Journal of Food Protection, Vol. 65, No. 6, 2002, Pages 911–917

Efficacy of Sanitation and Cleaning Methods in a Small Apple Cider Mill

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MS 01-438: Received 27 November 2001/Accepted 4 February 2002

ABSTRACT

The efficacy of cleaning and sanitation in a small apple cider processing plant was evaluated by surface swab methods as well as microbiological examination of incoming raw ingredients and of the final product. Surface swabs revealed that hard-to-clean areas such as apple mills or tubing for pomace and juice transfer may continue to harbor contaminants even after cleaning and sanitation. Use of poor quality ingredients and poor sanitation led to an increase of approximately 2 logs in aerobic plate counts of the final product. Reuse of uncleaned press cloths contributed to increased microbiological counts in the finished juice. Finally, using apples inoculated with *Escherichia coli* K-12 in the plant resulted in an established population within the plant that was not removed during normal cleaning and sanitation. The data presented in this study suggest that current sanitary practices within a typical small cider facility are insufficient to remove potential pathogens.

Before the 1990s, disease outbreaks attributed to the consumption of bacterial pathogens in unpasteurized juice were rare. For acidic juices such as apple cider and orange juice, low pH was presumed to inhibit or kill potential foodborne pathogens. Since 1991, however, several notable outbreaks of disease caused by *Escherichia coli* O157:H7 strains or isolates were associated with unpasteurized cider *(3–5, 14)*. In addition, several laboratories found acid tolerance in some *E. coli* O157:H7 strains *(2, 7, 10, 12)*. In response to the reported outbreaks, the U.S. Food and Drug Administration (FDA) initially required unpasteurized juices to state the possible risk of pathogen contamination on a label. Recently, the FDA issued regulations requiring the application of hazard analysis critical control point (HACCP) principles to juice production *(16)*. HACCP principles require that an HACCP procedure be completed for each specific process. After the hazard analysis, critical control points and control limits must be established for identified hazards. As a prerequisite for an appropriate HACCP plan, good manufacturing practices (GMPs) must be established; GMPs include cleaning and sanitation standard operating procedures (SSOPs) for the facility, equipment, and raw materials. At a minimum, processors must follow GMP regulations *(17)*. Some state agencies and Canada *(15)* have developed GMPs and SSOPs specific for cider production.

Although many producers have adopted GMPs and SSOPs for apple cider processing, how such procedures affect the microbial load of juice is unknown. Goverd et al. *(8)* first reported the incidence of coliforms and *Salmonella* spp. during the production of fermented apple cider in the United Kingdom. The cider production practices varied and included the use of ground-harvested (windfall) apples. Storage conditions of the harvested apples were not indicated. Sanitation ranged from "primitive" to "well-organized cleaning routines." Perhaps, not surprisingly, the apple ciders contained high coliform counts, as well as presumptive *E. coli* counts. Results did not indicate which ciders were produced using better practices. How production practices employing GMPs and SSOPs may affect microflora levels could not be discerned.

In 1997, the FDA conducted establishment inspections and examined cider from 237 manufacturers in 32 states *(15)*. In general, practices used for the harvest and production of the ciders were better than those reported by Goverd et al. *(8)*. Many producers used exclusively tree-picked apples from known sources. According to the findings, 67% of the firms had good sanitation, 27% were marginal, and only 4% were categorized as poor. Trends in the results of the study indicate that cider processing did not substantially change levels of indicator microorganisms between incoming raw ingredients and finished juice.

Although more limited in scope, Dingman *(6)* found that the overall occurrence of *E. coli* in apple cider manufactured in Connecticut was similar to that in the FDA report. Dingman also noted an association between the time

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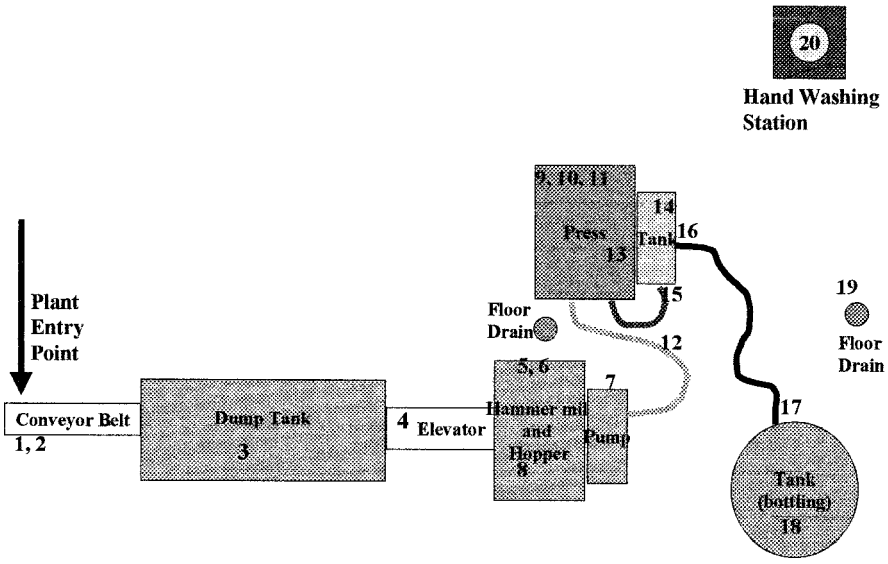

FIGURE 1. *Schematic diagram of a small apple cider processing facility. Numbers indicate the location of environmental sampling (swabs). Numbers correspond to locations shown in Table 1.*

of year of cider production and the occurrence of *E. coli.* Dingman attributed this time-related appearance to a common unidentified factor that occurred during cider production. GMPs and SSOPs were not discussed. In a report by Senkel et al. *(13),* changes in GMPs and SSOPs were examined by following the performance of 11 Maryland apple cider producers before and after receiving HACCP training. Standard plate counts and total coliform counts were unaffected by improved practices. However, there was a significant decrease in fresh cider samples that contained *E. coli.* Given the importance of *E. coli* as an indicator of failed sanitation *(9),* this latter finding suggests that improved practices can reduce the risk of foodborne illness in fresh apple cider. Although the Maryland study covered numerous types of interventions, specific practices were not examined in detail. Moreover, as noted in the study, other factors may have contributed to microbial reductions. Consequently, further controlled studies would help determine how specific control measures affect the composition and levels of microflora present in apple cider.

In the present study, the effects of GMPs and SSOPs in a small cider mill were examined. Typical GMPs and SSOPs for study followed recommendations that can be obtained from a variety of sources, including state and local health agencies and trade associations. One example is the Apple Hill Growers Association (Camino, Calif.), which published a quality assurance plan providing both production and processing guidance. These guidelines emphasize the need for quality ingredients from known sources, including the exclusive use of tree-picked fruit. The grading and inspection of fruit are recommended in addition to an established daily sanitation operating procedure. These guidelines provided the basis for the GMPs and SSOPs examined here.

To examine GMPs and SSOPs in a realistic environment, a commercial cider mill was located and leased for operation for two consecutive seasons. The evaluation included the specific effects of poor quality ingredients and the use of frequent versus infrequent sanitation and cleaning on the quality of the juice produced at the facility. The results should provide sufficient information to enable a processor to evaluate their current practices appropriately.

MATERIALS AND METHODS

Apple cider production. Apples were procured from local sources in the Placerville, Calif., area. Cider was produced in a small commercial facility that lacked environmental temperature controls, including heating and air conditioning (Fig. 1). The facility was capable of producing 757 liters (200 gal) of cider per hour with a five-person crew. Normal yields for this facility were approximately 3.78 liters (1 gal) of cider for every 5.44 kg (12 lb) of apples. No commercial product was produced in this plant for human consumption during the study.

Apples entered the building via a conveyor belt that transported the apples to a 1,340-liter (500-gal) dump tank containing potable municipal water. The water was tested according to state and federal regulations. Residence time in the tank was approximately 1 to 2 min. Each production run used approximately 85 kg (188 lb) of fruit. Apples were removed from the water tank by an elevator and macerated in a hammer mill containing a 1.27-cm (0.5-in.) sizing screen. The wet pomace (not yet pressed for juice extraction) was collected in a stainless steel hopper. The wet pomace was mixed by circulating it with a pump for 10 min before collecting it for analysis. The pomace was then transferred to a vertical rack and cloth press. The cider was extracted at a maximum pressure of 121 kg/cm^2 (1,720 psi). Expressed juice was collected in a covered stainless steel tank and subsequently pumped to a larger stainless steel tank for bottling. Cider in the holding and bottling tanks was stirred before collecting juice samples for microbiological and chemical analysis. After pressing, pomace (dry pomace) was thoroughly mixed before collecting the analytical sample.

Microbiological profiles. Incoming apples were sampled for microbiological profiles by collecting four groups of six randomly selected apples. Each group was placed in a large blender with an equal mass of 0.1% peptone (Difco, Ann Arbor, Mich.) and pureed for approximately 2 min. The puree was then diluted to appropriate levels with 0.1% peptone and plated on plate count agar (Difco) for monitoring naturally occurring aerobic microflora (APC) or brain heart infusion (BHI) agar (Difco) plus 100 μg/ml streptomycin (Sigma Chemical Co., St. Louis, Mo.) (BHIS) when apples inoculated with *E. coli* were used. The plates were incu-

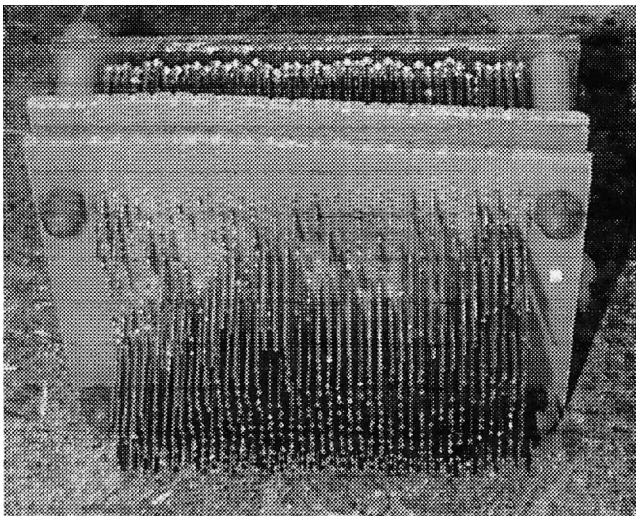

FIGURE 2. *Device used for replica plating of* E. coli *from petri dish to apple surfaces.*

bated 48 h at 32°C for APC or 24 h at 37°C for *E. coli*. The temperature of the puree was monitored after blending to ensure there was no increase (+2°C) over ambient during blending. Wet pomace was withdrawn at the hopper before pressing. Dry pomace was withdrawn from press cloths after pressing. Four samples were withdrawn at each sampling point. Pomace samples were diluted 1:10 (wt/vol) in peptone and homogenized in a blender for approximately 2 min. Pomace samples were plated as for apple puree. Triplicate juice samples (25 ml each) were removed from the collection tank after stirring. Dilutions were made, and then appropriate concentrations were plated as for apples and pomace. In some studies, yeast and mold populations were also examined on apple, pomace, and juice samples. Appropriate dilutions were made, and 1-ml aliquots were plated on Yeast and Mold Petrifilm (3M Company, Minneapolis, Minn.). Petrifilm plates were incubated at 25°C for 5 days.

Inoculation of apples. For studies using *E. coli*–inoculated apples, *E. coli* K-12 (ATCC 25253, a streptomycin-resistant strain) was transferred from stock to BHI broth (Difco) plus 100 μg/ml streptomycin and grown on a rotary shaker at 200 rpm overnight at 37°C in 2-liter Erlenmeyer flasks. Two methods of apple inoculation were used. The first technique was to submerge whole apples in a liquid culture containing 10^6 to 10^7 CFU/ml for 5 min. The apples were allowed to dry overnight at ambient temperature. For the second method, 10 ml of overnight culture was spread onto large-size petri plates (150 by 15 mm) containing BHIS and cultivated overnight at 37°C. The stationary culture was then transferred onto the surface of apples using a pin apparatus (35 by 35 pins on 0.32-cm centers) with multiple moveable pins to form to the surface of the apple (Fig. 2). Fifteen to 20 apples were inoculated from one plate. As with dipped apples, inoculated apples were held for overnight at ambient temperature before use.

Cleaning and sanitation. Normal cleaning and sanitizing of the equipment and facility consisted of an initial rinse with potable water to remove gross contamination, followed by spray washing with FoamClor, a commercial chlorinated alkaline product (Zep Company, Atlanta, Ga.) at 45°C. FoamClor was left in contact with equipment for 5 min before rinsing again with potable water. Next, the equipment and facility were sanitized with Zepamine, a quaternary amine disinfectant at pH 7.0 to 7.7 (Zep Company). Zepamine was left in contact with the equipment and facility overnight when sanitizing at the end of a production day. If production was to continue, then Zepamine was left in place for 5 min, followed by a potable water rinse before continued production. Cleaning and sanitizing were conducted before the first trial of the day, after each trial, and at the end of the production day, except when indicated. Water in the dump tank was changed whenever cleaning and sanitizing occurred. Excess pomace on press cloths was removed with a potable water rinse. The cloths were then washed in a commercial washer with commercial chlorine bleach and dried in a commercial drier. Press cloths were stored in a dry closed plastic container between uses.

GMP and SSOP efficacy. To determine the efficacy of GMPs and SSOPs, apples with visible damage but unknown history were used. Apples were not culled before production; visibly damaged, moldy, and unsound fruit was used. Cider was produced on 4 consecutive days. During this time, normal cleaning and sanitation procedures were performed once, at the end of production on the first day. Production took place in December. Soak water in the wash tank was not changed or chlorinated during this period, and equipment received only a quick rinse to remove gross debris after each successive processing day.

To determine carryover contamination and further assess the sufficiency of cleaning and sanitation SOPs, sound uninoculated apples were processed in trials alternated with inoculated apple trials. In addition, SSOPs between processing trials with inoculated and uninoculated apples were varied as follows: (i) no cleaning or sanitizer, press cloths reused from previous processing, (ii) no cleaning or sanitizer but press cloths changed between processing, and (iii) cleaning and sanitizer used and press cloths changed between processing. Results were analyzed with a one-way analysis of variance program in SAS Proc Glm (SAS Institute, Cary, N.C.). Multiple comparisons between means were tested by the Ryan-Elinot-Gabriel-Welsch test.

Environmental sampling. Environmental samples were obtained with commercial swabs following the manufacturer's directions (3M). Approximately 50 cm^2 per location was swabbed using the rayon-tipped applicator provided with the test vial. Samples were plated either on plate count agar for total APC or BHIS for *E. coli* K-12 to determine levels of microorganisms. Ambient temperature during each operating day was followed, and the high temperature was recorded. Environmental swab test results and their relationship to temperature were analyzed with the Cochran-Armitage linear trend test option in the SAS program Proc Multitest (version 8, SAS). Six temperature groups were created to eliminate zero cells in swab test results. The temperature group cut-off points were 15, 18, 21, 24, and 27°C.

Testing to determine airborne contamination was conducted before and during processing both with and without inoculated apples. Differences in populations before and after processing were compared to estimate the contribution of processing equipment to airborne contamination. Air sampling was performed by a single-stage N6 microbial sampler (Thermo Andersen, Smyrna, Ga.) with either plate count agar or BHIS plates. Air was drawn for 5-, 10-, and 15-min/plate intervals with a vacuum pump calibrated to draw 1 ft^3 (28.3 liter)/min. Plates were incubated as previously described.

RESULTS AND DISCUSSION

Results showing the effect on APC of apples before and after washing in the pomace and juice when GMPs or SSOPs were not employed are shown in Figure 3. Mean APCs on incoming fruit were 2.66 ± 0.37 log CFU/g and

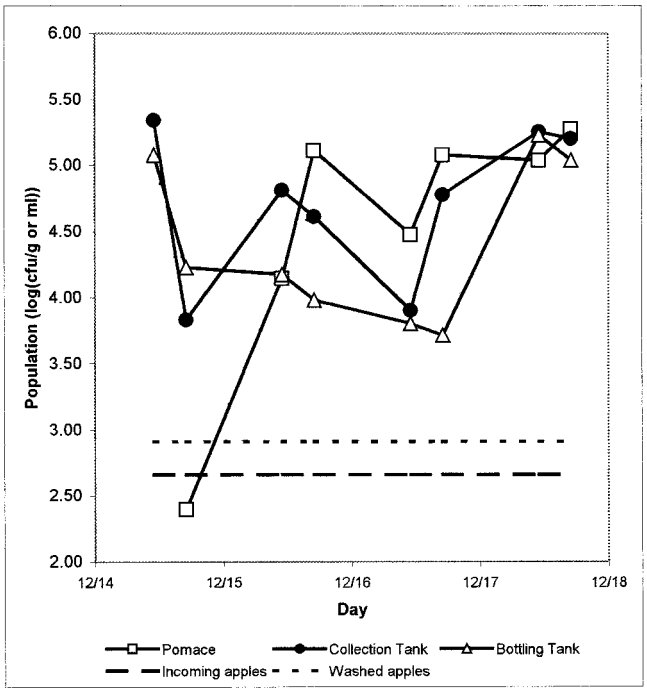

FIGURE 3. *Aerobic bacterial population in a cider plant lacking good manufacturing practices and standard sanitation operating procedures.*

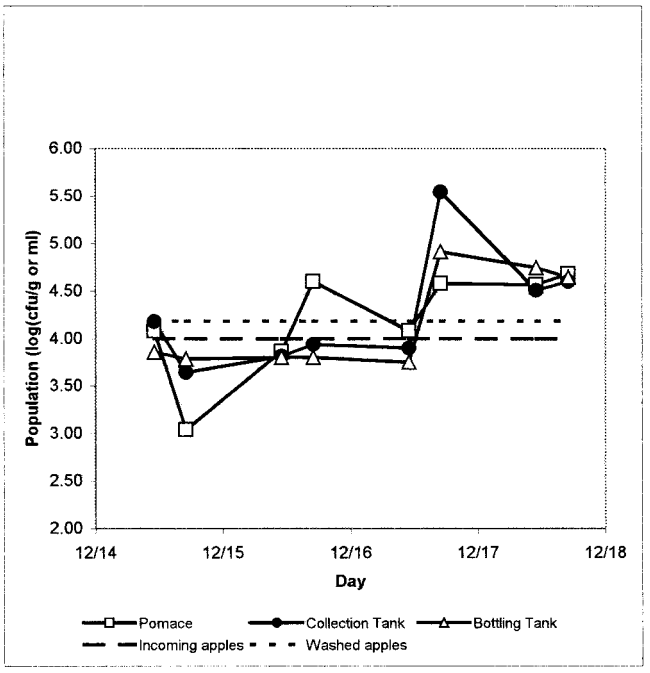

FIGURE 4. *Yeast and mold populations in a cider plant lacking good manufacturing practices and standard sanitation operating procedures.*

did not appear to reflect the apparent poor condition of the incoming ingredient. This level was at least 1 log less than Senkel et al. *(13)* reported for tree-picked apples. Pomace population levels rose throughout the trial period, increasing from log 2 to log 5. This increase may reflect increasing contamination transferred from unsanitized equipment. APC levels in juice from the collection and bottling tanks showed no obvious trend related to sanitation.

Yeast and mold populations were higher at 4.00 ± 0.12 log CFU/g, which may account for the poor appearance of the fruit (Fig. 4). Overall, levels of yeast and molds increased during the sampling period. Little has been published on yeast and mold populations on incoming apples used for juice processing. During the operation of this facility, yeast and mold populations of sound tree-picked apples averaged 2.74 ± 0.55 log CFU/g (n = 52). Therefore, it appears yeast and mold populations were elevated in this lot. In general, the microbiological quality of the juice should reflect the microbial load of the incoming apples. For yeast and mold populations, this appears to be valid. However, this is not true for total aerobic populations. For juice made from this lot of apples, total APC was as much as 2 logs higher than populations in apples. By comparison, Merker et al. *(11)* reported APC in juice from windfall apples at 5.0 ± 0.3 log CFU/ml and in juice from tree-picked, culled fruit at 2.9 ± 0.8 log CFU/ml. Results from this study would tend to be higher than even windfall apples, particularly as the week without sanitation progressed. The high level of microorganisms in the juice compared with the low level found on the apples would suggest that a substantial contribution in microflora was from contaminated equipment.

Results of analysis for airborne microbial populations indicate that before processing in the cider mill, total airborne APC was 0.85 CFU/liter of air. During processing, while the hammer mill was in operation, total airborne APC increased 100-fold to 90.6 CFU/liter of air. Inoculated apples were stored in the facility overnight before processing. Despite the overnight storage period, no streptomycin-resistant organisms were detected in the air before the processing of inoculated apples. However, during the processing of inoculated apples, 31.1 CFU/liter of streptomycin-resistant organisms were found, indicating substantial aerosol formation during operation. The production of aerosols could account for the spread of contamination from the fruit to the entire facility.

To determine the areas or equipment most likely to become a microbiological concern, environmental samples were taken before, during, and after the period in which poor sanitation was imposed. The environmental sampling scheme throughout the facility is shown in Figure 1. Results from this survey are shown in Table 1. Qualitative results are presented, rather than APC populations. In some locations, APC populations quickly exceeded expected numbers, despite low levels before processing; this resulted in confluent growth on plate count agar plates. Even after appropriate cleaning and sanitizing at the end of processing on the final production day, many areas retained high numbers of bacteria. These areas represent those that are more difficult to clean and sanitize and may have developed biofilms throughout the week.

To further assess microbiological problem areas, apples inoculated with *E. coli* K-12 were used to trace the probable path of areas likely to become contaminated and to determine if a nonpathogenic laboratory strain, deliberately introduced into the facility, could be removed by the use of

TABLE 1. *Identification of areas of microbiological concern identified by environmental sampling*[a]

Sampling location[b]	Day of continual operation							
	1		2		3		4	
	Pre	Post[c]	Pre	Post[d]	Pre	Post[d]	Pre	Post[c]
Conveyer belt seam (1)	−	++	−	++	+	+++	+	+
Metal edge and plastic curtain on conveyer (2)	−	+	−	+	−	+	−	+
Dump tank (3)	−	−	−	−	−	−	−	−
Elevator (4)	−	−	−	−	−	−	−	−
Hammer mill (5)	−	+	−	−	−	++	−	+++
Hammer mill screen (6)	−	−	−	−	−	−	−	−
Pomace pump (7)	−	++	−	−	−	−	−	+
Upper housing to hammer mill (8)	−	+	−	−	−	+++	+++	+
Press (9)	−	−	−	−	−	−	−	++
Press rack (10)	−	+	−	−	−	−	−	+++
Press tray (11)	−	++	−	−	−	−	−	+++
Tubing: pomace pump to press (12)	−	+	−	−	−	+	−	+++
Press mold (13)	−	+	−	+++	−	+++	+++	+++
Collection tank (14)	−	+	−	+++	−	+	−	+
Tubing: press to collection tank (15)	−	−	−	+	+++	+	−	+++
Transfer pump (16)	−	++	−	++	−	−	−	+
Bottling apparatus (17)	−	++	−	++	−	−	−	+++
Bottling tank (18)	−	++	−	++	−	+	−	+
Floor drain (19)	−	+	−	+	−	+++	+++	+++
Hand-washing faucet (20)	−	−	−	+	−	+	−	+

[a] Key: −, few colonies or no contamination (<0.1 CFU/cm^2); +, slight contamination (0.1 to 1.0 CFU/cm^2); ++, heavy contamination (1.0 to 10.0 CFU/cm^2); +++, likely hot spot (>10.0 CFU/cm^2).

[b] Numbers in parentheses correspond to sampling locations in Figure 1.

[c] Gross contamination removed before sampling; water rinse and sanitation postsampling.

[d] Gross contamination removed before sampling; water rinse only postsampling.

SSOPs. Areas where *E. coli* was recovered reflected a pattern similar to that found with naturally occurring aerobic populations (data not shown). During these initial assessments, appropriate sanitation reduced *E. coli* K-12 below detection. Furthermore, juice produced before the introduction of the organism and after appropriate plant sanitation did not contain detectable levels of *E. coli* K-12. Consequently, continued environmental sampling to monitor *E. coli* K-12 in subsequent studies focused on two areas of concern identified by these surveys, the hammer mill and transfer tubing.

Further environmental sampling for the presence of *E. coli* K-12 occurred only when the facility was in operation. Results are shown in Figure 5. Despite earlier data indicating complete removal of the *E. coli* K-12 with cleaning and sanitation, a small but persistent population of *E. coli* K-12 was detected in the facility. This persistent population resulted in juice in which there was a background population level of *E. coli* K-12 (1.06 ± 0.09 log CFU/ml), when previously, none was detected. This is in contrast with results found with the initial assessment of SSOPs in which the *E. coli* K-12 was no longer detectable in the environment. Furthermore, the contaminating population of *E. coli* K-12 increased with the high temperatures recorded in the processing plant and with the length of time of using inoculated apples. The association of increased levels of *E. coli* K-12 with increased temperatures was examined using the Cochran-Armitage linear trend test *(1).* Results indicate a statistically significant association between temperature and the probability of *E. coli* K-12 being detected (P = 0.0007). A nonparametric permutation trend test was performed on the same data, yielding a P-value of 0.001, confirming the parametric result. This association and persistence of the *E. coli* K-12 in the facility may have been due to environmental conditions normal during each production period. Initial assessments were conducted in February. During that month, typical evening temperatures were below freezing and remained low throughout the day. These extreme low temperatures would not promote growth or possible biofilm formation by contaminating microorganisms. In contrast, the temperatures recorded from May to August were considerably higher. The high temperatures recorded for those periods allow considerably more microbial growth than would temperatures below 0°C. Consequently, the difference in results found between initial assessments and subsequent studies is most likely due to favorable growth conditions and emphasizes the importance of environmental controls in a food processing facility.

The efficacy of SSOPs was further assessed by alternating production runs with inoculated and uninoculated fruit. Results of *E. coli* K-12 populations in juice from uninoculated fruit processed after contaminated apples (Table 2) are slightly higher than background. However, the increases shown were not statistically significant. The inclusion of a sanitizing step did not provide an additional benefit over a simple water rinse. In contrast, when press cloths

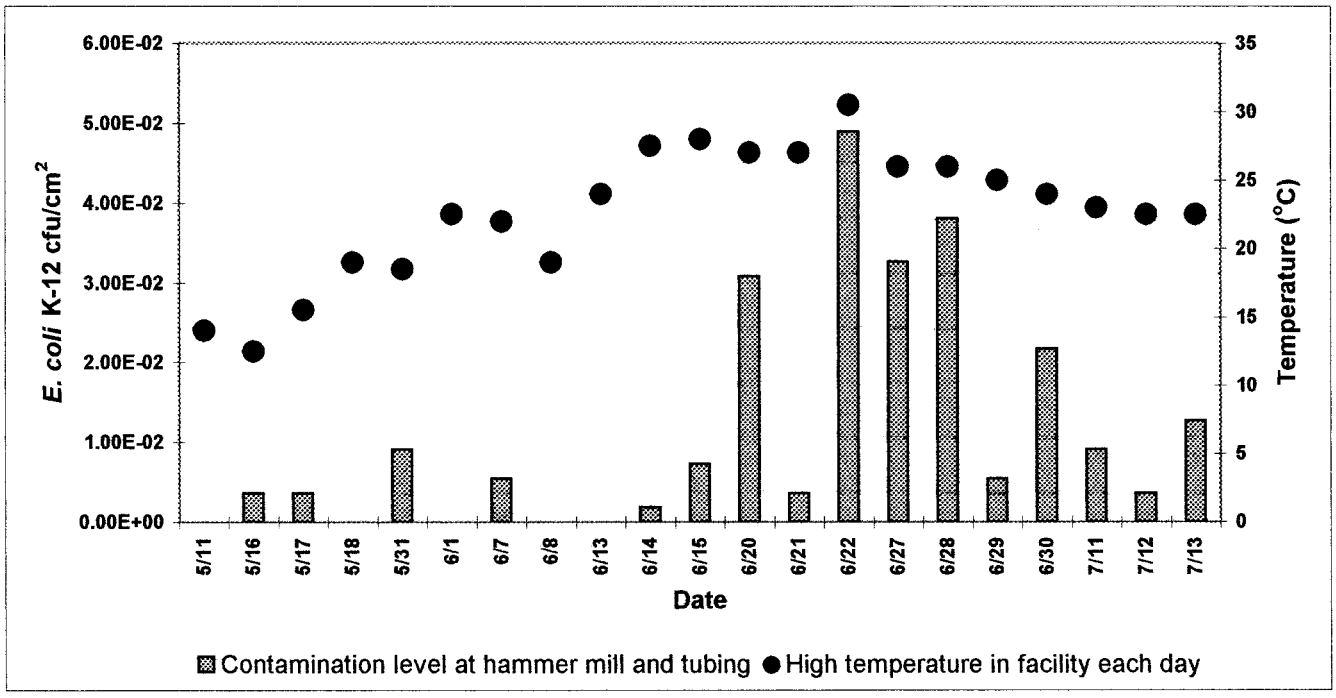

FIGURE 5. *Relationship between ambient temperature and* E. coli *K-12 contamination in an apple cider processing plant.*

commonly used in cider processing were not replaced between production runs in which inoculated apples preceded production runs with uninoculated apples, populations of *E. coli* K-12 increased nearly 3 log CFU/ml over background levels. Since most production facilities do not change press cloths throughout a production period, the substantial carryover observed here is of concern.

CONCLUSIONS

The efficacy and importance of good GMPs and SSOPs in a small commercial cider facility were examined in this work. Results indicated that, although useful in controlling levels of contamination in a facility, cleaning and sanitation alone may not ensure a safe product. Contamination of the facility as well as the final product may be attributed to the extensive production of aerosols, as indicated by the level of airborne microorganisms detected during operation of the facility, particularly when compared to numbers present when the plant was idle. Equipment must be designed for easy cleaning, to minimize contamination and to prevent biofilm formation. Covered collection tanks for juice likely reduced the incidence of contamination from airborne microorganisms, but they could not have eliminated it. Certain practices considered typical in a small cider facility, such as the reuse of press cloths, are clearly problematic and should be eliminated.

The detection of surface and airborne contamination points out the difficulties of controlling contamination once it has occurred. Unfortunately, once introduced into the facility, *E. coli* could clearly become established and difficult to remove. Diligence must be applied to prevent the initial contamination of a facility and must include a thorough knowledge of apple sources, harvest, and growing practices. Finally, the data presented in this study suggest that to ensure a safe product in this type of facility, the application of some postpressing intervention technology such as pasteurization may be advisable.

ACKNOWLEDGMENTS

This paper was supported by Cooperative Agreement no. FD-000431 from the FDA and the National Center for Food Safety and Technology. Additional support was received from the El Dorado County Department of Agriculture, the California Department of Public Health, and the University of California, Davis.

TABLE 2. *Carryover contamination between processing where inoculated apples are alternated with uninoculated apples*

	Control (background)	First pressing, inoculated apples	Second pressing, uninoculated apples
	Log(CFU/ml) ± SD *E. coli* K-12 in juice		
Background *E. coli* K-12	1.06 ± 0.09 A[a]		
Sanitizer, clean cloths		5.34 ± 0.36 B	1.70 ± 0.35 A
No sanitizer, clean cloths		5.63 ± 0.16 B	1.95 ± 0.13 A
No sanitizer, dirty cloths		5.21 ± 0.48 B	3.76 ± 0.25 C

[a] Each value represents an average of two separate pressings. Triplicate juice samples were drawn from each pressing. Different letters designate significant differences at $P < 0.05$.

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