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Microbiological Evaluation of Mechanically Harvested Citrus Fruit

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For Florida to effectively compete in the world citrus industry, significant reductions in harvesting costs will be necessary. Mechanical harvesting can be thought of a two-step process: removal of fruit from the tree, and retrieval of fruit from the ground and/or collection area. A citrus fruit pickup machine developed by OXBO International Corporation is being tested for performance and productivity. The machine is being evaluated for its picking up rate, efficiency, field capacity, and its efficiency for removing undesirable fruit and trash. The performance test is being conducted under different ranges of forward speed, orange variety, and grove conditions. This study evaluates the microbiological aspects of mechanically handled fruit with respect to fruit surface microflora. Three treatments were evaluated: hand-harvested fruit (control), mechanically harvested and dropped fruit picked up manually from the ground (MH/hand PU fruit), and mechanically harvested and dropped fruit picked up with the OXBO machine (MH/machine PU fruit). Microbial analysis included total plate count (TPC), acidophilic organisms, generic *Escherichia coli* (as an indicator of potential contamination), and *Salmonella.* Additionally, juice that was aseptically obtained from the three fruit samples was evaluated for microbiological quality. Finally, the approximate load of sand of the surface of the fruit was evaluated to determine potential impact on fruit handling equipment, which is known to be negatively impacted by the presence of abrasive foreign material such as sand. In general, within a particular harvest replicate, hand-harvested control fruit had similar microbial loads on the surface of the fruit as microbial loads on the MH/hand PU or MH/machine PU fruit. *Escherichia coli* and *Salmonella* were detected in three of the four replicates on fruit surface samples, but neither organism was obtained from any of the juice samples. There were substantial differences among the four replicate trials conducted in the 2006–07 season, which may have been due to a variety of factors, including differing grove care and floor conditions, weather, equipment sanitation, grove location, and tree/fruit treatments during production.

Various mechanical harvesters and pickup machines for citrus have been developed since 1970 (Whitney, 1995; Whitney and Sumner, 1977). Several systems incorporate a catch pan to collect fruit as it is being removed from the tree. However, there are other systems that drop the fruit directly to the grove floor for subsequent collection. The tractor-drawn canopy shaker detaches fruit and allows the fruit to fall to the ground. Trunk shakers and blowers have also been used to detach fruit from the tree for collection by either hand crews or pickup machines. Due to the large amounts of fruit and potential for hand labor shortages in the future, an efficient pickup machine would be preferred by the industry. Use of such machines would be also useful to collect fruit blown from the trees during hurricanes.

Much of the data collection on mechanical harvesting systems 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). There has been some work in the area of the impact of mechanical harvesting systems on fruit quality and presence of extraneous debris (Bora et al., 2006). However, there is little information about the microbiological effects of allowing harvested fruit to drop to the soil surface. What effects, if any, can be attributed to the pickup/cleaning portion of the system? Widespread adoption of particular mechanical systems will require demonstration that the system does not appreciably increase the microbial load on fruit vs. traditional harvesting systems. There is also increased sensitivity in the citrus production and processing industries to food safety risks, including the perceived risk of utilizing drop fruit, as a result of the foodborne disease outbreaks associated with fresh juices that occurred in the mid-1990s [US Food and Drug Administration (FDA), 1998b].

There are some data available on the overall prevalence of pathogens such as *Salmonella* on the surface and interior of fruit destined for processing (Parish et al., 2001). There are no recently published studies evaluating the effects of mechanical harvesting systems on *Salmonella* contamination rates on fruit, or on indicator organisms such as *Escherichia coli* that are linked with higher risks for pathogen contamination, except for a preliminary Florida study conducted in the 2005–06 citrus season (Goodrich et al., 2006). The objective of this work was to summarize the 2006–07 research results from the microbiological surface and internal evaluations of citrus fruit collected by a commercial-scale fruit pickup system and to estimate the sand surface level on the fruit.

Materials and Methods

Equipment and fruit sampling. The OXBO International Corporation (Clear Lake, WI) machine is a self-propelled rake, pickup, and cleaning system with suitable size and capacity to

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operate in modern Florida groves. This fruit pickup system is being evaluated for its performance in picking up desirable fruit from the ground and its efficiency in removing undesirable fruit and trash at various forward speeds, orange varieties, and grove conditions. This study evaluated fruit surface microbe loads from harvesting trials that were deemed successful in terms of overall machine performance. Four replicate trials spanning the 2006–07 season were the source of the fruit used in this study.

Seventy-five pieces of fruit per trial were collected, with 25 wholesome, non-defective fruit randomly selected from each of the three sample groups. Groups were identified as follows: control (hand picked from tree in a normal manner), MH (mechanically harvested from tree, then picked up by hand), and MH/PU (mechanically harvested from tree, then sampled from collection hopper after mechanical pickup).

Microbiological methods and reporting. Fruit were kept chilled for no longer than 24 h prior to analysis. Each piece of fruit was transferred to an individual, sterile whirl-pak bag using latex gloves. Thirty milliliters of 0.1% peptone buffer (Becton Dickinson, Sparks, MD) was poured over the orange in each plastic bag and was manipulated to remove surface microorganisms as previously described (Parish et al., 2001). Results for fruit surfaces are reported as colony-forming units (CFU) per milliliter of buffer. Following handling and testing of the fruit surfaces, interior fruit microbiological quality was assessed, again utilizing methods from Parish et al. (2001). Briefly, the same fruit were surface sterilized and aseptically juiced by hand through sterile cheesecloth into a sterile container, with parallel testing and results reported as CFU/mL juice.

All microbiological media were purchased from Becton Dickinson unless otherwise noted. Aerobic plate counts (APC) and acidophilic organism counts (AOC) were performed by making appropriate dilutions of the wash buffer, which was then spiral plated onto plate count agar (PCA) and orange serum agar (OSA), respectively. The PCA plates were incubated 24 h at 35 ºC (Morton, 2001) while the OSA plates were incubated 48 h at 30 ºC (Hatcher et al., 2001). After the appropriate incubation, numbers of colonies were counted and reported as CFU per fruit. Data were statistically evaluated using Excel software (Microsoft, Redmond, WA).

Due to time and expense constraints, assays of *E. coli* and *Salmonella* were performed on separate, pooled samples. Each pooled sample was from 5 mL buffer or juice aliquots from each orange sample or juice sample, which were mixed to yield one 25-mL sample for every five fruit. This resulted in five 25-mL samples for control fruit, MH, and MH/PU for each trial, and a total of 60 samples analyzed for *E. coli* and *Salmonella* over the entire study for the fruit surfaces and for the juice samples. *Escherichia 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, indicating the presence or absence of these organisms.

Assessment of sand level on fruit surfaces. To assess the effect of harvest method, if any, on fruit surface sand levels, 10 fruit samples each from the three test groups were rinsed with tap water. The wash water was allowed to settle, and was subsequently filtered. After drying, the mass of sample per grid section of the filter was measured. Results were reported as milligrams of sand per square centimeter.

Results and Discussion

Microbial populations were enumerated using PCA 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 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.

Fruit surface APCs and AOCs are shown in Table 1 for each of the four trials. We expected APCs to be significantly lower than for control fruit because control fruit were not in contact with the soil surface, the source of many microorganisms on agricultural products. Lower APCs for control fruit were not true for all of the trials. This result suggests that dropping fruit to the ground and/or picking it up mechanically does not always results in significantly higher microbial loads. It is important to note trials were conducted under different weather conditions. Soil moisture or other environmental factors may be very important in the ultimate number of microorganisms that adhere to fruit dropped on the ground. Trials 3 and 4 indicated that control fruit had fewer surface microorganisms than either of the mechanically handled fruit samples, but the machine pickup appeared to not have an effect on the overall surface microflora once the fruit was in contact with the ground. It is impossible to determine the reason for this particular result. Any fruit in contact with soil has the potential to become severely contaminated, although that was clearly not the case for many of the fruit that were picked up from the ground, either by hand or mechanically. However, this result emphasizes the need for proper grove floor observation and maintenance, as well as equipment cleaning and care. Both soil and machines are potential sources of contamination of fruit surfaces.

Table 1. Fruit surface microflora in log colony forming units (CFU) per milliter of orange wash for four trials of mechanical harvesting (MH) with and without pickup (PU) machines (n = 25 oranges). Different letters signify significant differences across treatments for an individual trial at the 95% confidence level.

Trial	Control	MH	MH/PU
	Log CFU/mL on PCA[z]		
1	3.6 ± 0.4 a	3.6 ± 0.6 a	3.5 ± 0.4 a
2	3.8 ± 0.4 a	4.3 ± 0.3 a	4.5 ± 0.4 a
3	3.0 ± 0.8 a	4.3 ± 0.4 b	4.4 ± 0.6 b
4	3.6 ± 0.4 a	4.7 ± 0.3 b	5.3 ± 0.5 b
	Log CFU/mL on OSA		
1	3.9 ± 0.6 a	3.8 ± 0.4 a	3.8 ± 0.6 a
2	4.1 ± 0.5 a	4.3 ± 0.4 a	4.4 ± 0.5 a
3	3.7 ± 0.4 a	4.5 ± 0.5 ab	4.6 ± 0.4 b
4	3.8 ± 0.3 a	4.8 ± 0.5 b	4.1 ± 0.5 ab

[z]PCA = plate count agar; OSA = orange serum agar.

Results of the OSA analysis followed the same general trend as those for APC. In general, there were no significant differences among the three treatment groups. None of the four trials indicated significantly greater acidophilic microbial loads for both of the fruit groups that contacted the soil vs. the hand-picked control. 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 (US FDA, 1998a). All of these factors may have impacted the results obtained from this study.

Table 2 summarizes the number of positive composite fruit surface samples that were evaluated for the presence/absence of *E. coli* and *Salmonella.* There were a total of seven positive *E. coli* enrichments over 60 samples, with Trial 1 demonstrating the most significant result for this indicator organism. It is known that wild pigs, particularly, are present in many Florida agricultural areas, and could lead to the observed result. Any fruit that contacts the ground (MH and MH/PU samples) would have a larger risk of contact with *E. coli.*

The overall level of *Salmonella* contamination on any raw agricultural product is quite low (2% to 3%; US FDA, 1998b), and the result of one out of 60 positive samples is not entirely unexpected. However, given the intimate contact of fruit from two of the groups with the soil surface, this result is an encouraging one in the development of harvesting/collection systems that rely on some sort of pickup system.

As discussed previously, while generic *E. coli* are not considered foodborne pathogens, their presence can be indicative of fecal contamination from warm-blooded animals. This is a situation that is linked with higher risk of concurrent contamination of agricultural products with human pathogens. Interestingly, fruit surface contamination by *E. coli* was not linked to the presence of *Salmonella* on fruit surfaces in this study.

Table 3 summarizes the data for juice obtained from the three samples of oranges over four replicate trials. It is well known that the interior of sound fruit and vegetables is generally free of large numbers of microorganisms, and results from this study support that generalization, with the juice in some cases containing levels of microorganisms at or below the level of detection. Table 4 summarizes the results of the indicator (*E. coli*) and pathogenic (*Salmonella*) organisms in the composite juice samples over the four trials. Despite the presence of these organisms on some of the fruit surfaces in three of four trials, they were not detected in the juice derived from the same fruit.

Results from the fruit surface sand assessment are summarized in Table 5. Differing results over the four trials may reflect differing weather and soil moisture conditions. There may also be significant differences in the sand level as a result of different harvesting practices. In Trial 2, for example, it appears substantially more sand is present on the MH/PU fruit than on the control of MH fruit. Visual assessments suggest that preliminary work should be continued to elucidate effects of harvesting, grove floor preparation, and weather on the overall fruit surface sand level.

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, 2006). However, incoming fruit to citrus processing plants is typically washed and sanitized, and the vast majority (>98%) of Florida-processed orange juice is pasteurized or similarly treated to inactivate spoilage enzymes and to

Table 2. Summary of fruit surface indicator (*Escherichia coli*) and pathogenic organisms (*Salmonella*) for four trials of mechanical harvesting (MH) with and without pickup (PU) machines (n = 5 enrichments), reported as positive samples.

Trial	Control	MH	MH/PU
	E. coli enrichments		
1	0	**3**	**3**
2	0	0	0
3	0	**1**	0
4	0	0	0
	Salmonella enrichments		
1	0	0	0
2	0	**1**	0
3	0	0	0
4	0	0	0

Table 3. Juice microflora in log colony forming units (CFU) per milliliter for four trials of mechanical harvesting (MH) with and without pickup (PU) machines (n = 25 juice samples). Different letters signify significant differences across treatments for an individual trial at the 95% confidence level.

Trial	Control	MH	MH/PU
	CFU/mL on APC[z]		
1	1.4 ± 2.2 a	0.3 ± 0.3 a	0.8 ± 2.7 a
2	>0.02 a	0.02 ± 0.1 a	0.06 ± 0.2 a
3	>0.02 a	1.1 ± 0.5 a	0.2 ± 0.6 a
4	0.3 ± 0.8 a	0.3 ± 0.6 a	2.0 ± 3.2 a
	CFU/mL on OSA		
1	0.3 ± 0.6 a	0.3 ± 0.5 a	0.6 ± 1.5 a
2	>0.02 a	>0.02 a	0.04 ± 0.2 a
3	>0.02 a	0.04 ± 0.2 a	0.04 ± 0.3 a
4	0.1 ± 0.4 a	0.1 ± 0.3 a	0.1 ± 4.5 a

[z]APC = aerobic plate counts; OSA = orange serum agar.

Table 4. Summary of juice indicator (*Escherichia coli*) and pathogenic organisms (*Salmonella*) for four trials of mechanical harvesting (MH) with and without pickup (PU) machines (n = 5 enrichments), reported as positive results.

Trial	Control	MH	MH/PU
	E. coli enrichments		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
	Salmonella enrichments		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0

Table 5. Sand collected from fruit surface for four trials of mechanical harvesting (MH) with and without pickup (PU) machines (n = 10 oranges).

Trial	Control	MH	MH/PU
	---------- mg·cm^{-2} ----------		
1	0.02	0	0.058
2	0.038	0.107	4.8
3	0.032	0.11	0.36
4	0.00	0.108	0.056

miocrobiologically stabilize the product. Wider adoption of mechanical harvest/pickup systems will be somewhat determined by the quality of fruit delivered to the processor. This quality includes potential microbiological contamination as well as the typical measures of machine yield and efficiency, and economics. A minor factor, but one of interest to the processors, is also the extent to which mechanically harvested or pickup fruit differs from control fruit in terms of surface sand levels. For these reasons, it is important to continue to collect fruit microbiological quality information for any harvest/collection system that promises commercial viability.

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