A Field Study of the Microbiological Quality of Fresh Produce^{†‡}

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ABSTRACT

The Centers for Disease Control and Prevention has reported that foodborne disease outbreaks associated with fruits and vegetables increased during the past decade. This study was conducted to characterize the routes of microbial contamination in produce and to identify areas of potential contamination from production through postharvest handling. We report here the levels of bacterial indicator organisms and the prevalence of selected pathogens in produce samples collected from the southern United States. A total of 398 produce samples (leafy greens, herbs, and cantaloupe) were collected through production and the packing shed and assayed by enumerative tests for total aerobic bacteria, total coliforms, total Enterococcus, and Escherichia coli. These samples also were analyzed for Salmonella, Listeria monocytogenes, and E. coli O157:H7. Microbiological methods were based on methods recommended by the U.S. Food and Drug Administration. For all leafy greens and herbs, geometric mean indicator levels ranged from 4.5 to 6.2 log CFU/g (aerobic plate count); less than 1 to 4.3 log CFU/g (coliforms and Enterococcus); and less than 1 to 1.5 log CFU/g (E. coli). In many cases, indicator levels remained relatively constant throughout the packing shed, particularly for mustard greens. However, for cilantro and parsley, total coliform levels increased during the packing process. For cantaloupe, microbial levels significantly increased from field through packing, with ranges of 6.4 to 7.0 log CFU/g (aerobic plate count); 2.1 to 4.3 log CFU/g (coliforms); 3.5 to 5.2 log CFU/g (Enterococcus); and less than 1 to 2.5 log CFU/g (E. coli). The prevalence of pathogens for all samples was 0, 0, and 0.7% (3 of 398) for L. monocytogenes, E. coli O157:H7, and Salmonella, respectively. This study demonstrates that each step from production to consumption may affect the microbial load of produce and reinforces government recommendations for ensuring a high-quality product.

The fresh fruit and vegetable industry has rapidly evolved during the past two decades. In the United States, increased awareness of the health benefits of eating fresh produce has contributed to a \$36.2 billion increase in retail and food-service sales from 1987 to 1997 (15). Furthermore, retailers' demand for year-round fresh produce has helped sustain the growing international trade market, ensuring consistent supplies to consumers during the off-season (16). Despite the nutritional and economic benefits of fresh produce, issues of public health concern have arisen. Although fruits and vegetables were associated with 0.5 to 4.2% of foodborne disease outbreaks from 1988 to 1997, the Centers for Disease Control and Prevention reported that the proportion of foodborne disease outbreaks associated with fruits and vegetables doubled from 1973 to 1987 and again from 1988 to 1991 (6, 7, 29). During this period, several changes occurred, including the discovery of newly identified pathogens, improvement of diagnostic methods,

and the advancement of foodborne disease surveillance systems (26).

A broad variety of fresh produce items, including cantaloupe, herbs, and leafy greens, has been linked to various pathogens (2, 26). Most well-characterized outbreaks have been caused by bacteria, namely *Salmonella, Escherichia coli* O157:H7, *Shigella*, and *Listeria monocytogenes*; a few outbreaks have also been linked to viruses such as hepatitis A virus and noroviruses, and parasites such as *Giardia lamblia* (2, 22).

Many factors can contribute to microbial contamination throughout production and packaging of fresh produce (2). These include contaminated irrigation or process water, the use of biosolids or manure for fertilization, poor worker hygiene, and poor equipment sanitation. To improve the safety of produce, the U.S. federal agencies responsible for food safety (i.e., U.S. Food and Drug Administration and the U.S. Department of Agriculture) published voluntary guidelines in 1998 entitled *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (32)*. The guide's primary purpose was to provide a framework for the identification and implementation of practices likely to decrease the risk for pathogenic microbiological contamination of produce, based on good agricultural practices and good manufacturing practices. Although the guide provides

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Commodity	n (%)	Field	Wash tank	Rinse	Conveyor belt	Box
Arugula	15 (4)	9	3	NA	NA^{a}	3
Cantaloupe	90 (23)	36	3	15	18	18
Cilantro	94 (24)	49	12	12	NA	21
Collards	12 (3)	6	NA	3	NA	3
Dill	12 (3)	6	NA	3	NA	3
Mustard greens	70 (18)	31	3	18	NA	18
Parsley	78 (20)	36	9	15	NA	18
Spinach	27 (7)	18	3	3	NA	3

TABLE 1. Summary of produce samples collected from each production and packing shed site

^a This step was not included in the process or no samples were collected.

general knowledge about potential pathways by which produce can become contaminated, systematic studies are lacking to identify critical points through the production-to-consumption continuum where contamination may occur.

To address these data needs, we sought to identify and further understand routes for potential microbial contamination of produce throughout production and packaging. The objectives of this study were threefold: (i) to monitor the microbiological quality of fresh produce from the field through the packing process by specifically enumerating various microbiological populations; (ii) to evaluate the prevalence of *L. monocytogenes, E. coli* O157:H7, and *Salmonella* on fresh produce; and (iii) to identify differences in microbiological quality between various produce items during production and packaging. The data reported here are part of a larger study to determine specific farm and on-site packaging practices that may be associated with microbial contamination of produce.

MATERIALS AND METHODS

Sample collection. The sampling site, located in the southern United States, comprised 13 farm locations and five packing sheds. Samples were collected from November 2000 through May 2002. Target commodities included produce items that are mostly eaten raw, except for collards and mustard greens (Table 1). Samples were taken sequentially, following the same crop from harvest throughout the packing shed. Samples designated as "field" included midseason crops, harvest samples, and samples collected at point of entry to the packing shed. Samples designated as "wash tank" and "rinse" were taken immediately after the wash and rinse step, respectively, at the packing shed. Samples labeled "box" were collected from boxes just before distribution. Cantaloupe samples were also taken directly off of the conveyor belt between the rinse step and box for distribution.

Two sets of composite samples (400 to 600 g each) of each produce commodity, except cantaloupe, were obtained from each location with hands protected by sterile, disposable gloves. Three cantaloupes were sampled from each location in the same manner. Samples were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson, Wis.). One of these composite sets was used for enumerative analyses and was numerically and alphabetically coded by the collection technicians to ensure anonymity. At the request of our scientific advisory committee, the other composite sample (intended for pathogen assay) was unmarked and therefore could not be traced after testing. All samples were immediately shipped on ice to our location at North Carolina State University by overnight courier. Microbial analyses were initiated within 24 h after sample collection. **Microbial indicator analysis.** Unless otherwise stated, all media were obtained from Becton Dickinson Laboratories (Sparks, Md.). Twenty-five–gram subsamples were weighed and diluted 1:10 in 0.1% peptone buffer. Three cantaloupe samples from each sample site were prepared by trimming rind (less than 0.5 cm deep) from melons with a sanitized paring knife and removing all visible mesocarp material. After homogenizing for 2 min at 230 rpm in a Stomacher 400 (Seward, Norfolk, UK), samples were processed to enumerate total aerobic bacteria (aerobic plate count [APC]), total coliforms, total *Enterococcus*, and *E. coli*. Assays for total aerobic bacteria, coliforms, and *E. coli* were done using aerobic count plate Petrifilm and coliform/*E. coli* Petrifilm plates (3M, Saint Paul, Minn.), respectively (9). Total enterococci were enumerated using KF Streptococcal agar (13).

Pathogen analysis. Three subsamples of 25 g each, originating from the composite sample intended for pathogen detection, were weighed and prepared for Salmonella, L. monocytogenes, and E. coli O157:H7 assays by the U.S. Food and Drug Administration Bacteriological Analytical Manual methods (1, 8, 14). For Salmonella detection, samples were homogenized in 225 ml of lactose broth, followed by incubation at 37°C for 24 h. One milliliter of the lactose preenrichment broth was then transferred to tetrathionate and selenite cystine broths and incubated at 37°C. After 18 to 24 h, samples were streaked to xylose lysine desoxycholate, bismuth sulfite, and hektoen enteric agar. Two or more typical colonies then were transferred to lysine iron agar and triple sugar iron agar slants, followed by Enterobacteriaceae Micro-ID (Remel, Lenexa, Kans.) for the generic identification of Salmonella. Presumptive Salmonella isolates were sent to the College of Veterinary Medicine at North Carolina State University for Vitek (bioMérieux, Hazelwood, Mo.) identification and subsequently shipped to the National Veterinary Services Laboratories (Ames, Iowa) for serotyping.

For *L. monocytogenes* detection, 25-g produce samples were incubated in *Listeria* enrichment broth at 30°C for 24 to 48 h. *Listeria* spp. then were isolated using Oxford agar and lithium chloride–phenylethanol-moxalactam agar, supplemented with esculin and ferric ammonium citrate (Sigma Chemical Company, St. Louis, Mo.). Typical colonies were analyzed for beta-hemolysis on 5% sheep blood agar (Remel), and colonies displaying betahemolysis were streaked on blood agar for the CAMP test, followed by *Listeria* Micro-ID (Remel) for speciation.

For *E. coli* O157:H7 detection, 25-g produce samples were first enriched in 225 ml of enterohemorrhagic *E. coli* enrichment broth at 37°C for 24 h followed by plating on sorbitol-MacConkey agar, supplemented with potassium tellurite and cefixime (Dynal, Lake Success, N.Y.). At least two presumptive colonies were screened for the presence of the O157 antigen using the commer-

Produce items	APC	Enterococci	Total coliforms	E. coli
Arugula	5.8 ± 1.0	2.1 ± 1.3	3.4 ± 1.2	0.7 ± 0.0
Cantaloupe	6.6 ± 1.0	4.1 ± 1.2	3.0 ± 1.3	1.5 ± 1.1
Cilantro	6.1 ± 1.1	1.9 ± 1.2	1.8 ± 1.2	0.8 ± 0.5
Collards	4.5 ± 1.0	1.3 ± 0.6	1.0 ± 0.7	0.7 ± 0.0
Dill	5.4 ± 0.6	3.6 ± 0.8	2.9 ± 1.0	0.7 ± 0.0
Mustard greens	6.2 ± 1.0	4.3 ± 1.3	2.4 ± 1.3	1.0 ± 0.9
Parsley	5.6 ± 1.0	2.5 ± 1.0	2.3 ± 1.1	1.0 ± 0.2
Spinach	5.8 ± 1.0	2.1 ± 0.9	1.5 ± 0.8	0.7 ± 0.0

TABLE 2. Microbial loads in various produce commodities^a

^{*a*} Values are log mean \pm standard deviation.

cial Prolex *E. coli* O157 latex test reagent kit (Pro-Lab Diagnostics, Round Rock, Tex.).

Statistics. Statistical analyses, including geometric means, standard deviations, ranges, and medians were conducted with Sigma Plot version 8.0 (SPSS, Chicago, Ill.). One-way analysis of variance tests were performed using Tukey comparisons to derive statistical differences (P < 0.05) of microbial levels between all sampling locations. To avoid underrepresentation and overrepresentation of sample counts, when enumerative results fell below the assay limit of detection, they were assigned a value halfway between zero and the assay detection limit (10, 27).

RESULTS

Sample collection. A total of 398 produce samples were collected during November 2000 through May 2002, originating from 13 farms and five packing sheds (Table 1). More than 80% of the produce items collected consisted of cantaloupe (23%), cilantro (24%), mustard greens (18%), and parsley (20%). Because of sampling limitations, smaller numbers of other produce items (arugula, collards, spinach, and dill) were collected.

Microbiological quality of produce. Total aerobic bacteria ranged from a geometric mean of 4.5 to 6.6 log CFU/g (Table 2). *Enterococcus* levels ranged from 1.3 to 4.3 log CFU/g, with cantaloupe and mustard greens having the highest levels. Geometric mean total coliform counts ranged from 1.0 to 3.4 log CFU/g. Overall geometric mean *E. coli* counts were low for most produce items (\leq 1.0 log CFU/g) and highest for cantaloupe (1.5 log CFU/g).

To identify critical points of contamination, further data analysis was done to compare microbial levels on produce associated with specific sampling locations (Figs. 1 through 4). Because of increased sample representation from cilantro, parsley, mustard greens, and cantaloupe, separate data analysis was limited to these commodities. For cilantro (Fig. 1), total APC levels increased from the field and throughout packing, with mean ranges of 5.7 log in the field to 6.7 log CFU/g in the samples obtained from boxes ready for distribution. Enterococcus levels remained consistently low, with levels ranging from 1.7 to 2.3 log CFU/g; however, there are slight increases throughout postharvest handling. Total coliforms increased significantly (approximately 1.4 log) (P < 0.05) from harvest through packing, with a rise occurring mainly at the rinse step. The levels of E. coli on cilantro were extremely low, typically below the lower limit of detection (<10 CFU/g).

In contrast, parsley showed a slight increasing trend throughout the packing shed for APC, enterococci, and total coliforms (Fig. 2). APC levels increased approximately 1.0 log CFU/g within the packing shed, from a mean of 5.2 log CFU/g at point of entry to 6.1 log CFU/g in the samples ready for distribution. This increase occurred at the rinse step, with APC levels remaining stable thereafter. Enterococci levels increased from a geometric mean of 2.1 log CFU/g from the field to 3.1 log CFU/g at the rinse step. Total coliform levels doubled after the rinse from levels at point of entry. Levels of *E. coli* were low, usually falling below the lower limit of detection.

Microbial levels on mustard greens, including APC, enterococci, coliforms, and *E. coli*, did not change significantly from the field through the packing process (Fig. 3). However, there was no indication that packing shed steps, such as water rinsing, reduced the microbial load on this product.

Concentrations of total enterococci, total coliforms, and *E. coli* on cantaloupes increased from harvest through packing (Fig. 4). APC levels remained constant from production and throughout packing, with a mean range of 6.4 log at point of entry to nearly 7.0 log CFU/g in the distribution box. Total enterococci increased significantly (P < 0.05) (approximately 1 log) between the rinse step and the conveyor belt. Total coliforms showed the same trend, with levels nearly doubling at the conveyor belt step. Interestingly, *E. coli* levels increased substantially from 0.8 log CFU/g for samples taken from the field to 2.5 log CFU/g for samples ready for retail distribution. As with enterococci and coliforms, these increases appeared to occur at the conveyor belt step.

Pathogen detection in fresh produce. All samples were analyzed for *L. monocytogenes, E. coli* O157:H7, and *Salmonella. Listeria monocytogenes* and *E. coli* O157:H7 were not detected in any of the 398 produce items tested. However, *Salmonella enterica* serovar Montevideo was detected on three cantaloupe samples, resulting in a prevalence of 0.8% for all produce items and 3.3% for cantaloupe alone.

DISCUSSION

Overall, the microbial quality of cilantro, parsley, and mustard greens was excellent. Despite the increase in total coliforms for cilantro and parsley, microbial loads remain

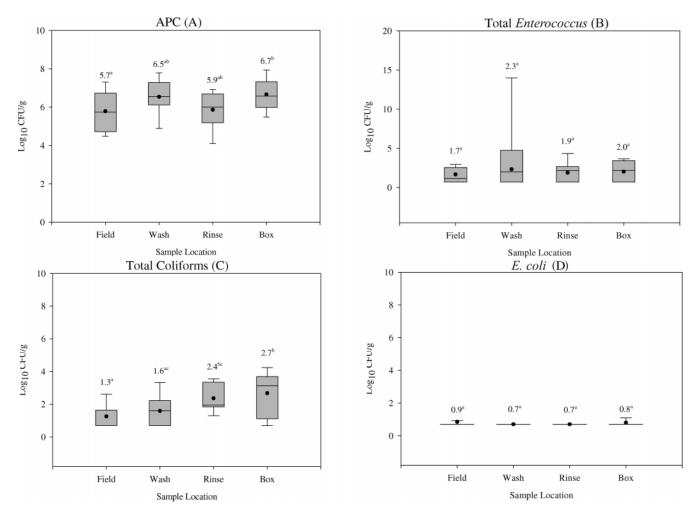


FIGURE 1. (A) APC, (B) total Enterococcus, (C) total coliforms, and (D) E. coli levels from cilantro collected from the field and various steps throughout the packing shed. The box plot indicates the 10th, 25th, 50th, 75th, and 90th percentiles. The number above each box plot indicates the geometric mean, also indicated by the black circle. Superscript letters indicate significant differences among the log means. Means that share the same superscript letter are not significantly different from one another; means with different superscript letters are significantly different (P < 0.05).

relatively constant during the packing process, and the levels of *E. coli* (which suggest fecal contamination) were extremely low. Moreover, no pathogens were detected in any of these produce items, either from the field or from the packing shed. These results are similar to those presented in the U.S. Food and Drug Administration's survey of domestic produce (*31*), which reported no *E. coli* O157:H7 and a low prevalence (<1%) of *Salmonella* among leafy greens.

Our results indicate that microbial loads on cantaloupes increased significantly during the packing process. Cantaloupes' characteristics can create challenges for maintaining a microbiologically sound product. The surface topography, known as the netting, may favor microbial attachment and complicate efforts aimed at reducing surface contamination. Furthermore, the pH range of the fruit itself (6.1 to 7.1) is suitable for microbial growth. The waxing procedure is used to improve appearance and reduce shrinkage or water loss (21). Because of the strong attachment characteristics of bacteria, particularly *Salmonella* (30), and the physical characteristics of the netting material, the wax may provide a barrier to further removal of microorganisms that might occur during washing at the retail and consumer levels. In our study 3 (3.3%) of 90 cantaloupe samples were contaminated with *S. enterica* serovar Montevideo. This result is similar to data reported in the U.S. Food and Drug Administration's domestic produce survey result, which reported 4 (2.4%) of 164 cantaloupe samples positive for *Salmonella* (*31*). Furthermore, a recent study by Castillo et al. (*5*) reported the low prevalence of *Salmonella* contamination on domestic (0.5%) and Mexican (0.3%) cantaloupes collected during production.

In general, our data are consistent with those of other studies that examined microbial levels on fresh produce items. Several investigators have reported similar levels of total aerobic bacteria on leafy green vegetables collected from both production and retail establishments (11, 17, 24, 28). For example, Ruiz et al. (24) found total aerobic bacteria levels ranging from 10^5 to 10^7 CFU/g on field samples, whereas levels on retail samples of leafy greens ranged from 10^4 to 10^6 CFU/g. However, the coliform and *E. coli* levels on leafy greens and herbs reported in our study were from 2 to 3 log CFU/g lower than those reported by Ruiz et al. (24).

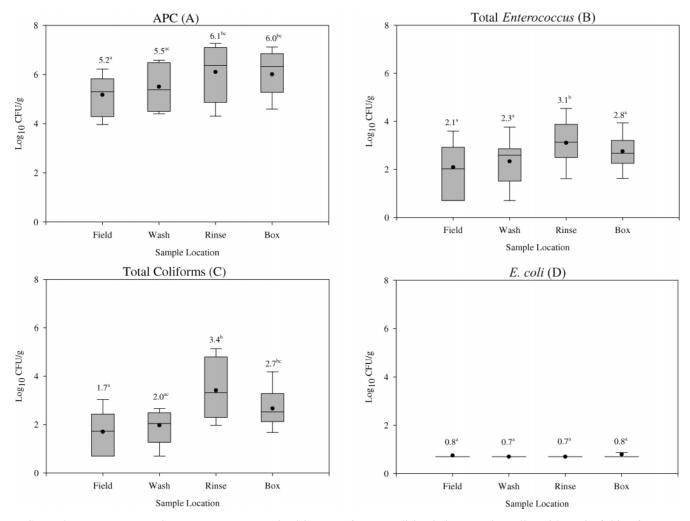


FIGURE 2. (A) APC, (B) total Enterococcus, (C) total coliforms, and (D) E. coli levels from parsley collected from the field and various steps throughout the packing shed. The box plot indicates the 10th, 25th, 50th, 75th, and 90th percentiles. The number above each box plot indicates the geometric mean, also indicated by the black circle. Means that share the same superscript letter are not significantly different from one another; means with different superscript letters are significantly different (P < 0.05).

Interestingly, only a few studies have characterized the change in microbial levels throughout the production and packaging of fresh produce. Geldreich and Bordner (12) reported a significant increase in the fecal coliform load for both root crops and leafy vegetables from field to market. In keeping with our results on various microbiological populations, Prazak et al. (23) found that packing sheds provided a suitable environment for the survival and proliferation of Listeria spp., particularly conveyor belts, where cross-contamination can occur between processing surfaces and cabbage. Likewise, Gagliardi et al. (10) concluded that a significant amount of contamination on cantaloupe occurs at the packing shed (during washing) rather than in the field or during harvest. Another study (5) found the frequency of E. coli among Mexican cantaloupes to increase at the packing shed, supporting the idea that the practice of washing melons after harvesting may increase the chance of fecal coliform contamination. If a limited number of products are contaminated, contamination may be spread over the entire lot during washes such as water dips, which are commonly used in produce packing sheds (4).

In general, these studies, along with the results presented here, suggest that microbiological levels can either increase or originate during the packing shed phase, perhaps affecting the shelf life of the product. However, attempts to correlate increased levels of microorganisms with spoilage have given conflicting results. High microbial counts on unstored lettuce were related to a short shelf life. However, product quality was negatively correlated with bacterial counts for shredded endive (20). Consequently, assuming that high microbial counts on some produce items in this study indicate low quality or reduced shelf life may not be appropriate. Furthermore, the health significance of high levels of APC, coliforms, and enterococci on produce is not clear, and we recognize that these microbial populations are not necessarily indicators relevant to food safety. Some coliforms (Klebsiella) are commonly associated with vegetable produce and can multiply under favorable environmental conditions, however (18).

Produce packing sheds often rely on a wash procedure after harvest to remove soil and debris, to reduce microbial levels, and to potentially increase the shelf life or quality of products. The use of sanitizers in the packing shed is

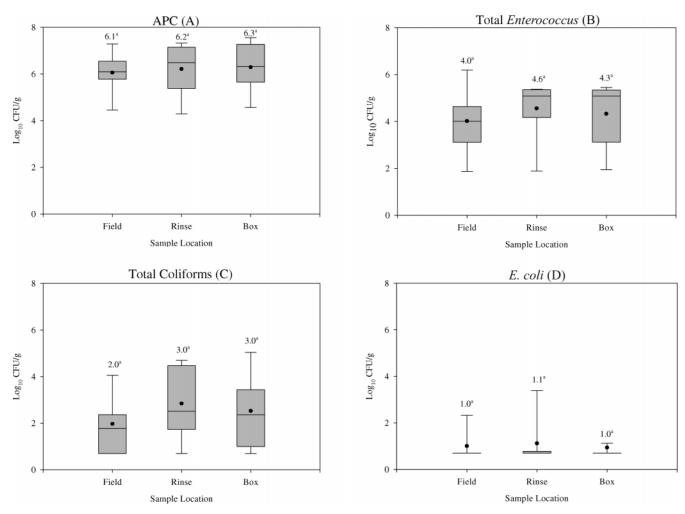


FIGURE 3. (A) APC, (B) total Enterococcus, (C) total coliforms, and (D) E. coli levels from mustard greens collected from the field and various steps throughout the packing shed. The box plot indicates the 10th, 25th, 50th, 75th, and 90th percentiles. The number above each box plot indicates the geometric mean, also indicated by the black circle. Means that share the same superscript letter are not significantly different from one another; means with different superscript letters are significantly different (P < 0.05).

perceived as an essential strategy to maintain clean wash and rinse water (32, 33). For cilantro and parsley, the level of total coliforms increased after the wash step (Figs. 1C and 2C). In both cases, the increase occurred during rinsing. Even though chlorine is an effective disinfectant for drinking and recreational waters and an effective surface disinfectant, it is less effective for reducing microbial loads on produce items. Chlorinated wash water generally will reduce microbial loads on produce by only 1 to 2 log units (4). Senter et al. (25) reported that chlorine had little effect on reducing microbial load on tomatoes. Although Beuchat and Brackett (3) found chlorine (200 to 250 μ g/ml) to be effective initially in reducing microbial loads on lettuce, after several days of storage, microbial levels increased significantly, and no significant differences could be found between microbial populations on lettuce washed with chlorinated water versus unchlorinated water. Li et al. (19) found that treatment of lettuce with 20 ppm chlorine at either 20 or 50°C did not result in significantly greater reductions in populations of E. coli O157:H7 compared with treatments in water without chlorine. The relative ineffectiveness of chlorine as a disinfectant for produce items also is evident in our study. For example, even though most packing sheds in our study used chlorine in wash water (data not shown), the results for mustard greens, herbs, and cantaloupe suggest that the use of chlorine did not reduce the microbial load on these products.

Equipment sanitation is another important consideration in controlling microbial contamination. The conveyor belt material used in many packing sheds consists of an abrasive, brush-like material, which may be difficult to thoroughly clean. We also saw carpeted surfaces in these sheds, which would be difficult to clean and could be reservoirs for microbes. Microbial levels increased on cantaloupe samples collected from conveyor belts. It is not clear whether these increases were due to contact with the conveyor belt or due to contact with workers' hands during sorting and grading before packing.

Even though packing sheds offer manageable ways of cleaning and packing produce under controlled conditions, the concept of field packing is worth revisiting for some products. Systematic studies comparing the quality of fieldpacked cantaloupes versus those packed in sheds are lacking. Packing in the field could decrease exposure to postharvest sources of contamination, such as dirty rinse water,

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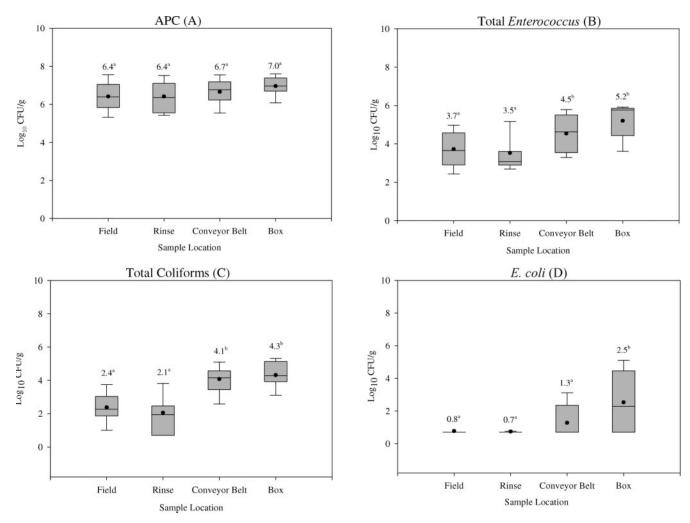


FIGURE 4. (A) APC, (B) total Enterococcus, (C) total coliforms, and (D) E. coli levels from cantaloupe collected from the field and various steps throughout the packing shed. The box plot indicates the 10th, 25th, 50th, 75th, and 90th percentiles. The number above each box plot indicates the geometric mean, also indicated by the black circle. Means that share the same superscript letter are not significantly different from each other; means with different superscript letters are significantly different (P < 0.05).

contact with dirty equipment, and additional human handling.

Although adherence to the *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* can address produce quality and safety issues during growing, harvesting, sorting, packing, and distribution, our study reinforces the frequently cited concept that every step from production to consumption will affect the microbial quality of produce. In fact, our results emphasize the importance of thorough sanitation measures, particularly during the packing shed phase, and indicate a need for careful evaluation of postharvest handling. Ultimately, individual growers and packers should examine their own processes and incorporate strategies for maintaining high-quality produce.

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