Contamination of Intact Apples after Immersion in an Aqueous Environment Containing *Escherichia coli* O157:H7†

R. L. BUCHANAN,*‡ S. G. EDELSON,‡ R. L. MILLER, AND G. M. SAPERS

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 98-175: Received 8 July 1998/Accepted 16 October 1998

ABSTRACT

The extent and location of *Escherichia coli* O157:H7 contamination after intact apples were immersed in cold (2°C) 1% peptone water containing approximately 3×10^7 CFU/ml was assessed using four apple varieties, Golden Delicious, McIntosh, Red Delicious, and Braeburn. Room temperature and refrigerated apples were used to determine the effect of temperature differential on *E. coli* infiltration. The highest levels of *E. coli* were associated with the outer core region of the apple, followed by the skin. Apples were subsequently treated by immersing them for 1 min in 2,000 mg/liter sodium hypochlorite, followed by a 1-min tapwater rinse. This treatment reduced pathogen levels by 1- to 3-log cycles but did not eliminate the microorganism, particularly from the outer core region. While *E. coli* was not detected in the inner core of most apples, warm fruit immersed in cold peptone water occasionally internalized the pathogen. The frequency and extent of internalization of the pathogen was less when cold apples were immersed in cold peptone water. Subsequent dye uptake studies with Golden Delicious apples indicated that approximately 6% of warm apples immersed into a cold dye solution accumulated dye via open channels leading from the blossom end into the core region. However, dye uptake did not occur when the dye solution was warmer than the apple.

Unpasteurized apple juice/cider has been associated recently with several outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS) caused by *Escherichia coli* O157:H7 *(2, 5, 6)*. Furthermore, an earlier outbreak of HUS associated with unpasteurized cider *(18)* is suspected of having been caused by enterohemorrhagic *E. coli*. Unpasteurized apple cider has also been implicated in outbreaks of salmonellosis and cryptosporidiosis *(4, 6)*. Laboratory investigations have demonstrated that *E. coli* O157:H7 can survive for extended periods in refrigerated apple juice/cider despite the beverage's acidic pH *(10, 12, 19)*. The use of preservative compounds such as potassium sorbate has been reported in some instances to increase the rate of acid inactivation, but the activities of these antimicrobials do not appear to be sufficient to assure elimination *(12, 19)*.

The National Advisory Committee on Microbiological Criteria for Foods has recommended that production of fruit juices should include treatments capable of producing a cumulative 5-log reduction in the levels of *E. coli* O157:H7 *(13)*. Thermal pasteurization of apple juice/cider can achieve this reduction readily *(17)*. While there are other potential means of reducing the levels of enteric pathogens in or on foods, there are few available data specific to apples *(14)*. This includes a lack of information on sources of contamination, location of the contamination on the fruit, the effectiveness of sanitizing agents, and the impact of processing steps for production of juices and ciders on levels of pathogenic microorganisms.

One of the keys to the selection of appropriate intervention steps for reducing bacterial levels is whether the bacteria of concern are restricted to the surface of the fruit. Internalization of pathogens would greatly reduce the effectiveness of surface treatments. The purpose of the current study was to characterize the extent and location of contamination when intact apples were exposed to an aqueous environment contaminated heavily with *E. coli* O157:H7, as might occur in dump tanks or flume water that were not hygienically maintained *(8)*. Factors examined included apple variety, temperature differentials between apples and the aqueous environment, and the effectiveness of a post-exposure chlorinated water rinse.

MATERIALS AND METHODS

Microorganism. *Escherichia coli* O157:H7 SEA13B88, which was isolated from unpasteurized apple juice associated with a 1996 outbreak of hemorrhagic colitis and HUS *(5)* was used throughout the study. The strain was obtained from the U.S. Food and Drug Administration. The organism was transferred bimonthly to fresh tryptic soy broth (TSB; Difco, Detroit, Mich.), incubated for 24 h at 37°C, and then stored at 2°C.

Inoculum. Four 250-ml Erlenmeyer flasks containing 50 ml of TSB with 1% dextrose (TSB supplemented with an additional 7.5 g dextrose/liter) was inoculated with 0.1 ml of the starter culture. The flasks were incubated without agitation at 37°C for 18 h. When grown in this acidogenic medium, *E. coli* O157:H7 cul-

* Author for correspondence. Tel: 202-205-5053; Fax: 202-401-7740; E-mail: rbuchana@bangate.fda.gov.

† Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

‡ Present address: U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 200 C-Street SW, Washington, DC 20204, USA.

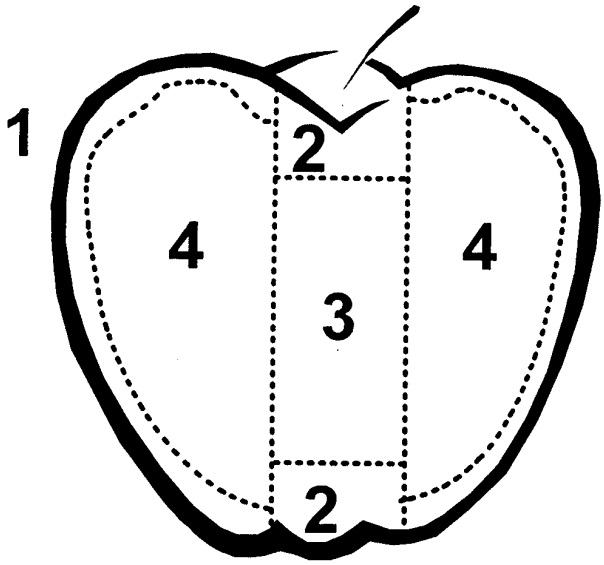

FIGURE 1. *Regions of apples examined for the presence of* E. coli *O157:H7. 1, Skin; 2, outer core; 3, inner core; 4, pulp.*

tures reach a final pH of approximately 4.6, yielding cells that were fully induced to pH-dependent, stationary-phase acid resistance *(3)*.

Uptake of *E. coli* O157:H7. Twelve apples of the appropriate variety were purchased at a local retail market the day prior to a trial. Golden Delicious apples were unwaxed, while the other varieties had been waxed at the packing house. Six of the apples and four 3-liter beakers containing 1.5 liters of sterile 1% peptone were pre-equilibrated for approximately 24 to 48 h to 2°C. The remaining six apples were kept at room temperature (22°C). A 2,000-mg/liter sodium hypochlorite solution was prepared by adding 38.1 ml of commercial bleach (5.25% sodium hypochlorite) to 961.9 ml of deionized water.

The prechilled 3-liter beakers were placed in ice-water baths and inoculated by adding one of the 50-ml starter cultures to each beaker. Three apples of either the refrigerated or room temperature sets were totally submerged in the contents of one of the beakers and allowed to stand with periodic gentle agitation for 20 min. The apples were then air dried under a biological hood (class IIA) for 30 min. A set of three apples of each temperature group was then surface sanitized by immersion in 1.0 liter of a 2,000-mg/liter sodium hypochlorite solution (22°C) for 1 min, followed by draining, immersion in 1.0 liter of tap water (22°C) for 1 min, and air drying.

Using a sanitized apple coring knife and cutting board, each apple was sectioned, producing a core and eight wedge-shaped segments (Fig. 1). Knives and cutting boards were sanitized by washing with brushing in an alkaline detergent, rinsing with tap water, soaking in a 2,000-mg/liter sodium hypochlorite solution for 1 min, rinsing with tap water, and air drying. Using a sanitized knife, approximately 1.5 cm was cut off from each end of the core. These segments were combined and designated as the outer core region. The remaining core segment was designated as the inner core. Using a second sanitized knife, the skin was separated from the body of the apple (i.e., pulp) to a depth of approximately 5 mm. Each apple portion (outer core, inner core, skin, and pulp) was placed in individual stomacher bags with filters (model 400, Seward, London, UK). The outer core and the inner core were diluted with sterile Butterfield's buffer using Diluflo diluent dispenser (model 800, Spiral Biotech, Bethesda, Md.) to obtain a 10^{-1} dilution. The outer core, inner core, and pulp samples were homogenized in a stomacher (model 400, Tekmar Co., Cincinnati; Ohio) for 1.5 min. The skin was homogenized for 3.0 min. Samples of the expressed liquid (5–10 ml) were transferred to sterile test tubes. After diluting as needed with sterile 1% peptone water, samples were surfaced plated onto duplicate MacConkey agar plates using the spiral plater (model 3000, Spiral Biotech). All plates were incubated at 37°C for 18 to 24 h and then enumerated using an automatic plate counter (Spiral Biotech).

Uninoculated control samples were handled in an identical manner except that no starter culture was added to the 1.5 liters of peptone water into which the apples were immersed.

Dye uptake. Two cases (113 apples each) of unwaxed Golden Delicious apples were obtained from a commercial supplier. One case was prechilled to 4°C, and the other was maintained at room temperature (21°C). Two 7-liter solutions of 0.1% red food dye no. 40 (FD&C red no. 40) were prepared; one prechilled to 9°C and the other kept at room temperature.

At the initiation of the trials, the 7 liters of dye solution was divided into two 3.5-liter portions and transferred to plastic tubs. For the trial where warm apples were immersed in cold dye solution, the plastic tubs containing the dye solution were held in ice-water baths. This maintained the temperature of the dye solution at 9 ± 1°C during immersion. For the trial with room temperature dye solutions, the temperature of the solution decreased to approximately 17 to 20°C as a result of the introduction of the cold apples and was re-equilibrated to room temperature between sets of apples.

The apples were immersed in the dye for 30 min in groups of 15 apples. The apples were continually dunked and agitated during the immersion period. After removal from the dye solution, the apples were rinsed under tap water and then dried with absorbent paper. A cotton swab was used to remove gently any water or dye solution retained in the blossom end cavity. The tap-water rinse was effective in removing excess dye from the stem end.

Each apple was then examined individually for uptake of dye into the skin. The apple was then cut in half starting at the blossom end and the core region examined for dye uptake. The responses were qualitatively divided into three classes: no, slight to moderate, or extensive uptake.

Statistical analyses. Means and standard deviations were determined using commercial spreadsheet software (Lotus 123, release 5.0, Lotus Development Corp.).

RESULTS

Uptake of *E. coli* O157:H7. Preliminary studies indicated that apples immersed in sterile peptone were unlikely to harbor *E. coli* (data not shown). Only in one instance was a single colony of a presumptive coliform observed. Accordingly, any *E. coli* detected in the inoculated samples were assumed to be the result of *E. coli* O157:H7 uptake or attachment.

The extent and location of *E. coli* O157:H7 contamination after immersion in an aqueous environment containing high populations of the pathogen were evaluated using Golden Delicious (Table 1), McIntosh (Table 2), Red Delicious (Table 3), and Braeburn (Table 4) apples. While differences among the four varieties were noted, the general trends were similar. The greatest extent of contamination on a per gram basis was consistently associated with the

TABLE 1. *Uptake of* E. coli *O157:H7 by cold (2°C) and warm (22°C) intact unwaxed Golden Delicious apples immersed in cold (2°C) peptone water containing approximately* 3×10^7 *CFU/ml*

		Skin		Pulp		Outer core		Inner core	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Warm apple	No rinse	3.67[a] ± 0.41	3.15–4.37	2.76 ± 0.46	2.04–3.42	5.03 ± 0.65	3.87–5.74	<2.75[b]	<2.00–4.35
	Chlorinated water	2.51 ± 1.14	1.30–4.19	<1.36[b]	<1.00–2.54	4.46 ± 1.20	3.00–5.75	<2.89[b]	<2.00–4.09
Cold apple	No rinse	4.04 ± 0.31	3.63–4.56	3.31 ± 0.30	2.93–3.63	5.47 ± 0.14	5.24–5.68	3.65 ± 0.25	3.28–4.05
	Chlorinated water	2.17 ± 0.55	1.60–2.99	<1.08[b]	<1.00–1.48	3.76 ± 0.86	2.60–5.15	<2.00	<2.00

[a] Log (CFU/g); results from six apples.
[b] One or more values below the lower limit of detection: inner and outer core: log (CFU/g) = 2.00. Skin and pulp: log (CFU/g) = 1.00.

TABLE 2. *Uptake of* E. coli *O157:H7 by cold (2°C) and warm (22°C) intact waxed McIntosh apples immersed in cold (2°C) peptone water containing approximately* 3×10^7 *CFU/ml*

		Skin		Pulp		Outer core		Inner core	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Warm apple	No rinse	<2.74[ab]	<2.00–3.63	<2.76[b]	<2.00–3.23	5.27 ± 0.27	5.01–5.55	<2.67[b]	<2.00–3.23
	Chlorinated water	<2.10[b]	<2.00–2.30	<2.00	<2.00	3.94 ± 0.06	3.88–4.00	<2.00	<2.00
Cold apple	No rinse	3.20 ± 0.44	2.70–3.48	<2.45[b]	<2.00–3.04	4.89 ± 0.34	4.50–5.14	2.99 ± 0.68	2.60–3.78
	Chlorinated water	<2.00	<2.00	<2.00	<2.00	3.14 ± 0.76	2.30–3.79	<2.00	<2.00

[a] Log (CFU/g); results from three apples.
[b] One or more values below the lower limit of detection: log (CFU/g) = 2.00.

TABLE 3. *Uptake of* E. coli *O157:H7 by cold (2°C) and warm (22°C) intact waxed Red Delicious apples immersed in cold (2°C) peptone water containing approximately 3 × 10^7 CFU/ml*

		Skin		Pulp		Outer core		Inner core	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Warm apple	No rinse	4.29[a] ± 0.06	4.23–4.33	3.18 ± 0.07	3.14–3.26	5.63 ± 0.23	5.47–5.89	3.81 ± 0.49	3.34–4.31
	Chlorinated water	<1.10[b]	<1.00–1.30	<1.10[b]	<1.00–1.30	3.97 ± 0.25	3.69–4.17	<2.51[b]	<2.00–3.23
Cold apple	No rinse	4.38 ± 0.43	4.12–4.76	3.39 ± 0.33	3.24–3.66	5.85 ± 0.17	5.74–6.04	4.11 ± 0.12	4.00–4.24
	Chlorinated water	2.19 ± 0.24	1.95–2.43	<1.20[b]	<1.00–1.60	4.30 ± 0.24	4.12–4.57	<2.42[b]	<2.00–2.78

[a] Log (CFU/g); results from three apples.
[b] One or more values below the lower limit of detection: inner and outer core: log (CFU/g) = 2.00. Skin and pulp: log (CFU/g) = 1.00.

TABLE 4. *Uptake of* E. coli *O157:H7 by cold (2°C) and warm (22°C) intact waxed Braeburn apples immersed in cold (2°C) peptone water containing approximately 3 × 10^7 CFU/ml*

		Skin		Pulp		Outer core		Inner core	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Warm apple	No rinse	4.54[a] ± 0.13	4.40–4.65	3.42 ± 0.37	3.16–3.84	5.67 ± 0.13	5.53–5.78	<3.22[b]	<2.00–4.02
	Chlorinated water	<1.10[b]	<1.00–1.30	<1.10[b]	<1.00–1.30	3.97 ± 0.25	3.69–4.17	<2.51[b]	<2.00–3.23
Cold apple	No rinse	4.46 ± 0.29	4.13–4.69	3.42 ± 0.41	3.17–3.89	5.65 ± 0.22	5.40–5.78	4.00 ± 0.42	3.53–4.31
	Chlorinated water	<1.56[b]	<1.00–2.38	<1.00	<1.00	<2.36[b]	<2.00–2.60	<2.00	<2.00

[a] Log (CFU/g); results from three apples.
[b] One or more values below the lower limit of detection: inner and outer core: log (CFU/g) = 2.00. Skin and pulp: log (CFU/g) = 1.00.

TABLE 5. *Effect of temperature differential between apples and water on the uptake of aqueous dye into the inner core region*

Temperature differential	Percent dye uptake by apples		
	None	Slight to moderate	Substantial
Warm apple/cold water	84.1 (95 of 113)	9.7 (11 of 113)	6.2 (7 of 113)
Cold apple/warm water	100 (113 of 113)	0 (0 of 113)	0 (0 of 113)

outer core region. The extent of contamination with apples that were immersed and dried without subsequent rinse steps was similar among the varieties—approximately 10^5 CFU/g. Sequentially rinsing the apples for 1 min with chlorinated water (2,000 mg/liter sodium hypochlorite) and then with tap water produced an average reduction in the extent of outer core contamination of 1- to 2-log cycles. However, some apples continued to harbor high populations of contamination despite the rinses. The levels of contamination retained after rinsing tended to be somewhat greater for three of the four varieties when warm apples were immersed in the cold peptone water.

The next greatest extent of contamination was associated with the skin (peel) of the apple. With the exception of the McIntosh apples, the levels were approximately 10^4 CFU/g. Rinsing the apples with chlorinated water produced a 1- to 3-log decline in *E. coli* levels, depending on the variety. Substantially fewer *E. coli* O157:H7 were detected on the skin of McIntosh apples, despite the fact that the extent of contamination of this variety's outer core was similar to the other varieties. No temperature effects were apparent.

The extent of contamination was lowest in the pulp. These low levels were virtually eliminated when the apples were rinsed, indicating that the somewhat higher levels in the unrinsed apples were caused by bacteria on the skin being driven into the interior when the apples were sectioned. The levels of *E. coli* in the inner core region were generally low and reduced further by rinsing. Again, this suggests that most of the *E. coli* O157:H7 associated with this section were translocated from the surface during cutting. However, a few inner core samples retained somewhat elevated *E. coli* levels despite the chlorinated rinse. This was restricted largely to apples that had been warm prior to immersion. This suggests that there may have been differences in the accessibility of *E. coli* to the inner core among individual apples, and that accessibility was enhanced when there was a positive temperature differential, i.e., the temperature of the apple was higher than the temperature of the cell suspension.

Dye uptake. The effect of temperature differentials between apples and the aqueous environment into which they are immersed was evaluated further by examining the uptake of FD&C red dye no. 40 by unwaxed Golden Delicious apples (Table 5). In the first instance, cold (4°C) apples were plunged into a warm (21°C) solution. Second, warm apples (22°C) were immersed in a cold (9°C) dye solution. No uptake of the dye into the inner core region was observed when cold apples were immersed in warm water. However, when warm apples were submerged in cold water, approximately 6% of the apples had substantial accumulation of the dye in the inner core region (Fig. 2). An additional 10% of the apples had small to moderate accumulation of the dye. In all cases, the dye entered through the blossom end of the apple. The apples displaying substantial dye uptake had easily discernable open channels into the core region. Some uptake of the dye was also observed through the skin, particularly in regions where the apple had been bruised or the skin punctured.

DISCUSSION

The immersion of apples in water is a common practice in the handling, cleaning, and processing of apples. Apples are often transported via flume systems and immersed for the removal of leaves and other debris. Typically, such water is chlorinated or otherwise treated. No published data appear to be available on the chlorine levels, temperatures, or organic loads that would likely be encountered in water used in apple handling, particularly in the initial dump tanks where contamination associated with the apple would tend to be greatest. Information is also unavailable on immersion times typical for cider production or apple-packing facilities. Goverd et al. *(8)* identified flume water was a potential source of coliforms, *E. coli,* and *Salmonella* spp. contamination or cross contamination during cider production. However, their study did not specify which, if any, of the facilities examined treated their flume water with antimicrobials.

The results of the current study indicate that if chlorination of water into which apples are immersed is insufficient to prevent cross contamination, then uncontaminated apples can be expected to take up *E. coli* O157:H7. The uptake of *E. coli* O157:H7 is not uniform; the site of greatest contamination was consistently the outer core region. While the *E. coli* O157:H7 uptake study did not differentiate between the stem and blossom regions, observations made during the dye uptake studies clearly indicated that the blossom end is the area of greatest concern. Concentration of pathogen cells in this region of the apple has serious implications in relation to the efficacy of sanitization treatments. Once this cavity filled with fluid, it became difficult to displace the fluid by rinsing, even when the blossom end of the apple was placed directly under a tap-water stream. This is also reflected in the presence of high levels of *E. coli* O157:H7 in the outer core in some apples after the rinse protocol (Tables 1 through 4).

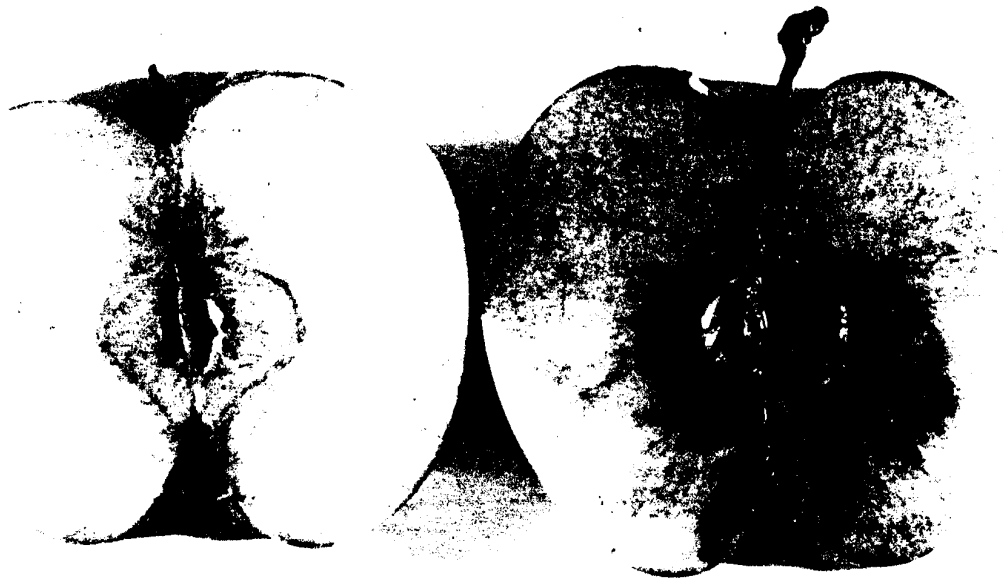

FIGURE 2. *Examples of apples that did and did not take up RD&C red dye no. 40 when warm apples were immersed in a cold solution.*

The importance of the apple's blossom end is further increased by the presence of open channels into the inner core region in a portion of the apples (Table 5). The incidence of such channels varies with apple variety and is also affected by how long the apple is held in storage *(16)*. The results clearly indicate that the potential for aspirating *E. coli* O157:H7 cells into the interior of the apple is increased by a temperature differential where the apple is warmer than the water into which it is immersed. Conversely, the immersion of cold apples into warmer water appears to prevent the infiltration of material into the core region. The infiltration of bacteria into the interior of foods due to the pressure differential produced during cooling has been well established for the handling of eggs *(9)* and tomatoes *(1, 20)*.

Treatment of the apples with 2,000 mg/liter sodium hypochlorite for 1 min reduced but did not totally eliminate *E. coli* O157:H7 on the skin of apples. The treatments were less effective for bacteria localized in the outer core region. Surface sanitization also produced a substantial decline in the levels of *E. coli* O157:H7 isolated from the inner core and pulp samples. However, in this instance it is more likely that the decrease is due to the elimination of bacteria on the surface of the apple, which were driven into the pulp and inner core region when the fruit was sectioned. Similar observations have been made with the transfer of salmonellae into tomatoes *(7, 11)*.

As indicated above, the factors affecting the extent and location of *E. coli* O157:H7 uptake by apples are similar to those affecting the uptake of salmonellae by tomatoes. Rushing et al. *(15)* identified the maintenance of dump tank water quality as one of the three critical control points in tomato packing houses. They recommended that the free chlorine levels be monitored and the water buffered to insure appropriate activity and the water temperature thermostatically maintained at 40°C to prevent infiltration and to enhance the biocidal activity of the chlorine. Overall, the current results suggest that immersion of apples into aqueous environments, particularly the dump tank, could play an important role as a potential source for contaminants. Conversely, through the use of surface sanitizers and temperature control to prevent infiltration, the dump tank may be a means of reducing the risks associated with production of unpasteurized juice/cider. However, it is important to note that some *E. coli* O157:H7 remained on inoculated apples, even when the temperature differential was unfavorable to infiltration and a 2,000-mg/liter sodium hypochlorite rinse was used. Additional research is needed to determine the effectiveness of these interventions under commercial conditions, to identify the optimal chlorine levels for sanitizing the surface of apples and preventing cross contamination, and to determine the optimal water temperatures for preventing infiltration. While these steps are not likely to eliminate *E. coli* O157:H7 contamination, they are likely to be important components of any integrated approach for reducing the risks associated of presence of pathogenic microorganisms in unpasteurized apple juice/cider.

ACKNOWLEDGMENTS

We gratefully acknowledge Gloria DiLullo, Angela Mattrazzo, and Mathew Evans for their assistance in conducting this research.

REFERENCES

1. Bartz, J. A., and R. K. Schowalter. 1981. Infiltration of tomatoes by bacteria in aqueous suspension. Phytophathology 71:515–518.
2. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M.Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. J. Am. Med. Assoc. 269:217–2220.
3. Buchanan, R. L., and S. G. Edelson. 1996. Culturing enterohemorrhagic *Escherichia coli* in the presence and absence of glucose as a simple means of evaluating the acid tolerance of stationary-phase cells. Appl. Environ. Microbiol. 62:4009–4013.
4. CDC (Centers for Disease Control and Prevention). 1975. *Salmonella typhimurium* outbreak traced to a commercial apple cider—New Jersey. Morbid. Mortal. Weekly Rep. 24:87–88.
5. CDC (Centers for Disease Control and Prevention). 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—October, 1996. Morbid. Mortal. Weekly Rep. 45:975.
6. CDC (Centers for Disease Control and Prevention). 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. Morbid. Mortal. Weekly Rep. 46:1, 4–8.
7. Gayler, G. E., R. A. MacCready, J. P. Reardon, and B. F. McKernan. 1955. An outbreak of salmonellosis traced to watermelon. Public Health Rep. 70:311–313.
8. Goverd, K. A., F. W. Beech, R. P. Hobbs, and R. Shannon. 1979. The occurrence and survival of coliforms and salmonellas in apple juice and cider. J. Appl. Bacteriol. 46:521–530.
9. ICMSF (International Commission of Microbiological Specifications for Foods). 1980. Microbial ecology of foods, vol. 2. Food commodities, p. 521–566. Academic Press, New York.
10. Leyer, G. J., L.-L. Wang, and E. A. Johnson. 1995. Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. Appl. Environ. Microbiol. 61:3752–3755.
11. Lin, C.-M., and C.-I. Wei. 1997. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. J. Food Protect. 60:858–863.
12. Miller, L. G., and C. W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Protect. 57:460–464.
13. NACMCF (National Advisory Committee on Microbiological Criteria for Food). 1997. Recommendations for controlling the transmission of pathogenic microorganisms in juices. USDA Food Safety and Inspection Service, Washington, D.C.
14. Parish, M. E. 1997. Public health and nonpasteurized fruit juices. Crit. Rev. Microbiol. 23:109–119.
15. Rushing, J. W., F. J. Angulo, and L. R. Beuchat. 1996. Implementation of a HACCP program in a commercial fresh-market tomato packinghouse: a model for the industry. Dairy Food Environ. Sanitation 16:549–553.
16. Smock, R. M., and A. M. Neubert. 1950. Apples and apple products, p. 30–34. Interscience Publishers, Inc., New York.
17. Splittstoesser, D. F., M. R. McLellan, and J. J. Churey. 1996. Heat resistance of *Escherichia coli* O157:H7 in apple juice. J. Food Protect. 59:226–229.
18. Steele, B. T., N. Murphy, G. S. Arbus, and C. P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. J. Pediatr. 101:963–965.
19. Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. 59:2526–2530.
20. Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment of chlorine. Appl. Environ. Microbiol. 61:2127–2131.