Washing with a Commercial Flatbed Brush Washer, Using Conventional and Experimental Washing Agents, in Reducing Populations of *Escherichia coli* on Artificially Inoculated Apples†

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ABSTRACT

Conventional and experimental washing formulations were applied with a commercial flatbed brush washer under conditions representative of commercial practice to determine their efficacy in decontaminating apples inoculated with a nonpathogenic *Escherichia coli* strain. Golden Delicious apples (18 kg) inoculated with *E. coli* were mixed with approximately 109 kg of uninoculated Fuji apples (distinctly different in appearance) in a wet dump tank containing 1,325 liters of water at 20°C for 15 min. The combined apples were washed in a flatbed brush washer with the following washing solutions: water at 20°C, water at 50°C, 200 ppm of chlorine (pH 6.4) at 20°C, 8% trisodium phosphate at 20°C, 8% trisodium phosphate at 50°C, 5% hydrogen peroxide at 20°C, 5% hydrogen peroxide at 50°C, 1% APL Kleen 245 at 50°C, and two-stage washing treatments using the combination of 1% APL Kleen 245 at 20 or 50°C followed by 5% hydrogen peroxide at 35 or 50°C. None of the washing treatments tested under the conditions of this experiment significantly reduced the *E. coli* populations on the inoculated apples or in cider made from these apples, probably as a consequence of the inability of this washing system to inactivate or remove the bacterial cells in inaccessible calyx and stem areas of apples. These results are important because they demonstrate the need for new fruit washing technology that can overcome this limitation. Also, there was no significant cross-contamination of the Fuji apples in the dump tank. Significant cross-contamination of cider, made with uninoculated apples, occurred in the hammer mill and/or the press cloth when these units were not sanitized following a trial with inoculated apples.

Outbreaks of foodborne illness have been associated with the presence of enterohemorrhagic *Escherichia coli* O157:H7, a pathogen capable of causing hemorrhagic colitis and hemolytic uremic syndrome, in unpasteurized apple cider (1–3). This microorganism has been reported to survive in refrigerated apple cider for extended periods (2, 12) and in low-pH synthetic gastric fluid (12). Such an acid resistance response challenged the widely held concept that unpasteurized apple cider was a safe food product because of its low pH of 3.3 to 4.1. Outbreaks associated with consumption of unpasteurized apple cider or juice have to date resulted in hundreds of cases of *E. coli* O157:H7 infections and one death (3, 4, 8). Also, outbreaks of *Salmonella* and *Cryptosporidium* infections have been associated with consumption of unpasteurized apple cider (1–4, 12).

Recent outbreaks of foodborne illness associated with consumption of fruit juices in general and apple cider in particular have led the U.S. Food and Drug Administration (FDA) to publish regulations proposing mandatory adoption of hazard analysis critical control point (HACCP) programs (4). The basic concept of an HACCP system is to

plan, control, and document the safe production of food. This system should include intervention steps and critical control points capable of producing at least a cumulative 5-log reduction in the population levels of target pathogens. An HACCP program is not a stand-alone system; it also requires the implementation of a sanitation standard operating procedure and current good manufacturing practices. In the case of apple cider, *E. coli* O157:H7 would be the logical target pathogen. Although thermal pasteurization is the most effective method for eliminating pathogens from cider, not every cider producer can afford to install a pasteurizer. Therefore, these producers have the option of implementing an HACCP program, which includes other potential intervention steps such as a policy of excluding “drops” and cleaning fruit with sanitizing agents (3, 7, 8) to attain a cumulative 5-log reduction in the levels of target pathogens in apple cider.

Laboratory washing studies of apples, using different sanitizing agents, were reported to produce up to 3-log reduction in the levels of *E. coli* (2, 9, 10). However, we have no knowledge of any studies of apple decontamination that used these sanitizing formulations and commercial washing equipment. Therefore, the objectives of this study were to elucidate the effect of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, on the bacterial load of apples artificially inoculated with a nonpathogenic *E. coli* strain; to determine the extent

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of cross-contamination in a dump tank; and to determine the importance of plant sanitation procedures in eliminating cross-contamination. Such information is essential in developing an HACCP program for minimizing the risk of foodborne illness associated with the consumption of unpasteurized apple cider.

MATERIALS AND METHODS

Microorganism, culture maintenance, and growth media.

E. coli ATCC 35695, a nonpathogenic streptomycin-resistant strain, was supplied by S. E. Keller (FDA, Summit-Argo, Ill.). Streptomycin (Sigma Chemical Co., St. Louis, Mo.) was added to all liquid and solid growth media used in this study to a final concentration of 20 µg/ml. The culture was maintained on brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.) at 4°C, with biweekly transfers to maintain cell viability. The growth medium used was tryptic soy broth (Difco). Media were prepared in distilled water according to the manufacturer's guidelines and autoclaved. The sterilized media were allowed to cool down to at least 52°C before the addition of filter-sterilized streptomycin solution.

Inoculum development, growth conditions, and apple inoculation.

A late-exponential-phase culture grown in tryptic soy broth at 37°C for 8 h was used to inoculate 20 liters of the same medium at a 0.01% (vol/vol) level. The culture was allowed to grow for 18 h, spun down at 9,500 × *g* for 5 min, washed once with 200 ml of sterile distilled water, and suspended in 75 liters of tap water to a final cell concentration of approximately 8.5 log CFU/ml. Unwaxed Golden Delicious apples, free of decay and punctures, were placed in a stainless steel basket and immersed in the 75-liter inoculum (9 kg per dip) for 5 min with occasional shaking. Apples were drained, placed on their sides in a large plastic bin to permit drainage from stem and calyx areas, and allowed to air dry at ambient temperature (approximately 10°C) overnight (approximately 20 h).

Assessment of bacterial viability. The bacterial suspensions were serially diluted in 0.1% (wt/vol) peptone (Difco) water and surface plated on duplicate BHIA plates. The plates were then incubated at 37°C for 18 to 24 h, and the survivors were enumerated. Cell densities were reported as log CFU per g or ml of sample.

Washing protocols and analytical procedures. Golden Delicious apples (18 kg) inoculated with *E. coli* were mixed with approximately 109 kg of uninoculated unwaxed Fuji apples (distinctly different in appearance) in a wet dump tank containing approximately 1,325 liters of tap water at 20°C, held in the dump tank with continued mixing for 15 min, and then conveyed to a flatbed brush washer (Model 1650, TEW Manufacturing Corp., Penfield, N.Y.) for washing. All washed apples were then conveyed from the brush washer, under water sprays (optional) for rinsing, to a hammer mill. Apples were then ground in the hammer mill, the pulp pumped to a hydraulic press and distributed on the press cloth in a frame, and the cider extracted. The cider was collected in a stainless steel tank. The entire cider production system was disassembled, rinsed with tap water, sanitized with FS Foam Chlor (Zep Manufacturing Co., Atlanta, Ga.), and rinsed again thoroughly between trials. The press clothes were sterilized, using an autoclave, and then washed between trials.

Unless otherwise indicated, all washing treatments were applied with the brush washer. The temperature of washing agents was adjusted by heating the washer reservoir with a propane burn-

er. The washing agents used in this study were as follows: (i) water at 20°C; (ii) water at 50°C; (iii) 200 ppm (wt/vol) of chlorine at 20°C, prepared from sodium hypochlorite (Clorox Co., Oakland, Calif.) and adjusted to pH 6.4 with citric acid; (iv) 8% (wt/vol) trisodium phosphate (Rhodia Inc., Cranbury, N.J.) at 20°C; (v) 8% trisodium phosphate at 50°C; (vi) 1% (vol/vol) APL Kleen 245, a commercial acidic surfactant formulation (Elf Atochem North America, Inc., Monrovia, Calif.) at 50°C; (vii) 5% (vol/vol) hydrogen peroxide (H₂O₂; EKA Chemicals, Marietta, Ga.) at 20°C; (viii) 5% H₂O₂ at 50°C; (ix) two-stage treatment using 1% APL Kleen 245 wash at 50°C followed by a 5% H₂O₂ rinse (applied as a spray rinse following the brush washer) at 35°C; and (x) two-stage treatment using 1% APL Kleen 245 (applied in the dump tank) at 20°C followed by a 5% H₂O₂ wash at 50°C. All wash treatments other than H₂O₂ treatments were followed by a water spray rinse.

Duplicate samples of both varieties, each comprising 6-apple composites, were collected before inoculation, after inoculation (Golden Delicious only), after the dump tank, and after the brush washer to determine population reductions that resulted from the washing treatments. Duplicate water samples were collected from the dump tank at the end of the 15-min holding time to determine the potential for cross-contamination in the dump tank. Duplicate cider samples were also collected from the juice collection tank to determine the *E. coli* population and the residual H₂O₂ concentration in the unpasteurized cider. Apple samples were weighed and the individual apples were cut in half and blended with a volume of sterile peptone water equal to the sample mass for 1 min in a 3.8-liter stainless steel blending container. Two samples of each blend were collected and filtered through a filter bag (Spiral Biotech, Inc., Bethesda, Md.) before assessing bacterial viability. Blender containers, cutting boards, and knives were sanitized between samples by dipping in 1,000 ppm of chlorine (pH 6.5) for 10 min, then rinsed with tap water.

Residual H₂O₂ concentrations in cider made from apples washed with 5% H₂O₂ solutions and from untreated controls were determined with a Reflectoquant test strip kit (EM Science, Gibbstown, N.J.). Concentrations were determined immediately following juice pressing and following 45-min incubation at room temperature.

Cross-contamination test. After a run with inoculated apples, the cider production system and the press cloth were rinsed thoroughly with water under pressure, but no sanitizing agent was used. Uninoculated Fuji apples (approximately 300 lb) were passed through the system and pressed into cider. Duplicate samples of apples and cider were collected as previously described (see above) for microbiological evaluation.

RESULTS AND DISCUSSION

An HACCP program is a system that is designed to identify potential health hazards (biological, chemical, and physical) and to establish strategies to prevent their occurrence. This system is based on seven principles (4), which are applicable to all phases of food production. In the case of unpasteurized apple cider, interventions should be made when possible to eliminate or control the target pathogen at all points in the growing, processing, storage, and distribution chain to achieve a cumulative 5-log reduction so that a safe product is delivered to the consumer (8). Failure at any one of these interventions during production of unpasteurized apple cider could significantly increase the risk of foodborne illness. Currently, the few available data spe-

TABLE 1. Efficacy of different sanitizing washes, applied with a commercial flatbed washer, in decontaminating artificially inoculated apples with nonpathogenic *E. coli* ATCC 35695^a

Treatment	Apple sample	<i>E. coli</i> concentration ^b				
		Before dump tank (log CFU/g)	After dump tank (log CFU/g)	After brush washer (log CFU/g)	In cider (log CFU/ml)	Dump tank water (log CFU/ml)
Water, 20°C	GD	5.49 ± 0.09	4.92 ± 0.37	4.81 ± 0.26	3.83 ± 0.15	NG
	Fuji	1.62	2.01	0.99		
Water, 50°C	GD	5.49 ± 0.09	5.04 ± 0.16	4.59 ± 0.08	3.73 ± 0.11	NG
	Fuji	1.62	1.81	1.15		
8% TSP, 20°C	GD	5.49 ± 0.09	5.02 ± 0.43	4.98 ± 0.03	3.57 ± 0.16	NG
	Fuji	1.62	NG	0.62		
8% TSP, 50°C	GD	5.49 ± 0.09	5.02 ± 0.08	4.75 ± 0.45	3.59 ± 0.06	NG
	Fuji	1.62	1.72	1.21		
5% H ₂ O ₂ , 20°C	GD	5.87 ± 0.07	5.47 ± 0.41	5.27 ± 0.09	4.13 ± 0.01	NG
	Fuji	0.69	NG	NG		
5% H ₂ O ₂ , 50°C	GD	5.87 ± 0.07	5.54 ± 0.31	5.50 ± 0.10	4.30 ± 0.72	NG
	Fuji	0.69	1.12	0.50		
200 ppm of Cl ₂ , 20°C	GD	5.87 ± 0.07	5.45 ± 0.05	5.64 ± 0.23	4.30 ± 0.10	NG
	Fuji	0.69	NG	0.62		
1% AK, 50°C ^c	GD	1.99 ± 0.15	1.72 ± 0.43	1.76 ± 0.26	1.24 ± 0.34	NG
	Fuji	1.27	1.16	NG		
CCT ^d	Fuji	0.69	NG	0.50	3.38 ± 0.01	ND
	GD	5.87 ± 0.07	5.49 ± 0.04	5.42 ± 0.50 ^e	4.58 ± 0.02	
Two stage: 1% AK wash, 50°C; 5% H ₂ O ₂ , spray rinse, 35°C	GD			5.19 ± 0.21 ^f		NG
	Fuji	0.69	0.80	NG ^e		
Two stage: 1% AK in dump tank, 20°C; 5% H ₂ O ₂ wash, 50°C	GD	4.18 ± 0.17	4.19 ± 0.10 ^e	3.88 ± 0.45 ^f	2.70 ± 0.04	NG
	Fuji	0.25	NG	NG		

^a TSP, trisodium phosphate; H₂O₂, hydrogen peroxide; Cl₂, chlorine; AK, APL Kleen 245; CCT, cross-contamination test; GD, inoculated Golden Delicious apples; Fuji, uninoculated Fuji apples; NG, no growth was detected on BHIA medium. ND, not determined.

^b Mean ± SD of four determinations. SD was not calculated for Fuji samples since more than one determination resulted in no growth on BHIA medium.

^c Initial *E. coli* cell concentration in the inoculum preparation was lower than expected, which resulted in a bacterial load less than 5 log CFU/g on the inoculated apples.

^d Only uninoculated Fuji apples were used in this trial. Cider production system and press cloth were rinsed thoroughly with water only (no sanitation step was used) following a trial with inoculated apples before the CCT treatment.

^e Bacterial load following wash with AK in two-stage treatment.

^f Bacterial load following wash with H₂O₂ in two-stage treatment.

cific to apples are insufficient to develop a validated HACCP plan for apple cider production. This includes lack of information on introduction and transmission of *E. coli* O157:H7 into apple cider (3–5). Therefore, research is needed to determine the effectiveness of these interventions under commercial conditions so that an effective HACCP plan can be developed to ensure a safe unpasteurized product. In this study, we evaluated washing as an intervention to reduce levels of *E. coli* O157:H7, using a nonpathogenic surrogate strain, in a commercial cider mill operated by the FDA at Placerville, Calif.

Efficacy of washing agents in decontaminating apples. The results of washing trials are shown in Table 1. Golden Delicious apples were inoculated with approximately 5 log CFU/g of streptomycin-resistant *E. coli* (Table 1). The uninoculated control Fuji apples (before dump tank) appeared to contain low levels of streptomycin-resistant organisms (Table 1). Standard deviation for Fuji samples was not calculated (Table 1) since more than one determination

showed no growth on BHIA medium. In contrast to results obtained with laboratory-scale washing studies (2, 9–11, 13), none of the washing treatments tested in the cider mill trials significantly reduced the bacterial population on the inoculated apples (Table 1). This is probably due to one or more of the following factors: short (fixed) exposure time (25 s) in the brush washer used in this study; failure of the brush washing agents to reach inaccessible regions of apples near the calyx and stem, where bacteria are believed to adhere (2); and resistance of adhering bacteria to detachment. Exposure time of fruits to the various washing solutions during laboratory-scale trials were 1 to 5 min compared with the 25 s reported herein. This may explain the reduction in efficacy of the washing agents observed in this study compared with that observed in laboratory-scale trials. Results from this laboratory indicated that *E. coli* cells on artificially inoculated Golden Delicious apples were mainly attached to the calyx and the stem areas compared with the outer skin of the apples (9). When these



FIGURE 1. Scanning electron microscopy (SEM) image ($\times 10,000$) showing biofilm formation by *E. coli* cells in the calyx area of artificially inoculated Golden Delicious apples. Apples were inoculated by immersion and allowed to air dry at 4°C for 3 days, dissected, and treated for SEM imaging.

apples were washed with 5% H_2O_2 at 50°C , bacterial survival was confined primarily to the calyx and stem areas (9). Furthermore, internalization studies of apples inoculated with *E. coli* (2) showed that this microorganism can penetrate into the core of the apple through the calyx area following immersion in a bacterial suspension. Thus, such internalized bacterial cells would be inaccessible to the inactivation and removal action of the washing agents. Sapers et al. (9) showed that holding the inoculated apples to dry overnight following immersion in the bacterial suspension (see "Materials and Methods") resulted in firm attachment of the bacterial cells to the apples. Also, they showed that attachment of bacterial cells to apples resulted in a sharp reduction in the efficacy of water washing. Whether this is a reflection of adhesion of bacterial cells to the apple surface or incorporation into a protective biofilm is not clear. Preliminary data from scanning electron microscopy showed that *E. coli* cells attached to the calyx and stem areas of the apple were incorporated into biofilms (Fig. 1). A biofilm is characterized by the production of extracellular polymeric substances. Extracellular polymeric substances play an important role in cell adhesion to surfaces, producing firm anchorage, and are critical for the survival of the bacterial cells in a hostile environment (6), such as that of the surface of an apple.

Cider produced from contaminated raw material contained approximately 1 log fewer bacteria than the washed

inoculated apples (Table 1) largely because of dilution by juice from the comixed uninoculated Fuji apples, although some retention of *E. coli* by the press cloth may have occurred (see below).

Evaluation of cross-contamination. Dump tanks have been reported to be a potential source of cross-contamination in fruit and vegetable processing lines (1, 2). The uninoculated Fuji apples showed no significant increase in the levels of *E. coli* after the dump tank in all wash trials (Table 1). Also, no survivors were recovered from the water samples collected from the dump tank (Table 1). This could indicate that no significant cross-contamination occurred in the dump tank under the present experimental conditions. This is a reflection of the firm attachment of *E. coli* cells to apple surfaces during the 18- to 24-h interval between inoculation and introduction of apples into the dump tank (see above). Cross-contamination in the dump tank might have occurred if apples were inoculated shortly before addition to dump tank when the cells were less firmly attached to the apple (9). This experimental condition might simulate the disintegration of a heavily contaminated, decayed apple during unloading of a fruit shipment or bin into the dump tank.

A cross-contamination test was performed to evaluate the relation between the efficacy of the sanitation procedure and possibility of cross-contamination. This protocol resulted in minimal contamination of apples taken from the dump tank and brush washer but high-level contamination ($3.4 \log \text{CFU/ml}$) in the cider (Table 1), suggesting that cross-contamination of apples occurred in the hammer mill and/or press cloth. Therefore, an inadequate cleaning and sanitation program has the potential to lead to accumulation of particulates and bacterial cells on equipment surfaces and subsequent biofilm formation, thereby increasing the risk of contamination. Since removal of biofilms is a very difficult and demanding process (6), development of a sanitation standard operating procedure and rigorous clean-up schedules is required.

Hydrogen peroxide residues in apple cider. Apples treated with 5% H_2O_2 were intentionally not subjected to a final water rinse (see "Materials and Methods") to provide a residual concentration of H_2O_2 in the finished product (9, 10) that would be available to inactivate surviving *E. coli* cells when the apples were ground in the hammer mill. Residual H_2O_2 concentrations in freshly prepared cider made from apples washed with 5% H_2O_2 solutions were small (25 to 85 ppm) and dissipated to background levels (4 to 9 ppm, resulting from uncontrolled mixing of endogenous enzymes and substrates during the disintegration of untreated apples in the hammer mill) within 45 min. This residual H_2O_2 had little or no effect on the level of microbial decontamination in cider.

CONCLUSIONS

(i) Brush washing with a flatbed brush washer, under the current experimental conditions, cannot decontaminate apples that contain attached *E. coli* cells, even if potent antimicrobial agents are added to the wash solution.

(ii) No significant cross-contamination in the dump tank was detected, but cross-contamination might be expected with more recent raw material contamination or if contaminated, decayed apples fell apart in the dump tank.

(iii) Cross-contamination within the cider mill processing line due to an inadequate sanitation procedure could result in significant product contamination.

(iv) Residual hydrogen peroxide in cider from treated apples rapidly decomposed to background levels and would, therefore, not limit use of this solution as an antimicrobial wash.

(v) New technology that improves contact between contaminating bacteria and the antimicrobial wash, accomplishes surface pasteurization, or physically removes bacteria from the apples or cider is required.

(vi) Although the interventions tested herein resulted in no reduction in the bacterial load, they still should be included as integral components of any HACCP program to be developed for reducing the risks of foodborne illness associated with consumption of unpasteurized apple cider. Such interventions would probably reduce bacterial levels when contamination was more recent than the 20-h interval used herein.

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REFERENCES

1. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204-216.
2. Buchanan, R. L., S. G. Edelson, R. L. Miller, and G. M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J. Food Prot.* 62:444-450.
3. De Roeber, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9:321-347.
4. Food and Drug Administration. 1998. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. *Fed. Regist.* 63(79):20450-20486.
5. Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl. Environ. Microbiol.* 65:1-5.
6. Kumar, C. G., and S. K. Anand. 1998. Significance of microbial biofilms in food industry: a review. *Int. J. Food Microbiol.* 42:9-27.
7. Lang, M. M., S. C. Ingham, and B. H. Ingham. 1999. Verifying apple cider plant sanitation and hazard analysis critical control point programs: choice of indicator bacteria and testing methods. *J. Food Prot.* 62:887-893.
8. McLellan, M. R., and D. F. Splittstoesser. 1996. Reducing risk of *E. coli* in apple cider. *Food Technol.* 50:174.
9. Sapers, G. M., R. L. Miller, M. Jantschke, and A. M. Mattrazzo. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. *J. Food Sci.*, in press.
10. Sapers, G. M., R. L. Miller, and A. M. Mattrazzo. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. *J. Food Sci.* 64:734-737.
11. Somers, E. B., J. L. Schoeni, and A. C. L. Wong. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. *Int. J. Food Microbiol.* 22:269-276.
12. Uljas, H. E., and S. C. Ingham. 1999. Combinations of intervention treatments resulting in 5-log₁₀-unit reductions in numbers of *Escherichia coli* O157:H7 and *Salmonella typhimurium* DT104 organisms. *Appl. Environ. Microbiol.* 65:1924-1929.
13. Zhuang, R.-Y., and L. R. Beuchat. 1996. Effectiveness of trisodium phosphate for killing *Salmonella montevideo* on tomatoes. *Lett. Appl. Microbiol.* 22:97-100.