Working Title: Evaluation of pre- and post-processed tomatoes.

Microbial evaluation of pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses

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Abstract

Prevention of microbial cross-contamination during postharvest handling is an important step to minimize microbial food safety hazards. Dump tanks and flume systems are widely used in states like Florida to transfer/wash tomatoes, and are one of the most critical points where cross-contamination may occur. Some processors in states such as New Jersey, New York and California utilize dry dump systems, with or without overhead spray bars, to process tomatoes, while others states such as Maryland field-pack tomatoes. This study was conducted in 2013 and 2014, from five growing regions in Florida and New Jersey each and from four growing regions in Maryland. A total of 1600 and 1597 composite samples were analyzed for aerobic plate count (APC), and total coliforms (TC) and generic *E. coli* (EC), respectively, from both pre- and post-processed tomatoes. Seventeen samples for APC and 72 for TC had counts outside the countable range and failed to provide any valid result, and were not included in the final data sets. The least square mean (LSM) value of APC for all samples (both pre- and post-processed) was $6.8 \log_{10}$ CFU/tomato ($n=1583$), whereas the LSM for TC counts was $4.9 \log_{10}$ CFU/tomato ($n=1438$). Ninety out of 1528 (5.9%) and 1498 out of 1597 (93.8%) samples had TC and EC counts below the detection limit of $1.3 \log_{10}$ CFU/tomato, respectively. APC and TC counts in post-processed samples were significantly lower ($p<0.0001$) than those in the pre-processed samples. There was no significant difference ($p=0.1011$) in the occurrence of generic EC pre- and post-process. There were significantly higher ($p<0.0001$) APC and TC on samples collected in 2014 than 2013, while the EC levels showed no significant differences between years. TC counts varied significantly ($p<0.0001$) by different growing seasons, with highest counts in summer,

over a two-year period, while APC varied significantly ($p < 0.0001$) in summer and fall vs. winter and spring. APC and TC counts were positively correlated. Tomatoes from FL had significantly lower APC and TC ($p < 0.0001$) than those from NJ and MD. Despite the potential for increasing microbial contamination resulting from improperly maintained water systems, many packinghouses will continue using existing washing practices to prevent cross-contamination.

Keywords: Tomato, flume, dump tank, processing, packinghouse

1. Introduction

Leafy greens, tomatoes, melons, sprouts, and berries have all been frequently associated with foodborne illness outbreaks (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). The bacterial pathogen typically associated with these foods include Shiga toxin-producing *E. coli* (STEC), *Salmonella* spp., *Shigella* spp., and *Listeria monocytogenes* (Brackett, 1999; Frank et al., 2011; Park et al., 2015; Reller, Nelson, Mølbak, & Mintz, 2006). Between 1998 and 2008, of the 21 vine-stalk vegetable-associated outbreaks, 19 (90%) were attributed to tomatoes (Jackson, Griffin, Cole, Walsh, & Chai, 2013). Once tomatoes are harvested, care must be followed to prevent direct and/or cross-contamination of the crop during sorting, washing, packing and shipping. Dump or flume tank systems are widely used in commercial tomato packinghouses (Zhou, Luo, Turner, Wang, & Schneider, 2014). Other washing methods, such as spray brush-beds, can reduce microbial loading as well as prevent cross-contamination on tomato surfaces (Chang and Schneider, 2012; Tomás-Callejas et al., 2012), while some tomatoes are field-packed and are not washed at all. Several foodborne illness outbreaks associated with fruits and vegetables (Chaidez, Moreno, Rubio, Angulo, & Valdez, 2003; Sivapalasingam et al., 2004; Scallan et al., 2011; Steyn, Cameron, Brittin, & Witthuhn, 2011), including tomatoes (Bennett, Littrell, Hill, Mahovic, & Behraves, 2015; CDC, 2011; Reller, Nelson, Mølbak, & Mintz, 2006; Taylor, Kastner, & Renter, 2010) have been traced back to packing operations, and more research is needed to characterize the microbial control measures in packinghouse operations.

78 Researchers have explored the efficiency of numerous physical, chemical, and
79 biological methods for reducing the microbiological load of produce (Parish et al., 2003;
80 Tomás-Callejas et al., 2012; Zhou, Luo, Nou, Lyu, & Wang, 2015). Flume-tanks,
81 overhead spray-applied sanitizers and hydrocooling are all used to control microbial
82 contamination. The use of sanitizers in post-harvest flume and dump tanks
83 reduces cross-contamination (Chang and Schneider, 2012) and is the critical step in the
84 tomato packinghouse processing (Rushing, Angulo, & Beuchat, 1996). Flume and dump
85 tanks may be preferred as risk of bruising of the produce surface is comparatively less
86 than other methods (Gereffi, Sreedharan, & Schneider, 2015; Zhou, Luo, Turner, Wang,
87 & Schneider, 2014), however, flume and dump tanks may also be less effective in
88 controlling cross-contamination compared to overhead spray-applied sanitizers (Chang
89 and Schneider, 2012). The water used in flume and dump tank systems can also
90 become a point of cross-contamination for spoilage organisms and plant pathogens,
91 which may lead to quality loss and decay, as well as human pathogens that can cause
92 outbreaks of foodborne diseases (Harris et al., 2003). The potential accumulation
93 of microbes in dump tank water as well as the need to disinfect this water to minimize
94 quality defects in produce was identified as early as 1932 (Baker and Heald, 1932) and
95 continues to be reported (Suslow et al., 2003; Zhou, Luo, Nou, Lyu, & Wang, 2015).
96 Washing efficiency varies with factors such as commodity, wash system type, soil type,
97 contact time, detergent, water temperature, and wash water quality, especially if
98 recycled or untreated prior to reuse (Parish et al., 2003). Disinfectant chemicals are used
99 in wash water to provide an effective barrier to cross-contamination (Parish et al., 2003;

100 Park, Gray, Oh, Kronenberg, & Kang, 2008; Zhou, Luo, Turner, Wang, & Schneider,
101 2014).

102
103 Many post-harvest operations rely on copious water contact during fruit unloading and
104 washing (Tomás-Callejas et al., 2012). A single piece of contaminated produce can
105 potentially cross-contaminate a large amount of clean product, resulting in an increased
106 risk of foodborne illness (Danyluk and Schaffner, 2011). The accumulation of organic
107 matter in flume/dump tanks can cause a decline in sanitizer concentration, allowing
108 pathogen survival (Zhou, Luo, Turner, Wang, & Schneider, 2014), leading some packers
109 to employ single pass water applications such as spray bars, or field pack product to
110 eliminate washing altogether.

111
112 Although many studies have evaluated farm-related factors influencing the microbial
113 contamination of produce (Allen et al., 2013; Bohaychuk et al., 2009; Park et al., 2013,
114 2014, 2015; Mukherjee, Speh, & Diez-Gonzalez, 2007; Mukherjee, Speh, Dyck, & Diez-
115 Gonzalez, 2004; Mukherjee, Speh, Jones, Buesing, & Diez-Gonzalez, 2006; Orozco et
116 al., 2008; Strawn et al., 2013a, b), only a few (Benjamin et al., 2013; Gereffi,
117 Sreedharan, & Schneider, 2015; Izumi, Poubol, Hisa, & Sera., 2008a; Izumi, Tsukada,
118 Poubol, & Hisa, 2008b) have examined the bacterial counts on produce as affected by
119 factors other than farming method. This study presents data gathered over two years
120 (2013 and 2014) on tomatoes collected from 14 growing regions in Florida (5), Maryland
121 (4) and New Jersey (5) to evaluate the risk associated with post-harvest processing of
122 tomatoes in commercial packinghouses. Most Florida packers utilize flume-tanks,

though a small number of packers use brush roller systems, or a combination of the two. The typical sanitizing agent utilized in these systems was sodium hypochlorite (NaOCl) and/or peroxyacetic acid (PAA). Packing facilities studied in Maryland did not wash their tomatoes or use chemicals in their post-harvest processing, typical of many small and medium growers in the state. Growers in New Jersey use flume-tank, spray bars, and/or brush rollers, with the primary chemical treatment being PAA or NaOCl. This study may help to identify areas for improvement in packinghouse operational procedures that could reduce the risk of potential microbial contamination.

2. Material and Methods

2.1. Sampling sites and procedure

Tomatoes were collected from packinghouses located at 14 growing regions shown in the Figure 1. Tomatoes were harvested when green in color in Florida, red in Maryland, and pink to red in New Jersey. Growers at two of the four sampling locations in Maryland field packed their tomatoes (a common practice among small/medium growers), while at the other two locations they packed the tomatoes after using a dry-brush line. Twenty composite samples consisting of five tomatoes each ($n=100$) were collected pre- and post-processing from each site during each visit. Pre-processed tomatoes were aseptically sampled directly from the tomato field at locations, where growers field-packed their tomatoes. At other locations tomatoes were collected from 10 field bins or baskets (two composite samples from each bin or basket) or a single gondola (sampling from different locations around the perimeter). Post-processed composite samples were collected from boxes immediately after harvesting and packing (at locations which field-

packed tomatoes) or after processing and packing at the remaining sampling locations. All samples were placed in sterile plastic bags (15"x20"; Thermo Fisher Scientific, Pittsburgh, PA), and were stored on ice, transported to the laboratory and analyzed within 24 h of collection.

2.2. Microbiological analysis

One hundred ml of 0.1% (w/v) sterile peptone water (PW) (Thermo Fisher Scientific, Waltham, MA) was added to the sterile sample bags and each tomato was rubbed for 60s to remove surface bacteria. Sodium thiosulfate (0.6% w/v) (Thermo Fisher Scientific, Waltham, MA) was added to PW to quench any residual sanitizer activity. Bacterial enumeration was performed by 10-fold serial dilution in 0.1% (w/v) PW. One hundred μ l of each dilution was spread plated onto plate count agar (PCA) (Thermo Fisher Scientific, Waltham, MA) to determine total mesophilic aerobic plate counts (APC) and on CHROMagar™-ECC (DRG International, Inc., Mountainside, NJ) to determine total coliform (TC) and generic EC counts. PCA plates were incubated at 30°C for 48 h and CHROMagar™-ECC plates were incubated at 37°C for 24h. The lowest limit for detection of microbes was 1.3 log₁₀ CFU/tomato and 25-250 CFU/plate was considered as the countable range for any dilution.

2.2.1. Presumptive generic EC confirmation

Blue colonies on CHROMagar™-ECC, denoting *E. coli*, were confirmed using one of the three following methods.

2.2.1.1. Selective media

Colonies were streaked onto MacConkey agar (MAC) (Thermo Fisher Scientific, Waltham, MA) and eosin methylene blue agar (EMB) (Thermo Fisher Scientific, Waltham, MA) and incubated at 35°C for 18-24 h. Colonies on MAC with red/pink color and/or metallic green sheen on EMB were considered confirmed *E. coli*. One or more additional tests were performed to further characterize isolated colonies, as indicated below.

2.2.1.2. Phenotypic characterization (FL only)

API® 20E™ (BioMérieux, Durham, NC) profiles were used as per manufacturer's instructions. One blue colony from the CHROMagar™ was tested from each presumptive positive composite sample. Each colony was re-grown for 18-24 h on tryptic soy agar (TSA) (Thermo Fisher Scientific, Waltham, MA). A single colony was resuspended in 0.85% (w/v) NaCl (BioMérieux, Durham, NC) and subsequently inoculated onto the API® 20E™ strip and incubated at 36±2°C. Results were read after 24-48 h and analyzed using apiweb™ database (<https://apiweb.biomerieux.com/>) to identify each isolate.

2.2.1.3. BD Enterotube™ II test (NJ only)

Biochemical identifications of isolated colonies on MAC were performed using the BBL® Enterotube™ II (Becton Dickinson, Sparks, MD). A single blue colony from the CHROMagar™ was tested by re-streaking on TSA and incubating for 18-24 h. A single colony was selected and used to inoculate the Enterotube™ II, and the results were determined using the BBL® code database.

2.3. Statistical analysis

Multi-factorial linear models for the $\log_{10}$ -transformed responses (bacterial counts) for states (Florida, Maryland and New Jersey), sampling years (2013 and 2014), seasons (spring, summer, fall and winter) and treatment (pre- and post-processed) were determined utilizing SAS[®] (ver. 9.3; SAS Institute Inc., Cary, NC, USA). Adjustment for multiple comparisons was performed by Tukey-Kramer method. Least square mean (LSM) was used to compare different conditions. Correlation between APC and TC, and incidence of *E. coli* were analyzed by MS-Excel. Statistical significance (α) for all tests was set at 0.05, unless otherwise stated.

3. Results

The LSM value of APC for all samples (both pre- and post-processed) were 6.8 $\log_{10}$ CFU/tomato (n=1583). Seventeen samples (16 from MD and 1 from NJ) had counts outside the countable range (25-250 CFU/plate). The APC counts ranged from 2.3 to 12.1 $\log_{10}$ CFU/tomato with median of 6.9 $\log_{10}$ CFU/tomato, while 72 samples from MD had counts outside the countable range and thus failed to give any valid result. Ninety out of 1528 (5.9%) and 1498 out of 1597 (93.8%) valid samples had total coliform and generic EC below the detection limit of 1.3 $\log_{10}$ CFU/tomato, respectively (Table 1). APC and total coliform counts were positively correlated ($p < 0.0001$). The post-processed samples generally had lower counts of APC and TC than pre-processed samples for both years (Tables 1 & 2). The number of samples with EC over the detection limit for post-processed samples was approximately double ($p < 0.0001$) of

that seen for pre-processed samples (Tables 1 & 4) in 2014. Florida and New Jersey showed a similar increase in occurrence (% of incidence) of EC in post-processed samples.

The post-processed APC and TC counts were significantly lower ($p < 0.0001$) than pre-processed counts (Table 2). There were no significant differences noted for EC results (Table 2). There were significantly higher ($p < 0.0001$) APC and TC in samples collected in year 2014 than 2013, while EC levels were not significantly different between 2013 and 2014 (Table 2). Table 2 shows post-processed APC were significantly higher ($p < 0.0001$) in samples collected in summer and fall compared to spring. Post-processed tomatoes sampled in winter had significantly lower APC ($p < 0.0001$) than the other three seasons (Table 2). Pre-processed APC in tomatoes collected during spring and winter were significantly lower than that of the other two seasons. Seasonal variance yielded significant ($p < 0.0001$) differences in TC counts in both pre- and post-processed samples. Counts of TC in pre- and post-processed samples were always significantly different ($p < 0.0001$) in each season and in between seasons (Table 2).

Maryland had significantly higher ($p < 0.0001$) APC followed by New Jersey and Florida (Table 3). TC counts were significantly higher in New Jersey samples ($p < 0.0001$) than in samples from Maryland and Florida (Table 3). A frequency distribution of overall count ranges of microbial loads is shown in Figure 2. Counts for APC and TC were significantly lower ($p < 0.0001$) in Florida tomatoes vs. tomatoes from the other two states. EC counts

and incidence were higher in post-processed samples from Florida and New Jersey although the difference was not statistically significant ($p>0.05$).

4. Discussion and Conclusion

Many packinghouse operations use process-water as a means of moving products via flumes, prevent cross-contamination, and to clean the produce. It is therefore essential that packinghouses evaluate their postharvest practices, and ensure the minimal potential for cross-contamination by foodborne pathogens, especially whenever water contact is involved.

Improper use of sanitizers in a packinghouse dump-tank and/or flume system may open pathways for pathogens to transfer from contaminated to uncontaminated tomatoes (López-Gálvez, Allende, Selma, & Gil, 2009; Allende et al., 2007; Gil, Selma, López-Gálvez, & Allende, 2009). A study by Zhou et al. (2014) measured dump tank water quality parameters in three separate tomato packinghouses, where organic matter content in the water was quantified by chemical oxygen demand (COD). Zhou et al. (2014) showed that water quality in tomato packinghouses had COD levels as high as 800 ppm at the end of a six-hour shift, although these researchers did not examine the increased potential of cross-contamination associated with high levels of organic loading. Gereffi et al. (2015) showed a significant reduction of *Salmonella* from washing even when no sanitizers were used. However, these authors also showed that uninoculated tomatoes were often cross contaminated, especially when water organic

loadswere high.This same study also showed that the proper maintenance of sanitizer levels in flumes is essential.

Any produce washing system should be designed to reduce the microbial load that the product has acquired in the field or at least prevent an unacceptably high microbial load after postharvest washing. Populations of APC and TC were significantly lower ($p<0.0001$) in processed tomatoes compared to the unprocessed ones (Table 1), in contrast to an earlier study by Tomás-Callejas et al. (2012) where populations of total aerobic mesophilic bacteria (TAM) and coliforms were not significantly different ($p>0.05$) or were significantly higher ($p<0.05$) in washed vs. unwashed tomatoes. Senter et al. (1985) noted that total plate counts (TPC) and Enterobacteriaceae counts increased by 27.5% and 138.5%, respectively on tomatoes washed with chlorine (90 to 140 ppm) compared to unwashed tomatoes. Counts for APC and TC counts were positively correlated ($p<0.0001$) in the present study.However, there was no such correlation between EC and APC, either between processed and unprocessed tomatoes, or among tomatoes from different years or places.There were significantly higher ($p<0.0001$) APC and TC observed in 2014. Further investigationsare needed to determine if the higher occurrence of APC and TC seen in 2014has a cause or is chance variation. Occurrence of significantly higher ($p<0.0001$) APC and coliforms in warmer (summer and fall) seasons compared to the colder (spring and winter) seasonswas similar to a study by Castro-Ibáñez et al. (2015) that reported significantly higher ($p<0.05$) coliform counts on baby spinach when ambient temperature increased from 5-10°C to 15-20°C. Holvoet et al. (2014) also observed a small,but significant correlation between temperature and *E.*

coli numbers on lettuce. The higher microbial loads on Maryland tomatoes could be attributed to the practice of field packing and/or lack of post-harvest produce washing with a sanitizer, which could remove the microbes from the tomato surfaces.

When properly implemented, produce washing may prevent cross-contamination of foodborne pathogens in flume-tank water in addition to reducing pathogens present on produce surfaces. A reduction in the total microbial load that results from the use of a washing system may increase the safety of the washed product resulting in reduced costs and health impacts associated with the microbial contamination while increasing the overall quality and shelf life of the final product. Conversely, washing also has the potential of enhancing cross-contamination if not properly implemented.

In general, the prevalence of generic EC observed on tomatoes from the three states surveyed was low. This suggests that fecal contamination from the field and the processing environment was minimal. The low levels of generic EC that were observed on the tomatoes were not effectively removed by post-harvest rinsing in the flume/dump tank and/or spray bar systems observed in this study. In a limited number of packinghouses surveyed, the number of generic EC increased post-processing. Since packinghouses often employ other procedures (e.g., waxing, sorting, boxing), collection of data after these steps would give more information on the impact of these practices have on the final product. To better assess the impact of post-harvest practices, more work is needed. Lastly, studies on the relationship between specific variables such

304 as ambient temperature and initial microbial loading would also give insight on how to
305 more efficiently implement control measures during produce handling.

306

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**Table 1: Percentage of positive samples ($\geq 1.3 \log_{10}$ CFU/tomato)* analyzed by
microbe type and year**

Year	2013		2014		Total Samples
	Pre-processed	Post-processed	Pre-processed	Post-processed	
Aerobic Plate Count	99.4 (360)	98.1 (360)	99.3 (440)	98.7 (440)	98.9 (1600)
Total Coliforms	94.7 (339)	83.8 (327)	99.1 (430)	96.5 (432)	94.1 (1528)
<i>Escherichia coli</i>	7.5 (360)	6.7 (357)	3.4 (440)	7.5 (440)	6.2 (1597)

*Numbers in parenthesis represent total number of samples analyzed

Table 2: Comparison of microbial loads in pre- and post-processed tomatoes by year and season

Conditions Treatment	APC*	TC	EC
Pre-processed	7.12±0.03 _A	5.13±0.04 _a	2.56±0.22 _m
Post-processed	6.42±0.03 _B	4.70±0.05 _b	2.67±0.22 _m

Year				
2013	Pre-processed	7.02±0.04 _B	4.97±0.07 _b	2.19±0.31 _m
	Post-processed	6.26±0.04 _D	4.58±0.07 _c	2.27±0.32 _m
2014	Pre-processed	7.19±0.04 _A	5.26±0.06 _a	2.89±0.30 _m
	Post-processed	6.55±0.04 _C	4.80±0.06 _{bc}	3.03±0.31 _m

Season				
Summer	Pre-processed	7.50±0.04 _A	5.92±0.07 _a	2.66±0.25 _m
	Post-processed	6.86±0.04 _C	5.58±0.07 _b	2.63±0.25 _m
Spring	Pre-processed	6.25±0.06 _D	3.86±0.10 _d	2.03±0.53 _m
	Post-processed	5.31±0.06 _E	3.63±0.10 _{ed}	2.69±0.56 _m
Winter	Pre-processed	6.45±0.10 _D	3.09±0.16 _e	ND
	Post-processed	4.94±0.10 _F	1.73±0.16 _f	ND
Fall	Pre-processed	7.30±0.05 _B	5.32±0.08 _b	3.44±0.24 _m
	Post-processed	6.92±0.05 _C	4.87±0.09 _c	2.91±0.15 _m

*Reported values are LSM ± SE, Values with same letters within a column are not significantly different

APC = Aerobic plate count; TC = Total coliform; EC = *E. coli*; ND = Not done

Summer (June-Aug); Fall (Sept-Nov); Winter (Dec-Feb); Spring (Mar-May)

Table 3: Microbial loads on tomato surfaces (pre- and post-processed) from different states

States	Condition [Total Samples (N)]	APC*	TC	EC
Florida	Pre-processed (360)	6.62±0.04 _C	4.38±0.06 _c	2.20±0.43 _m
	Post-processed (360)	5.76±0.04 _D	4.08±0.06 _d	2.66±0.45 _m
Maryland	Pre-processed (140)	7.73±0.06 _A	5.24±0.12 _b	1.74±0.40 _m
	Post-processed (137)	7.53±0.07 _{AB}	5.06±0.13 _b	2.27±0.26 _m
New Jersey	Pre-processed (300)	7.43±0.04 _B	5.98±0.07 _a	3.06±0.29 _m
	Post-processed (300)	6.70±0.04 _C	5.29±0.07 _b	2.80±0.29 _m

*Reported values are LSM ± SE, Values with same letters within a column are not significantly different

Table 4: Occurrence of EC [incidence % = number of positive case (n)/actual number of samples analyzed in a category (N)x100] on tomatoes in different conditions.

Conditions		Positives (n)/Total Samples (N)/	<i>E. coli</i> incidence (%)
Year			
2013	Pre-processed	28/360	7.78
	Post-processed	25/357	7.00
2014	Pre-processed	15/440	3.41*
	Post-processed	31/440	7.05

Season			
Summer	Pre-processed	30/360	8.33
	Post-processed	29/357	8.12
Spring	Pre-processed	10/160	6.25
	Post-processed	2/160	1.25
Winter	Pre-processed	ND/60	ND
	Post-processed	ND/60	ND
Fall	Pre-processed	3/220	1.36
	Post-processed	25/220	11.36

State			
Florida	Pre-processed	7/360	1.94
	Post-processed	12/360	3.33
Maryland	Pre-processed	13/140	9.29
	Post-processed	12/137	8.76
New Jersey	Pre-processed	23/300	7.67
	Post-processed	32/300	10.67

*Z-Score and Exact Measures of Association has been used to find the statistical significance of incidence. (<http://www.openepi.com/PersonTime2/PersonTime2.htm>).

Numbers in boldfaced italics within a year or season are significantly different (p<= 0.05).

Summer (June-Aug); Fall (Sept-Nov); Winter (Dec-Feb); Spring (Mar-May)

502 (A)

504 (B)

507 **Figure 2:**

508

509 Figure Legends:

510 Figure 1. Tomato sampling sites in New Jersey, Maryland (A) and Florida (B). Locations
511 are marked with balloon. The maps were created via ZeeMaps
512 (<https://www.zeemaps.com/>)

513

514 Figure 2. Pre-wash (black bars) and post-wash (grey bars) bacterial counts for tomatoes.
515 (A) Frequency of total aerobic plate counts. (B) Frequency of total coliform counts,
516 where samples with no detectable coliforms are shown as 0 log CFU/tomato. (C) *E. coli*
517 counts and where pre (n=756) and post (n=742) samples with no detectable *E. coli* are
518 omitted to allow better visualization of the countable data.