Microbiological Evaluation of Mechanically Harvested Citrus Fruit

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Significant reductions in harvesting costs will be necessary in coming years for Florida to continue to compete in the international citrus industry. Two steps are involved in the mechanical harvesting (MH) of citrus fruit: removal of fruit from the tree and collection of fruit (by a catch-frame device or retrieval of fruit from the ground). This study evaluates the microbiological aspects of mechanically handled fruit with respect to fruit surface microflora and corresponding fruit juice microflora. Four replicates of mechanical harvesting were performed, including two catch-frame (CF) replicates and two pick-up machine (PU) replicates. For each replicate, three treatment groups were evaluated: 1) hand-harvested fruit (control); 2) ground fruit (picked up directly from ground following canopy shaking); and 3) mechanically harvested (MH) fruit (collected from the OXBO pick-up machine or from the goat following collection on a catch frame). Microbial analysis included a total plate count (TPC), an acidophilic organism count (OSA), and generic Escherichia coli and Salmonella testing on pooled samples of five oranges. Juice samples, from each fruit, were subjected to the same tests. The amount of sand found on the surface of fruit was measured. Hand-harvest control fruit generally had fewer microbes on the surface of the fruit than either ground or MH fruit on both TPC and OSA. Juice corresponding to the control fruit also generally had fewer microbes on both TPC and OSA than the juice corresponding to ground or MH fruit. However, no real trends can be attributed to the harvest method for all runs. Generic E. coli was detected in 10 pooled orange samples (five CF and five PU) and Salmonella spp. was not detected in any of the pooled fruit or juice samples. Sand levels on PU MH samples were significantly higher than those found on CF MH, ground, or control fruit.

Since the 1970s, development of various mechanical harvesters and pick-up machines for citrus has been explored (Whitney, 1995; Whitney and Sumner, 1977). Two general systems for fruit collection are commonly seen including either 1) using a catch frame (CF) to collect fruit as it is falling from the tree, or 2) dropping the fruit directly to the grove floor for future collection. To detach the fruit from the tree, a tractor drawn canopy shaker is often used; however, trunk shakers and blowers have also been used. If fruit is not caught in a catch frame, collection by either hand crews or pick-up (PU) machines can occur. Due to the large amounts of fruit and potential for hand labor shortages in the future, efficient CF or PU machines are desired by the industry. Use of PU machines would also be useful to collect fruit blown from the trees during hurricanes.

Within the citrus production and processing industries, sensitivity to food safety risks exists as a result of Salmonella outbreaks associated with fresh orange juice that occurred in the mid-1990s (Vojdani et al., 2008). The impact of a new harvesting system, that may place fruit in contact with the grove floor, needs to be explored to quell food safety fears that may prevent mechanical harvesting from moving forward. Much of the data collection on mechanical harvesting systems currently consists of yield, performance, and efficiency studies, as well as the effect of tree shaping and grove design (Roka and Rouse, 2004; Whitney et al., 1986). Data are available on the overall prevalence of pathogens such as Salmonella on the surface of oranges destined for processing (Parish et al., 2001), and some information about the microbiological effects of mechanical harvesting is available from the two previous years of this study (Goodrich et al., 2006; Goodrich-Schneider et al., 2007).

The objective of this work is to summarize the 2007–08 research results from this ongoing study (begun in 2005–06) evaluating the microbiological surface and juice microflora of citrus fruit collected by PU and CF mechanical harvesting systems and the amount of sand present in mechanically harvested samples.

Materials and Methods

FRUIT SAMPLING. Four replicates of mechanical harvesting were performed through the 2007–08 harvest season, including two catch-frame replicates and two pick-up machine replicates. For each replicate, three treatment groups were evaluated: 1) hand-harvested fruit (control); 2) ground fruit (picked up directly from ground following canopy shaking); and 3) mechanically harvested (MH) fruit (collected from the OXBO pick-up machine or from the goat following collection on a catch frame). The OXBO International Corp. (Clear Lake, WI) PU machine is a self-propelled rake, pick-up, and cleaning system. Within each group of fruit (control, ground, and MH), 75 oranges were collected, and 25 wholesome, non-defective fruit were randomly selected from each group for analysis.

MICROBIOLOGICAL METHODS AND REPORTING. All microbiological media were purchased from Becton Dickinson (Sparks, MD) unless otherwise noted. Fruit were stored at 4 °C for no longer than 24 h prior to analysis. Each orange was transferred to an individual, sterile whirl-pak bag using latex gloves. Thirty milliliters of sterile 0.1% peptone buffer was poured over the

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orange in each plastic bag and was manipulated using the rub-shake-rub technique to remove surface microorganisms (Parish et al., 2001).

Aerobic plate counts (APC) and acidophilic counts (AOC) were performed by making appropriate dilutions of the wash buffer in sterile 0.1% peptone and spiral plating onto plate count agar (PCA) and orange serum agar (OSA), respectively. When necessary, to increase the limit of detection, four spread plates of 0.25 mL each of the lowest dilution were prepared. The PCA plates were incubated 24 h at 35 °C (Morton, 2001) while the OSA plates were incubated 48 h at 30 °C (Hatcher et al., 2001). After the appropriate incubation, numbers of colonies were counted and reported as colony forming units (CFU) per fruit. Data were statistically evaluated using Excel software (Microsoft, Redmond, WA).

Due to time and expense constraints and the low expected frequency of isolation, assays of E. coli and Salmonella were performed on separate, pooled samples. Each pooled sample resulted from 5-mL buffer aliquots from each orange sample which were mixed to yield one 25-mL sample for every five fruit. This resulted in five 25-mL samples for control, ground, and MH for each replicate trial, and a total of 60 samples analyzed over the entire study.

E. coli and Salmonella detection was performed by adding each 25-mL composite sample to appropriate media according to Parish et al. (2001). The VIP Salmonella test kit (BioControl, Bellevue, WA) was used as specified by the manufacturer for the Salmonella assay, while the E-Colite™ test kit (Charm Sciences, Lawrence, MA) was used to detect the presence of generic E. coli. Appropriate negative and positive controls were run to ensure performance of test kits. Results were reported as the number of positive composite samples.

Following testing of the fruit surface, all samples were stored at 4 °C for 18 ± 2 h. Oranges were then placed in 85 °C water for 2 min to sterilize the surface of the fruit and juices by hand through cheesecloth into a sterile container. Parallel microbial testing was done for juice samples and is reported as CFU/mL juice and as presence or absence of E. coli and Salmonella.

SAND METHODS AND REPORTING. Due to processor concerns with regards to the amount of sand present in mechanically harvested samples, experiments were undertaken to quantify the influence of mechanical harvesting on sand levels. Within the three test groups previously described, 10 fruit samples were selected and washed with tap water. The wash water was allowed to settle and was filtered. The filter was then weighed to calculate the mg of sand per cm² of the fruit surface.

Results and Discussion

Microbial populations were enumerated using APC media. This test is also described as “Total plate count” or “Standard plate count” and, in this case, represents the number of microorganisms on the surface of the orange that are capable of growing into viable colonies aerobically and at warm temperatures. The APC gives a general indication of the overall microbial load on or in a food product. Similarly, the AOC count represents the number of microorganisms on the surface of the orange that are capable of growing into viable colonies under more acidic conditions than PCA (acidophiles; Hatcher et al., 2001). OSA is the typical media used in citrus processing quality control laboratories in order to enumerate the acidophilic organisms in the environment or in the product that are capable of surviving and growing in juice-like conditions.

In general, control fruit had fewer microbes on the surface of the fruit when compared to ground and MH fruit from both harvesting methods; however, no real trends can be attributed to the harvest method for all runs (Table 1). Significantly lower APC counts for control fruits are often expected as these fruits were not in contact with the soil surface, the source of many microorganisms on agricultural products. However, this was not true for all trials in 2007–08, and is consistent with results reported for PU fruit in 2005–06 and 2006–07. This result suggests that dropping fruit to the ground and picking it up mechanically or dropping it into a catch frame does not necessarily result in higher microbial loads. Moisture, other environmental variables, such as soil type and cover crop, or general grove maintenance may ultimately be very important in the total number of microorganisms that adhere to the dropped fruit.

Results of the AOC analysis follow the same general trend as those for APC (Table 1). In general, there are no significant differences among the three treatment groups. Many factors contribute to the surface microflora of a raw agricultural product. These include production practices, natural ecology of the fruit/microorganism system, equipment sanitation, geography and climate, and hygiene of harvest and packinghouse personnel. All of these factors may have impacted the results obtained from this study.

E. coli was detected in five of the pooled PU MH samples and in one of the CF Ground samples and four of the CF MH samples (Table 2). High isolation frequencies of E. coli for both the PU and CF samples indicate a potential for cross contamination from these surfaces and highlights the need for adequate cleaning and sanitation of the machinery. No Salmonella was detected this study.

Table 1. Fruit surface microflora in log colony forming units (CFU) per orange for four trials of mechanical harvesting, two with pick-up (PU) machines and two with catch frames (CF) (n = 25 oranges).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU 1</td>
<td>5.7 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>PU 2</td>
<td>4.4 ± 0.2</td>
<td>6.0 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>CF 1</td>
<td>5.4 ± 0.6</td>
<td>6.2 ± 0.7</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>CF 2</td>
<td>5.4 ± 0.6</td>
<td>5.6 ± 0.8</td>
<td>6.0 ± 0.8</td>
</tr>
</tbody>
</table>

Table 2. Summary of fruit surface indicator (E. coli) and pathogenic organisms (Salmonella) for four trials of mechanical harvesting, two with pick-up (PU) machines and two with catch frames (CF) (n = 5 enrichments).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PU 2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>CF 1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>CF 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

Salmonella enrichments

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PU 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CF 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CF 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
year, as opposed to the previous two harvest seasons where at least one Salmonella isolate was identified. The overall level of Salmonella contamination on any raw agricultural product is quite low (2% to 3%; U.S. FDA, 1998b), and the result of 0 positive samples is not entirely unexpected.

In all cases, juice samples contained significantly less microflora than the corresponding fruit, oftentimes being at or below the limit of detection (Table 3). For both APC and AOC in fruit juice, no real trends can be attributed to the harvest method. The interior of sound fruits harbor few microbes as is apparent by these results. The indicator organism E. coli and Salmonella were not detected in any of the juice samples, despite the presence of these organisms on the fruit surface in some samples (Table 4).

No indication that fruit which come in contact with the ground, pick-up, or catch-frame machinery are consistently and significantly higher in surface or corresponding juice microflora is indicated by the results of the four trials run during 2007–08. While generic E. coli are not considered foodborne pathogens, their presence can be indicative of fecal contamination, and the high isolation frequency from all composite samples in one PU and four of five composite samples in one CF harvest indicate a potential for cross-contamination. Any fruit in contact with soil has the potential to become contaminated. Many factors contribute to the surface microflora of a raw agricultural product. These include production practices, natural ecology of the fruit/microorganism system, equipment sanitation, geography and climate, and hygiene of harvest and packinghouse personnel (U.S. FDA, 1998a). All of these factors may have impacted the results obtained from this study.

Due to processor concerns with regards to the amount of sand present in mechanically harvested samples, experiments were undertaken to quantify the influence of mechanical harvesting on sand levels. Average results of sand collection are shown in Table 5 for PU and CF trials. Data indicate that a significantly higher level of sand is seen on the MH PU samples, indicating that fruit surface sand levels are affected by PU but not CF harvesting.

There is practical importance to the surface microflora of oranges delivered to the processor. Contamination of raw materials is listed as the second most serious food safety problem in the food processing industry, after deficiencies in employee training (Sertkaya et al., 2006). Fruit coming into citrus processing plants is typically washed and sanitized prior to juicing, and the vast majority (>98%) of Florida-processed orange juice is pasteurized or similarly treated to inactivate spoilage enzymes and to microbiologically stabilize the product. Wider adoption of MH PU or CF systems will be influenced by the quality of fruit delivered to the processor, including potential for microbiological contamination as well as the typical measures of machine yield and efficiency, and economics.

### Table 3. Juice microflora in log colony forming units (CFU) per mL for four trials of mechanical harvesting, two with pick-up (PU) machines and two with catch frames (CF) (n = 25 juice samples).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU/mL on APC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PU 1</td>
<td>0.02 ± 0.03</td>
<td>0.06 ± 0.2</td>
<td>0.03 ± 0.2</td>
</tr>
<tr>
<td>PU 2</td>
<td>0.02 ± 0.00</td>
<td>0.1 ± 0.5</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td>CF 1</td>
<td>&gt; 0.02</td>
<td>0.2 ± 0.6</td>
<td>0.09 ± 0.4</td>
</tr>
<tr>
<td>CF 2</td>
<td>0.05 ± 0.2</td>
<td>0.02 ± 0.0</td>
<td>0.2 ± 0.5</td>
</tr>
</tbody>
</table>

### Table 4. Summary of juice indicator (E. coli) and pathogenic organisms (Salmonella) for four trials of mechanical harvesting (MH), two with pick-up (PU) machines and two with catch frames (CF) (n = 5 enrichments).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli enrichments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PU 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PU 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CF 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CF 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

### Table 5. Sand collected from the fruit surface of mechanical harvesting using pick up (PU) and catch frames (CF) (n = 20 oranges).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Control</th>
<th>Ground</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU/mL on OSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PU 1</td>
<td>&gt; 0.02</td>
<td>0.06 ± 0.2</td>
<td>&gt; 0.02</td>
</tr>
<tr>
<td>PU 2</td>
<td>&gt; 0.02</td>
<td>0.1 ± 0.5</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>CF 1</td>
<td>&gt; 0.02</td>
<td>0.2 ± 0.6</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>CF 2</td>
<td>&gt; 0.02</td>
<td>&gt; 0.02</td>
<td>0.1 ± 0.4</td>
</tr>
</tbody>
</table>

### Literature Cited


