Journal of Food Protection, Vol. 71, No. 1, 2008, Pages 46–51

Washing Practices on the Microflora on Georgia-Grown Cantaloupes

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MS 07-050: Received 29 January 2007/Accepted 21 August 2007

ABSTRACT

In recent years, several foodborne illness outbreaks have been associated with the consumption of cantaloupe. Cantaloupes can be contaminated with pathogens anywhere from the field to the packing line. In the United States, cantaloupes are handled and packed differently in each state. Georgia-grown cantaloupes are brought to sheds, washed, and packed, whereas California-grown cantaloupes are field packed. In this study, the microbiological status of cantaloupes produced by four Georgia growers that use various washing and packing practices was assessed to determine the influence of these different practices. The facilities were visited four times during the harvest season. Aerobic bacteria, *Escherichia coli,* and coliforms on these Georgia-grown cantaloupes were enumerated in transport trailers, after washing, and after packing. Samples also were analyzed for the presence of *Salmonella* and *E. coli* O157:H7. In sheds 1 and 4, a chlorinated dump tank was used to wash melons. In sheds 2 and 3, heated water with chlorine was used in the dump tanks. Although there was a significant reduction ($P < 0.05$) in the populations of the aerobic bacteria and *E. coli* between the transport trailer and the dump tank for sheds 1 and 4, the reduction was less than 0.5 log CFU/cm^2. The temperatures of the water in the dump tanks at sheds 2 and 3 were not high enough to effectively reduce the microbial populations evaluated. Populations on the melons increased slightly (<0.5 log CFU/cm^2) after the melons were removed from the dump tank, suggesting possible contamination after washing.

Foodborne diseases are widespread and continue to be a public problem, both in developed and developing countries. In industrialized countries, the percentage of people suffering from foodborne disease has been reported as up to 30% according to the World Health Organization *(10).* In the United States, 76 million cases of foodborne diseases resulting in 325,000 hospitalizations and 5,000 deaths are estimated to occur each year. Economic loss due to productivity loss, medical cost, and food recalls amount to $6.9 billion in just the United States *(8).*

The risk of foodborne disease depends on the type of food, its production source, how it is prepared and handled, and the consuming host's resistance to the infectious agent. As these factors change, the epidemiology of foodborne diseases also necessarily changes. The relationship between cardiovascular disease and consumption of saturated fat has led many Americans to stop consuming the traditional meat-and-potato diet that accompanied the postwar boom of the 1950s. The new American diet emphasizes fruits, vegetables, and grains and deemphasizes meats and foods with a high fat content. Another influence on our eating habits is the fact that, in general, people today tend to lead more hurried lives than in the past. In addition to the rapid rise in popularity of fast food restaurants, consumers are demanding more take-home, ready-to-eat foods. Grocery stores are providing a variety of in-store-prepared foods, including ready-to-eat prepackaged fresh fruits and vegetables *(6).*

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The increased amount of fresh produce consumed has been accompanied by a corresponding rise in the number of reported cases of foodborne disease linked to produce. Fourteen outbreaks involving cantaloupe have been reported in the last 13 years. More than half of the outbreaks involved melons that were cut but not consumed immediately, allowing pathogens to grow on the sugar-rich interior of the fruit *(4).*

Contamination of produce such as cantaloupes can occur anywhere along the farm-to-fork pathway *(3).* Sources of contamination include irrigation water, runoff water from livestock farms adjacent to fields and orchards, manure, wash water, handling by workers, contact with contaminated surfaces, and feces of rodents and ruminants *(5).* Contamination of the skin on the cantaloupe can be a food safety problem. Even when the skin itself is not eaten, contamination can be spread to the edible part and the fruit can cross-contaminate other foods and food preparation areas *(2).* It is important to determine whether cantaloupes are being contaminated in the field or after harvest. California growers field pack cantaloupes for distribution, whereas Georgia cantaloupes are brought to packing sheds to be packaged *(6).* Because handling practices differ in regions of the United States, it is important to specify microbial-related issues for each region. Four Georgia cantaloupe growers were chosen for the present study because they use different washing and packing methods. The microbiological status of the cantaloupes from each grower was assessed to determine the influence of these differing practices. From the cantaloupes, aerobic bacteria, coliforms, and *Escherichia coli* were enumerated and the presence of *Sal-*

monella and *E. coli* O157:H7 was evaluated. The levels of heterotrophic bacteria, coliforms, and *E. coli* in the dump tank water used in the cantaloupe processing lines also were determined.

MATERIALS AND METHODS

Field sampling. Cantaloupes were sampled from four south Georgia farms and packing sheds (sheds 1 through 4) at weekly intervals for 4 weeks during the growing season in 2005. After harvesting, the cantaloupes were brought to packing sheds, washed, sorted, and hand packed into shipping boxes. Sheds 1 and 3 were certified as following good agricultural practices (GAPs), whereas sheds 2 and 4 were not certified. Sheds 1 and 4 utilized dump tanks (approximately 95,000 liters). Trailers from the field were backed into the dump tank of chlorinated water, and the cantaloupes were floated off the trailer. At sheds 2 and 3, cantaloupes were side-dumped off trailers into smaller dump tanks (approximately 19,000 liters) that contained heated water and chlorine. The water was heated at shed 2 up to a mean temperature of 46.3°C (maximum of 57.2°C, minimum of 41.3°C), and that at shed 3 had a mean of 34.3°C (maximum of 36.7°C, minimum of 24.6°C). After leaving the dump tank 1 to 5 min after entry, the cantaloupes were taken by conveyor belts through brushes and sprayers and then sized for packing. Samples were obtained from the cantaloupes in three places along the processing line. Twenty cantaloupes were picked randomly and aseptically off of the trailer before washing and placed in sterile bags. Twenty cantaloupes also were randomly chosen from the group of melons leaving the dump tank, and 20 more were chosen from the shipping boxes. Of the 20 cantaloupes collected at each location, 10 were collected in the morning approximately 2 to 3 h after start up, and the other 10 were collected early in the afternoon. Once the cantaloupes were placed in bags, 200 ml of 0.1% peptone water (Bacto peptone, Difco, Becton Dickinson, Sparks, Md.) was added, and the bagged contents were shaken vigorously by hand for 1 min. The cantaloupes were then aseptically removed from the bag and placed back in the dump tank. The bag containing the peptone was labeled and placed in a cooler with ice until further analysis could take place in the lab approximately 4 h later. Sampling was repeated four times (replications): once each week at each shed for four consecutive weeks.

Microbiological analysis. The peptone rinses were analyzed for aerobic plate counts (APCs). Serial dilutions were prepared, and 1 ml of the dilutions was pipetted onto APC Petrifilm (3M Microbiology Products, St. Paul, Minn.) plates in duplicate and incubated at 37°C for 48 h. The number of CFU on the Petrifilm was then determined as per the manufacturer's instructions. Peptone rinses also were analyzed for coliforms and *E. coli.* For all replications, 1 ml of peptone rinse was removed from the serial dilutions already prepared, pipetted onto coliform/*E. coli* Petrifilm plates in duplicate, and incubated at 35°C for 48 h. The number of CFU on the Petrifilm was then determined as per the manufacturer's instructions. To determine the presence or absence of *Salmonella* and *E. coli* O157:H7, 1 ml from each bag of peptone was transferred into 9 ml of lactose broth (Difco, Becton Dickinson) for *Salmonella* enrichment and into 9 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) modified to contain 10.0 g/liter casamino acids, 1.5 g/liter bile salts no. 3, 6.0 g/liter dibasic anhydrous sodium phosphate, and 1.35 g/liter potassium phosphate for *E. coli* O157:H7 enrichment. Both enrichment broths were incubated at 37°C for 24 h. After incubation, portions of the modified TSB cultures were streaked onto sorbitol MacConkey agar (Oxoid, Basingstoke, UK) plates, which were incubated at 37°C for 24 h and then examined for the presence of representative colonies. Subcultures also were made from lactose broth into selenite cystine (Difco, Becton Dickinson) and Rappaport-Vassiliadis R10 (Difco, Becton Dickinson) broths and incubated at 37 and 42°C, respectively, for 24 h. After incubation, portions were streaked onto bismuth sulfite agar (Difco, Becton Dickinson), brilliant green agar (Difco, Becton Dickinson), and XLT-4 agar (Difco, Becton Dickinson) for possible *Salmonella* isolation. After 24 h at 35°C, plates were examined for the presence of representative colonies. Presumptive positive colonies were streaked onto triple sugar iron agar slants (Difco, Becton Dickinson) and lysine iron agar slants (Difco, Becton Dickinson) and incubated at 35°C for 48 h. Presumptive *Salmonella* and *E. coli* O157:H7 isolates were identified using latex agglutination tests (Oxoid) as per the manufacturer's instructions.

Microbiological analysis of dump tank water. Water for the enumeration of heterotrophic plate counts (HPCs) and counts of *E. coli* and coliforms was collected aseptically using a 500-ml scoop attached to a 1.1-m handle. Two samples, one in the morning and one in the afternoon, were collected from the dump tanks immediately after cantaloupes were dumped. At each sampling time, 120 ml of water from the dump tanks was put into sample cups containing a sodium thiosulfate tablet to inactivate available chlorine in the water. The SimPlate (IDEXX Laboratories, Inc., Westbrook, Maine) method was used for the HPCs from these water samples as per the manufacturer's instructions. The Colisure test kit (IDEXX) was used to detect total coliforms and *E. coli* in the sample water as per the manufacturer's directions.

Physical properties of dump tank water. Measurements of various water parameters were made directly from the dump tank water while cantaloupes were being collected. Free available and free total chlorine measurements were taken as per the manufacturer's instructions with a hand-held chlorine meter (model HI 95711, Hanna Instruments, Woonsocket, R.I.). The oxygen reduction potential (ORP) was determined with a hand-held QuiKcheK ORP pocket meter (model 108, Thermo Orion, Beverly, Mass.). The pH of the water was measured with a QuiKcheK pocket pH meter (model 106, Thermo Orion). The temperature of the water was taken with a hand-held QuiKcheK pocket temperature meter (model 110, Thermo Orion). The chemical oxygen demand (COD) was measured as per the manufacturer's directions with a COD meter (Thermo Orion).

Statistical analysis. The mean APCs, *E. coli* counts, and coliform counts from the cantaloupes collected from the transport trailers, dump tanks, and packing areas of the processing line at four sheds during four collecting trips were log transformed. For the APCs and coliform counts, an analysis of variance of main effects (packing shed, sampling time, sampling location at the shed, and sampling trip) and all the interactions was conducted using the PROC GLM procedure in the Statistical Analysis System (Statistical Analysis Systems Institute, Cary, N.C.) to determine whether there were significant differences ($P < 0.05$). The data for *E. coli* counts were analyzed using a logistic regression analysis in the SAS software, which allows for comparison between samples in the event that large percentages yield undetectable levels of *E. coli* (<15 CFU/cm^2). This approach models the probability of a sample containing undetectable levels of the organism.

RESULTS AND DISCUSSION

APCs from cantaloupes. The APCs for cantaloupes sampled in the morning were not different ($P > 0.05$) from

TABLE 1. *Mean populations of mesophilic aerobic bacteria on surfaces of cantaloupe sampled from the transport trailer, dump tank, and packing stages at four different packing sheds in Georgia*

	Mean aerobic bacteria (log CFU/ml of rinse)[b]		
Shed no.[a]	Transport trailer	Dump tank	Packing
1	6.88 A	6.76 B	6.76 B
2	6.83 A	6.89 A	7.00 B
3	6.92 A	6.91 A	7.15 A
4	6.83 A	6.65 B	6.83 A

[a] Shed 1, chlorinated water, farm GAP certified; shed 2, chlorinated and heated water, farm not GAP certified; shed 3, chlorinated and heated water, farm GAP certified; shed 4, chlorinated water, farm not GAP certified.

[b] Within the same row, means with different letters are significantly different ($P \leq 0.05$). SD = 0.48.

those of cantaloupes sampled in the afternoon, so the total microbial aerobic populations for all four trips were averaged (Table 1). Microbial populations on cantaloupes sampled from the packing boxes from sheds 1 and 4, at which chlorinated treatments were used, were <0.5-log lower than those on cantaloupes from sheds at which heat and chlorine treatments were used (sheds 2 and 3). Compared with populations on cantaloupes in the transport trailers, aerobic bacteria and coliform numbers decreased slightly after washing in the dump tank at two of the four sheds (sheds 1 and 4).

Free available chlorine can be an effective reducer of microbial populations on produce *(1).* Effectiveness is achieved when there is at least 2 ppm free chlorine available *(1),* as occurred in sheds 1 and 4. However, aerobic bacterial populations after packing at sheds 1 and 4 were approximately the same as those on the unwashed cantaloupes.

Cantaloupes from shed 3 had a significantly higher microbial population ($P \leq 0.05$) coming off of the transport trailers than after packaging. As for shed 2, exposing cantaloupe to water temperatures between 25 and 57°C for 5 to 10 min did not result in a significant reduction in microbial populations. For sheds 2 and 3, aerobic bacterial populations were slightly higher on cantaloupes in packing boxes than on cantaloupes on the transport trailers.

***E. coli* and coliform counts from cantaloupe samples.** *E. coli* and coliforms from the cantaloupes were enumerated to determine potential fecal contamination. For 83% of the cantaloupes sampled, <15 CFU/ml *E. coli* was detected in the rinsate. The results for *E. coli* were analyzed by comparing the proportion of the samples with <15 CFU/ml to the proportion of the samples with ≥15 CFU/ml. When sheds 1 and 4 were compared individually with sheds 2 and 3, there was a significant difference in the presence of *E. coli* ($P \leq 0.05$) (Table 2). There were also significant differences ($P \leq 0.05$) in *E. coli* populations between the transport trailers, dump tanks, and packing boxes at all four sheds (Table 2).

Results for coliforms were similar to those for *E. coli.*

TABLE 2. *Proportion of cantaloupes that were positive for the presence of* E. coli *(>15 CFU/ml of rinse) compared with proportion of melons that had undetectable levels (<15 CFU/ml)*

	Ratio of detectable:undetectable *E. coli* in samples[b]		
Shed no.[a]	Transport trailer	Dump tank	Packing
1	0.114 XB	0.129 YB	0.072 ZB
2	0.125 XA	0.200 YA	0.329 ZA
3	0.000 XA	0.133 YA	0.583 ZA
4	0.128 XB	0.088 YB	0.138 ZB

[a] Shed 1, chlorinated water, farm GAP certified; shed 2, heated chlorinated water, farm not GAP certified; shed 3, heated chlorinated water, farm GAP certified; shed 4, chlorinated water, farm not GAP certified.

[b] Within the same row, numbers followed by different letters (X, Y, or Z) are significantly different ($P \leq 0.05$). Within the same column, numbers followed by different letters (A or B) are significantly different ($P \leq 0.05$).

Coliform populations from sheds 1 and 4 were significantly different ($P \leq 0.05$) from those from sheds 2 and 3 (Table 3). The coliform populations increased on cantaloupes from sheds 1 and 4 between the dump tank and the packing boxes. In this study, the *E. coli* and coliform counts decreased when exposed to chlorine in the dump tank water but then increased after leaving the dump tank. This suggests that the belts and other contact surfaces may not be clean and are recontaminating the cantaloupes after they leave the dump tank.

Isolation of *E. coli* O157:H7 and *Salmonella.* Rinse water from the cantaloupes was enriched for the isolation of *E. coli* O157:H7 and *Salmonella. E. coli* O157:H7 was not found in any cantaloupe samples. *Salmonella* was detected in the rinsate of 1 of 900 cantaloupes sampled from the transport trailers.

HPCs from dump tank water. HPCs were determined from the dump tank water at each packing shed for each sampling time (Table 4). Microbial populations ranged from 15 most probable number (MPN)/ml of water from the dump tank at shed 4 to >355,000 MPN/ml from the dump tank at shed 2. There were increases in HPCs as the day progressed from the morning to the afternoon for samples from the dump tank water at shed 2. For shed 4, HPCs were consistent in the dump tank water during all four trips At shed 3, the HPCs of the dump tank water decreased considerably between the morning and afternoon sampling during the first trip but remained relatively consistent for trips 2 and 3. No samples were collected during the fourth trip to this shed because the shed was not in operation.

***E. coli* and coliform counts from dump tank water.** *E. coli* populations in the dump tank water ranged from <1.0 MPN/ml to >2,419.6 MPN/ml at all the sheds. Shed 4 had the lowest *E. coli* counts in the dump tank water, and the highest counts were from shed 2.

Coliform counts also were greater in the dump tank water than in the rinsate samples for cantaloupes collected from the trailers and the packing boxes. Coliform popula-

TABLE 3. *Frequency of distribution of coliform bacteria on surfaces of cantaloupes from the transport trailer, dump tank, and packing boxes at four different packing sheds in Georgia*

Sample area, shed no.[a]	No. of coliform-positive rinse samples at:					
	<1 log CFU/ml	1–2 log CFU/ml	>2–3 log CFU/ml	>3–4 log CFU/ml	>4–5 log CFU/ml	>5 log CFU/ml
Transport trailer						
Shed 1	44	10	10	3	3	0
Shed 2	42	18	8	8	3	1
Shed 3	43	5	7	2	3	0
Shed 4	56	8	7	5	1	3
Dump tank						
Shed 1	50	9	10	1	0	0
Shed 2	11	32	15	16	5	1
Shed 3	25	12	17	4	2	0
Shed 4	54	8	9	3	6	0
Packing						
Shed 1	32	17	4	12	5	0
Shed 2	6	14	24	11	22	2
Shed 3	0	0	5	21	33	1
Shed 4	23	12	21	4	9	11

[a] Shed 1, chlorinated water, farm GAP certified; shed 2, chlorinated and heated water, farm not GAP certified; shed 3, chlorinated and heated water, farm GAP certified; shed 4, chlorinated water, farm not GAP certified.

tions in the dump tank water ranged from <1.0 MPN/ml to >2,419.6 MPN/ml. With the exception of one sampling trip, shed 4 had the lowest overall coliform population of the four sheds. Shed 1 had higher coliform counts during the first trip. The highest dump tank water coliform populations were found in sheds 2 and 3.

Sheds 1 and 4 had lower numbers of heterotrophs, *E. coli,* and coliforms in the dump tank water. The total resid-

TABLE 4. *Heterotrophic plate counts from samples collected during four sampling trips from dump tank water used in four cantaloupe packing sheds*

Sample time, shed no.[a]	Heterotrophic plate counts (MPN/ml)			
	Morning		Afternoon	
	Mean	95% confidence limit	Mean	95% confidence limit
Trip 1				
Shed 1	209	159–273	2,990	2,290–3,900
Shed 2	50,700	37,100–69,500	>73,800	>47,600 to >114,600
Shed 3	>73,800	>47,600 to >114,600	342	
Shed 4	324	248–425	156	117–207
Trip 2				
Shed 1	>738	>476 to >1,146	ND[b]	
Shed 2	623	432–899	>738	>476 to >1,146
Shed 3	>738	>476 to >1,146	>738	>476 to >1,146
Shed 4	>738	>476 to >1,146	231	177–302
Trip 3				
Shed 1	19	10–36	355	270–466
Shed 2	>7,380	>47,600 to >114,600	>7,380	>47,600 to >114,600
Shed 3	1,000	730–1,390	>7,380	>47,600 to >114,600
Shed 4	26	15–45	56	38–84
Trip 4				
Shed 1	90	64–126	30	18–51
Shed 2	55,500	39,800–77,500	355,000	270,000–466,000
Shed 3	ND		ND	
Shed 4	17	8–33	15	7–30

[a] Shed 1, chlorinated water, farm GAP certified; shed 2, chlorinated and heated water, farm not GAP certified; shed 3, chlorinated and heated water, farm GAP certified; shed 4, chlorinated water, farm not GAP certified.

[b] ND, measurement not done.

TABLE 5. *Oxygen reduction potentials (ORPs) in the morning and afternoon samples of cantaloupe dump tank water with* E. coli *counts >15 CFU/ml (*n *= 900)*

Shed no., sampling time[a]	No. of samples with *E. coli* counts >15 CFU/ml	ORPs (mV)	
		Morning	Afternoon
Shed 1			
Trip 1	3	ND[b]	ND
Trip 2	2	660	660
Trip 3	3	685	678
Trip 4	1	685	693
Shed 2			
Trip 1	3	ND	ND
Trip 2	4	270	310
Trip 3	5	151	193
Trip 4	4	310	176
Shed 3			
Trip 1	1	ND	ND
Trip 2	2	685	385
Trip 3	5	683	295
Trip 4	ND	ND	ND
Shed 4			
Trip 1	0	ND	ND
Trip 2	6	680	669
Trip 3	0	704	696
Trip 4	1	690	694

[a] Shed 1, chlorinated water, farm GAP certified; shed 2, chlorinated and heated water, farm not GAP certified; shed 3, chlorinated and heated water, farm GAP certified; shed 4, chlorinated water, farm not GAP certified.

[b] ND, measurement not done.

ual chlorine added to the water would have killed many microbes coming from the shed's water supply. The water supply for both of these sheds came from ponds that are fed by deep wells. During the course of the day, the microbial numbers varied at both sheds, in some cases increasing and in other cases decreasing.

Physical properties of the dump tank water. The data indicate a correlation between the ORP and the number of *E. coli* present in the dump tank water. As the ORPs increased, the *E. coli* population decreased for all trips to sheds 1 and 4 with the exception of one trip to shed 4 (Table 5). An effective ORP for killing or inhibiting microorganisms is between 650 to 700 mV *(1).* For dump tank water, a pH above 7.5 will have a lower concentration of active chlorine, and a pH below 4.0 causes the chlorine to turn into gas. The pH values for dump tank water from sheds 1 and 4 averaged 8.2 and 8.3, respectively, and these tanks had 3.0 and 4.0 ppm free chlorine, respectively, available for effectiveness. The pH of the dump tank water was not adjusted by the packing shed operators.

Microbial populations also are affected by COD. Untreated wash and chill water is generally rich in organic matter, which will decrease the effectiveness of chlorine in the water *(1).* Sheds 1 and 4 had low CODs in the dump tank water. The reduction of microbial populations in the water at sheds 1 and 4 could be explained by the combination of the free chlorine, an ORP near 700 mV, a low COD, and a pH just outside of normal.

The heated water treatment in shed 2 was not as effective as the chlorine used at sheds 1 and 4 for reducing microbial contamination. At high enough temperatures, microbes on cantaloupe surfaces will be killed. A 4.4-log reduction of *Salmonella* on cantaloupes was found after exposure to water heated to 97°C for 60 s *(9).* Solomon et al. *(7)* found that heat treatments at 85°C for 90 s resulted in a reduction of *Salmonella* up to 4.7 log CFU. The equipment in shed 2 took approximately 2 h to heat the water, which averaged 43.2°C, and the temperature was very hard to regulate and maintain once up to 2,500 cantaloupes per load at ambient temperature (approximately 32°C) were added to the water. To be effective, the water temperature would have to be increased to higher than desired and would have to be monitored during the processing hours to ensure a lethal treatment temperature was reached. Increasing the water temperature also would increase energy costs and thus total processing expense. Thus, thermal treatment of melon surfaces in field packing operations appears impractical.

Microbial populations on cantaloupes from shed 3 increased slightly after cantaloupes left the dump tank and were packed. Shed 3 utilized hot chlorinated water for the dump tank treatments of the cantaloupe. When a certain amount of heat, approximately 82°C, is used in combination with chlorine, the chlorine turns to gas and is no longer effective as an antimicrobial *(1).* Although the water temperature in shed 3 did not reach this level, temperatures may have been high enough to gas off some of the chlorine added to the dump tank. The water temperature at shed 3 reached 57°C, which was not sufficient to reduce microbial numbers on the cantaloupe surfaces. During three of four sampling trips, there was sufficient free available chlorine at this shed to be effective for reducing microbial numbers. However, the total chlorine levels decreased as the day progressed, which suggests that the chlorine was not maintained at a consistent level during the day. The results of this study indicate that there is no reason to use both heat and chlorine in the dump tank. Chlorine alone will reduce microbial populations, as seen at sheds 1 and 4.

Cantaloupes collected during three steps of the processing line at sheds 2 and 3 had significantly higher microbial populations ($P \leq 0.05$) than did those collected from sheds 1 and 4. This finding indicates that chlorine alone is more effective than the heat plus chlorine treatments used at these sheds. However, the chlorine did not prevent an increase in microbial numbers on cantaloupes after they left the dump tank. Sanitary conditions down line from the dump tank should be monitored to prevent additional microbial contamination. Conveyor belts should be washed and sanitized, and workers should follow proper hand-washing techniques. Further studies should be focused on the sanitary conditions of conveyor belts and on worker hygiene to determine why microbial populations increased on cantaloupes after they left the dump tank.

ACKNOWLEDGMENTS

The material in this study was based on work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture project Fresh Produce Food Safety Training Program, and on curriculum developed in the Southeast and as part of the Georgia Agriculture Experiment Station project no. 2003-511110-01730. The authors thank Dr. Jaxk Reeves (Department of Statistics, University of Georgia, Athens, Ga.) for his assistance in designing and conducting the statistical analysis of the data.

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