

TRANSFER AND INACTIVATION OF *SALMONELLA* DURING POST-HARVEST
PROCESSING OF TOMATOES

By

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ABSTRACT

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Salmonella outbreaks have been historically linked to tomatoes, with cross-contamination during post-harvest processing having become a major public health concern. In response, a series of studies were developed to assess the extent to which dump tanks, conveyors, slicers, and dicers can spread *Salmonella* and other microorganisms.

An evaluation of the microbiological quality of tomatoes and dump tank water was conducted during three visits to a local tomato packinghouse. At the beginning of whole-day processing and after 3 h of operation, bacteria and yeast/mold populations decreased < 2 logs on tomatoes, with these microbial counts greatly impacted by changes in organic load and sanitizer concentration during washing. When the spread of *Salmonella* was assessed during washing of tomatoes with various sanitizers and subsequent conveying in a pilot-scale packing line, $\sim 90\%$ of the original *Salmonella* inoculum transferred to sanitizer-free water. Acidified chlorine yielded the greatest *Salmonella* reduction on tomatoes (3.1 log CFU/g). After processing with sanitizers, *Salmonella* populations decreased to non-detectable levels (< 0.2 log CFU/100 cm²) on the equipment surfaces. Thereafter, *Salmonella* transfer was assessed during conveying of tomatoes with plastic, foam, or brush rollers. Overall, cross-contamination was greatest using foam, followed by plastic and brush rollers ($P < 0.05$). After 5 inoculated tomatoes were roller conveyed, 24 and 76% of all uninoculated subsequently conveyed tomatoes were cross-contaminated with *Salmonella* of 10 - 100 and 1 - 10 CFU/tomato, respectively, compared to 8% of 25 tomatoes using brush rollers.

The next two studies focused on *Salmonella* transfer during slicing and dicing of tomatoes. For tomato slicing, one red round tomato was inoculated with *Salmonella* Typhimurium LT2 (~ 5 log CFU/g) and sliced using a manual or electric slicer, followed by 20 uninoculated tomatoes, all of which yielded quantifiable numbers of *Salmonella* after slicing. The quantitative data was fitted to a two-parameter exponential model ($Y = A \cdot e^{(B \cdot X)}$). While significantly higher ($P \leq 0.05$) percentages of *Salmonella* were transferred to wet (12.2%) as opposed to dry tomatoes (1.1%), with the variety of tomato also impacting transfer, post-contamination hold time, processing temperature and tomato slice thickness did not significantly impact the overall percentage of cells transferred. When one 0.9 kg batch of inoculated Roma tomatoes (~ 5 log CFU/g) was mechanically diced, followed by ten batches of uninoculated tomatoes, all uninoculated tomatoes yielded *Salmonella*, with populations exponentially decreasing from 3.3 to 1.1 log CFU/g. Flume tank washing in sanitizer-free water or water containing 80 ppm peroxyacetic acid, 80 ppm mixed peracid, or 80 ppm total chlorine decreased the *Salmonella* populations on diced tomatoes 1.3 ± 0.2 , 2.3 ± 0.3 , 2.4 ± 0.4 , and 2.4 ± 0.1 log CFU/g, respectively. Spray sanitation on conveyor belts proved to be an effective way to enhance safety of diced tomatoes, with electrolyzed water being especially attractive due to its relatively low cost and ease of preparation.

Finally, the impact of temperature, pH, and wash water organic load on *Salmonella* morphology and early-biofilm formation was assessed on different surfaces encountered in tomato packing houses. Both pH and temperature significantly affected the surface hydrophobicity of *Salmonella*. Early-biofilm formation on tomatoes was significantly affected by both time ($P = 0.0004$) and temperature ($P < 0.0001$). After 6 d, early-biofilms consistently developed on stainless steel and HDPE surface, with the former being more evenly distributed.

To my parents, Xizeng Wang and Guirong Liu,
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KEY SYMBOLS AND ABBREVIATIONS

CA	citric acid
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit(s)
COD	chemical oxygen demand
CSC	cellular surface charge
CSH	cell surface hydrophobicity
CSPI	Center for Science in the Public Interest
d	day(s)
ERS	Economic Research Service
FDA	Food and Drug Administration
FSMA	Food Safety Modernization Act
g	grams
GHP	good hygiene practices
GMP	good manufacturing practices
h	hour(s)
HDPE	high density polyethylene
kg	kilogram
L	liters
lb	pounds
MAB	mesophilic aerobic bacteria
min	minutes(s)
ml	milliliter(s)
MRD	maximum recovery diluent

ORP	Oxidation/reduction potential
PAA	Peroxyacetic acid
PBS	Phosphate Buffered Saline
ppm	Parts per million
PVC	polyvinyl chloride
RMSE	root mean square error
s	second(s)
SD	standard deviation
SDW	sterile deionized water
SE	standard error
TDS	total dissolved solids
TSAYE	Trypticase Soy Agar with 0.6 % Yeast Extract
TSB	tryptic soy broth
TSBYE	Trypticase Soy Broth with 0.6 % Yeast Extract
US	United States of America
USDA	United States Department of Agriculture
YM	yeast/mold
ZP	zeta potential
μl	microliter(s)
μm	micron(s)

INTRODUCTION

Due to various health benefits associated with tomatoes, annual per capita consumption in the United States has increased to 8.1 kg (17.9 lb) for fresh and 31.2 kg (68.7 lb) for processed tomatoes (21, 30, 69, 125). However, fresh tomatoes also have been linked to human salmonellosis, with 35 tomato-related outbreaks, including 5324 cases of illness documented in the United States from 1990 to 2013. Among all 35 outbreaks, 12 involved more than 100 cases of illnesses. Different varieties of tomatoes including round, Roma, and grape tomatoes, as well as multiple *Salmonella* serotypes, were responsible for these outbreaks (33). In addition, multiple tomato recalls were issued each year due to cross-contamination of *Salmonella*. According to the U.S. Food and Drug Administration, from 2011 to 2013, 15 tomato recalls were issued due to *Salmonella* contamination (158). Outbreaks and recalls not only pose a health risk to the public, but also damage the tomato industry.

Tomatoes can become contaminated with *Salmonella* at various points during the farm-to-fork continuum. In the pre-harvest environment, contaminated irrigation water, soil, fertilizer, birds, and other wildlife are all possible sources of contamination (12, 17, 54, 103, 117). During post-harvest processing, contamination can be further spread during contact with contaminated wash water and equipment surfaces (120, 187). In addition, the packinghouse is normally constructed with a simple roof and concrete floor, which makes it an attractive location for birds and other rodents (54).

Multiple *Salmonella* outbreaks have been traced to tomato packinghouses and producers of fresh-cut tomatoes (42, 145), which reinforces the need to minimize the spread of *Salmonella* during post-harvest processing. Multiple studies have shown that *Salmonella* can easily transfer to and from tomatoes and tomato contact surfaces under laboratory conditions (24, 83, 108). Brar

and Danyluk (24) investigated *Salmonella* transfer between gloves (single- or re-usable) and tomatoes through single or subsequent contacts and showed that clean reusable gloves transferred higher levels of *Salmonella* to the first few tomatoes touched than did single-use gloves and dirty reusable gloves. However, investigations of *Salmonella* transfer during pilot-scale processing have been limited. For fresh-cut processing, only one recent published study is currently available, which described the transfer of norovirus during slicing of tomatoes (143). Therefore, *Salmonella* transfer during fresh-cut processing (slicing/dicing) of tomatoes also needs close investigation, along with the impact of processing variables on *Salmonella* transfer.

Sanitizer application is recommended for washing of tomatoes to minimize the risk of microbial hazards. Currently, different sanitizers such as sodium hypochlorite, peroxyacetic acid, mixed peracid, aqueous ClO_2 , and aqueous ozone are being added to the dump tank for a 2-min contact time with the tomatoes to be washed (2, 14, 56, 94, 116, 156). A number of studies have investigated the efficacy of various sanitizers against *Salmonella* under laboratory conditions, with 1 to 3 logs reductions observed during washing of tomatoes (23, 25, 56, 81, 136, 184). However, the efficacy of sanitizers against *Salmonella* under pilot-scale conditions has not been evaluated thoroughly. Furthermore, investigation of sanitizer applications during fresh-cut processing of tomatoes, especially under pilot-scale conditions, is also of great importance, since high organic loads are normally encountered during slicing and dicing.

When conditions permit, foodborne pathogens can attach to food-contact surfaces, colonize, and form biofilms (8, 132). Biofilm formation is normally determined by the interaction between three main components: bacteria cells, attachment surface, and the surrounding medium (50, 52, 150). Dourou et al. (51) showed that attachment of *E. coli* O157:H7 to beef-contact surfaces was influenced by the type of soiling substrate and

temperature, with higher attachment observed during cold storage at 4 °C. Although several studies investigated *Salmonella* biofilms on surfaces including stainless steel, plastic, and glass, most of these studies were conducted in well-defined laboratory media, with the incubation environment also favorable for bacterial growth. Therefore, it is of interest to evaluate attachment and biofilm formation by *Salmonella* on commonly used equipment materials, based on practical environmental parameters.

It is hypothesized that 1) *Salmonella* can be transferred in quantifiable numbers during post-harvest washing and conveying of tomatoes; 2) sanitizer efficacy under pilot-scale processing differs from the bench-top conditions; 3) transfer of *Salmonella* can be impacted by different processing variables associated with slicing and dicing; and 4) attachment and early-biofilm formation by *Salmonella* on equipment surface materials is affected by the temperature and substrate.

The ultimate goal of this research was to collect quantitative data on *Salmonella* transfer during post-harvest processing, for subsequent risk assessments and to enhance the current understanding of *Salmonella* inactivation and biofilm formation during tomato processing. Thus, this dissertation includes five primary objectives: 1) quantify the transfer of *Salmonella* during washing and conveying of tomatoes; 2) assess the efficacy of different sanitizer treatments against *Salmonella* during pilot-scale processing of tomatoes; 3) evaluate the impact of different processing variables on *Salmonella* transfer during tomato slicing; 4) quantify the spread of *Salmonella* during simulated commercial production of diced tomatoes; and 5) assess the effect of temperature, pH, and substrate on *Salmonella* morphology, physicochemical characteristics, attachment, and biofilm formation on different surface materials encountered in the tomato industry.

CHAPTER 1:

Review of Pertinent Literature

1.1 TOMATO INDUSTRY

In the American diet, tomatoes (*Lycopersicon esculentum*) have now become the fourth most commonly consumed fresh vegetable behind potatoes, head lettuce, and onions, and the most frequently consumed canned vegetable, with an average of 8.1 kg (17.9 lb) of fresh and 31.2 kg (68.7 lb) of processed tomatoes consumed annually (30). Tomatoes are a good source of lycopene, vitamins C and E, soluble fiber, carotenoids, and polyphenols, which provide various human health benefits (21, 69, 125). A connection has been found between increased tomato consumption and reduced risk of both cardiovascular disease and prostate cancer (30).

Based on the most recent data from the USDA ERS (Economic Research Service) the United States is the second largest tomato producer worldwide, with fresh and processed tomatoes accounting for more than \$2 billion in annual sales (162). Fresh-market tomatoes primarily come from 20 states, with California and Florida accounting for almost two-thirds of total planted acreage, followed by Virginia, Georgia, Ohio, Tennessee, North Carolina, New Jersey, and Michigan. In addition to the most common field-grown red round tomatoes, plum (Roma) tomatoes, grape, and cherry tomatoes are also consumed fresh and widely available in supermarkets (162).

Tomatoes destined for processing normally contain a higher percentage of soluble solids (5 – 9%), which is ideal for soups, sauces, catsup, and paste (16). Compared to fresh-market tomatoes that are normally hand-picked, processing tomatoes are most commonly machine-harvested. The processed tomato industry has been slowly moving toward the western region of the United States, with California now the leader for processed-tomato products (162). In 2008, California produced 12 million tons of processing tomatoes, which accounted for 89% of all tomatoes grown in the US. Despite the large volume produced, processing tomatoes are

responsible for a smaller share of the U.S. total crop value than fresh-market tomatoes due to their relatively lower price (4).

1.2 TOMATO RELATED OUTBREAKS AND RECALLS IN THE US

It is estimated that one in six Americans (total 48 million people) contracts a foodborne illness annually, with 128,000 hospitalizations and 3,000 deaths (34). The Center for Science in the Public Interest (CSPI) analyzed 10,409 foodborne disease outbreaks that were reported to CDC between 2002 to 2011 and found that foodborne illness outbreaks decreased by 42% from 2002 to 2011. By food category, produce accounted for the highest number of outbreaks, with 667 (17% of total) outbreaks resulting in 23,748 cases of illnesses (24% of total) (31). While both the number of outbreaks and illnesses caused by produce consumption showed a downward trend across the decade, a spike in the number of produce-related illnesses in 2008 occurred due to one large multi-state outbreak related to jalapeno and serrano peppers that sickened ~ 1,500 people (35). It also has to be mentioned that the tomato industry was damaged tremendously in summer 2008 due to CDC's initial announcement of tomatoes as the vehicle for *Salmonella* Saintpaul infections.

According to the information from CSPI, within the produce category, tomatoes were responsible for 4% of all produce-linked outbreaks from 1998 to 2006. Among all tomato-related outbreaks, *Salmonella* was the leading cause of infection and continues to pose a risk to consumers and damage the tomato industry (32, 43, 72, 75, 145). From 1990 to 2013, 35 tomato-related outbreaks, including 5324 cases of illness, were documented in the United States. Among all 35 outbreaks, 12 involved more than 100 cases of illnesses. The tomato varieties involved covered a wide range, including round, Roma, and grape tomatoes, with various *Salmonella* serotypes identified (33).

According to the U.S. Food and Drug Administration, from 2011 to 2013, 15 tomato recalls were also issued due to *Salmonella* contamination. In early February of 2014, 790 boxes of fresh tomatoes were recalled by a Florida tomato packer due to possible *Salmonella* contamination. Then in mid-September, Taylor Farms Pacific, Inc. also voluntarily recalled specific lots of Roma tomatoes for the same reason. Similar to the outbreaks, different tomato varieties, including round, Roma, and grape tomatoes, were involved, with the number of recalls involving grape and cherry tomatoes showed rising trend (158). With the passage and implementation of the Food Safety Modernization Act (FSMA), more tomato recalls will likely be mandatorily issued by FDA in the future. Both outbreaks and recalls can significantly affect consumer confidence and also economically damage the tomato industry.

1.3 SALMONELLA AND SOURCE OF CONTAMINATION

The genus *Salmonella* belongs to the family *Enterobacteriaceae* and is composed of facultatively anaerobic, oxidase-negative, catalase-positive, Gram-negative, rod-shaped bacteria. Most *Salmonella* strains are motile and ferment glucose with production of both acid and gas. Depending on the somatic (O) and flagellar (H) antigens, *Salmonella* spp. can be classified into different serovars (serotypes). As of 2008, 2,579 serovars have been identified, with 58.9% of serovars belonging to the species *enterica* subspecies *enterica* which contains most of the serovars responsible for foodborne disease in humans (41, 44). As a leading cause of foodborne bacterial illnesses in humans, *Salmonella* spp. has long been related to foodborne outbreaks worldwide, covering a wide range of foods, such as raw milk, ice cream, cheese, pork, poultry, eggs, nuts, and peanut butter (20, 44, 60, 106, 127). In the last decade, fresh fruits and vegetables became important vehicles of human salmonellosis, with major outbreaks having been traced to fresh tomatoes, lettuce, mixed salads, bean sprouts, alfalfa sprouts, and cantaloupe (44, 59, 74,

144). Unlike other foodborne organisms, many different *Salmonella* serovars have been associated with foodborne illness.

In terms of *Salmonella* serovars related to tomatoes, according to the CSPI outbreak database, 15 different serovars were implicated in outbreaks during 1990 to 2011 in the US, with *Salmonella* Newport being the leading serovar, followed by *Salmonella* Javiana and Typhimurium (Table 1.1). A recent study on the fitness of *Salmonella* serovars in tomato plants showed that *Salmonella* Newport and Javiana were dominant in sandy loam soil, while *Salmonella* Montevideo and Newport were more prevalent on leaves and blossoms (186). It was also observed that *Salmonella* Typhimurium had a poor rate of survival in all of the plant parts examined, which indicated that postharvest contamination routes are more likely in *S. Typhimurium* contamination of tomato fruit (186). Although it was not until 2011 that *S. enterica* was first identified to cause *Salmonella* outbreaks in tomatoes in the US tomatoes (33), *Salmonella enterica* has been isolated from wetlands, ditches, and ponds in or near tomato fields in 2005 (68). Barak and Liang (13) showed that *S. enterica* contaminated soil can lead to contamination of the tomato phyllosphere, with presence of the bacterial plant pathogen - *X. campestris* pv. *Vesicatoria*, which is beneficial for *S. enterica* multiplication. In addition, colonization of tomato plants by *Salmonella enterica* is cultivar dependent and the type 1 trichomes of tomato plants are the preferred colonization site compared to stomata (11). Therefore, proper selection of tomato cultivars and good management of irrigation water are critical to reducing *Salmonella* contamination in tomatoes.

Salmonella contamination of tomatoes can come from both the pre-harvest and post-harvest environment. In the pre-harvest environment, contaminated irrigation water, soil, fertilizer, birds, and other wildlife are all possible sources (12, 17, 54, 103, 117). Since most

Salmonella strains that infect domestic or wild animals are pathogenic to humans, prevalence and survival of such pathogens in the tomato production environment can lead to serious cross-contamination (17). In one previous study, a wide range of wildlife, including sparrows, towhee, crows, feral pigs, coyotes, deer, elk, opossums, and skunks, tested positive for *Salmonella* (66). In addition, domestic animals raised in confinement, such as cattle, yielded *Salmonella* at rates up to 100% (96, 149). Microbial quality of water in the pre-harvest environment should be well maintained to reduce the risk of cross contamination during crop irrigation and spray application (118). A survey showed that more than 7.1% of the surface water samples in California were positive for *Salmonella* (66). However, prevalence of *Salmonella* in the Southeastern U.S. (North Carolina, Georgia, Florida) was more than 10 times higher than in California, with 96% of all samples positive for *Salmonella* (131). It has been shown that sewage effluents, agricultural runoff, and feces from wild animals and birds are the major sources of *Salmonella* contamination in aquatic environments (3, 19, 85). Furthermore, agricultural soil used for tomato production, especially if amended with improperly treated fertilizers, can be a source of *Salmonella* contamination for tomatoes and water sources (10, 82). In one study *Salmonella* survived up to 45 d in soil, with the population of *Salmonella* on tomatoes in contact with the soil increasing by 2.5 log CFU per tomato during 4 d of storage at 20 °C and remaining constant for an additional 10 d (71). Therefore, appropriate treatment and intervention steps to reduce the prevalence of *Salmonella* contamination in soil become critical to minimize the overall risk in the pre-harvest environment.

After harvesting from the field, tomatoes are normally transported to the packinghouse for post-harvest washing and packing. During post-harvest processing, *Salmonella* contamination can be introduced from wash water or wildlife such as birds (54). Further spread of

contamination can occur through contact with contaminated wash water and equipment surfaces including roller conveyors and the waxing and packing tables (120, 187). In addition, the packinghouse is normally constructed next to the field with a simple roof and concrete floor, which makes it an attractive location for birds and other rodents (54). *Salmonella* outbreaks have been traced back to tomato packinghouse in the past. For instance, in the summer of 2004, a single Roma tomato-packinghouse in Florida was the source of three salmonellosis outbreaks involving 561 cases of illnesses in the United States and Canada (36). Two years later, another tomato packinghouse in Ohio was linked to a similar outbreak, which involved 190 culture-confirmed cases of *Salmonella* Typhimurium infection in 21 states (145). Such packinghouse-related outbreaks highlight the need for improved microbial reduction strategies to better ensure tomato safety.

Table 1.1: *Salmonella* serovars related to tomatoes that involved in outbreaks during 1990 to 2011 in the US (based on CSPI outbreaks database) (33).

Product	Serovar	Year	Cases	States	Location
Tomato	S. Javiana	1990	176	Multi-state	School
Tomato	S. Montevideo	1993	100	Multi-state	Private home; school
Tomato	S. Baildon	1998	86	Multi-state	Multiple locations
Tomato	S. Thompson	2000	43	Multi-state	Private home
Tomato	S. flexneri	2001	886	New York	Restaurant/deli
Tomato	S. Javiana	2002	3	Massachusetts	Restaurant/deli; restaurant/theme park
Tomato	S. Newport	2002	510	Multi-state	Restaurant/deli; school; hospital
Tomato, "Grape"	S. Newport	2002	7	Connecticut	Private home
Tomato, "Roma"	S. Javiana	2002	159	Florida	Hotel; restaurant/deli
Tomato	S. Virchow	2003	11	California	Multiple locations
Tomato, "Roma"	S. Braenderup	2004	137	Multi-state	Restaurant/deli; private home
Tomato, "Roma"	S. Anatum, S. Group D, S. Javiana, S. Muenchen, S. Thompson, S. Typhimurium	2004	429	Multi-state	Restaurant/deli
Tomato, "Roma"	S. Braenderup	2005	84	Multi-state	Restaurant/deli
Tomato, beefsteak	S. Newport	2005	52	Multi-state	Restaurant/deli
Tomato	S. Typhimurium	2006	8	Maine	Unknown
Tomato	S. Berta	2006	16	Pennsylvania	Nursing home; Hospital
Tomato	S. Newport	2006	115	Multi-state	Restaurant or deli
Tomato	S. Typhimurium	2006	192	Multi-state	Private home
Tomato, beefsteak	S. Newport	2007	65	Multi-state	Restaurant or deli; Private home
Tomato	S. Typhimurium	2007	23	Minnesota	Restaurant or deli
Tomato	S. Newport	2007	10	New York	Unknown or undetermined

Table 1.1 (cont'd)

Tomato; Avocado; Guacamole; Cilantro	S. Newport	2007	46	District of Columbia	Restaurant or deli; Private home
Tomato, salad, green	S. Braenderup	2008	12	Iowa	Restaurant or deli
Tomato	S. Saintpaul	2009	21	Michigan	Private Home; Restaurant - "Fast- food"(drive up service or pay at counter); Restaurant - Sit- down dining
Tomato	S. Newport	2010	27	Rhode Island	Restaurant - Sit- down dining
Tomato	S. Newport	2010	16	Washington	Private Home
Tomato	S. Javiana	2010	30	Multi-state	Private Home; Restaurant - "Fast- food"(drive up service or pay at counter)
Tomato	S. enterica	2011	10	New York	Unknown
Tomato	S. enterica	2011	166	Multi-state	Unknown

1.4 POST-HARVEST PROCESSING OF TOMATOES

Depending on the tomato variety, post-harvest processing might be slightly different. However, the major post-harvest processes include packing in the packinghouse and fresh-cut/value-added processing (Figure 1.2). Sometimes, repacking and distribution operations are also necessary to meet the market demand (157). While it is well known that *Salmonella* contamination can occur during post-harvest processing, a better understanding of the processes involved is critical for effectively preventing and minimizing microbial hazards associated with tomatoes.

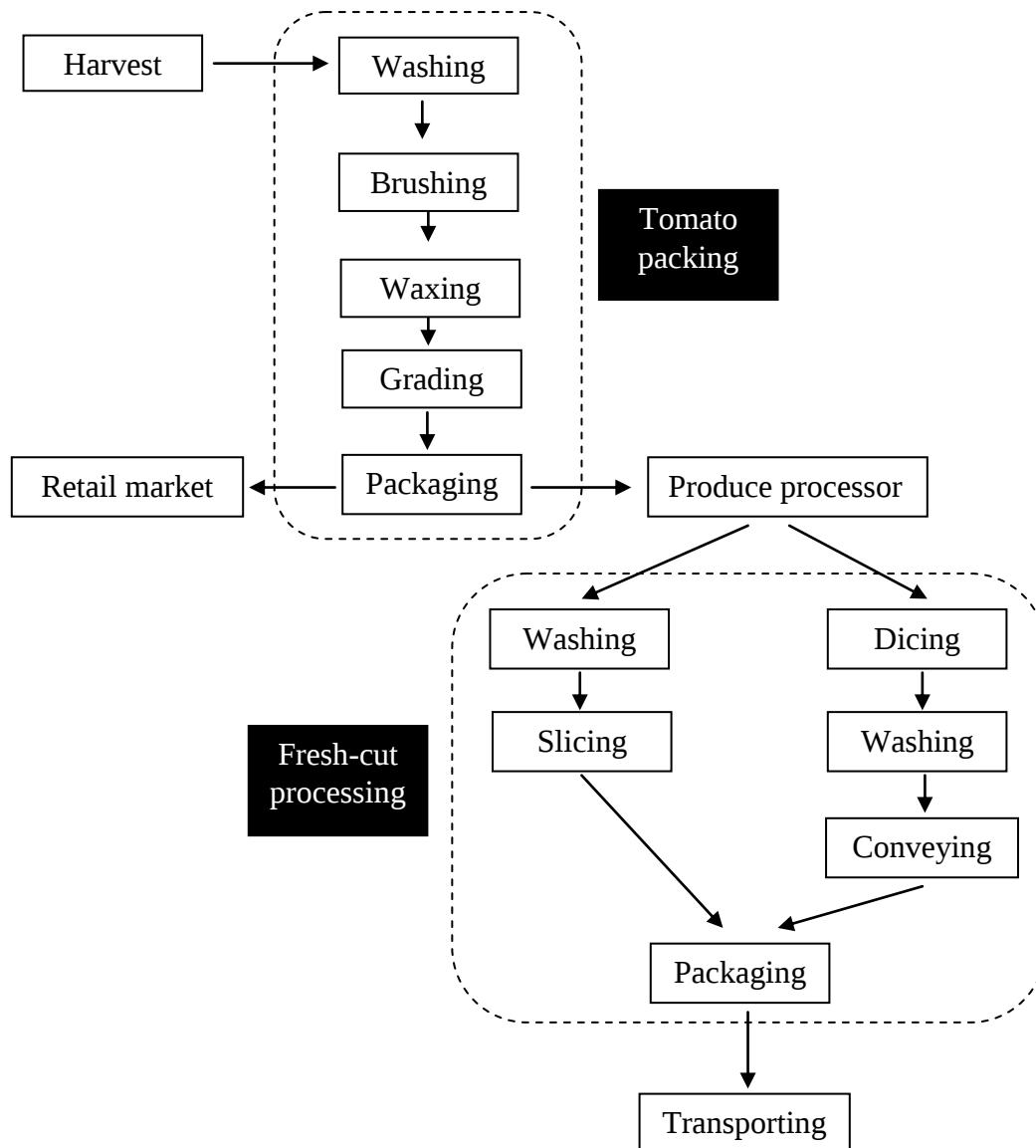


Figure 1.1: Post-harvest processing of tomatoes (packing and fresh-cut processing).

During field harvesting, tomatoes are normally collected in large plastic bins and later transported to the packinghouse for washing and packing. A large dump tank containing wash water is used to create a cushion to prevent bruising and to wash off soil and other field debris from the tomatoes. Two min of washing in dump tank water containing a chemical sanitizer (e.g., chlorine, peroxyacetic acid) is recommended by the FDA to minimize the spread of

contaminants (157). In this same guidance document, it is also recommended that the water temperature be at least 6.6 °C (10 °F) above the pulp temperature of the tomato and that the pH and oxidation-reduction potential (especially when chlorine is used in water) be monitored to ensure proper sanitizer efficacy (157).

After dump-tank washing, tomatoes are typically brushed and dried with the aid of an over-head fan. In some cases, foam-rubber roller conveyors (or in combination with an air-blast drier) can also be used to remove residual water after washing (109). After drying, a food-grade wax often is applied as a fine mist to coat the tomato surface. Then, tomatoes are normally graded and separated by size on multiple sizing belts. For tomato varieties like Roma or grape tomatoes, grading is not necessary. After grading, tomatoes are delivered to a table by plastic roller conveyors for manual packing. Depending on the variety and size, tomatoes may be packed in various containers, such as a 9.07 kg (20-lb) two-layer flat box, 11.3 kg (25-lb) loose carton, or a 6.8 kg (15-lb) flat. Currently, cardboard cartons are still widely used for tomato packing. Proper labeling also should be applied to all containers to accurately represent the commodity name, packinghouse, production date, and lot number (109, 157).

After packing, the boxed tomatoes are transported from the packinghouse either to processors for further processing or directly to the retail market. The common practices among produce processors may include repacking or fresh-cut/value-added processing (more detailed information can be found in section 1.7). For the repacking process, similar guidelines such as employee hygiene, record tracking and lot labeling should be followed to minimize cross contamination and maintain traceability (157).

1.5 BACTERIAL TRANSFER DURING POST-HARVEST PROCESSING

Once present in the processing environment, foodborne bacteria can readily transfer during post-harvest processing. While this is not a new concept, very little information exists on the numbers of foodborne bacteria transferred during processing. Hence, a better understanding of bacterial transfer will be critical to the development of science-based transfer models for risk analysis and development of more effective sanitation programs.

Allen et al. (5) assessed the survival of *Salmonella* on fresh tomatoes and selected packing materials including stainless steel, polyvinyl chloride (PVC), sponge rollers, conveyor belts, and unfinished oak wood surfaces during 28 d under various temperature/relative humidity conditions. Overall, *Salmonella* populations remained detectable on tomatoes after 28 d regardless of environmental conditions. Stainless steel, PVC, and wood surfaces supported the survival of *Salmonella* for more than 28 d, with populations declining to undetectable levels on sponge rollers and conveyor belts by day 7 and day 21, respectively. This study clearly illustrated the ability of *Salmonella* to survive in processing environments and the potential for spread during processing.

Multiple studies have shown that *Salmonella* can easily transfer between produce and surfaces under laboratory conditions (24, 83, 108). When Moore et al. (108) evaluated the extent to which *Campylobacter jejuni* and *Salmonella* Typhimurium transferred from stainless steel to lettuce, high numbers (up to 66% of the original inoculum) of bacteria transferred to lettuce even 1 to 2 h after surface contamination. Similarly, Jensen et al. (83) determined the transfer rates for *Salmonella* and *E. coli* O157:H7 between fresh-cut produce (celery, lettuce, carrot, and watermelon) and common kitchen surfaces, including ceramic, stainless steel, glass, and plastic. Higher percentages of bacteria transferred from freshly inoculated surfaces to produce items with

the direction of transfer greatly influencing the transfer rate. In addition, Brar and Danyluk (24) investigated *Salmonella* transfer between gloves (single- or re-usable) and tomatoes through single or subsequent touches and showed that both clean and dirty reusable gloves transferred similar numbers of salmonellae during a single contact. However clean reusable gloves transferred higher levels of *Salmonella* to the first few tomatoes touched than did single-use and dirty reusable gloves. Although all of these studies highlight the great potential of *Salmonella* transfer under bench-top conditions, more pilot-scale studies are needed to simulate industry practices for a more enhanced understanding of bacterial transfer during post-harvest processing.

Due to the complexity of scaling up bench-top studies, there has been only limited work on pilot-scale processing of produce. At Michigan State University, Buchholz et al. (29) quantified *E. coli* O157:H7 transfer from leafy greens to equipment surfaces during simulated pilot-scale commercial processing, which included shredding, conveying, flume washing without a sanitizer, shaker table dewatering, and centrifugal drying. Overall, ~90% of the *E. coli* O157:H7 inoculum transferred to the wash water during processing, with highest populations remaining on the conveyor and shredder, followed by the centrifugal dryer, flume tank, and shaker table after processing. Another study conducted by the same group tracked the transfer of *E. coli* O157:H7 from 9.07 kg (20 lb) of inoculated radicchio (10^6 CFU/g) used as a colored surrogate for iceberg lettuce to 907 kg (2000 lb) of iceberg lettuce during shredding, conveying, flume washing without a sanitizer, shaker table dewatering, and centrifugal drying. Overall, the contaminated product continually spread during leafy green processing long after the contamination event (a short video detailing the process can be seen at: <http://www.youtube.com/watch?v=8mSKdjxauTw>). During processing, the inoculated radicchio spread to all 907 kg (2000 lb) of iceberg lettuce, with 94, 1.3, 0.8 and 0.5% of the radicchio,

respectively, recovered from pound 1 to 500, 501 to 1000, 1001 to 1500 and 1501 to 2000.

Microbial analysis of radicchio-free iceberg lettuce showed that these same groupings contained mean *E. coli* O157:H7 populations of 1.69, 1.22, 1.10 and 1.11 log CFU/g. After processing, several hundred pieces of radicchio still clung to the various equipment surfaces, with contamination most prevalent on the conveyor (9.8 g), followed by the shredder (8.3 g), flume tank (3.5 g) and shaker table (0.1 g) (27). Compared to leafy green processing, no reports are currently available to describe *Salmonella* transfer during post-harvest processing of tomatoes, especially at the pilot-plant scale.

1.6 SANITIZER APPLICATION FOR TOMATO PROCESSING

Similar to leafy green processing, the use of sanitizers is recommended for processing of tomatoes, to minimize the risk of cross contamination during washing. Traditionally, the sanitizer is added to the dump tank water for 2 min of contact with the upcoming tomatoes, with overhead spray-sanitation drawing more attention recently. Previous studies showed that overhead sanitizer spraying combined with brushing provided an effective physical scrubbing and is capable of decreasing *Salmonella* populations up to 5 logs on tomatoes (38, 120). Despite multiple advantages of spray sanitation, which include greater efficacy and lower water and sanitizer usage, most tomato processors still rely on conventional dump-tank washing. Chemical sanitizers commonly used for tomato washing include sodium hypochlorite, peroxyacetic acid, mixed peracid, aqueous ClO₂, aqueous ozone, and electrolyzed water (2, 14, 56, 94, 116, 156). Chlorine-based sanitizers have been most commonly used in the fresh produce industry due to their strong antimicrobial activity, relatively low cost, and minimal negative impact on product quality. The efficacy of chlorine-based sanitizers is enhanced at pH of 6.0 to 6.5, with citric acid normally added to the water as an acidulant (122, 135). T-128, a recently developed chlorine

acidifier and stabilizer, has also proven effective in decreasing the rate of free chlorine depletion in the presence of soil (95, 112). Addition of surfactants to chlorine solutions can also improve contact with the microbial surface, and thus enhance efficacy (146). Despite its broad spectrum of antimicrobial activity, the application of chlorine in produce washing has led to multiple concerns, including potential carcinogenic residues, hazardous byproducts, equipment corrosion, and decreased efficacy in the presence of high organic loads (65, 115, 154). Therefore, various alternatives to chlorine have been developed for the produce industry.

Electrolyzed water (also known as electrolyzed oxidizing water), which is also based on hypochlorous acid, has received much attention since its approval by the US Environmental Protection Agency in 2002 (90, 134, 183). Unlike other chlorine-based sanitizers that must be purchased, the hypochlorous acid found in electrolyzed water is generated on site during electrolysis of water containing sodium chloride, with an available chlorine level as high as 100 ppm attained at $\text{pH} < 3.0$. Compared to traditional chemical sanitizers, electrolyzed water has multiple advantages, including ease of generation and low cost. However, its strong acidity and high free chlorine concentration can cause chlorine gas emission, corrosion to metals and degradation of synthetic resins. If not continuously maintained by electrolysis, electrolyzed water also will rapidly lose its antimicrobial activity (80). Thus, when electrolyzed water is used, the electrolysis process needs to be continuously monitored to ensure efficacy.

Peroxyacetic acid (PAA) has been widely used in the produce industry since its approval by FDA (21 CFR 173.315) at concentrations ≤ 80 ppm (135). Although also a strong oxidizing agent like chlorine, PAA efficacy is only minimally impacted by changes in pH and organic load of the wash water (47, 55). Baert et al. (9) assessed the efficacy of sodium hypochlorite and peroxyacetic acid against murine norovirus, *Listeria monocytogenes*, and *Escherichia coli*

O157:H7 on shredded iceberg lettuce and in residual wash water and showed that unlike NaOCl, the effectiveness of peroxyacetic acid was not influenced by the presence of organic material. In addition, peroxyacetic acid can also be mixed with other acids to improve efficacy. One previous study showed that a peroxyacetic acid/octanoic acid mixture (Tsunami 200) was more effective in reducing yeasts and molds in produce wash water than peroxyacetic acid alone (77). In this study, “mixed peracid” was used to represent this mixture obtained from a commercial sanitizer - Tsunami 200 (Ecolab, St. Paul, MN).

Aqueous ClO₂ (21CFR173.300) and ozone (21CFR173.368) have been approved by FDA for use in the food industry due to their highly effective, broad spectrum antimicrobial activity, especially at low concentrations. Typically, ClO₂ and ozone must be generated on-site and mixed with water to reach the appropriate concentration – 4 to 5 ppm for ClO₂ and 2 to 3 ppm for ozone (79, 152, 153). Despite their advantages over hypochlorite, ClO₂ is not very stable and can become explosive at partial pressures greater than 120 mm Hg (135). Similarly, ozone is highly unstable, corrosive to equipment, and can become toxic to workers at concentrations greater than 0.1 ppm in air (160). Therefore, ClO₂ and ozone should only be used in well-ventilated areas and monitored extensively to avoid potential hazards to workers.

During post-harvest processing of tomatoes, effective dump tank washing is critical to eliminate potential biological hazards and maximize end product safety. Several studies have investigated the efficacy of various sanitizers against foodborne pathogens, *Salmonella* in particular, during washing of tomatoes, with pathogen reductions of 1 to 3 logs having been reported on tomatoes using dip or spray treatments under laboratory conditions (23, 25, 56, 81, 136, 184). However, the ability of these same sanitizers to maintain their efficacy against

Salmonella under conditions that more closely resemble commercial processing remains unknown.

Proper design, cleaning, and sanitizing of conveyor belts remains a problem for the food industry, with development of biofilms on equipment surfaces also hindering sanitizer efficacy and causing consistent cross-contamination (7, 84). In addition, continuous spraying of conveyor belts with a sanitizer can provide a further decrease in bacteria cross-contamination. When McCarthy and Burkhardt (2012) evaluated the efficacy of electrolyzed oxidizing water against *Listeria monocytogenes* and *Morganella morganii* on conveyor belt surfaces under laboratory conditions, exposure to EO water for a minimum of 5 min assisted in the removal of biofilms and food residue, with continuous or intermittent spraying of food processing equipment (e.g., conveyor belts, slicers) reducing or preventing further biofilm formation (100). In another study conducted by our group, spraying a food-grade sanitizer on a pilot-scale conveyor belt decreased populations of *L. monocytogenes* > 5 logs on the belt surface (182). As the only process between flume-tank washing and final packaging of dice tomatoes, it is necessary to maintain “clean” conveyor belts to avoid the spread of foodborne pathogens during conveying. Therefore, more research will be needed on spray application of sanitizers in the diced tomato industry.

1.7 SLICING AND DICING OF TOMATOES

Commercial preparation of fresh-cut produce invariably involves shredding (e.g., lettuce, cabbage), slicing (e.g., tomatoes, onions, cucumbers, melon), or dicing (e.g., tomatoes, onions, celery, green pepper, melon), with or without prior washing. The market for sliced/diced tomatoes among large restaurant chains and commercial cafeterias has continually grown due to their increased convenience (91). Unlike mechanical shredding of leafy greens, which is a relatively uniform process across the industry, commercial practices employed for slicing of

fresh produce vary from completely manual (e.g., hand-held knives) to semi-manual (e.g., hand-operated slicers) to automated, depending on the processor and the product, with much of this work being extremely repetitive and labor-intensive due to the human element. Commercial slicing of tomatoes still heavily relies upon the use of manual counter-top slicers, with electric and mechanical slicers also available on the market (53, 99). Although many different brands of manual slicers are currently available, most are based on a similar design in which tomatoes are individually pushed through a stationary set of equally spaced horizontal blades. Prior to slicing, tomatoes are normally washed in a sanitizer solution to decrease the microbial load on the surface; however, the slicer won't typically be cleaned and sanitized until the end of processing. Consequently, any foodborne pathogens surviving the sanitizer treatment can potentially transfer to the slicer, leading to cross-contamination of subsequent products.

Dicing, the last of three main unit operations for preparing fresh-cut produce, is best suited for products having a firm texture, such as Roma tomatoes, onions, celery, and peppers. Unlike slicing, mechanical dicing requires that the product be cut in three directions (the last two being perpendicular) as opposed to a single direction to obtain cubes. For tomato dicing, Roma tomatoes are normally cored to remove the stem portion before dicing, to avoid causing damage to the dicer blades and compromising the final product quality. Depending on customer needs, various types of products, such as $\frac{3}{4}$, $\frac{1}{4}$, $\frac{3}{8}$, or $\frac{1}{8}$ inch tomato dices, can be obtained (161). Compared to mechanical dicing, both manual and electric dicers have remained unpopular for industrial processing and are inherently more complex in their design (176), raising increased concerns in regard to cross-contamination during normal operation as well as cleaning and sanitizing afterwards.

Unlike tomato slicing, which is conducted after washing, diced tomatoes often are flume washed after dicing (Figure 1.1). This washing step not only reduces the microbial load on diced tomatoes, but also helps to remove tomato seeds and the internal “jelly” portion. Sanitizers can be added to the wash water, with chlorine most commonly used in the industry. After washing, the diced tomatoes are dewatered using a shaker table and transported on a conveyor belt to the packaging machine. Consequently, commercial production practices are far more complex for diced as opposed to sliced tomatoes and require additional attention to ensure food safety.

1.8 BACTERIAL TRANSFER DURING SLICING/DICING

It is well known that bacteria can transfer between different batches of product during slicing or dicing, which leads to expanded cross-contamination. Both spoilage and pathogenic microorganisms, such as *Salmonella*, *L. monocytogenes* and *Escherichia coli* O157:H7, can be transferred from the intact outer surface of the product (e.g., skin or rind) to multiple cut surfaces and subsequently grow, resulting in spoilage or a potentially hazardous situation. Regardless of the cutting process, contact between the product and blade as well as other food contact surfaces of the shredder, slicer, or dicer will lead to extended transfer of microorganisms that can seriously compromise both the quality and safety of large volumes of product produced during extended production runs. While some cross-contamination during cutting is for all practical purposes unavoidable, the extent of microbial transfer needs to be better controlled given the recent surge in foodborne outbreaks and recalls traced to an ever widening range of products, which includes lettuce, spinach, celery, onions, tomatoes and cantaloupe among others. Progress in this area can only come through a better understanding of those factors that can affect bacterial transfer during processing, with such new knowledge leading to improved equipment designs and the development of science-based transfer models for risk assessments.

A number of previous studies have focused on the transfer of various foodborne pathogens during slicing/mincing/grinding of meat products, such as deli meat (39, 128, 138, 139, 140, 141, 169, 170), “gravad” salmon (1), beef fillet (121), and pork (107). This growing interest in bacterial transfer during processing of meat products, especially deli meat, was prompted by several high-profile *Listeria* outbreaks associated with deli meats (58, 67), during which foodborne pathogens transferred extensively during the slicing process. Therefore, most research has focused on assessing bacterial transfer during slicing of various products, with the experimental designs including blade-to-product (bacteria transferred from inoculated blade to uninoculated product) and product-to-blade-to-product transfer (bacteria transferred from inoculated product to slicer blade then to uninoculated product). However, product-to-blade-to-product transfer best represents the real world scenario during slicing and dicing. Most transfer studies showed that a higher initial inoculation level led to more extended transfer, with detectable levels of bacteria still observed up to 200 slices after initial contamination (139). However, due to the nature of bacterial transfer, large variations within replicates were observed for most of the transfer studies, particularly at lower initial inoculation levels.

Bacterial transfer is a complicated process, with many factors affecting the spread of microorganisms during slicing. For instance, Vorst et al. (169) reported significantly greater ($P < 0.05$) transfer of *Listeria* to the table and back plate of a mechanical delicatessen slicer when 4.5 as opposed to 0 kg of force was applied against the product during slicing. In addition, the higher fat and lower moisture content of certain products such as salami can potentially prolong *L. monocytogenes* transfer during slicing (169). In another study, inoculum level, temperature and attachment time significantly affected the total number of *L. monocytogenes* cells transferred during slicing of salmon (1). However, Buchholz et al. (29) failed to observe a significant impact

of either shred size or post-inoculation hold time on *E. coli* O157:H7 transfer during pilot-scale processing of iceberg lettuce. Sheen and Hwang (140) summarized the factors affecting microbial transfer, which include the microorganism (e.g., strain, age, inoculum size, attachment/adhesion ability), food composition (e.g., moisture, fat content, formulation), food surface texture (e.g., homogeneity, roughness), blade profile (e.g., material, size, sharpness, cutting speed), cutting force, and the environmental condition (e.g., temperature). Despite the complexity and difficulty, more research is needed to assess the impact of different factors/variables on bacterial transfer during slicing, which will ultimately provide critical quantitative data for mathematical modeling and help fill the data gaps in current risk assessments.

1.9 MODELING OF BACTERIA TRANSFER DURING SLICING/DICING

In most of the aforementioned transfer studies, various mathematical models (empirical or semi-empirical) were developed based on the experimental transfer data to describe the bacterial spread during processing. Pérez-Rodríguez et al. (128) modeled the transfer of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during slicing of cooked pork and discovered that the populations of both pathogens decreased logarithmically from initial inoculum levels of 8 and 6 log cfu/blade, respectively, with 20 slices being obtained. When fitted to the transfer data, both log-linear and Weibull model yielded good fits, with R^2 values > 0.73. Aarnisalo et al. (1) modeled the transfer of *L. monocytogenes* during slicing of salmon using an exponential model ($y = a \cdot e^{(-x/b)}$), which assigned slice number as the independent variable (x) and *L. monocytogenes* concentration (log CFU/g) on each slice as the dependent variable (y). The model fit the data from different test conditions and was suitable for predicting *L. monocytogenes* transfer during slicing of salmon. Similarly, multiple studies conducted by Sheen

et al. (138, 139, 140, 141) evaluated the transfer of common foodborne pathogens (including *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*) during slicing of deli meats (e.g., ham, salami), after which models were selected to describe the transfer behavior. Generally, two scenarios, bacterial transfer from an inoculated blade to previously non-contaminated product and from an inoculated product to a previously non-contaminated product via the blade, were assessed. For the first scenario, the experimental data showed a good fit to a power model - $Y = A \cdot X^B$, with X and Y being the slice number and log CFU/slice, respectively. The authors hypothesized that the interaction of cutting force (i.e., tangential stress and radial stress on the circular blade tends to pull the microbes away) and friction (the ham surface in contact with blade tends to retain them) during slicing resulted in the 'irregular' pattern of microbial transfer on the slices. However for the second scenario, an exponential decay model - $Y = C \cdot \text{Exp}^{(-X/D)}$ provided a better fit to the transfer data and showed a less steep decay than the first scenario. Although it under-estimated initial transfer to the first slice, the model was able to predict the extent of cross-contamination during continued slicing.

Published work on the spread of microbial contaminants during slicing of tomatoes is currently confined to a single study involving norovirus transfer during slicing that showed norovirus was able to spread from one inoculated tomato used to contaminate the slicer to all 28 subsequently sliced tomatoes with these data fitted to a logarithmic model (143). However, the author did not assess the impact of any of the factors/variables on virus transfer. Furthermore, as the leading foodborne pathogen for tomatoes, the transfer of *Salmonella* during tomato slicing is of great importance to be evaluated. In addition, no transfer model has been developed to describe pathogen transfer during dicing of tomatoes.

1.10 *SALMONELLA* ATTACHMENT AND BIOFILM FORMATION ON SURFACES

A biofilm is an assemblage of microbial cells that is irreversibly attached to a surface and enclosed in a matrix of primarily polysaccharide material (50). It is well known that foodborne bacteria can attach to surfaces and eventually form a layer of biofilm after a multi-step phase, which includes the initial adhesion, extracellular polysaccharide (EPS) secretion, and the final mature phase. Biofilm formation in food processing facilities has become a major food safety concern. Once the biofilm is formed, bacteria under the EPS layer will become inaccessible to chemical sanitizers and more resistant to various environmental/processing stresses, leading to prolonged contamination of product during processing (142, 148, 164). Biofilm formation is normally determined by the interaction between three main components: bacteria cells, attachment surface, and the surrounding medium (50, 52, 150).

As the initial phase, bacterial attachment to surfaces is critical to overall colonization and biofilm formation and is influenced by multiple factors such as surface charge, surface hydrophobicity, surface roughness and presence of flagella/fimbriae (50). The attachment between bacteria and surfaces has proven to be a complicated process, which partially depends on various reversible and subsequently irreversible interactions (78, 97). The initial reversible stage is mostly determined by cell physicochemical properties, such as cell surface hydrophobicity (CSH) and cellular surface charge (CSC) (159, 165, 189). The physicochemical properties of bacteria cells can be affected by various factors, such as temperature, pH, nutrient, and strain serotype. For instance, Chia et al. (40) observed a clear difference in bacterial hydrophobicity between *Salmonella* Sofia and other *Salmonella* serovars, with no significant differences between isolates. Similarly, another study also indicated that attachment of *Campylobacter* to abiotic surfaces significantly correlated with cell surface hydrophobicity ($P \leq$

0.007), but not with surface charge ($P > 0.507$) (111). While multiple studies have investigated the impact of bacterial serotype, it is also necessary to assess the impact of common environmental parameters on the dynamic physicochemical changes that occur in bacteria.

Another important component of biofilm formation is the attachment surface. The properties of the attachment surface, such as roughness, wettability (also defined as hydrophilicity), and cleanability, are significant factors that can affect biofilm formation. In food processing facilities, commonly encountered materials include stainless steel, plastic, glass, rubber, and cement. The degree of bacterial attachment to different materials becomes critical for biofilm establishment and the design of sanitation programs. When Rogers et al. (133) assessed biofilm formation of *Legionella pneumophila* on polybutylene, chlorinated polyvinyl chloride, and copper surfaces, less biofilm was formed on copper than plastic, with the use of copper tubing in water systems helping to limit colonization by *L. pneumophila*. Similarly, researchers also discovered that the capacity to support biofilm growth progressively increased from glass to stainless steel, polypropylene, chlorinated PVC, unplasticized PVC, mild steel, polyethylene, ethylene-propylene, and latex (102, 164). However, the interactions between bacteria and surfaces must also be considered when attempting to predict the extent of biofilm formation.

Lastly, the surrounding medium (also known as substrate) can also affect biofilm formation by bacteria in processing environments. For instance, Dourou et al. (51) compared the impact of three soiling substrates including sterile tryptic soy broth (TSB), unsterilized beef fat-lean tissue (1:1 [wt/wt]) homogenate (10% [wt/wt] with sterile distilled water), and unsterilized ground beef on attachment and biofilm formation by *E. coli* O157:H7 on food contact surfaces found in beef processing facilities. *E. coli* O157:H7 attachment to beef-contact surfaces was influenced by the type of soiling substrate, with the fat-lean tissue homogenate supporting

greater attachment than TSB and ground beef. However, the presence of skim milk and milk proteins, such as casein and lactalbumin, can significantly decrease the attachment of *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas fragi*, *Salmonella*, *L. monocytogenes*, and thermophilic bacilli to stainless steel surfaces (15, 76, 124, 180). While many previous studies were conducted in well-controlled laboratory media, it is of even greater importance to assess bacterial attachment and biofilm formation under those parameters encountered in food processing environments.

1.11 OVERALL GOALS AND OBJECTIVES

As an important step, post-harvest processing of tomatoes play a critical role in minimizing potential microbial risks and ensuring overall food safety. However, limited scientific data, such as quantitative transfer and bacteria inactivation data, is currently available for the overall risk assessment. Therefore, two major components transfer and inactivation of *Salmonella* during post-harvest processing of tomatoes, were selected as the main focus of this study. Both of the two post-harvest processing, tomato packing and fresh-cut processing (slicing and dicing), were evaluated. Specifically, chapter 2, chapter 3, and chapter 4 focused on tomato packing, with chapter 5 and chapter 6 focused on tomato slicing and dicing, respectively. In addition, a fundamental research was conducted to assess the impact of temperature, pH, and substrate on *Salmonella* attachment and early-biofilm formation on multiple surfaces, which was reported in chapter 7.

CHAPTER 2:

Microbial Cross-Contamination of Tomatoes during Washing with a Peroxyacetic Acid- Based Sanitizer in a Commercial Packinghouse

2.1 OBJECTIVE

The objective of this study was to evaluate the microbiological quality of tomatoes and dump tank water during industrial-scale processing in a commercial packinghouse.

2.2 MATERIALS AND METHODS

2.2.1 Overall experimental design

During three visits to one Michigan tomato packinghouse, various tomato, water, equipment surface and brush samples were collected during 4 h of processing. All samples were analyzed for mesophilic aerobic bacteria and yeast/mold. Water samples from the dump tank were also assessed for sanitizer concentration, oxidation/reduction potential, pH, temperature, chemical oxygen demand, and total solids. A pilot-scale tomato packing line also was used at Michigan State University to evaluate the efficacy of 50 ppm peroxyacetic acid against mesophilic aerobic bacteria and yeast/mold on unwashed tomatoes obtained from the same packer (Figure 2.1).

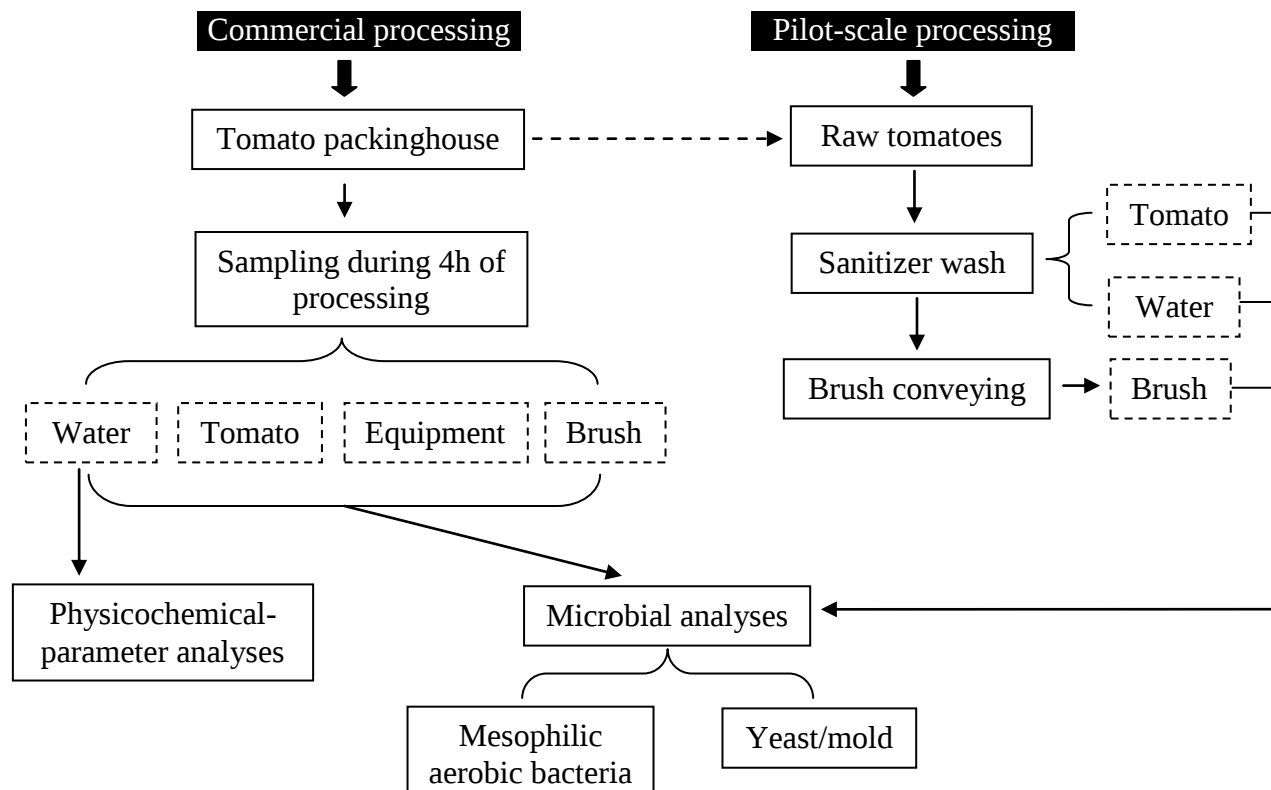


Figure 2.1: Overall experimental design of the study (chapter 2).

2.2.2 Tomato packinghouse

A small seasonal tomato packinghouse located in southwestern Michigan was selected for sampling on three separate days. At this facility, field-grown tomatoes were dumped from large bins into the dump tank, and washed in water containing approximately 50 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN) for 3 min. Thereafter, the tomatoes were conveyed by a short step-conveyor to a set of brush rollers for further cleaning and drying with the help of an over-head fan, followed by spray waxing in combination with brush rollers. Finally, the tomatoes were conveyed via a roller conveyor to the sorting table for packing (Figure 2.2).

2.2.3 Tomato samples collection

During each of three visits, four tomatoes (~900 g) were collected from three locations (tomato bin, dump tank, and after brushing/conveying) 0, 0.5, 1, 2, and 3 h after processing and placed in Whirl-Pak[®] bags (Nasco, Fort Atkinson, WI) containing 500 ml of neutralizing buffer (Difco, BD, Sparks, MD).

2.2.4 Water samples collection

Similarly, duplicate water samples (50 ml) were collected from the dump tank after 0, 0.5, 1, 2, and 3 h of processing. One set of water samples were immediately assessed on-site for physicochemical parameters after collection. The sanitizer concentration was tested using peracid/peroxide test kit (Kit #311, Ecolab, St. Paul, MN). The oxidation/reduction potential (ORP) was measured using ORPTestr 10 (Oakton, Vernon Hills, IL). The pH and temperature was measured using pHTestr 30 (Oakton, Vernon Hills, IL). Additional samples were brought back to the laboratory and analyzed for chemical oxygen demand (COD), total solids, mesophilic aerobic bacteria (MAB) and yeast/mold (YM). COD (mg O₂ per L of solution) was quantified using Hach Digestion Reactor Method 8000 (Hach, Loveland, CO) and the total solids was

measured by drying 10 ml of wash water in a pre-heated and pre-weighed crucible in a drying oven (Model 625-A, Precision Scientific Inc, Chicago, IL) set at 103 ± 2 °C for 2 h to determine the mass of solids in suspension (46).

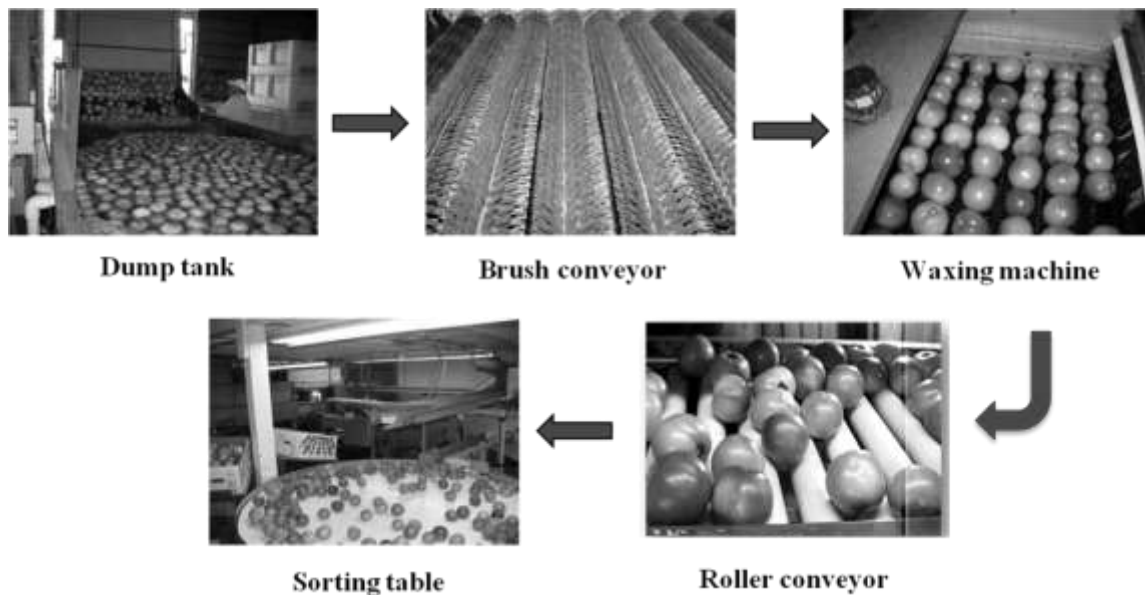


Figure 2.2: The commercial tomato packing line in a local tomato packinghouse.

2.2.5 Equipment and brush samples collection

Since the packinghouse workers took a break every two hours, equipment samples were collected at 2-h interval. After 0, 2 and 4 h of operation during each visit, 4 equipment surface (100 cm^2) and 2 brush samples (~ 220 bristles/bunch) were obtained using 1-ply composite tissues (Kimwipes[®], Kimberly-Clark Corp., Roswell, GA) and placed in Whirl-Pak[®] bags containing 15 ml of neutralizing buffer (171). The composite tissues were moistened with 1 ml of neutralizing buffer and folded twice to wipe the surface of processing equipment or the bristle of brush rollers.

2.2.6 Pilot-scale processing

Additional unwashed tomatoes (~5 kg) obtained from the same packer were transported to Michigan State University. Within 7 d, tomatoes were washed in 50 L of wash water containing 50 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN) in a plastic container for 2 min and then brush-washed using a pilot plant-scale brush roller conveyor (Figure 2.3), which was custom-made using styrene brushes in the Department of Biosystems & Agricultural Engineering at Michigan State University. Two set of tomato, water, and brush samples were collected as previously described before and after treatment, and quantitatively analyzed for MAB and YM.

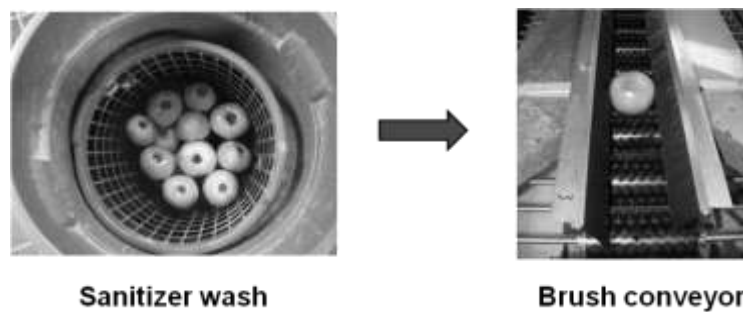


Figure 2.3: Immerse-washing container and brush conveyor for tomato processing in the pilot-scale facility at Michigan State University.

2.2.7 Microbial analyses

Tomato samples were hand-rubbed in the Whirl-pak[®] bag for 2 min, appropriately diluted, and then surface-plated on Standard Method Agar (Difco, BD) and acidified Potato Dextrose Agar (Difco, BD) with or without membrane filtration (Milipore Corporation, Billerica, MA) to enumerate MAB and YM, respectively. Water samples were appropriately diluted and surface-plated to enumerate MAB and YM, with or without membrane filtration depending on the microbial level of samples. Specifically, if the preliminary results showed that low number of *Salmonella* existed in the sample, membrane filtration was applied to quantify the population. However, if high number of *Salmonella* existed in the sample and the direct plating can quantify

the population, no membrane filtration was used. This similar procedure was applied for the following chapters. Equipment and brush samples were homogenized by stomaching (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 260 rpm and then similarly analyzed for MAB and YM. The MAB plates were incubated at 37 °C for 24 h before enumeration, whereas the YM plates were placed at room temperature for 3 d before enumeration.

2.2.8 Statistical analysis

MAB and YM populations were converted to log CFU per g, per ml, and per 100 cm² for the tomato, water, and surface samples, respectively. Analysis of variance and the Tukey-Kramer HSD test were performed using JMP 11.0 (SAS Institute Inc., Cary, NC). Statistical significance was set at $\alpha = 0.05$.

2.3 RESULTS

2.3.1 Tomato samples

As shown in Table 2.1, tomato samples collected from the dump tank and after brush roller conveying generally had lower MAB and YM populations, compared to those collected before processing. The log reductions for MAB and YM during processing were calculated as the difference between the initial and brush roller conveyed tomatoes. For trip 1, initially (at the very beginning of processing) and after 3 h of commercial operation, MAB/YM populations decreased 0.3/1.2 and 0.2/0.6 log CFU/g, respectively. For trip 2, initially and after 3 h of commercial operation, MAB/YM populations decreased 1.6/1.7 and -0.2/-0.1 log CFU/g, respectively. For trip 3, initially and after 3 h of commercial operation, MAB/YM populations decreased 1.1/1.7 and 0.5/-0.5 log CFU/g, respectively. When the same tomatoes were processed in 50 ppm of peroxyacetic acid under the pilot-scale conditions, MAB and YM populations decreased 1.2 and 0.9 log CFU/g after brush roller conveying, respectively.

2.3.2 Water samples

As shown in Figure 2.4, MAB and YM populations in the dump tank water ranged from 1.1 ± 1.0 to 2.8 ± 0.8 and 2.2 ± 0.5 to 3.2 ± 0.2 log CFU/ml, respectively (original data in Appendix A, Table A1.1). However, the populations at different sampling times were not statistically different ($P > 0.05$). No statistical differences were observed for each of the physicochemical parameters measured over time (Table 2.2). While COD and ORP values slightly decreased during 3 h of processing and the total solids slightly increased from 0.0035 ± 0.0012 to 0.0051 ± 0.001 g/10ml, no significant ($P > 0.05$) difference was observed. Although the processing facility attempted to maintain peroxyacetic acid concentration at 50 ppm, the

sanitizer levels observed during the three visits were 13 ± 3.3 , 52 ± 2.7 , and 72 ± 11.6 ppm (Table 2.3).

2.3.3 Equipment and brush samples

As shown in Figure 2.5A, MAB populations increased significantly ($P \leq 0.05$) on equipment surfaces after 2 and 4 h of operation. YM populations on the equipment surfaces ranged from 2.5 ± 0.2 to 3.6 ± 0.2 log CFU/100 cm², with the highest populations observed after 2 h of operation (original data in Appendix A, Table A1.2). While the brush samples yielded slightly higher MAB and YM populations after 2 and 4 h of processing, as shown in Figure 2.5B, no significant ($P > 0.05$) differences were observed between samples collected at different points (original data in Appendix A, Table A1.3).

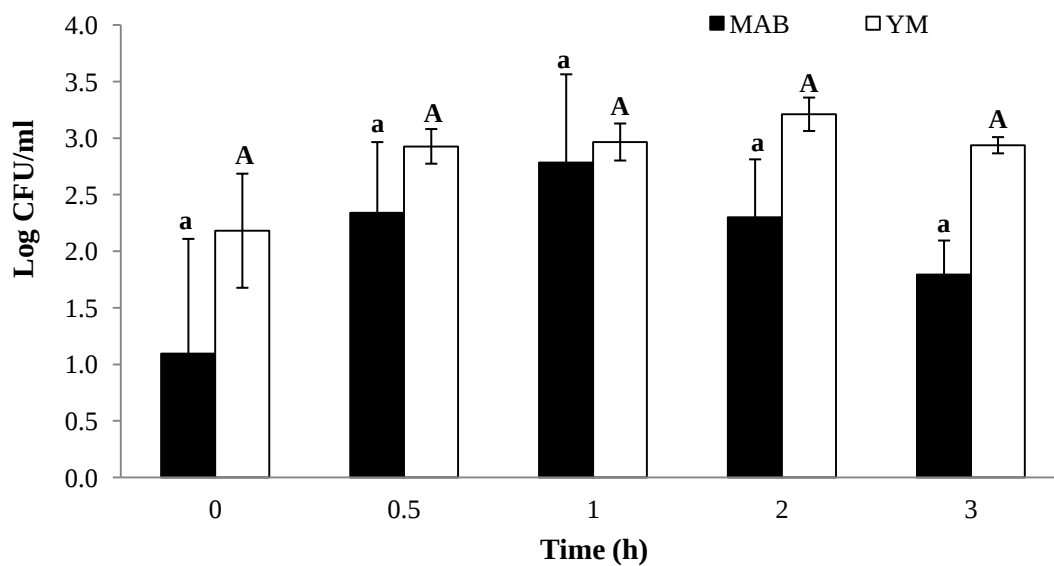


Figure 2.4: Mean ($\pm$ SE) microbial populations in the dump tank water collected after 0 (at the beginning of operation), 0.5, 1, 2, and 3h of operation in a commercial tomato packinghouse ($n = 3$). Means with the same letters for MAB (mesophilic aerobic bacteria) values are not significantly different ($P > 0.05$). Means with the same capital letters for YM (yeast/mold) values are not significantly different ($P > 0.05$).

2.3.4 Pilot-scale processing

After pilot-scale processing, MAB and YM populations on tomatoes decreased 1.2 ± 0.8 and $0.9 \pm 0.3 \log \text{CFU/g}$, respectively (Table 2.1). For the wash water, the MAB and YM populations were below the limit of detection (0.04 CFU/ml) before washing. After 2 min of washing, MAB and YM populations in the water increased 0.2 ± 0.9 and $0.9 \pm 0.3 \log \text{CFU/ml}$, respectively. Similarly, after brushing, numbers of MAB and YM populations on the brushes increased 2.4 ± 0.2 and $2.2 \pm 0.1 \log \text{CFU/bunch}$ (Table 2.4).

Table 2.1: Microbial (MAB and YM) population of tomato samples collected during 3-h processing.

Tomato samples (log CFU/g)		0HR0		HR0.5		HR1		HR2		HR3	
		MAB ^a	YM ^b	MAB	YM	MAB	YM	MAB	YM	MAB	YM
Trip 1 (13 ± 3.3 ppm)	Initial	3.8	3.6	4.2	3.7	4.3	4.7	4.9	3.0	3.6	3.1
	Dump tank	3.5	3.2	4.2	3.5	3.8	3.2	4.6	4.0	3.7	3.0
	After brushing	3.5	2.4	3.3	3.1	3.1	2.6	3.6	2.5	3.4	2.5
	Log reduction ^c	0.3	1.2	0.9	0.6	1.2	2.1	1.3	0.5	0.2	0.6
Trip 2 (52 ± 2.7 ppm)	Initial	4.4	4.1	4.5	3.6	4.1	3.3	4.2	4.2	3.9	3.7
	Dump tank	3.9	2.8	2.9	2.7	4.1	2.5	2.9	2.5	4.1	4
	After brushing	2.8	2.4	2.9	2.3	3.0	2.4	3.3	3.0	4.1	3.8
	Log reduction	1.6	1.7	1.6	1.3	1.1	0.9	0.9	1.2	-0.2	-0.1
Trip 3 (72 ± 11.6 ppm)	Initial	4.5	4.2	4.5	4.0	4.3	3.8	3.6	3.7	3.9	3.2
	Dump tank	3.9	3.0	3.9	3.5	4.6	4.1	4.1	3.5	3.5	2.7
	After brushing	3.4	2.5	3.8	2.8	3.8	2.8	3.6	3.2	3.4	3.7
	Log reduction	1.1	1.7	0.7	1.2	0.5	1.0	0.0	0.5	0.5	-0.5
Pilot-scale (50 ± 1.5 ppm)	Initial	5.4	4.8	NA ^d	NA	NA	NA	NA	NA	NA	NA
	Dump tank	4.7	4.1	NA	NA	NA	NA	NA	NA	NA	NA
	After brushing	4.2	3.9	NA	NA	NA	NA	NA	NA	NA	NA
	Log reduction	1.2	0.9	NA	NA	NA	NA	NA	NA	NA	NA

^a MAB: mesophilic aerobic bacteria.

^b YM: yeast/mold.

^c Log reduction = Initial – After brushing.

^d NA: not applicable.

Table 2.2: Mean ($\pm$ SE) physicochemical parameters of the dump tank water during 3-h processing (n = 3).

Physicochemical parameter (Ave $\pm$ SE)	Sampling point (h)				
	0	0.5	1	2	3
Temperature (°C)	16.9 $\pm$ 1.2A ^a	17.1 $\pm$ 0.8A	16.2 $\pm$ 1.1A	16.9 $\pm$ 1.2A	16.6 $\pm$ 1.4A
pH	6.1 $\pm$ 0.3A	6.2 $\pm$ 0.4A	6.2 $\pm$ 0.4A	6.3 $\pm$ 0.3A	6.5 $\pm$ 0.3A
COD (mg/L)	400 $\pm$ 78.3A	383 $\pm$ 66A	390 $\pm$ 65.5A	385 $\pm$ 47.4A	383 $\pm$ 33.5A
ORP (mV)	412 $\pm$ 24.3A	408 $\pm$ 22A	403 $\pm$ 18.6A	399 $\pm$ 24.9A	399 $\pm$ 22.7A
Total solids (g/10 ml)	0.0035 $\pm$ 0.0012A	0.0037 $\pm$ 0.0012A	0.0044 $\pm$ 0.0014A	0.0048 $\pm$ 0.0012A	0.0051 $\pm$ 0.001A

^a Means with the same letters in the same row are not significantly different ($P > 0.05$).

Table 2.3: Sanitizer concentrations (ppm) of the dump tank water during 3-h processing for three trips.

Trip	Sanitizer concentrations (ppm)					Average	SD
	0	0.5	1	2	3		
Trip 1	10	10	13	12	18	13	3.3
Trip 2	55	50	50	55	50	52	2.7
Trip 3	56	65	74	81	84	72	11.6

Table 2.4: Mean ($\pm$ SE) microbial populations for water and brush samples during pilot-scale processing (n = 3).

Sample		MAB ^a	YM ^b
Water (log CFU/ml)	Before processing	ND ^d	ND
	After processing	0.2 $\pm$ 0.9	0.9 $\pm$ 0.3
	Log increase ^c	0.2 $\pm$ 0.9	0.9 $\pm$ 0.3
Brushes (log CFU/bunch ^e)	Before processing	0.6 $\pm$ 0.1	0.1 $\pm$ 0.07
	After processing	3.0 $\pm$ 0.2	2.3 $\pm$ 0.1
	Log increase	2.4 $\pm$ 0.2	2.2 $\pm$ 0.1

^a MAB: mesophilic aerobic bacteria.

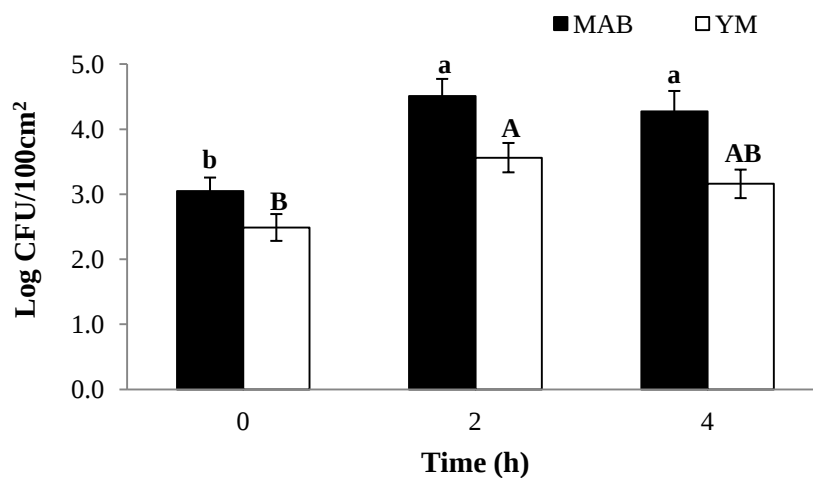
^b YM: yeast/mold.

^c Log increase = After processing – Before processing.

^d “ND”: not detected at the limit of detection of 0.04 CFU/ml.

^e “log CFU/bunch”: due to the difficulty of measuring the surface area of brushes, the whole bunch of the bristles were wiped to sample the surface microbial population.

(A)



(B)

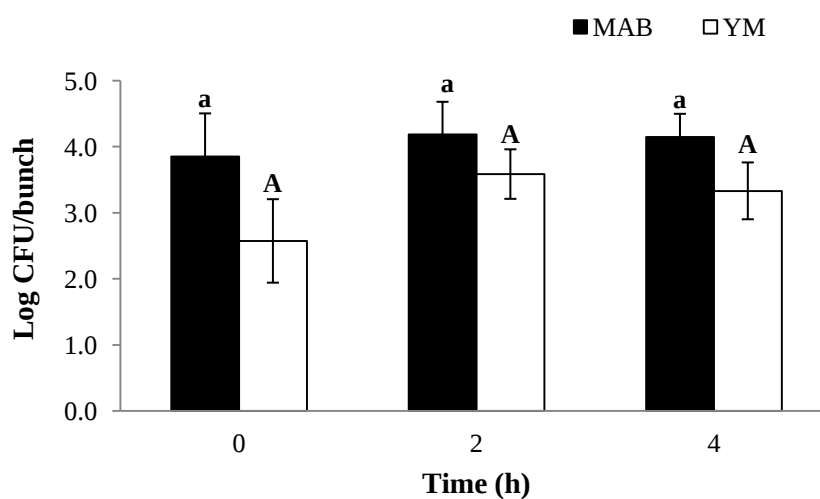


Figure 2.5: Mean ($\pm$ SE) microbial population on equipment surfaces (A) and brushes (B) after 0 (at the beginning of operation), 2, and 4h of operation in a commercial tomato packinghouse ($n = 3$). Means with the same letters on MAB (mesophilic aerobic bacteria) values are not significantly different ($P > 0.05$). Means with the same capital letters on YM (yeast/mold) values are not significantly different ($P > 0.05$).

2.4 DISCUSSION

Three trips were made to a local tomato packinghouse, which claimed to use 50 ppm peroxyacetic acid as the target level in the wash water during processing. However, the actual sanitizer levels varied significantly, ranging from 13 to 72 ppm during three separate visits. Although the dump tank was equipped with an automatic sanitizer replenishing unit, the target level of 50 ppm peroxyacetic acid was not maintained during continued processing, with the operator performing only random checks.

After dump tank washing, the tomatoes passed through a series of brush rollers for drying. In this study, multiple tomato, water, and equipment samples were collected from dump tank and other locations, with log reductions for MAB and YM calculated. Regardless of the sanitizer level, the log reductions were highly variable and were not significantly different from each other during 3 h of operation, ranging from -0.5 to 2.06 logs. This wide variation of sanitizer efficacy was also observed from a previous study that assessed the efficacy of chlorine dioxide in reducing total aerobic mesophilic bacteria and total coliforms with reductions on tomatoes ranging from -2.2 to 1.0 logs (155). More consistent results were obtained during pilot-scale processing, with average reductions of 1.2 and 0.9 logs reductions for MAB and YM, respectively. However, pilot-scale processing was only performed for 2 min without extended operation, due to the limited quantity of tomatoes obtained from the packinghouse. This scenario can be considered as the most ideal situation for dump tank washing and brush drying, since the water was free of an organic load and the brush rollers contained only low numbers of microorganisms.

In addition to tomatoes, water samples were also collected from the dump tank for both physicochemical and microbial analysis. Although an increasing trend was observed, average

microbial populations in the water samples were not statistically different due to large variations between the three visits. Although no significant difference was observed, the total solids levels, which directly relate to organic load in the wash water, slightly increased during 3 h of operation. Several previous studies using either total dissolved solids (TDS) or turbidity to reflect the dynamic change in organic matter in wash water showed similar increasing trends during extended operation times (155, 187). Based on current industry practices, ORP is still widely used as a critical control point for assessing sanitizer efficacy during washing. However, the ORP change was a poor indicator of sanitizer efficacy in the current study. Similarly, Davidson et al. (46) reported that reduced sanitizer efficacy during lettuce processing generally correlated to increases in total solids, chemical oxygen demand and turbidity, with total solids being the best predictor. However, total solid analyses are time-consuming and therefore remain impractical for the industry.

Contact between equipment surfaces and tomatoes remains critical for cross-contamination. While previous studies did not assess the microbial populations on equipment and brushes, the dynamic changes in microbial populations on surfaces from the packinghouse were assessed in this study. Initial surface populations before start-up were surprisingly high on the equipment and brushes and continually increased after processing commenced. Since there was no additional control after dump tank washing, once the equipment and brush surfaces were contaminated, they can serve to contaminate subsequent tomatoes. Better strategies need to be developed to minimize contamination. One such option is the combined use of overhead sanitizer spraying and brushing, which has proven to effectively reduce *Salmonella* on tomatoes (120). In addition, more effective equipment sanitation strategies should be implemented after processing.

In this study, only one tomato packinghouse was investigated. Despite this limitation, the findings from this study provide some first-hand information to enhance our understanding of current industry practices and also aid in the development of experimental designs for future studies. Based on the current findings, maintaining effective sanitizer levels in tomato dump tanks during washing may be a challenge at small seasonal packinghouses. More effective microbial intervention strategies are also needed to minimize cross-contamination from dump tank water and roller conveyors as the organic load increases during continued operation of the packing line.

CHAPTER 3:

Efficacy of Various Sanitizers against *Salmonella* during Simulated Commercial Processing of Tomatoes

3.1 OBJECTIVE

The objective of this study was to assess the efficacy of six sanitizer treatments against *Salmonella* during pilot-scale processing of tomatoes.

3.2 MATERIALS AND METHODS

3.2.1 Overall experimental design

Six different sanitizer treatments - (1) 40 ppm peroxyacetic acid, (2) 40 ppm mixed peracid, (3) 40 ppm available chlorine alone or acidified to pH 6.0 with (4) citric acid (CA) or (5) T-128, and (6) electrolyzed water containing 40 ppm available chlorine at pH 6.7 - were evaluated in triplicate for their efficacy against *Salmonella* during simulated commercial packing of tomatoes, with sanitizer-free water serving as the control. During and after treating 11.3 kg of *Salmonella*-inoculated red round tomatoes (~6 log CFU/g) in a dump tank for 2 min, various tomato, water, and equipment surface samples were collected and analyzed for numbers of surviving salmonellae (Figure 3.1).

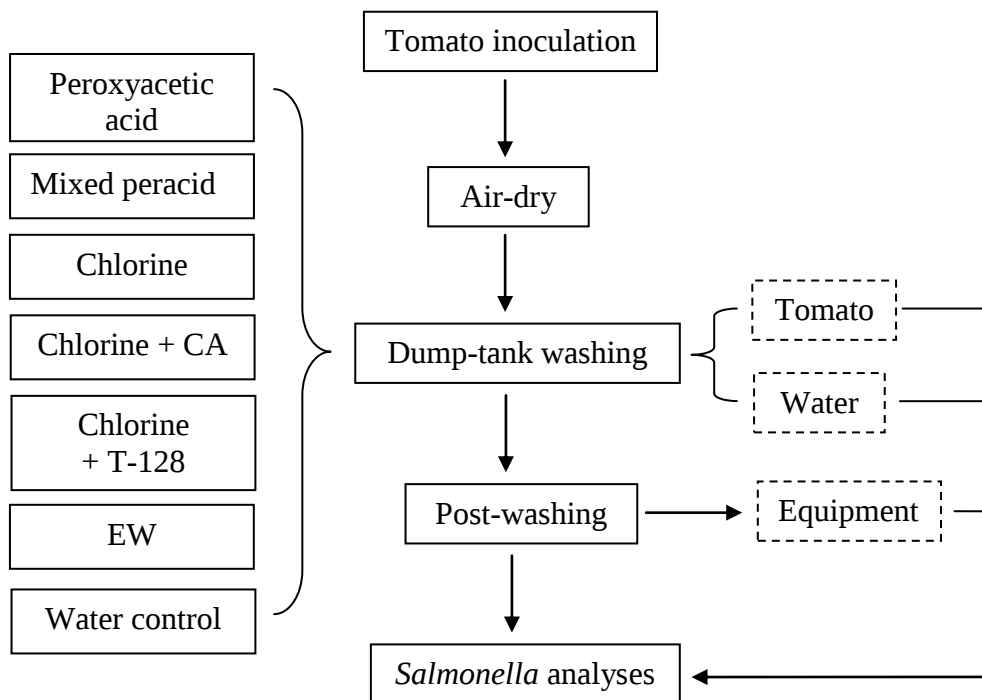


Figure 3.1: Overall experimental design of the study (chapter 3).

3.2.2 Tomatoes

Red round, unwaxed tomatoes (*Solanum lycopersium* L.) (~260 g/tomato) were obtained from a local distributor (Mastronardi Produce Ltd., Livonia, MI), stored at 4 °C, and used within 7 d of delivery.

3.2.3 *Salmonella* strains

Salmonella Typhimurium LT2 – an avirulent strain, was obtained from Dr. Michelle Danyluk (University of Florida, Gainesville, FL) and stored at -80 °C in trypticase soy broth containing 0.6% (wt/vol) yeast extract (TSBYE, BD, Sparks, MD) and 10% (v/v) glycerol. Working cultures were prepared by streaking the stock culture onto trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, BD). After 24 h of incubation at 37 °C, a single colony was subjected to two successive transfers (24 h/37 °C) in 9 ml of TSBYE with a loop of this *S. Typhimurium* LT2 culture then transferred to 2 L of TSBYE and incubated at 37 °C for 18 to 20 h before use.

3.2.4 Inoculation of tomatoes

On the day of the experiment, 11.3 kg (25 lbs) of tomatoes were divided into small batches (~1.5 kg/batch) and immersed in the *Salmonella* suspension (~10⁹ CFU/ml) for 2 min with gentle agitation to ensure uniform inoculation, followed by 2 h of air drying in a biosafety hood at ~23 °C. Thereafter, two tomatoes were randomly selected immediately before processing to determine the initial inoculation level.

3.2.5 Processing equipment

The pilot-scale commercial leafy green processing line described by Buchholz *et al.* (29) was modified for tomato processing in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory at MSU. This processing line modified for tomatoes

included a water recirculation tank (~1,000-liter capacity), dump tank (3.6 m; ~130-liter capacity; Heinzen Manufacturing Inc., Gilroy, CA), and a specially designed 1.5 m × 0.4 m plastic roller conveyor (174). The water tank containing 890 L of sanitizer solution was connected by a hard plastic discharge hose (4.5 m × 0.1 m) to the dump tank through a centrifugal pump (model XB754FHA, Sterling Electric, Inc., Irvine, CA). A custom-made stainless steel screen attached to the end of dump tank was used to retain the tomatoes for longer washing (Figure 3.2).



Figure 3.2: Pilot-scale tomato processing line: (A) water tank, (B) dump tank, (C) roller conveyor, and (D) stainless steel retention screen.

3.2.6 Sanitizer treatments

Six sanitizers were evaluated: (1) 40 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), (2) 40 ppm mixed peracid (Tsunami 200, Ecolab), (3) 40 ppm available chlorine (XY-12, Ecolab), (4) 40 ppm available chlorine from XY-12 acidified to pH of 6.0 with citric

acid (Sigma Aldrich, St. Louis, MO), (5) 40 ppm available chlorine from XY-12 acidified to pH of 6.0 with T-128 (New Leaf Food Safety Solutions, Salinas, CA), and (6) 40 ppm available chlorine at pH of 6.7 from an electrolyzed water generator (Model PathoSans, Spraying Systems Co., Glendale Heights, IL), with sanitizer-free water serving as the control. Sanitizer treatments 1, 2, and 3 were prepared by adding the appropriate volume of sanitizer to 890 L of water in the water recirculation tank. The concentrations of peroxyacetic acid/mixed peracid and chlorine were measured using a peracid/peroxide test kit (Kit #311, Ecolab, St. Paul, MN) and chlorine test kit (Kit #321, Ecolab, St. Paul, MN), respectively. For treatments 4 and 5, after adding XY-12 to achieve 40 ppm of available chlorine in the water recirculation tank, citric acid or T-128 solution was added, respectively, to adjust the pH to 6.0. For treatment 6, the appropriate volume of electrolyzed water from the anode hose of the generator (chlorine level of 80 ppm at pH of 2.5) was added to the water recirculation tank and diluted with fresh tap water to obtain a wash solution containing 40 ppm of available chlorine at pH 6.7. The chlorine level was tested as described for treatment 3. The pH values were confirmed with a pH meter (Oakton Waterproof pHTestr 30, Vernon Hills, IL). The temperature of the all wash solution was also measured for each treatment.

3.2.7 Tomato processing and sample collection

Inoculated red round tomatoes (~11.3 kg) were manually dumped into the dump tank and washed for 2 min. The wash solution was centrifugally pumped (model XB754FHA, Sterling Electric, Inc., Irvine, CA) into the dump tank at a flow rate of ~15 L/s. After washing, the tomatoes were released from the dump tank, roller conveyed, and collected in a plastic basket. Two tomatoes (~500 g) and individual water samples (400 ml) were collected at 15-s intervals during 2-min washing and immediately added to Whirl-Pak[®] bags (Nasco, Fort Atkinson, WI)

containing 200 ml of sterile Difco Neutralizing Buffer (BD, Franklin Lakes, NJ) and 10 ml of 38× concentrated Neutralizing Buffer, respectively. Another tomato sample was collected from the plastic basket. After processing and draining the system, ten dump tank (D1 – D10; 100 cm²/sample), four water tank (W1 – W4; 100 cm²/sample), and six roller conveyor (R1 – R6; 350 cm²/sample) surface samples were collected as shown in Figure 3.3 using one-ply composite tissues moistened with 1 ml of sterile Difco Neutralizing Buffer (171). Preliminary testing showed that this volume of Difco Neutralizing Buffer was sufficient to neutralize any residual sanitizer remaining in the tomato, water, and equipment surface samples.

3.2.8 Microbiological analyses

All tomato samples were hand-rubbed in Whirl-Pak[®] bags for 2 min, appropriately diluted in sterile 1% (w/v) phosphate buffer (8.5 g/L NaCl, 1.44 g/L Na₂HPO₄, and 0.24 g/L KH₂PO₄, J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ), and then surface-plated on trypticase soy agar (BD) containing 0.6% yeast extract (BD), 0.05% ferric ammonium citrate (Sigma) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS) with or without 0.45 µm-membrane filtration (Milipore Corporation, Billerica, MA) to quantify *Salmonella* (limit of detection: 8 CFU/g). Similarly, the water samples were either appropriately diluted in phosphate buffer and plated on TSAYE-FS or filtered through a 0.45 µm-membrane to quantify *Salmonella* (limit of detection: 0.0025 CFU/ml). Equipment surface samples (tissues) were homogenized by stomaching (Stomacher 400 Circulator, Seward, Worthington, UK) in 15 ml of Difco Neutralizing Buffer for 1 min at 260 rpm and then similarly analyzed for salmonellae (limit of detection: 1.5 CFU/100 cm² for the dump tank and water tank; 0.43 CFU/100 cm² for roller conveyor). Plates were incubated at 37 °C for 24 h, after which all black colonies were

counted as *S. Typhimurium* LT2. Selected colonies were confirmed as *Salmonella* using the Neogen Reveal[®] 2.0 kit (Neogen Corporation, Lansing, MI).

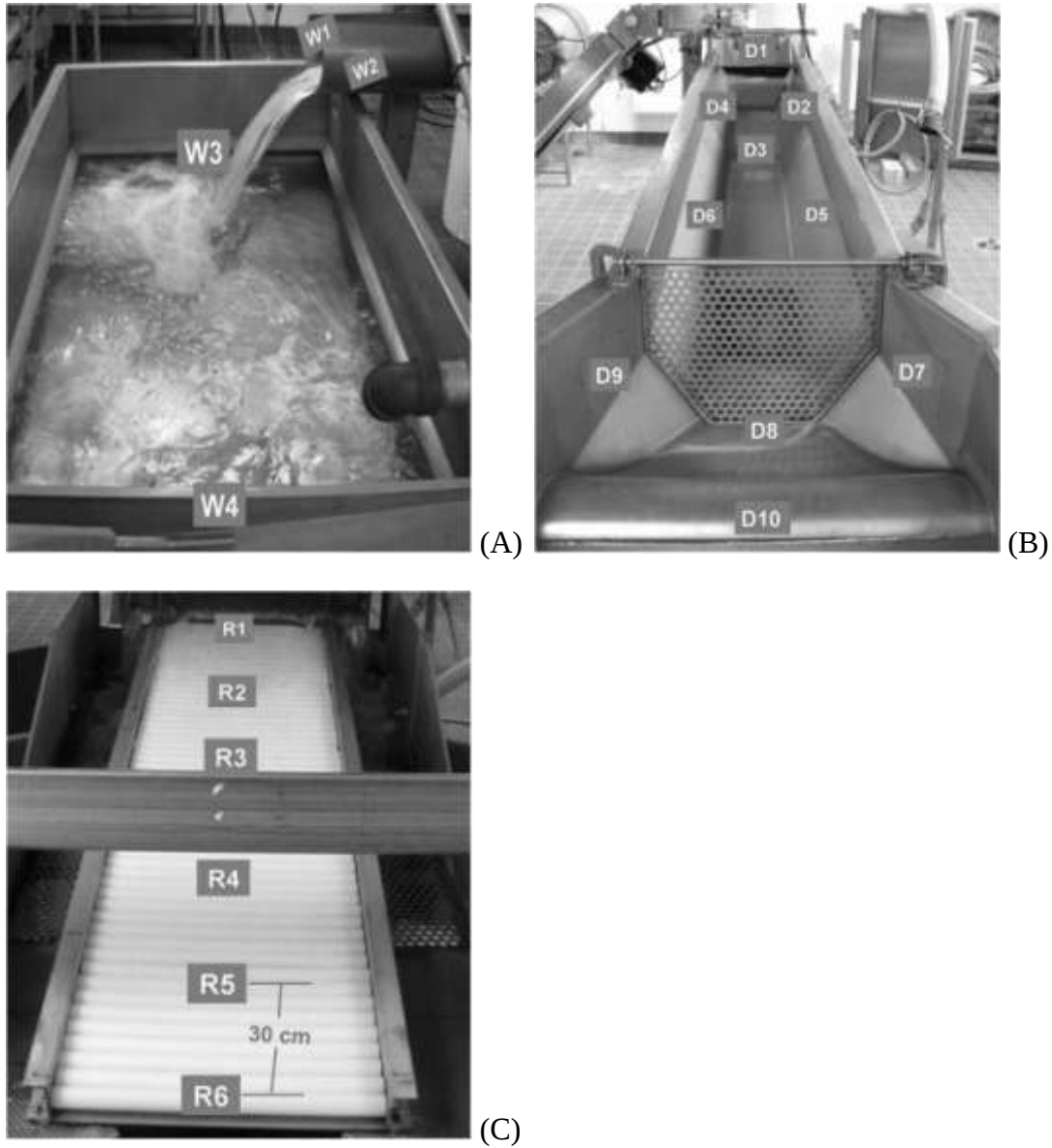


Figure 3.3: Equipment surface sampling locations for: (A) water tank (W1 – W4), (B) dump tank (D1 – D10), (C) roller conveyor (R1 – R6).

3.2.9 Statistical analysis

All experiments were performed in triplicate. *Salmonella* populations were converted to log CFU per gram, per ml, or per 100 cm² for tomato, water, and equipment surface samples, respectively. Analysis of variance and the Tukey-Kramer HSD test were performed using JMP 10.0 (SAS Institute Inc., Cary, NC). Statistical significance was set at $\alpha = 0.05$.

3.3 RESULTS

3.3.1 Tomatoes

Salmonella populations on the inoculated tomatoes before processing were statistically similar for the water control and six sanitizer treatments ($P > 0.05$), ranging from 5.3 ± 0.1 to 5.8 ± 0.2 log CFU/g (Table 3.1). After contacting the wash water in the dump tank, *Salmonella* populations were significantly lower on the tomatoes ($P \leq 0.05$). However, washing and subsequent roller drying, no significant differences in numbers of salmonellae for samples collected at different time-points were observed between any of the treatments except chlorine + CA ($P > 0.05$). All six sanitizer treatments were more effective ($P \leq 0.05$) in reducing *Salmonella* than the water control (1.2 ± 0.0 log CFU/g), with chlorine + CA yielding a significantly greater reduction for *Salmonella* on tomatoes (3.1 ± 0.1 log CFU/g) as compared to electrolyzed water (2.1 ± 0.4 log CFU/g), chlorine (2.1 ± 0.3 log CFU/g), and chlorine + T-128 (2.0 ± 0.2 log CFU/g). Mixed peracid and peroxyacetic acid yielded similar results ($P > 0.05$) with *Salmonella* reductions of 2.5 ± 0.4 and 2.5 ± 0.1 log CFU/g, respectively (Table 3.1).

3.3.2 Water sample

Except for peroxyacetic acid at 15 s, the other five sanitizer treatments all yielded significantly lower ($P \leq 0.05$) *Salmonella* populations than the water control (Table 3.2). After 2 min of washing, all six sanitizer treatments yielded significantly lower ($P \leq 0.05$) numbers of salmonellae compared to water alone (3.0 ± 0.3 log CFU/ml). Across all treatments, *Salmonella* populations were highest after 15 s and decreased thereafter. Using water, peroxyacetic acid, chlorine, electrolyzed water, and chlorine + T-128, significantly lower levels ($P \leq 0.05$) of *Salmonella* were recovered after 30 to 120 s, compared to after 15 s. However, no significant difference in *Salmonella* populations ($P > 0.05$) was observed for chlorine + CA at the eight time

points, with *Salmonella* levels ranging from -0.9 ± 0.8 to -2.5 ± 0.2 log CFU/ml (Table 3.2).

Since only 2-min washing was conducted, the sanitizer concentrations after washing was not significantly changed from the initial concentration (Table 3.3).

Table 3.1: Mean ($\pm$ SD) *Salmonella* populations (log CFU/g) on tomatoes collected during 2-min (at 15 s interval) washing with 6 sanitizer treatments in dump-tank and after processing (n = 3).

Time (s)	Water	Chlorine + T-128	Chlorine	EW	Peroxyacetic Acid	Mixed Peracid	Chlorine + CA
0	a ^x 5.6 $\pm$ 0.1 A	a 5.8 $\pm$ 0.2 A	a 5.7 $\pm$ 0.3 A	a 5.4 $\pm$ 0.1 A	a 5.7 $\pm$ 0.1 A	a 5.6 $\pm$ 0.2 A	a 5.3 $\pm$ 0.1 A
15	b 4.5 $\pm$ 0.2	b 4.1 $\pm$ 0.2	b 3.8 $\pm$ 0.4	b 3.8 $\pm$ 0.3	b 4.2 $\pm$ 0.0	b 3.7 $\pm$ 0.3	b 3.6 $\pm$ 0.4
30	b 4.4 $\pm$ 0.3	b 3.7 $\pm$ 0.8	b 3.0 $\pm$ 0.2	b 3.8 $\pm$ 0.4	b 3.9 $\pm$ 0.2	b 3.3 $\pm$ 0.4	bc 3.3 $\pm$ 0.3
45	b 4.5 $\pm$ 0.2	b 3.7 $\pm$ 0.4	b 3.0 $\pm$ 0.4	b 3.7 $\pm$ 0.5	b 3.9 $\pm$ 0.5	b 3.2 $\pm$ 0.4	bcd 2.7 $\pm$ 0.3
60	b 4.5 $\pm$ 0.2	b 3.9 $\pm$ 0.3	b 3.9 $\pm$ 0.4	b 3.8 $\pm$ 0.2	b 3.9 $\pm$ 0.2	b 3.3 $\pm$ 0.4	bcd 3.3 $\pm$ 0.8
75	b 4.4 $\pm$ 0.3	b 3.9 $\pm$ 0.1	b 3.8 $\pm$ 0.4	b 3.8 $\pm$ 0.5	b 3.1 $\pm$ 0.0	b 3.7 $\pm$ 0.2	bcd 2.8 $\pm$ 0.4
90	b 4.5 $\pm$ 0.2	b 3.5 $\pm$ 0.2	b 3.5 $\pm$ 0.8	b 3.8 $\pm$ 0.3	b 3.7 $\pm$ 0.1	b 3.3 $\pm$ 0.2	bcd 3.0 $\pm$ 0.5
105	b 4.2 $\pm$ 0.1	b 3.9 $\pm$ 0.2	b 3.8 $\pm$ 0.5	b 3.5 $\pm$ 0.3	b 3.6 $\pm$ 0.8	b 3.3 $\pm$ 0.5	bcd 2.7 $\pm$ 0.6
120	b 4.4 $\pm$ 0.3	b 3.6 $\pm$ 0.4	b 3.7 $\pm$ 0.8	b 3.6 $\pm$ 0.4	b 3.5 $\pm$ 0.5	b 2.8 $\pm$ 0.5	d 2.1 $\pm$ 0.3
After process	b 4.4 $\pm$ 0.1	b 3.8 $\pm$ 0.1	b 3.6 $\pm$ 0.6	b 3.3 $\pm$ 0.4	b 3.2 $\pm$ 0.3	b 3.1 $\pm$ 0.2	cd 2.2 $\pm$ 0.2
Log reduction ^z	1.2 $\pm$ 0.0 C ^y	2.0 $\pm$ 0.2 B	2.1 $\pm$ 0.3 B	2.1 $\pm$ 0.4 B	2.5 $\pm$ 0.1 AB	2.5 $\pm$ 0.4 AB	3.1 $\pm$ 0.1 A

^xMeans with the same letters in the same column are not significantly different ($P > 0.05$).

^yMeans with the same capital letters in the same row are not significantly different ($P > 0.05$).

^zLog reduction = *Salmonella* population at “0” – *Salmonella* population “After process”.

Table 3.2: Mean ($\pm$ SD) *Salmonella* populations (log CFU/ml) in flume water during 2-min (at 15 s interval) washing of 11.3 kg tomatoes inoculated at ~ 6 log CFU/g (n=3).

Time (s)	Water	Peroxyacetic Acid	Mixed Peracid	Chlorine	EW	Chlorine + CA	Chlorine + T-128
15	a ^x 4.1 $\pm$ 0.4 A ^y	a 4.0 $\pm$ 0.1 A	a 1.7 $\pm$ 0.2 B	a 0.5 $\pm$ 0.2 C	a -0.8 $\pm$ 0.5 D	a -0.9 $\pm$ 0.8 D	a -0.4 $\pm$ 0.2 CD
30	b 2.8 $\pm$ 0.3 A	b 1.9 $\pm$ 0.1 B	ab 1.2 $\pm$ 0.3 B	b -1.0 $\pm$ 0.6 C	bc -2.2 $\pm$ 0.2 D	a -2.3 $\pm$ 0.2 D	b -2.3 $\pm$ 0.2 D
45	b 2.5 $\pm$ 0.3 A	c 1.4 $\pm$ 0.3 B	abc 1.0 $\pm$ 0.1 B	b -1.8 $\pm$ 0.3 C	bc -2.2 $\pm$ 0.2 C	a -2.1 $\pm$ 0.6 C	b -2.5 $\pm$ 0.2 C
60	b 3.1 $\pm$ 0.3 A	cd 1.1 $\pm$ 0.1 B	bcd 0.7 $\pm$ 0.2 B	b -1.3 $\pm$ 0.4 C	b -1.5 $\pm$ 0.2 C	a -1.8 $\pm$ 0.5 C	b -2.0 $\pm$ 0.5 C
75	b 3.1 $\pm$ 0.3 A	de 1.0 $\pm$ 0.1 B	bcd 0.5 $\pm$ 0.2 B	b -1.2 $\pm$ 0.3 C	bc -2.1 $\pm$ 0.2 CD	a -2.2 $\pm$ 0.7 D	b -1.8 $\pm$ 0.1 CD
90	b 3.0 $\pm$ 0.4 A	de 0.8 $\pm$ 0.1 B	cd 0.4 $\pm$ 0.4 B	b -1.4 $\pm$ 0.4 C	bc -2.1 $\pm$ 0.3 C	a -2.5 $\pm$ 0.2 C	b -2.1 $\pm$ 0.7 C
105	b 3.0 $\pm$ 0.3 A	de 0.8 $\pm$ 0.0 B	cd 0.3 $\pm$ 0.3 B	b -1.5 $\pm$ 0.2 C	c -2.3 $\pm$ 0.0 C	a -1.7 $\pm$ 0.8 C	b -1.9 $\pm$ 0.5 C
120	b 3.0 $\pm$ 0.3 A	e 0.6 $\pm$ 0.2 B	d 0.1 $\pm$ 0.0 B	b -1.7 $\pm$ 0.3 C	bc -2.1 $\pm$ 0.3 C	a -2.2 $\pm$ 0.4 C	b -2.3 $\pm$ 0.4 C

^xMeans with the same letters in the same column are not significantly different ($P > 0.05$)

^yMeans with the same capital letters in the same row are not significantly different ($P > 0.05$)

^zLimit of detection (LOD): -2.6 log CFU/ml

Table 3.3: Mean ($\pm$ SD) concentration (ppm) change before and after 2-min washing for 6 sanitizer treatments (n=3).

Treatment	Sanitizer concentration (ppm)					
	Peroxyacetic Acid	Mixed Peracid	Chlorine	EW	Chlorine + CA	Chlorine + T-128
Before washing	40 $\pm$ 0.5 A ^x	40.5 $\pm$ 1.0 A	40 $\pm$ 0.0 A	40.7 $\pm$ 0.6 A	40 $\pm$ 0.0 A	40 $\pm$ 0.0 A
Post washing	38 $\pm$ 0.8 A	37 $\pm$ 1.0 A	35.5 $\pm$ 0.7 A	39.3 $\pm$ 1.2 A	39.3 $\pm$ 0.8A	38 $\pm$ 1.0 A

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

3.3.3 Equipment surface sample

After 2 min of washing in water alone, *Salmonella* was recovered from all 20 surface samples, with populations ranging from 1.0 ± 0.8 to 3.2 ± 0.6 log CFU/100 cm². For the dump tank, *Salmonella* populations tended to be higher in the four surface samples (D1, D3, D8, and D10) from the bottom as opposed to the six samples collected from the side walls. However, all four sampling locations of the water recirculation tank yielded similar numbers of salmonellae ($P > 0.05$), with populations ranging from 1.2 ± 0.6 to 2.2 ± 0.6 log CFU/100 cm². Similarly, no significant difference in numbers of *Salmonella* were observed for the six roller conveyor surfaces ($P > 0.05$), with populations ranging from 1.4 ± 0.4 to 2.9 ± 0.3 log CFU/100 cm² (Figure 3.4) (original data in Appendix B, Table B1.1). However, compared to the water control, all six sanitizer treatments successfully reduced *Salmonella* on equipment surfaces to levels below the limit of detection (0.2 log CFU/100 cm²).

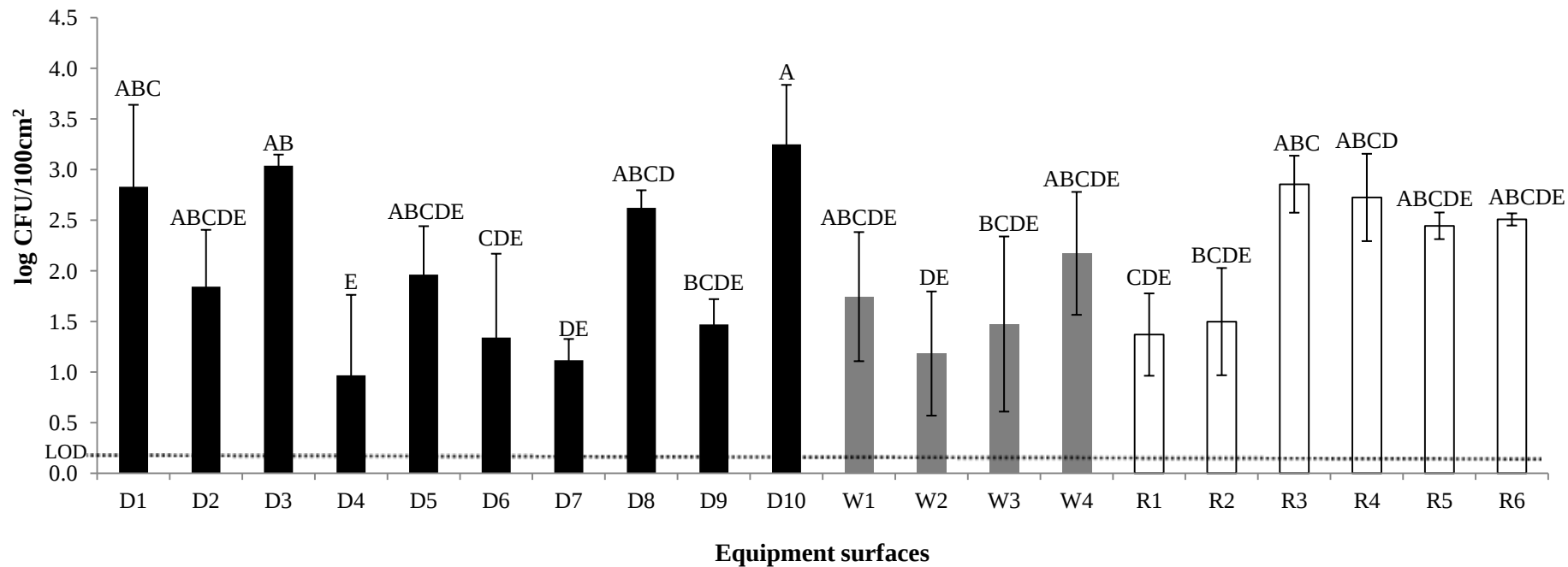


Figure 3.4: Mean ($\pm$ SD) *Salmonella* populations (log CFU/100cm²) on equipment surfaces (Dump tank: D1 to D10; Water tank: W1 to W4; Roller conveyor: R1 to R6) after washing 11.3 kg of inoculated tomatoes (~ 6 log CFU/g) with water alone ($n=3$). LOD (limit of detection): 0.2 log CFU/100cm². Means with the same letters are not significantly different ($P > 0.05$).

3.4 DISCUSSION

Since this study was conducted in a non-biosafety pilot-plant environment, an avirulent strain of *Salmonella* (*S. Typhimurium* LT2) with deficient *rpoS* gene was needed to eliminate the potential production of infectious aerosols during processing. However, based on our previous work, this avirulent strain behaved similarly to several virulent *Salmonella* strains in terms of growth in laboratory media, attachment in the microtiter plate assay, survival on tomatoes, and sensitivity to chlorine and peroxyacetic acid (174).

Dump tank washing has remained a critical step in tomato packinghouses for the removal of soil and microbial contaminants. Multiple studies have shown that immersing warm tomatoes in cold water can lead to internalization of bacteria (18, 49, 188). The FDA has long recommended that the water temperature be maintained at least 5.5 °C (10 °F) above the tomato pulp temperature to minimize internalization (157). In packinghouses, field-harvested tomatoes are typically held in a cooling room to decrease the pulp temperature before washing. However, the water can also be heated as an alternative. Although no specific cooling step was used in the present study, the water and tomato temperatures were 17 ± 1.5 and 22 ± 0.5 °C, respectively, which negated the risk of internalization during washing (181).

In the absence of a sanitizer, washing fresh produce in water alone typically reduces microbial populations only ~ 1 log, as reported in various bench-top (70, 113, 136) and pilot-scale studies (28, 45). In this work, a 1.2 log CFU/g reduction in *Salmonella* was observed on tomatoes after washing in water alone for 2 min, with *Salmonella* populations increasing to ~ 3 log CFU/ml in the wash water during washing. Therefore, chemical sanitizers are normally added to the wash water, with their concentration frequently monitored to minimize cross-contamination from the water during continued processing (173). When the production line was

drained after processing, a layer of residual water that remained on the equipment surface contained the *Salmonella* population recovered by sampling. Some locations on various pieces of equipment harbored significantly higher numbers of salmonellae, which may eventually lead to the re-design of the equipment for enhanced sanitization, such as selection of more hydrophobic material and spray sanitation at specific locations. Maintaining an appropriate level of sanitizer in the wash water is also crucial for minimizing *Salmonella* contamination on equipment surfaces.

Chlorine- and peroxyacetic acid-based sanitizers have been most widely used for commercial dump tank and flume washing of fresh produce, including tomatoes. Based on our experience with an area tomato packer (173), an active ingredient concentration of 40 ppm was chosen for each sanitizer treatment to assess efficacy. Compared to the water control, all six sanitizer treatments showed significantly better efficacy in reducing *Salmonella* on tomatoes ($P \leq 0.05$), with chlorine + CA being the only treatment to exceed a 3 log reduction. When Felkey *et al.* (56) assessed the efficacy of a chlorine wash against *Salmonella* at different sites on tomatoes, a 2 min exposure to 150 ppm free chlorine at pH 6.5 achieved a 6.4 and 1.9 log reduction on the intact surface and stem scar regions, respectively. In our study, tomatoes were exposed to a lower level of chlorine (40 ppm), with the log reductions for *Salmonella* quantified on the whole tomato, as opposed to quantifying the *Salmonella* reduction on stem scar and intact surface area separately. Overall, *Salmonella* populations decreased 3.1 logs on intact whole tomatoes using chlorine + CA.

Compared to chlorine + CA, the other three chlorine-based sanitizer treatments (EW, chlorine, and chlorine + T-128) yielded significantly lower log reductions for *Salmonella* on tomatoes. Previous studies also showed that T-128 did not enhance the efficacy of chlorinated

wash solutions against microbial pathogens on iceberg lettuce (95, 112). When Park *et al.* (123) used electrolyzed water containing 37.5 ppm available chlorine at pH of 2.06 to treat grape tomatoes inoculated with *E. coli* O157:H7, *S. Typhimurium*, or *L. monocytogenes*, all three pathogens decreased > 5 log CFU/g after a 1 min treatment. This difference could originate from the lower pH (the pH of the electrolyzed water is 6.7 vs pH of 2.06), means of inoculation (e.g., dip-inoculation vs spot-inoculation) and or method of exposure (e.g., immersion vs spray). Although inexpensive to produce, the relatively slow rate of generation for electrolyzed water may limit its usefulness for large-scale processors.

The efficacy of peroxyacetic acid-based sanitizers against foodborne pathogens has been investigated under laboratory conditions on multiple types of produce, including alfalfa seeds (26), lettuce (114), tomatoes (38), and mung bean sprouts (110). In this study, both peroxyacetic acid and mixed peracid decreased *Salmonella* ~ 2.5 log on tomatoes. In a previous study evaluating an overhead sanitizer-spray and brush roller system, a 60 s spray treatment with 80 ppm peroxyacetic acid decreased *Salmonella* populations > 5 log on tomatoes (38). Although this overhead sanitizer-spray system provides several benefits over dump tank washing, potential brush roller damage to the final product along with treatment consistency should be further evaluated before implementation in tomato packinghouses.

In this study, the four chlorine-based sanitizer treatments showed significantly greater ($P \leq 0.05$) efficacy against *Salmonella* in the wash water as compared to the peroxyacetic/peracid-based treatments and water control. Another study from our laboratory showed similar efficacy of several chlorine-based sanitizer treatments against *E. coli* O157:H7 in leafy green wash water without any appreciable organic load (45). However, the efficacy of chlorine-based sanitizers is sharply decreased in the presence of soil, debris, and leached organic material from produce that

can accumulate in the wash water during extended processing (6, 65, 94, 185). Therefore, the results from this study represent the “best-case” scenario in terms of pathogen control in wash water and on tomatoes and equipment surfaces. Future studies incorporating various types and levels of organic material in the wash water are also needed to simulate the range in water quality encountered during tomato packing.

CHAPTER 4:

***Salmonella* Transfer during Pilot-Plant Scale Washing and Roller Conveying of Tomatoes**

4.1 OBJECTIVE

The objectives of this study were to 1) assess the impact of roller type on *Salmonella* transfer during conveyance of tomatoes using a pilot-scale packing line and 2) evaluate the effect of sanitizer application on *Salmonella* cross-contamination.

4.2 MATERIALS AND METHODS

4.2.1 Overall experimental design

Red round tomatoes inoculated with *Salmonella* Typhimurium LT2 (avirulent) at 4 log CFU/g were washed and then conveyed across a roller conveyor (plastic, foam or brush roller) to contaminate the roller surface. Then, 25 uninoculated tomatoes were passed over the contaminated roller conveyor to assess subsequent *Salmonella* transfer. The impact of adding various chemical sanitizers to the dump tank water on *Salmonella* transfer was also evaluated. All experiments were performed in triplicate (Figure 4.1).

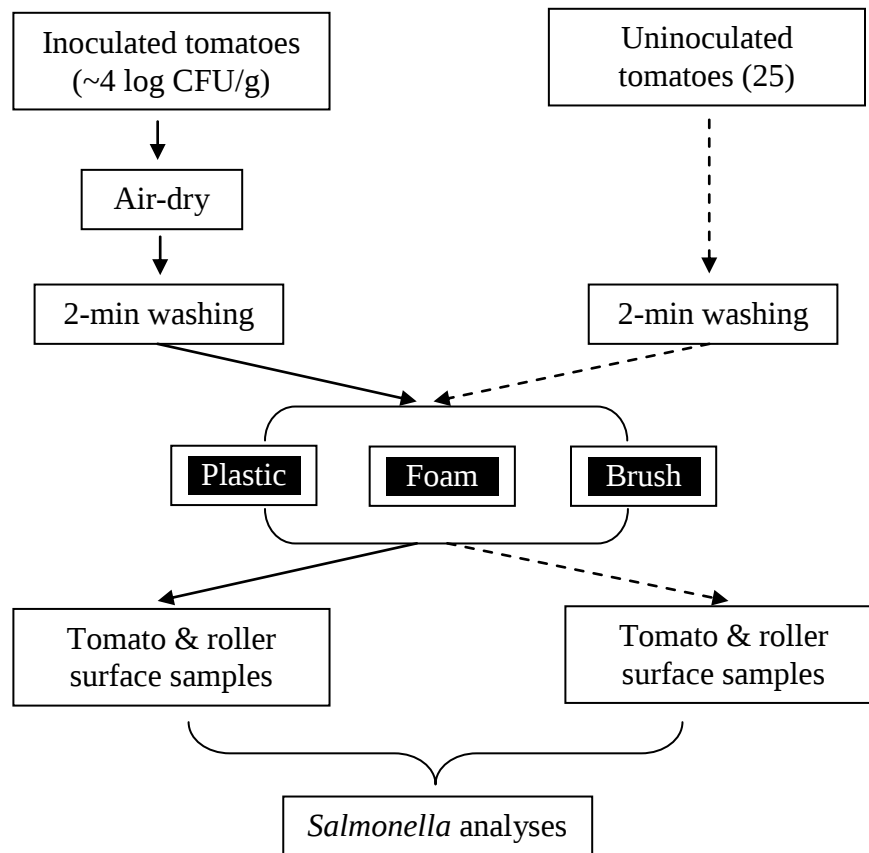


Figure 4.1: Overall experimental design of the study (chapter 4).

4.2.2 Tomatoes

The same tomatoes as described in 3.3.2 were used for this study.

4.2.3 *Salmonella* strains

Salmonella Typhimurium LT2 – an avirulent strain obtained from Dr. Michelle Danyluk (University of Florida, Gainesville, FL) was used for all roller conveyor work. In preliminary work, the growth, attachment, and survival characteristics of this avirulent strain were compared to three virulent strains - *Salmonella* Montevideo MDD22 (tomato outbreak, human isolate), *Salmonella* Poona MDD237 (cantaloupe outbreak, human isolate), and *Salmonella* Newport MDD314 (tomato outbreak, environmental isolate) (Dr. Lawrence Goodridge, Colorado State University, Fort Collins, CO). All strains were stored at -80 °C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. Working cultures were prepared by streaking the stock culture onto trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, Becton, Dickinson and Company). After incubation at 37 °C for 24 h, a single colony was subjected to two successive transfers (24 h/37 °C) in 9 ml of TSBYE before use.

4.2.4 *Salmonella* growth, attachment, survival, and sanitizer sensitivity

Growth of *Salmonella* was assessed in flasks containing 100 ml of TSBYE that were separately inoculated with serial dilutions of each of the four overnight *Salmonella* cultures (3 flasks/culture) so as to contain $\sim 10^2$ CFU/ml. One ml aliquots taken initially and after 2, 4, 6, 8, 10, 12, 14, and 24 h of incubation without shaking at 37°C were appropriately diluted in sterile phosphate buffer (8.5 g/liter NaCl, 1.44 g/liter Na₂HPO₄, and 0.24 g/liter KH₂PO₄), and plated on TSAYE containing 0.05% ferric ammonium citrate (Sigma Chemical Co., St. Louis, MO) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS). After

incubation at 37 °C for 24 h, white colonies with black centers were counted as *Salmonella* with this same medium used later for all roller conveyor experiments. The generation times for *Salmonella* growth were calculated by comparing the time in minutes to the log CFU/ml, based on the population increase from 0 to 12 h.

A modified microtiter plate assay was conducted in triplicate to assess attachment of the four aforementioned *Salmonella* strains (20). Each strain was serially diluted to $\sim 10^2$ CFU/ml in TSBYE (Becton, Dickinson and Company). After vortexing, 200 μ l of the diluted cell suspension was added to triplicate wells of a 96-well untreated polystyrene microtiter tissue culture plate (Flat Bottom BD Falcon, Franklin Lakes, NJ). Three wells per plate containing 200 μ l of sterile TSBYE served as negative controls. After 4 d of incubation at 23 °C, the microtiter plate wells were emptied, rinsed three times with sterile phosphate buffer to remove unattached cells, and then allowed to air-dry. The remaining cells were fixed to the well by adding 200 μ l of 99% methanol (Fisher Chemicals, Fair Lawn, NJ) with the methanol decanted after 15 min. After air drying and adding 200 μ l of 2% crystal violet (Remel, Lenexa, KS), the wells were thoroughly rinsed 5 min later with sterile deionized water and air-dried. The remaining dye was solubilized in 160 μ l of 33% (v/v) glacial acetic acid (Sigma Chemical Company), with optical densities (OD) read at 570 nm using a Synergy HT-I Microplate Reader (Bio-Tek Instruments Inc. Winooski, VT).

Salmonella survival was assessed on red round tomatoes that were dip-inoculated in triplicate with *S. Typhimurium* LT2 (avirulent) or a 3-strain cocktail of *S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314 to contain ~ 5 log CFU/g. Tomatoes were collected and quantitatively analyzed for salmonellae after 2, 24, 48, 72, 96, 120, and 144 h of storage at 25 °C.

Sensitivity of avirulent *S. Typhimurium* LT2 and the virulent 3-strain *Salmonella* cocktail to peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN) and chlorine (Ecolab) was also assessed. Briefly, 1 ml of an overnight TSBYE culture of *S. Typhimurium* LT2 or the 3-strain cocktail containing ~ 9 log CFU/ml was added to 30 ml of water containing either 60 ppm peroxyacetic acid or 50 ppm of free chlorine. After 1 min of exposure, 1 ml of sanitizer solution was collected and added to tubes containing 9 ml of concentrated neutralizing buffer (Difco, BD). *Salmonella* populations pre- and post-treatment were determined as described below with the log reductions then calculated.

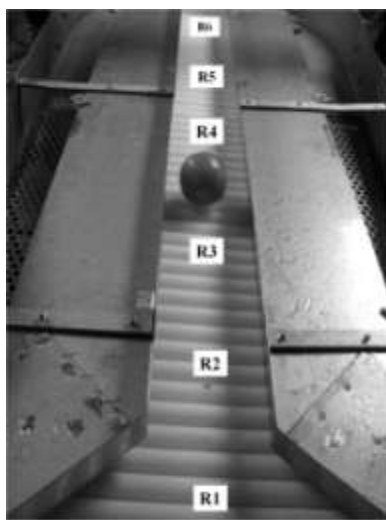
4.2.5 Inoculation of tomatoes

The same inoculation procedure as described in 3.3.4 was used for this study.

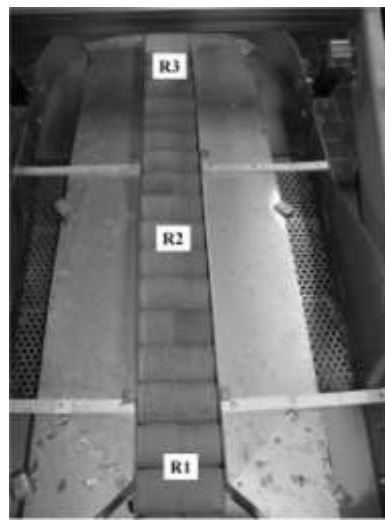
4.2.6 Processing equipment

A pilot-scale commercial leafy green processing line as described by Buchholz et al. (29) was modified for tomato processing in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory (Michigan State University, East Lansing, MI). This line included a water tank (~1,000-liter capacity), dump tank (3.6 m; ~130-liter capacity; Heinzen Manufacturing Inc., Gilroy, CA), and a specially designed 1.5 m × 0.4 m roller conveyor. The water tank containing 890 L of tap water (~15 °C) with or without sanitizer was connected by a hard plastic discharge hose (4.5 m × 0.1 m) to the dump tank through a centrifugal pump (model XB754FHA, Sterling Electric, Inc., Irvine, CA). As shown in Figure 3.2, a custom-made stainless steel screen was attached to the end of dump tank to retain the tomatoes for longer washing. Three types of roller conveyors were designed by the Department of Biosystems & Agricultural Engineering (Michigan State University) to contain plastic (53 rollers; polyethylene; Alro Plastics, Jackson, MI), foam (19 rollers; Latex; Filtrona Porous

Technologies, Colonial Heights, VA), or brush (26 rollers; Styrene; TEW Manufacturing Corp., Penfield, NY) rollers. In order for single file conveying of tomatoes, the roller conveyors were narrowed to a width of 13.5 cm by attaching two stainless steel barriers on either side (Figure 4.2). Due to different size of roller conveyor, the plastic and foam roller conveyors were connected immediately after the dump tank, while the motorized brush roller conveyor was free-standing and operated at a rotation speed of 100 rpm.



(A)



(B)



(C)

Figure 4.2: Three types of roller conveyor: (A) plastic, (B) foam, and (C) brush roller conveyor.

4.2.7 Tomato processing and sample collection

Ten inoculated red round tomatoes (~2.5 kg) were manually dumped into the dump tank and washed for 2 min in sanitizer-free water. After washing, these tomatoes were collected in a plastic basket and conveyed single file across the roller conveyor, followed by 25 uninoculated tomatoes that previously-washed in sanitizer-free water. Two previously inoculated tomatoes (~500 g) were collected after 2 min of washing and after roller conveying, with each of the 25 uninoculated tomatoes collected individually in Whirl-Pak[®] bags (Nasco, Fort Atkinson, WI) after conveying. Based on the roller configuration for the different conveyors, 6 plastic (R1 – R6; 118 cm²/sample), 3 foam (R1 – R3; 362 cm²/sample), and 3 brush roller (R1 – R3; 4 bunches of bristles/sample) surface samples as shown in Figure 4.2 were collected from adjacent rollers before and after conveying uninoculated tomatoes using one-ply composite tissues moistened in 1 ml of phosphate buffer (171). The plastic and foam rollers were wiped with composite tissue thoroughly to recover all the residual waters on the surface. However, for the brush roller, composite tissues were used to cover the entire bunch of bristles and scrub multiple times to recover bacteria. Since it was hard to measure the surface area of brushes, the *Salmonella* population was reported as log CFU/bunch of bristles. The purpose of evaluation of equipment surface population was to compare the cross-contamination of *Salmonella* on three types of roller conveyors during tomato conveying.

4.2.8 Impact of chemical sanitizers on *Salmonella* transfer

Two commonly used commercial sanitizers - 40 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN) and 40 ppm free chlorine from XY-12 (Ecolab) adjusted to pH of 6.0 with citric acid (CA), were added to the dump tank water to assess the impact of sanitizer washing on *Salmonella* transfer during tomato conveying. In addition to the same initial population level of

inoculated tomatoes ($\sim 4 \log \text{ CFU/g}$), a higher *Salmonella* level of $\sim 6 \log \text{ CFU/g}$ was also evaluated to illustrate the impact of sanitizer treatment on *Salmonella* transfer during conveying. Tomato and roller surface samples were collected in Whirl-Pak[®] bags containing neutralizing buffer (Difco, BD) following the same sampling procedure as previously described.

4.2.9 Microbiological analyses

Microbiological analysis was followed as described in 3.3.8. The medium used was xylose lysine tergitol-4 agar (XLT-4; Neogen Corporation, Lansing, MI).

4.2.10 Statistical analysis

Statistical analysis was performed as described in 3.3.9. In addition, the *Salmonella* population (CFU/tomato) on 25 individual uninoculated tomatoes was calculated and the transfer coefficient of total *Salmonella* transferred from inoculated tomatoes to 25 uninoculated tomatoes was calculated as described below.

$$\text{Transfer coefficient} = \frac{\text{Salmonella population transferred to 25 uninoculated tomatoes}}{\text{Salmonella population on the original inoculated tomatoes}} \times 100$$

4.3 RESULTS

4.3.1 *Salmonella* growth, attachment, survival, and sanitizer sensitivity

Similar growth rates were observed for all four *Salmonella* strains ($P > 0.05$), with generation time of 104.9, 100.8, 102.8, and 101.3 min for *S. Typhimurium* LT2, *S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314, respectively (Appendix C, Table C1.1). In the microtiter plate attachment test, except for *S. Poona* MDD237, *S. Typhimurium* LT2 and the other two virulent strains showed similar attachment ($P > 0.05$), with average OD values of 0.377 ± 0.074 , 0.104 ± 0.041 , 0.051 ± 0.004 , and 0.04 ± 0.019 for *S. Poona* MDD237, *S. Newport* MDD314, *S. Montevideo* MDD22, and *S. Typhimurium* LT2, respectively (Appendix C, Figure C1.1). *S. Typhimurium* LT2 and the 3-strain virulent *Salmonella* cocktail survived similarly ($P > 0.05$) on tomatoes during 6 d of incubation at 25°C, with populations ranging from 4.8 ± 0.1 to 5.4 ± 0.2 and 4.8 ± 0.0 to 5.5 ± 0.2 log CFU/g, respectively (Appendix C, Figure C1.2). Similar sensitivity was seen in the sanitizer study ($P > 0.05$) with *S. Typhimurium* LT2 and the 3-strain virulent *Salmonella* cocktail decreasing 5.7 ± 0.2 and 5.9 ± 0.2 log CFU/ml and 3.6 ± 0.2 and 3.5 ± 0.1 log CFU/ml after a 1 minute exposure to peroxyacetic acid and chlorine, respectively (Appendix C, Table C1.3).

4.3.2 *Salmonella* populations on inoculated tomatoes

After dip inoculation and air drying, tomatoes to be conveyed using foam, plastic, and brush rollers had initial *Salmonella* populations of 4.0 ± 0.1 , 3.8 ± 0.1 , and 4.2 ± 0.2 log CFU/g, respectively, which decreased ($P < 0.05$) by 0.7, 0.8, and 1.1 log CFU/g after 2 min of washing in water alone (Figure 4.3) (original data in Appendix C, Table C1.4). However, statistically similar populations ($P > 0.05$) ranging from 3.0 to 3.2 CFU/g were observed after conveying, regardless of the type of roller used.

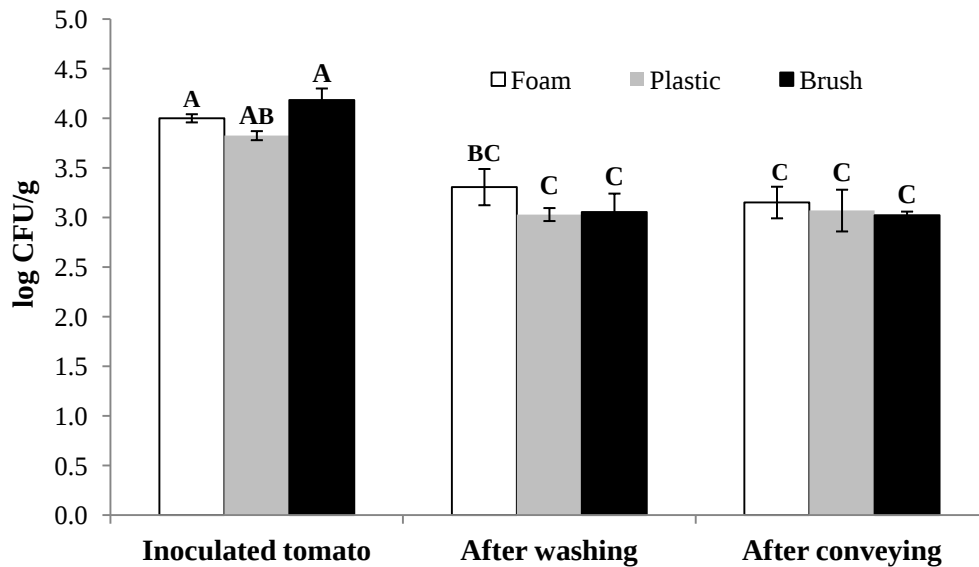


Figure 4.3: Mean ($\pm$ SD) *Salmonella* populations (log CFU/g) of inoculated tomatoes before processing, after washing, and after conveying with three roller conveyors (foam, plastic, or brush) ($n = 3$). Columns with the same letters are not significantly different ($P > 0.05$).

4.3.3 *Salmonella* transfer to uninoculated tomatoes

The numbers of salmonellae transferred from contaminated roller conveyors to 25 uninoculated tomatoes are shown in Figure 4.4 (original data in Appendix C, Table C1.5). After traversing over the contaminated foam rollers, 24 of 25 (96%) previously uninoculated tomatoes yielded *Salmonella* populations > 100 CFU/tomato (high level). Less transfer was seen using plastic rollers with 5 (20%) and 20 (80%) of the previously uninoculated tomatoes cross-contaminated with *Salmonella* at 10 - 100 (medium level) and 1 - 10 (low level) CFU/tomato, respectively. In contrast, using brush rollers only 2 of 25 (8%) uninoculated tomatoes were cross-contaminated with low level of *Salmonella* (Table 4.1). Although a small percentage of the *Salmonella* population transferred to uninoculated tomatoes, the transfer coefficient for foam rollers (0.18 ± 0.09) was significantly higher ($P < 0.05$) as compared to plastic (0.013 ± 0.008) and brush rollers (< 0.001).

Table 4.1: The percentage of uninoculated tomatoes contaminated with different levels of *Salmonella* after conveying through roller conveyors that contaminated with *Salmonella* and the transfer coefficient for three types of roller conveyors.

Roller type	Tomatoes contaminated with different levels of <i>Salmonella</i> (%)				Transfer coefficient (Mean $\pm$ SD %) ^b
	< 1 ^a (LOD)	1 - 10 (Low)	10 - 100 (Medium)	> 100 (High)	
Foam	0	0	4	96	0.18 $\pm$ 0.09 A
Plastic	0	76	24	0	0.013 $\pm$ 0.008 B
Brush	92	8	0	0	< 0.001 B

^a Below limit of detection (LOD) of 1 CFU/tomato.

^b Transfer coefficient = (Total *Salmonella* population on 25 uninoculated tomatoes) / (Total *Salmonella* population on inoculated tomatoes conveyed through roller conveyor). Means with the same letters are not significantly different ($P > 0.05$).

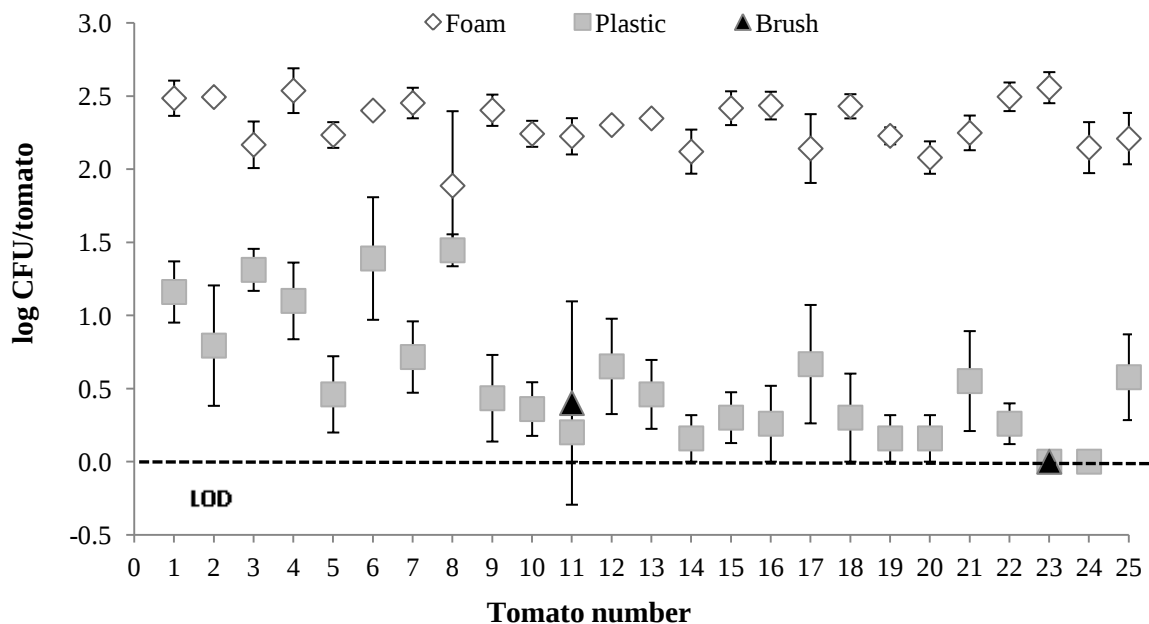


Figure 4.4: Mean ($\pm$ SE) *Salmonella* populations transferred to 25 uninoculated tomatoes after conveying through three different types (foam, plastic, and brush) of roller conveyor that contaminated with *Salmonella* from ~2 kg inoculated tomatoes ($n = 3$).

4.3.4 *Salmonella* transfer to roller conveyor surfaces

In order to assess *Salmonella* transfer from inoculated tomatoes to the rollers and then to uninoculated tomatoes, roller surface samples were collected before and after conveying the 25

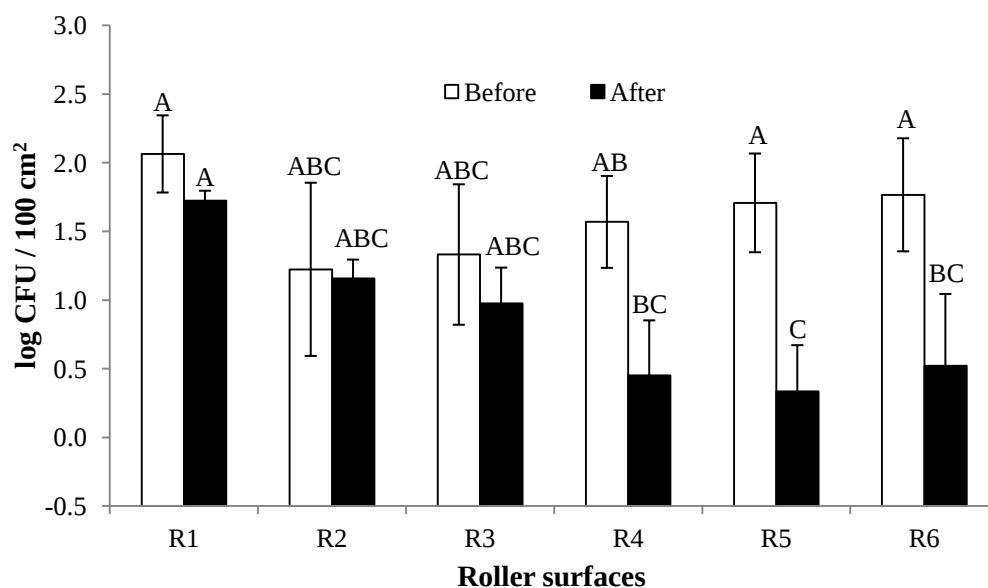
uninoculated tomatoes. For plastic rollers, all 6 surface samples yielded *Salmonella* after single file conveying inoculated tomatoes with populations ranging from 1.2 ± 0.6 to 2.1 ± 0.3 log CFU/100 cm². Numbers of salmonellae decreased after conveying 25 uninoculated tomatoes with reductions at each sampling location ranging from 0.0 to 1.4 log CFU/100 cm² (Figure 4.5A) (original data in Appendix C, Table C1.6). Compared to plastic, the foam rollers retained higher numbers of *Salmonella* (2.1 ± 0.2 to 2.4 ± 0.1 log CFU/100 cm²) with no significant ($P > 0.05$) reductions seen for foam rollers after conveying 25 uninoculated tomatoes (Figure 4.5B) (original data in Appendix C, Table C1.7). In contrast, none of the brush roller samples collected before and after conveying yielded detectable levels of *Salmonella* (< 1.5 CFU/surface sample).

4.3.5 Impact of sanitizer application on *Salmonella* transfer

Regardless of roller type, adding a chemical sanitizer to the dump tank water significantly decreased ($P < 0.05$) *Salmonella* recovery from both inoculated (initial population at 4 log CFU/g) and uninoculated tomatoes during conveying, with all 25 previously uninoculated tomatoes below the limit of detection (1 CFU/tomato). When the initial *Salmonella* inoculum was increased to 6 log CFU/g, peroxyacetic acid at 40 ppm effectively reduced *Salmonella* transfer, with only 4 (16%), 8 (32%), and 6 (24%) of the 25 uninoculated tomatoes cross-contaminated with low level of *Salmonella* for foam, plastic, and brush roller, respectively (Figure 4.6A and Table 4.2) (original data in Appendix C, Table C1.8). Similar contamination rates were seen using chlorine + CA at 40 ppm (pH 6.0) with plastic and brush rollers, with 7 (28%) and 6 (24%) of the 25 uninoculated tomatoes cross-contaminated with low level of *Salmonella*, respectively. However, after foam roller conveying, 2 (8%) and 19 (76%) of the 25 uninoculated tomatoes were cross-contaminated with medium and low level of *Salmonella*, respectively (Figure 4.6B and Table 4.2) (original data in Appendix C, Table C1.9). Hence,

chlorine was less effective in controlling *Salmonella* on foam as compared to plastic and brush rollers.

(A)



(B)

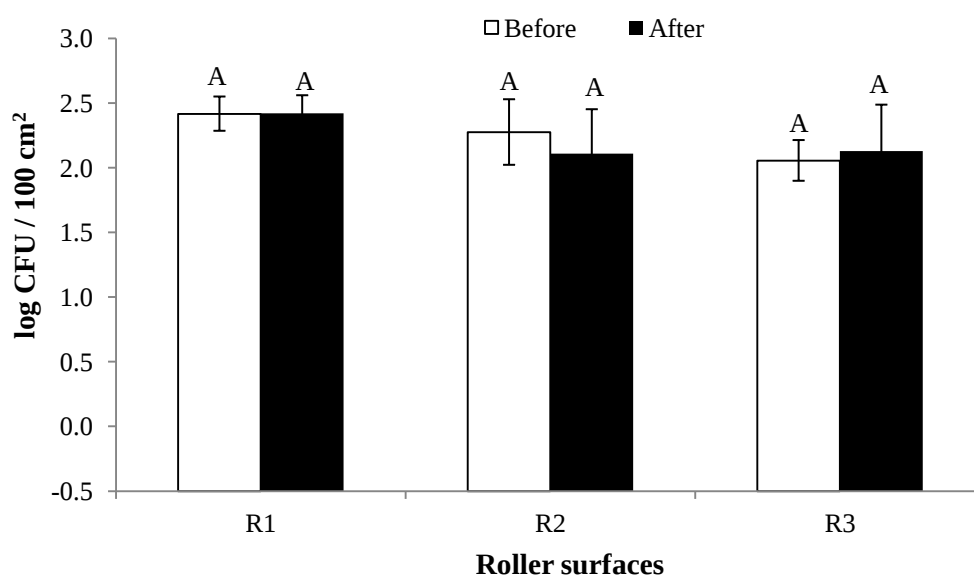


Figure 4.5: Mean ($\pm$ SE) *Salmonella* populations recovered from roller (A: plastic roller; B: foam roller) surfaces before and after conveying 25 uninoculated tomatoes ($n = 3$). Six (R1 – R6) and three (R1 – R3) surface samples were sampled for plastic and foam rollers, respectively. Columns with the same letters are not significantly different ($P > 0.05$).

Table 4.2: Tomatoes contaminated with different levels of *Salmonella* after conveying sanitizer-washed tomatoes and the transfer coefficient for three types of roller conveyors.

Roller type	Tomatoes contaminated with different levels of <i>Salmonella</i> (%)								Transfer coefficient (Mean ± SD %) ^c	
	< 1 ^a		1 – 10		10 – 100		> 100		T-100	XY-12
	(LOD)		(Low)		(Medium)		(High)			
	T-100 ^b	XY-12	T-100	XY-12	T-100	XY-12	T-100	XY-12		
Foam	84	16	16	76	0	8	0	0	< 0.001	< 0.001
Plastic	68	72	32	28	0	0	0	0	< 0.001	< 0.001
Brush	76	76	24	24	0	0	0	0	< 0.001	< 0.001

^a Below the of detection (LOD) of 1 CFU/tomato.

^b T-100: Tsunami 100 at 40 ppm; XY-12: 40 ppm chlorine at pH of 6.0.

^c Transfer coefficient = (Total *Salmonella* population on 20 uninoculated tomatoes) / (Total *Salmonella* population on inoculated tomatoes conveyed through roller conveyor). Means with the same letters are not significantly different ($P > 0.05$).

(A)

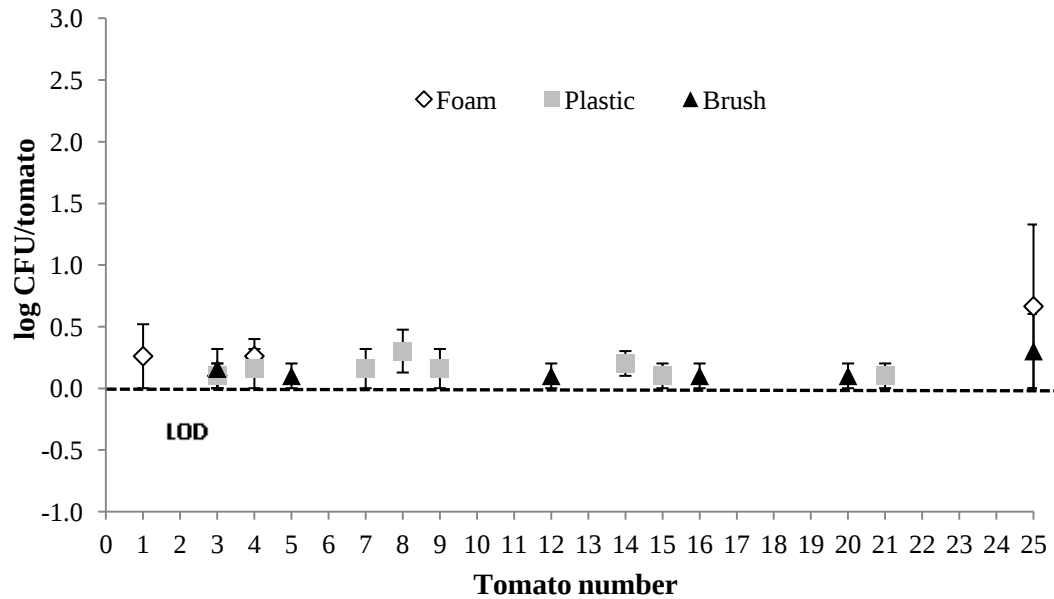
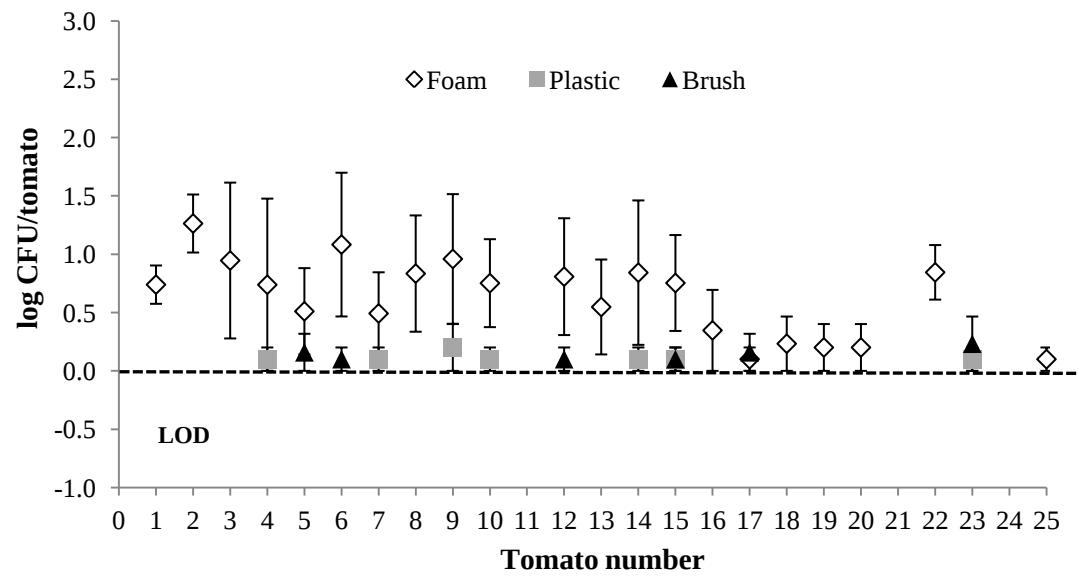


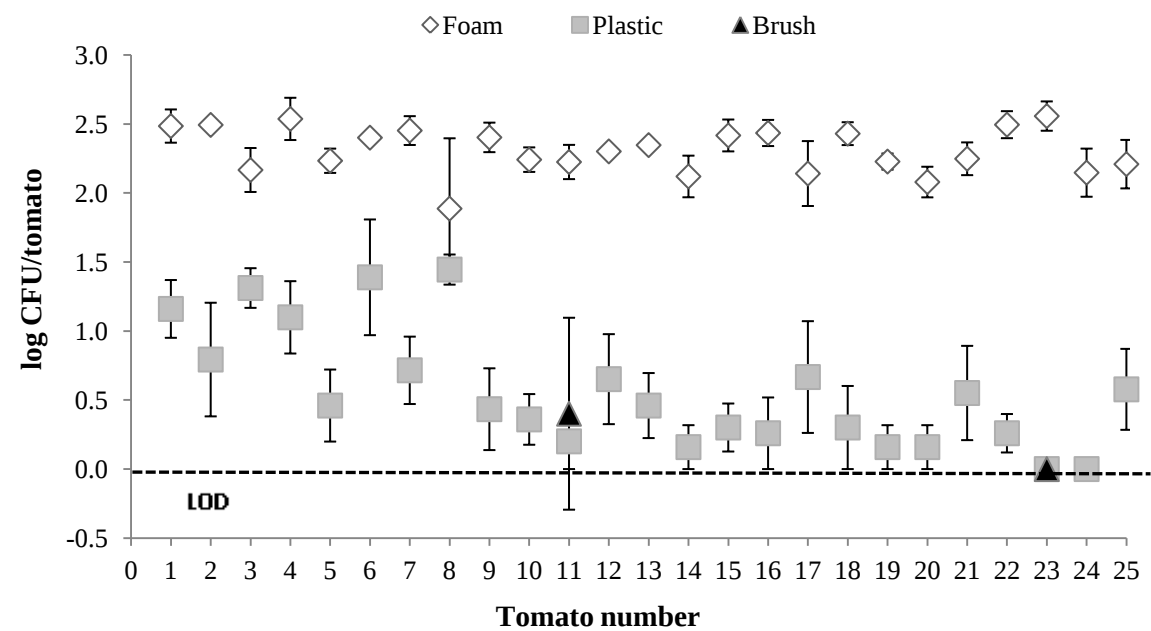
Figure 4.6: Mean ($\pm$ SE) *Salmonella* populations transferred to 25 uninoculated tomatoes through three different types of roller conveyor that contaminated with *Salmonella* from ~2 kg inoculated tomatoes (~ 6 log CFU/g) that previously washed with sanitizer (A: 40 ppm peroxyacetic acid; B: 40 ppm chlorine + CA; C: Water control) (n = 3).

Figure 4.6 (cont'd)

(B)



(C)



4.4 DISCUSSION

An avirulent *rpoS*-deficient strain of *Salmonella* (*S. Typhimurium* LT2) (151), was used by necessity to eliminate the generation of infectious aerosols during tomato processing. Except for virulent *S. Poona* MDD237, which showed significantly stronger attachment, avirulent *S. Typhimurium* LT2 behaved similarly to the virulent *Salmonella* strains in terms of growth in laboratory media, attachment in the microtiter plate assay, survival on tomatoes, and sensitivity to chlorine and peroxyacetic acid. Decreased bacterial attachment strength to a surface can potentially increase the rate of transfer. Therefore, any surrogate organisms chosen for transfer work should be similar to their pathogenic counterparts in terms of attachment ability.

During tomato processing, different types of rollers are used for different purposes. Brush rollers are normally used to facilitate physical scrubbing and uniform waxing with overhead wax sprayers (119). Foam or sponge rollers are most helpful in removing excess water during drying, while plastic rollers are commonly used to convey waxed tomatoes to the sorter and packing table, with both of these rollers able to minimize bruising and injury (5). When Pao et al. (120) investigated the combined use of brush washing and ClO_2 spraying to minimize *Salmonella enterica* transfer during tomato processing, brushing tomatoes with inoculated polyethylene roller brushes at a speed of 85 rpm for 10 s resulted in *Salmonella* contamination levels of $5.7 \pm 0.1 \log \text{CFU/cm}^2$ on the tomato surface. Compared to the water control ($2.1 \pm 0.6 \log \text{CFU/cm}^2$), spray washing with 5 ppm ClO_2 for 10 s decreased cross-contamination by $4.7 \pm 0.2 \log \text{CFU/cm}^2$. Another study conducted by Chang and Schneider (38) evaluated the efficacy of chemical sanitizers to inactivate *Salmonella* on tomatoes during washing with an overhead sprayer and nylon brush rollers (180 rpm) under laboratory conditions with *Salmonella* populations similarly decreasing up to 5 logs on tomatoes. Compared to conventional flume

washing, such overhead spray systems can produce greater pathogen reductions with less sanitizer and water use. However, one potential disadvantage is that inadequate contact between the fruit and brushes rotating in a single direction may decrease pathogen control.

While the combined use of brush rollers and chemical sanitizers can reduce microbial contamination during tomato washing, any pathogens that survive due to decreased sanitizer efficacy can be transferred during product conveying. Plastic and foam rollers are more likely to result in cross-contamination since these rollers are normally used without an overhead sprayer. In this study, inoculated tomatoes were flume-washed for 2 min before roller conveying to simulate commercial processing. To avoid *Salmonella* cross-contamination from the wash water, 25 uninoculated tomatoes were washed first followed by the inoculated tomatoes. After washing in sanitizer-free water, *Salmonella* populations decreased ~1 log CFU/g on inoculated tomatoes as previously reported for tomatoes and other types of fresh produce (29, 38, 136). Therefore, the actual *Salmonella* population on inoculated tomatoes at the start of roller conveying was ~ 3 log CFU/g.

For all three types of roller conveyors, no significant reductions in *Salmonella* were seen on inoculated tomatoes after roller conveying. However, a previous study conducted by Pao et al. (120) showed that tomato brushing combined with spray washing (5.0 ml/s per tomato for 10 s) decreased *Salmonella* populations by 3.2 logs. Similarly, Chang and Schneider (38) also obtained a 3-log reduction in *Salmonella* after brushing inoculated tomatoes for 60 s with a specially designed brush roller system that combined overhead spraying with brush roller conveying. Therefore, overhead spraying can facilitate *Salmonella* removal and inactivation on tomatoes. In addition to water spraying, both of these studies obtained *Salmonella* reductions of up to 5 logs when the overhead spray system contained commercial sanitizers. However in our

study, no overhead spray was applied. Future research should assess the impact of overhead spray on *Salmonella* transfer during the conveying process.

After conveying inoculated tomatoes, plastic and foam roller conveyors were cross-contaminated with *Salmonella* at different levels (Figures 4.5A and 4.5B). The potential for these contaminated rollers to further cross-contaminate 25 uninoculated tomatoes was then assessed. Greatest cross-contamination of uninoculated tomatoes was seen using foam followed by plastic and brush rollers with only 2 of 25 brush roller-conveyed tomatoes cross-contaminated with *Salmonella* at 1 - 10 CFU/tomato. These differences are likely due, in part, to variations in roller surface configuration and surface roughness. The soft porous surface of foam rollers is designed for dewatering and protecting tomatoes. However, foam rollers readily absorb bacteria-laden water and become highly-contaminated, further spreading *Salmonella* to subsequently processed tomatoes as seen in Figure 4.5B. After conveying 25 uninoculated tomatoes, foam rollers remained highly-contaminated with *Salmonella*. A previous study conducted by Merritt et al. (101) showed that the porous structure of foam can readily entrap bacteria, leading to colonization; whereas plastic rollers are smooth, dense and nonabsorbent. Therefore, significantly less *Salmonella* transfer was seen using plastic as compared to foam rollers, with *Salmonella* populations on the 25 uninoculated tomatoes gradually decreasing (Figures 4.4). Minimal cross-contamination occurred during brush roller conveying of tomatoes due to limited contact between bunches of bristles and the tomatoes during conveying. However, these brush rollers were far cleaner compared to those used in commercial packinghouses. Additional work from our laboratory showed that brush rollers used for commercial waxing of tomatoes were heavily contaminated with mesophilic aerobic bacteria and yeast/mold populations of 2.39 and

2.17 log CFU/bunch of bristles, respectively (173), with wax on these bristles leading to bacterial entrapment and further cross-contamination.

In commercial packinghouses, various chemical sanitizers are routinely used to minimize cross-contamination during washing as discussed in several federal (157) and state guidance documents (57) for the industry. In this study, both sanitizer treatments effectively prevented *Salmonella* cross-contamination during roller conveying, with *Salmonella* counts on all 25 uninoculated tomatoes below the limit of detection. However, use of 40 ppm free chlorine with foam rollers led to substantial cross-contamination of uninoculated tomatoes due to entrapment and protection of *Salmonella* from the sanitizer within the roller pores and sequestration of residual chlorine.

This is the first report to quantitatively assess the impact of different types of roller conveyors on *Salmonella* transfer during conveying of tomatoes. These findings are needed to develop science-based transfer models for risk assessments. Proper use of an appropriate sanitizer during tomato processing and packing is essential to prevent cross-contamination. Additional research regarding different tomato conveyance materials, such as stainless steel or antimicrobial-amended HDPE, and conveyance methods, such as UV-light tunnel, should provide new insights for GAP (Good Agricultural Practices) improvements that can enhance the microbial safety of tomatoes.

CHAPTER 5:

Transfer of *Salmonella* during Mechanical Slicing of Tomatoes as Impacted by Multiple Processing Variables

5.1 OBJECTIVE

The objectives of this study were to 1) assess the spread of *Salmonella* from inoculated tomatoes to various surfaces of manual and electric slicers as well as to subsequently uninoculated tomatoes during slicing and 2) quantify the impact of post-contamination hold time, tomato surface wetness, slicing room temperature, slice thickness, tomato variety, and pre-wash treatment on *Salmonella* transfer during manual slicing of tomatoes.

5.2 MATERIALS AND METHODS

5.2.1 Tomatoes

The same tomatoes as described in 3.3.2 were used for this study.

5.2.2 *Salmonella* strains

The same *Salmonella* Typhimurium LT2 strain as described in 3.3.3 was used for this study.

5.2.3 Inoculation of tomatoes

Tomatoes were dip-inoculated as described in 3.3.4 to contain ~ 5 log CFU/g *Salmonella*.

5.2.4 Tomato slicer

Two different slicers were evaluated - a manual slicer (Model 943, Prince Castle Inc., Carol Stream, IL) and an electric slicer (Model 350, Edlund Company, Inc., Burlington, VT). Using Glo-GermTM powder as previously described by Vorst et al. (171), the major tomato contact areas of the manual slicer included the blade, back plate, and bottom plate (Figure 5.1A), whereas the blade, pusher, and side plate were identified for the electric slicer (Figure 5.1B).

5.2.5 *Salmonella* transfer to individual tomato slices

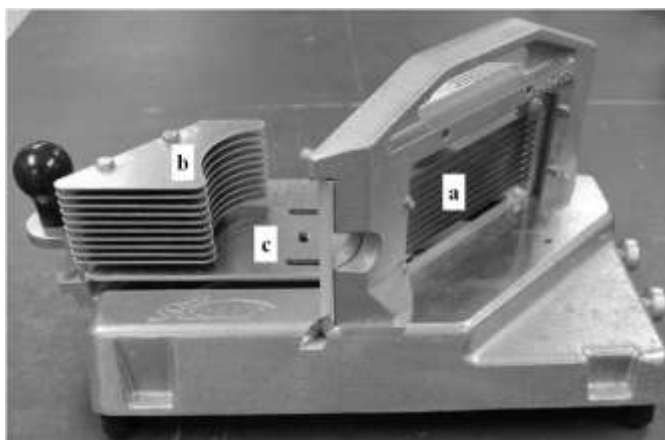
The distribution of *Salmonella* on individual tomato slices was assessed by slicing one inoculated tomato. Each of the nine slices (5.7 mm thickness) obtained was collected in a separate Whirl-Pak[®] bag (Nasco, Fort Atkinson, WI) containing 20 ml of phosphate buffer, weighed, and then quantitatively analyzed for *Salmonella*. Thereafter, one uninoculated tomato was sliced using the same slicer with all nine slices similarly analyzed. Based on these results, only the top, middle, and bottom slices were collected and analyzed in subsequent experiments, with the resulting *Salmonella* populations for the three slices then averaged to estimate the *Salmonella* concentration (log CFU/g) for the whole tomato. In a hand contamination study

(143), the author similarly analyzed tomato slices 3, 5, and 7 for norovirus. However, the distribution of natural surface contaminants on tomatoes is most likely non-uniform.

5.2.6 *Salmonella* transfer to the slicer

Transfer of *Salmonella* to the previously identified product contact areas of the manual and electric slicer was assessed after slicing inoculated as well as uninoculated tomatoes. Briefly, after slicing one inoculated tomato, the blade, back plate, and bottom plate of the manual slicer as well as the blade, pusher and side plate of the electric slicer were individually sampled using one-ply composite tissues moistened with 1 ml of sterile phosphate buffer (171). Similarly, after slicing one inoculated followed by 20 uninoculated tomatoes, the same product contact locations from the two slicers were sampled to quantify *Salmonella*.

(A)



(B)

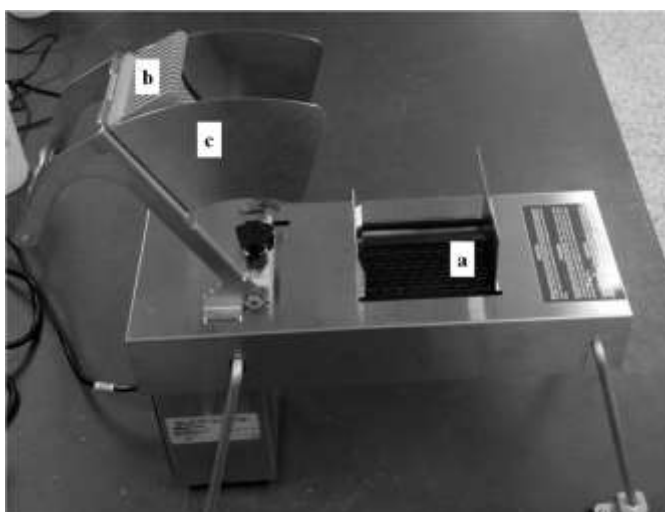


Figure 5.1: Components of the manual slicer A: (a) blade; (b) back plate, (c) bottom plate and electric slicer B: (a) blade, (b) pusher, and (c) side plate.

5.2.7 *Salmonella* transfer from individual slicer components to tomatoes

The contribution of individual components to *Salmonella* transfer was also evaluated. In order to assess *Salmonella* transfer from the contaminated back and bottom plates of the manual slicer, one inoculated tomato was sliced, after which the blade of slicer was removed, sanitized, and replaced, followed by slicing 20 uninoculated tomatoes thereafter. Similarly, the number of salmonellae transferred from the blade was determined by slicing one inoculated tomato,

sanitizing the back and bottom plates and then slicing 20 uninoculated tomatoes. For the electric slicer, the same protocol was followed to assess *Salmonella* transfer from the blade and the pusher and side plates. In all cases, the top, middle and bottom slices were collected from each tomato and analyzed for numbers of salmonellae. Considering that the limits of detection (LOD) differed due to varying tomato weights, the final LOD for *Salmonella* was calculated as the average of all LODs for the three replicates. For subsequent statistical analyses, individual tomato slices that were positive for *Salmonella* by enrichment were assigned a count of 1 CFU for the slice sample that contains top, middle and bottom slices, with the total *Salmonella* population calculated based on the total tomato weight. Enrichment-negative tomatoes were assigned a *Salmonella* population of 1 CFU/tomato. The assignment to enrichment-positive samples can potentially under-estimate the real *Salmonella* population transferred to tomatoes. However, for the enrichment-negative samples, this assignment can potentially lead to over-estimate of the real *Salmonella* population transferred to tomatoes.

5.2.8 Impact of different processing variables on *Salmonella* transfer

Six processing variables as shown in Table 5.1 were evaluated for their impact on *Salmonella* transfer by slicing one inoculated followed by 20 uninoculated tomatoes under the following conditions: 1) Post-contamination hold time - after slicing one inoculated tomato, 0 or 30 min elapsed before the slicer was again used to slice twenty uninoculated tomatoes, 2) Tomato wetness - dip-inoculated tomatoes were sliced immediately or after 2 h of drying followed by 20 uninoculated tomatoes that were either dipped in sterile water or dry, 3) Processing room temperature - slicing was conducted in a walk-in cold room at 4 or 10°C or at room temperature (23 °C) with all tomatoes tempered to the temperature of the room before slicing, 4) Slice thickness - three different sets of blades were used to generate tomato slices that

were 5.7 mm (1/4"), 4.8 mm (3/16") or 9.5 mm (3/8") thick, 5) Tomato variety - Rebelski and Bigdena in addition to Torero were evaluated, and 6) Pre-wash - both inoculated and uninoculated tomatoes were washed for 2 min in tap water or 100 ppm of chlorine (XY12, Ecolab, St. Paul, MN) adjusted to pH 6.0 with citric acid before slicing with unwashed tomatoes serving as the control. In order to assess the differences between different tomato varieties, the peak force and free liquid percentage was also measured. For the peak force, the peak force appeared during tomato cutting were measured using a texture analyser (TA-XT2i, Texture Technologies Corp., Scandain, New York). For the free liquid measurement, tomato slices were cut from the whole tomato, weighed, and faced down to pre-weighed two layers of 1-ply composite tissues (Kimwipes[®], Kimberly-Clark Corp., Roswell, GA). After 2-min, the composite tissues were weighed again and the liquid absorbed was calculated. Then, the free liquid percentage was calculated using the weight of liquid divided by the total weight of corresponding tomato slices.

Table 5.1: Six variables evaluated for their impact on *Salmonella* transfer.

Variable	Control	Condition 1	Condition 2
Post-contamination hold time (min)	0	30	--
Tomato surface wetness	Dry	Wet	--
Processing temperature (°C)	23	10	4
Tomato slice thickness (mm)	5.7	4.8	9.5
Tomato variety	Torero	Rebelski	Bigdena
Wash treatment	No wash	Tap water	Chlorine

5.2.9 Microbiological analyses

Tomato samples collected in Whirl-pak[®] bags containing 20 ml of phosphate buffer solution or 50 ml of lactose broth were weighed, homogenized by stomaching (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 300 rpm, and then surface-plated on trypticase soy agar (BD) containing 0.6% yeast extract (BD), 0.05% ferric ammonium citrate (Sigma) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS)

with or without 0.45 µm-membrane filtration (Milipore Corporation, Billerica, MA) to quantify *Salmonella* (limit of detection: 20 and 50 CFU/sample for one and three tomato slices, respectively). Equipment surface sample tissues were homogenized by stomaching in 30 ml of phosphate buffer for 1 min at 300 rpm and then similarly analyzed for salmonellae (limit of detection: 3 CFU/slicer part). Plates were incubated at 37 °C for 24 h after which all black colonies were counted as *S. Typhimurium* LT2. Along with the plates, tomato samples in Whirl-Pak® bags containing lactose broth were incubated at 37 °C for 24 h to enrich for *Salmonella*. When *Salmonella* was not detected by plating or filtration, xylose lysine tergitol-4 (XLT-4; Neogen Corporation, Lansing, MI) plates streaked from the previous lactose broth-enriched sample were analyzed for the presence or absence of *Salmonella* after 24 h of incubation at 37°C. Selected colonies were confirmed as *Salmonella* using the Neogen Reveal® 2.0 kit (Neogen Corporation, Lansing, MI).

5.2.10 Statistical analysis

All experiments were performed in triplicate. Average *Salmonella* populations were calculated from three replicates and then converted to log CFU per gram, per slicer part, or per tomato for the *Salmonella* distribution, surface transfer, and tomato transfer studies. For the *Salmonella* distribution and surface transfer studies, analysis of variance and the Tukey-Kramer HSD test were performed using JMP 11.0 (SAS Institute Inc., Cary, NC). Statistical significance was set at $\alpha = 0.05$. Two-parameter exponential decay curves were fitted to the *Salmonella* transfer (log CFU/tomato) data for tomatoes using Eq. (1):

$$Y = A \cdot \text{Exp} (B \cdot X) \quad (1)$$

where Y (dependent variable) is the log CFU/tomato transferred and X (independent variable) is the order number for the specific uninoculated tomato that was sliced. A and B are twomodel

parameters. The parameter $\pm$ standard error for each replicate and the root mean square error (RMSE) of the model were obtained using JMP 11.0. In addition, the percentage of the *Salmonella* population transferred from one inoculated to 20 uninoculated tomatoes was calculated as in Eq. (2)

$$\%T = \frac{\text{Salmonella population transferred to 20 uninoculated tomatoes}}{\text{Salmonella population on the original inoculated tomato}} \times 100 \quad (2)$$

Analysis of variance and the Tukey-Kramer HSD test were performed using JMP to compare percent transfer and the decay parameter for the different processing variables.

5.3 RESULTS

5.3.1 *Salmonella* transfer to individual tomato slices

After slicing the inoculated tomato (4.9 ± 0.1 log CFU/g), populations on the nine tomato slices ranged from 4.6 ± 0.2 to 5.5 ± 0.3 log CFU/g, with significantly fewer ($P \leq 0.05$) salmonellae transferred to interior slices 4 through 7 compared to those closer to or including the blossom and stem ends (Figure 5.2) (original data in Appendix D, Table D1.1). Since the inoculated tomato was dip-inoculated with *Salmonella*, this difference was likely due to the relatively larger surface area for top and bottom slices than the middle slices. However, when one inoculated tomato was sliced followed by one uninoculated tomato, *Salmonella* populations on the previously uninoculated tomato ranged from 2.1 ± 0.3 to 3.3 ± 0.2 log CFU/g and were not significantly different ($P > 0.05$). This difference was likely due to the fact that consistent similar contact areas were contacted by the subsequent uninoculated tomatoes, with relatively smaller contact areas for top & bottom slices and larger contact areas for the middle slices. In the meantime, the middle slices weighed more than the top and bottom slices. When the *Salmonella* population was calculated to log CFU/g for each individual tomato slices, no significant difference was observed for 9 uninoculated tomato slices.

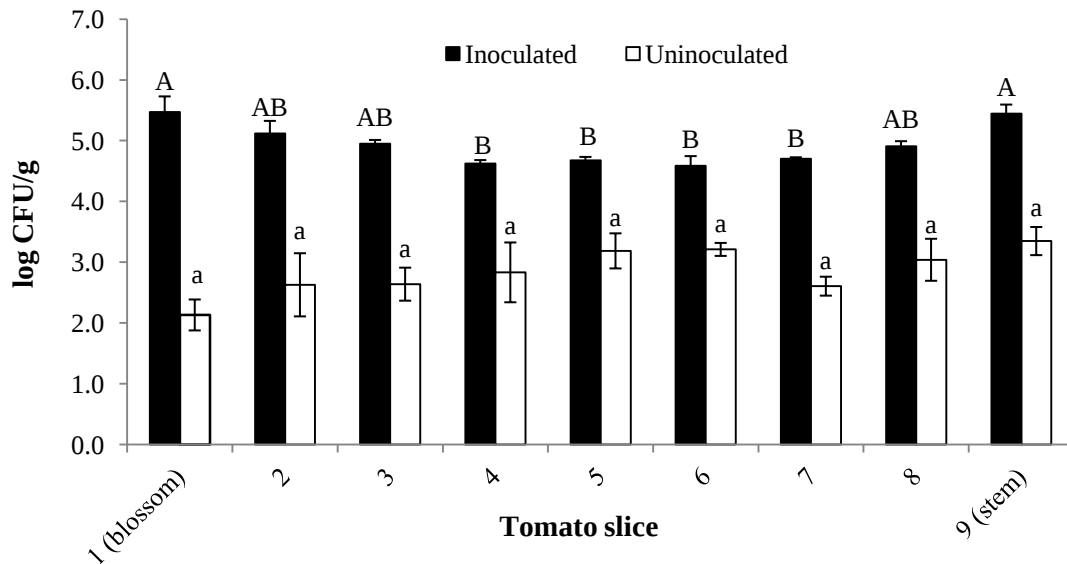
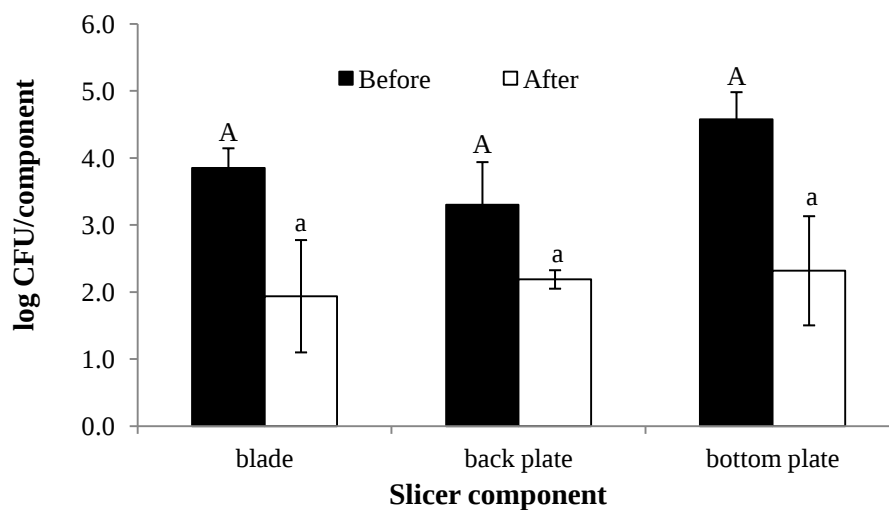


Figure 5.2: Mean ($\pm$ SE) *Salmonella* distribution on nine tomato slices from inoculated and uninoculated tomatoes (1: top slice is the blossom end; 9: bottom slice is the stem end) after slicing with the manual slicer ($n = 3$). Means with the same capital letters for inoculated tomato slices are not significantly different ($P > 0.05$). Means with the same letters for uninoculated tomato slices are not significantly different ($P > 0.05$).

5.3.2 *Salmonella* transfer to the slicer

After slicing one inoculated tomato on the manual slicer, the blade, back plate, and bottom plate yielded statistically similar *Salmonella* populations ($P > 0.05$) of 3.8 ± 0.3 , 3.3 ± 0.6 , and 4.6 ± 0.4 log CFU/part, respectively (Figure 5.3A) (original data in Appendix D, Table D1.2). When 20 uninoculated tomatoes were subsequently sliced, numbers of salmonellae decreased to 1.9 ± 0.8 , 2.2 ± 0.1 , and 2.3 ± 0.8 log CFU/part, with statistically similar populations again seen ($P > 0.05$) for the three components of the manual slicer. Similarly, for the electric slicer, no significantly different *Salmonella* populations ($P > 0.05$) were observed on the blade, pusher, and side plate either before or after slicing 20 uninoculated tomatoes (Figure 5.3B) (original data in Appendix D, Table D1.3).

(A)



(B)

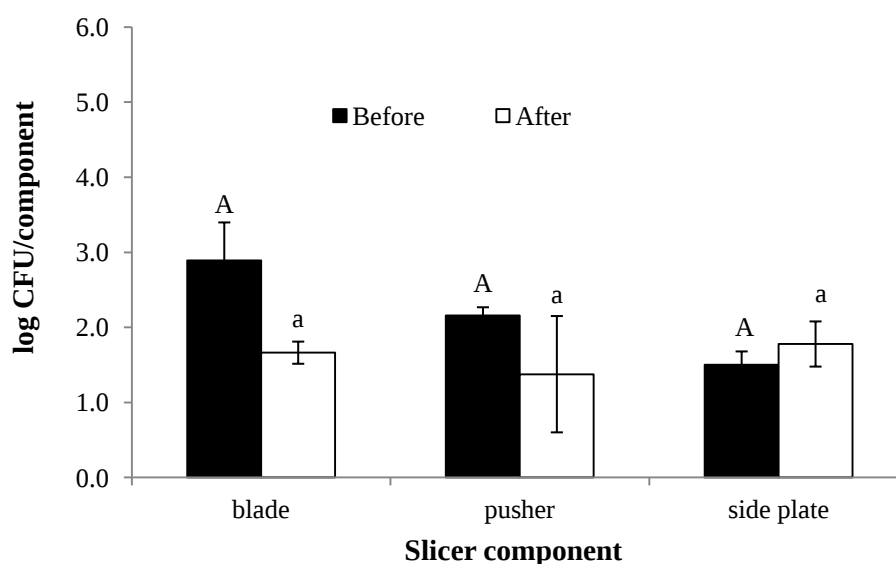


Figure 5.3: Mean ($\pm$ SE) *Salmonella* distribution on different components of the manual slicer (A) and electric slicer (B) before and after slicing 20 uninoculated tomatoes ($n = 3$). Means with the same capital letters for surface population before slicing 20 uninoculated tomatoes are not significantly different ($P > 0.05$). Means with the same letters for surface population after slicing 20 uninoculated tomatoes are not significantly different ($P > 0.05$).

5.3.3 *Salmonella* transfer from the slicer to tomatoes during sequential slicing

After slicing one *Salmonella*-inoculated tomato (4.9 ± 0.1 log CFU/g) followed by 20 previously uninoculated tomatoes in three separate trials, 58 of 60 (96.7%) tomatoes yielded *Salmonella* – 47 by direct plating and 11 by enrichment (Figure 5.4) (original data in Appendix D, Table D1.4). When these results were fitted to a previously published two-parameter exponential decay model (140), *Salmonella* populations decreased 3.1, 2.8, and 2.4 log CFU/tomato in trials 1, 2, and 3 after slicing 20 uninoculated tomatoes. Similar transfer decay rates of -0.047, -0.052, and -0.054, were observed for trials 1, 2, and 3, respectively, with the root mean square error (RMSE) of 0.42 log CFU/tomato indicating a relatively good fit.

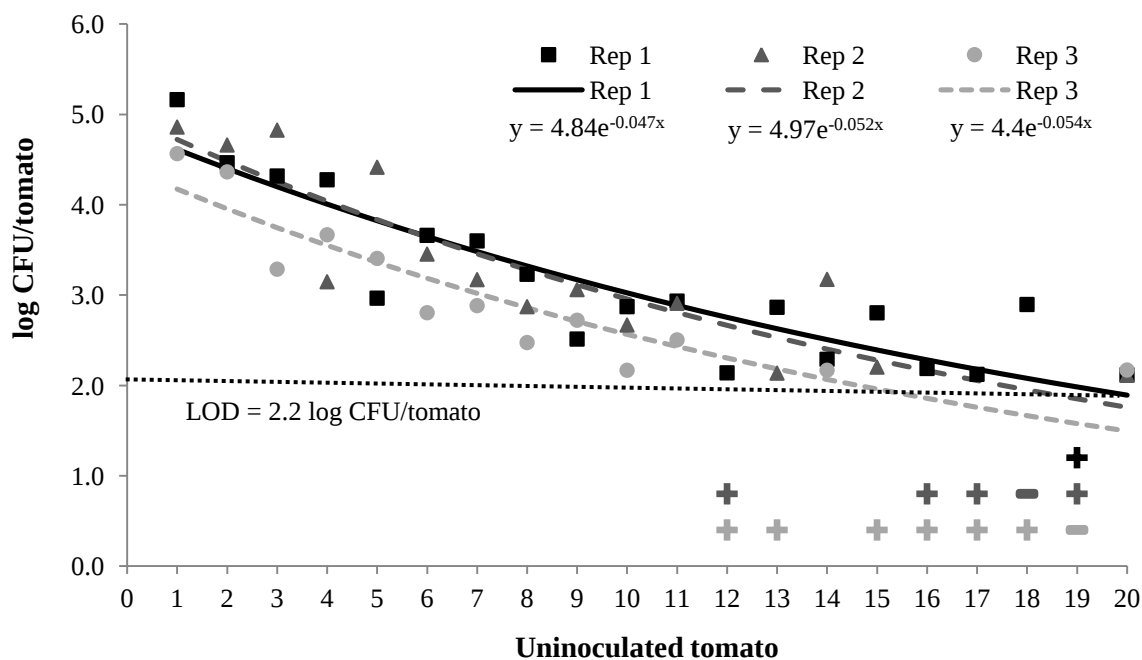
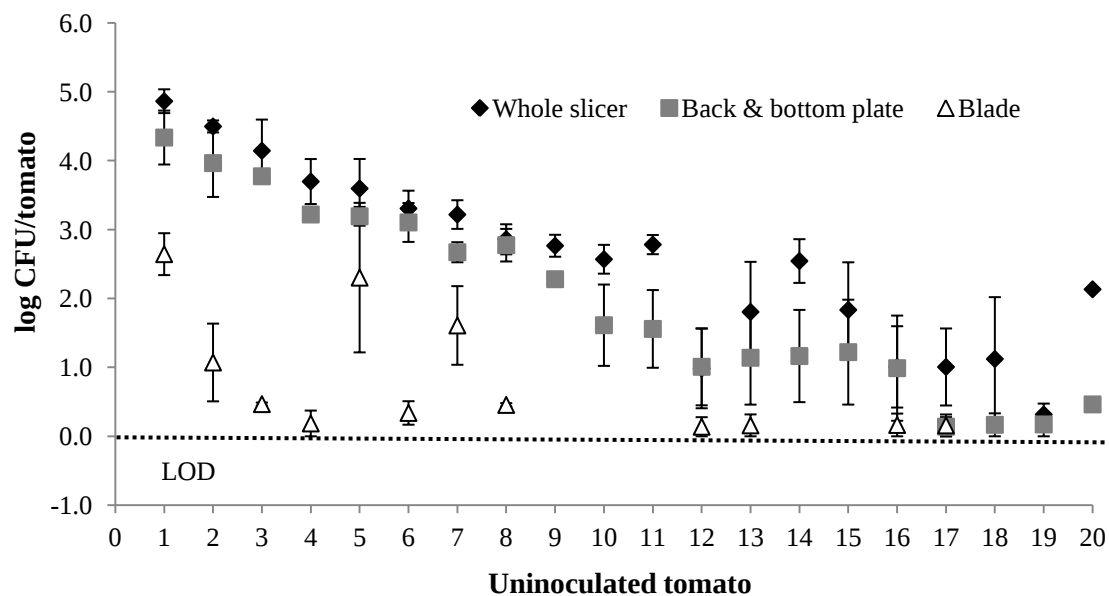


Figure 5.4: *Salmonella* transfer from one inoculated tomato to twenty uninoculated tomatoes via the manual slicer (control). Rep 1, Rep 2, and Rep 3 are three replicates of the study. “+” in the figure means tomato sample was positive after enrichment and “-” means tomato sample was negative after enrichment. The quantitative transfer data (without the enrichment result) of three replicates were fitted to the two-parameter exponential decay model separately.

5.3.4 *Salmonella* transfer from different slicer components

In order to assess the contribution of individual slicer components to cross-contamination, the numbers of *Salmonella* transferred from specific parts of the slicer to uninoculated tomatoes during slicing were quantified. It has to be noted that, for the comparison purpose, 1 CFU was assigned to the tomato samples that were enrichment-positive and the total *Salmonella* population on the corresponding whole tomato (log CFU/tomato) was calculated. For the tomato samples were enrichment-negative, 0 log CFU/tomato was assigned. Therefore, the limit of detection for this study was 0 log CFU/tomato. When the blade alone on the manual slicer was contaminated, *Salmonella* transferred to only 12 of 20 uninoculated tomatoes, with these low numbers likely due to the relatively lower initial *Salmonella* population on blades and the fact that the juices produced during slicing create an “washing effect” on blades (Figure 5.5A) (original data in Appendix D, Table D1.5). However, *Salmonella* spread to all 20 uninoculated tomatoes when the entire slicer or the back and bottom plates were contaminated before slicing. The total number of salmonellae transferred from the contaminated blade set to 20 uninoculated tomatoes (3.4 ± 0.4 log CFU), was significantly lower ($P \leq 0.05$) than for the contaminated back and bottom plates (4.7 ± 0.3 log CFU) and the whole slicer (5.2 ± 0.2 log CFU). The back and bottom plates were the primary contributors to *Salmonella* transfer during manual slicing. However, the blade was the primary contributor to *Salmonella* transfer during tomato slicing with the electrical slicer (Figure 5.5B) (original data in Appendix D, Table D1.6).

(A)



(B)

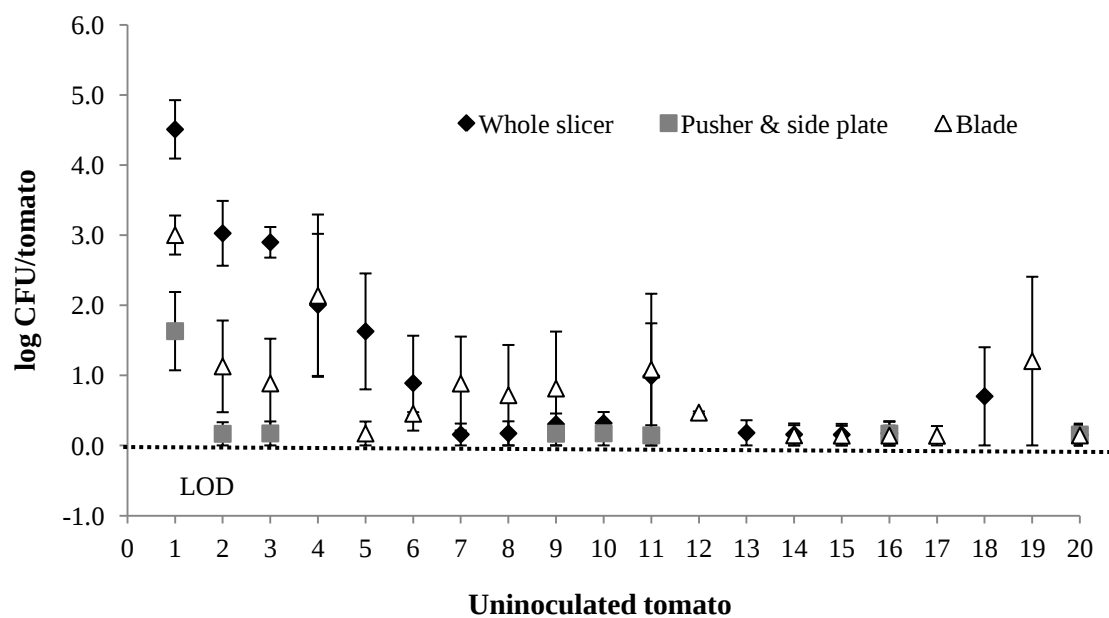


Figure 5.5: Mean ($\pm$ SE) *Salmonella* population transferred from one inoculated tomato to 20 uninoculated tomatoes through different parts of manual (A) and electric (B) slicer during slicing ($n = 3$). LOD: limit of detection.

5.3.5 *Salmonella* transfer impacted by different processing variables

For each of the six processing variables assessed, a previously described two-parameter exponential decay model was fitted to *Salmonella* populations obtained during the slicing of 20 uninoculated tomatoes. Model parameters and the root mean square error (RMSE) are shown in Table 5.2, along with the percent transfer for *Salmonella*. Post-contamination hold time, processing temperature and tomato slice thickness did not significantly ($P > 0.05$) impact the transfer decay rate (parameter B) or the overall percentage of salmonellae transferred. When the tomato surface was wet, a significantly lower ($P \leq 0.05$) transfer decay rate (-0.028 ± 0.002) was observed compared to dry tomatoes (-0.051 ± 0.002), with a significantly higher ($P \leq 0.05$) percentage of *Salmonella* transferred to wet ($12.2 \pm 2.4\%$) as opposed to dry tomatoes ($1.1 \pm 0.5\%$). When different tomato varieties were assessed, significantly lower ($P \leq 0.05$) transfer decay rates and *Salmonella* transfer percentages were observed for Rebelski and Bigdena as compared to Torero. When the tomatoes were washed for 2 min in tap water before slicing, no significant differences in the transfer decay rate or transfer percentage were observed compared to the control. However, all 20 uninoculated tomatoes were negative for *Salmonella* when a 100 ppm chlorine wash treatment was applied for 2-min washing on tomatoes before slicing. The RMSE for all processing variables ranged from 0.17 to 0.56 log CFU/tomato, which supported the exponential decay model. The original transfer data for each variable was presented in Appendix D, from Table D1.7 to Table D1.12.

Table 5.2: Transfer model parameters (A and B) and percent transfer of *Salmonella* from inoculated tomato for six processing variables (n = 3).

Processing variables		A $\pm$ SE (log CFU/tomato)	B $\pm$ SE	RMSE ¹ (log CFU/tomato)	% Transfer $\pm$ SE
Post-contamination hold time (min)	0	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002A ²	0.42	1.1 $\pm$ 0.5a ³
	30	4.84 $\pm$ 0.14	-0.044 $\pm$ 0.007A	0.38	1.0 $\pm$ 0.5a
Tomato surface wetness	Dry	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002B	0.42	1.1 $\pm$ 0.5b
	Wet	6.78 $\pm$ 0.03	-0.028 $\pm$ 0.002A	0.17	12.2 $\pm$ 2.4a
Processing temperature (°C)	23	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002A	0.42	1.1 $\pm$ 0.5a
	10	3.32 $\pm$ 0.55	-0.060 $\pm$ 0.051A	0.33	0.1 $\pm$ 0.1a
	4	4.33 $\pm$ 0.76	-0.076 $\pm$ 0.038A	0.35	0.6 $\pm$ 0.3a
	5.7	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002A	0.42	1.1 $\pm$ 0.5a
Tomato slice thickness (mm)	4.8	4.77 $\pm$ 0.05	-0.036 $\pm$ 0.003A	0.25	0.7 $\pm$ 0.2a
	9.5	3.59 $\pm$ 0.34	-0.024 $\pm$ 0.012A	0.56	0.2 $\pm$ 0.1a
Tomato variety	Torero	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002B	0.42	1.1 $\pm$ 0.5a
	Rebelski	3.32 $\pm$ 0.12	-0.011 $\pm$ 0.006A	0.45	0.3 $\pm$ 0.0b
	Bigdena	3.28 $\pm$ 0.46	-0.015 $\pm$ 0.015A	0.46	0.1 $\pm$ 0.1b
	No wash	4.74 $\pm$ 0.17	-0.051 $\pm$ 0.002A	0.42	1.1 $\pm$ 0.5a
Wash treatment	Tap water	4.56 $\pm$ 0.21	-0.068 $\pm$ 0.012A	0.37	2.7 $\pm$ 1.5a
	Chlorine	--	--	--	0.0 $\pm$ 0.0

¹ RMSE: root mean square error for the exponential decay model.

² Means with the same capital letters for the same processing variable are not significantly different ($P > 0.05$).

³ Means with the same letters for the same processing variable are not significantly different ($P > 0.05$).

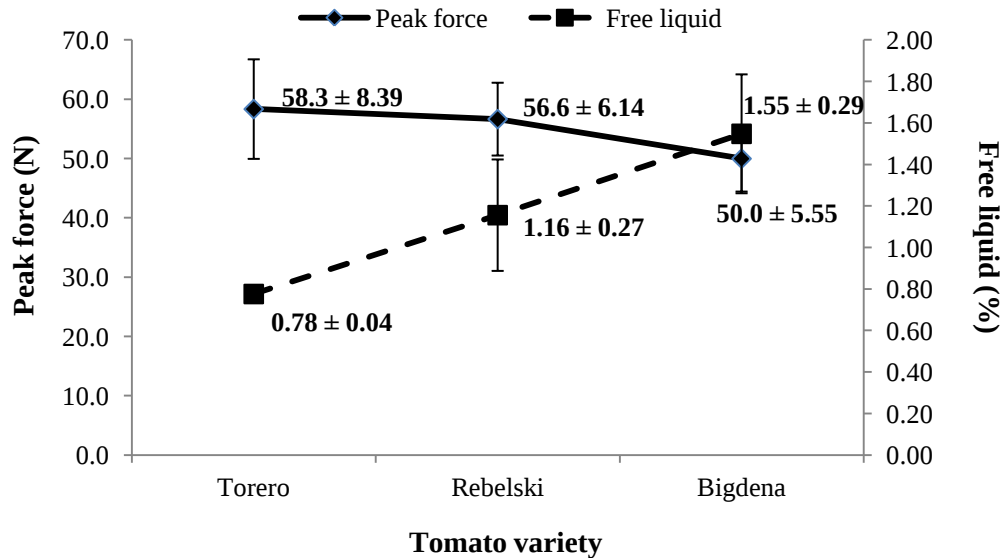


Figure 5.6: Mean ($\pm$ SE) peak force (N) and free liquid percentage (%) of three different tomato varieties including Torero, Rebelski, and Bigdena (n = 3).

5.4 DISCUSSION

The components of different types of slicers have been previously assessed for their role in bacterial transfer. When delicatessen meats were sliced using a commercial slicer, Vorst et al. (2006b) found that after slicing *L. monocytogenes*-inoculated turkey breast ($\sim 10^5$ CFU/cm²) with an application force of 4.5 kg, ~ 2.5 log CFU of *L. monocytogenes* transferred to the various slicer components including the table, back plate, guard, blade, and collection area. However, in the absence of an application force, significantly lower numbers of *Listeria* transferred to the table and back plate.

Various exponential, logarithmic, semi-logarithmic and Weibull models have been used to describe *L. monocytogenes* transfer during slicing of salmon (1), *E. coli* O157:H7 and *Staphylococcus aureus* transfer during slicing of cooked meat (128) and norovirus transfer during slicing of tomatoes (143). The R^2 value was normally obtained from Excel and has been most often used to describe the goodness of fit for the model. However, root mean square error (the square root of the variance) more properly represents the deviation of data fitting to the model. Therefore, in this study, RMSE was used, with the values for all variables being less than 0.5 (Table 5.2), meaning that the difference between prediction value and actual values are within 1 log. In this study, *Salmonella* populations on 20 previously uncontaminated tomatoes similarly decreased in all three trials as confirmed by the similar transfer decay rates (parameter B).

Opposite results were obtained for the electric slicer, with more consistent transfer to 20 uninoculated tomatoes seen from the blade (3.6 ± 0.4 log CFU) compared to the pusher and side plate (1.8 ± 0.4 log CFU) (Figure 5.5B). The pusher (equivalent to the back plate of the manual slicer) of the electrical slicer is made of high density polyethylene compared to the back plate of

the manual slicer, which is stainless steel. The side plate (equivalent to the bottom plate of the manual slicer) of the electric slicer, which is designed to keep the tomato inside the “cutting pocket” during slicing, also serves to minimize tomato contact during slicing. In addition to the equipment material, the cutting action of the electric slicer, which includes motorized blade movement and manual pushing, is different from the manual slicer, which solely relies on manually pushing the tomato through the blades.

Pathogen transfer during slicing of foods is a complex process. In this study, six variables were evaluated individually for their impact on *Salmonella* transfer during mechanical slicing of tomatoes. Except for surface wetness and tomato variety, no significant difference ($P > 0.05$) in the transfer decay rate (parameter B) or overall percent transfer was observed for the other four processing variables. These observations are in partial agreement with those of Aarnisalo et al. (1) who evaluated the impact of inoculum level, processing temperature and attachment time on *L.monocytogenes* transfer during slicing of salmon fillets. For these two studies, the wet and dry surfaces of inoculated tomatoes corresponded to the shorter (10 min) and longer (2.5 h) attachment times, respectively, in the salmon slicing study. In both of these studies, greater transfer was seen from wet as opposed to dry surfaces. However, in contrast to salmon slicing, changing the processing room temperature did not impact the extent of *Salmonella* transfer during tomato slicing. In both studies, the pathogen of interest decreased exponentially during slicing, leading to the development of corresponding transfer models for each scenario. However, *Listeria* transfer from an inoculated blade to salmon was far lower (0.00011 to 0.17%) than that seen for *Salmonella* in our tomato study (0.07 to 12.21%). These results were also likely influenced by the method of blade inoculation, which differed between the two studies.

The tomato variety significantly impacted *Salmonella* transfer during slicing. Further analysis of the three tomato varieties (Torero, Rebelski and Bigdena) indicated that Torero tomatoes, which yielded greater transfer, had a tougher texture and lower water content compared to the other two varieties (Figure 5.6). The free liquid released during slicing can potentially “wash off” attached bacteria from the blade, resulting in less bacteria transfer to subsequent tomatoes. These observations are also supported by the earlier work of Vorst et al. (169) who reported less transfer of *L. monocytogenes* during mechanical slicing of delicatessen turkey breast compared to salami, with similar findings also reported for delicatessen hams containing different levels of water.

In summary, the tomato variety along with the degree of ripeness, are important considerations when attempting to minimize potential cross-contamination during slicing. When possible, the tomato surface should also be dry before slicing. Given ongoing concerns regarding the safety of sliced tomatoes, the slicer should be intermittently disassembled, cleaned and sanitized to avoid the potential spread of bacterial pathogens during extended use. Such practical guidelines should be of interest to the fresh-cut produce industry, with these findings also useful in the development of future science-based transfer models for risk assessments.

CHAPTER 6:

Transfer and Sanitizer Inactivation of *Salmonella* during Simulated Commercial Dicing and Conveyance of Tomatoes

6.1 OBJECTIVE

The objectives of this study were to 1) quantify *Salmonella* transfer during simulated commercial dicing of tomatoes, 2) assess the efficacy of three sanitizer treatments against *Salmonella* during flume tank washing of diced tomatoes and 3) determine the efficacy of four sanitizers against *Salmonella* using a custom dual belt conveyor system during conveyance of diced tomatoes.

6.2 MATERIALS AND METHODS

6.2.1 Overall experimental design

Three key steps in the commercial production of diced tomatoes were simulated using pilot-scale equipment – dicing, washing, and conveying. During dicing, transfer of *Salmonella* from one batch of inoculated tomatoes to ten batches of uninoculated tomatoes as well as various surfaces of the dicer was quantified. Thereafter, three sanitizer treatments were assessed for the efficacy against *Salmonella* on diced tomato during flume washing, with the diced tomatoes, flume water, and flume tank surfaces evaluated for surviving salmonellae. Finally, four sanitizer treatments were evaluated against *Salmonella* on a dual-track conveyor equipped with a novel spray washing system. All experiments were conducted in triplicate.

6.2.2 Tomatoes

Unwaxed Roma tomatoes (*Solanum lycopersicum* 'Roma'; ~160 g/tomato) were obtained from a local distributor (Mastronardi Produce Ltd., Livonia, MI), stored at 4 °C to maintain good quality, and used within 7 d of delivery.

6.2.3 *Salmonella* strains

The same *Salmonella* strain as described in 3.3.3 was used for this study.

6.2.4 Inoculation of tomatoes

Tomatoes were dip-inoculated as described in 3.3.4 to contain ~ 5 log CFU/g *Salmonella*.

6.2.5 Tomato dicing

A mechanical dicer (Model H-A, Urschel Laboratories, Inc., Valparaiso, IN) (Figure 6.1) was used to assess *Salmonella* transfer, with one batch (0.9 kg) of uninoculated Roma tomatoes initially diced (5.7 mm) to prime the dicer, followed by one 0.9 kg batch of inoculated Roma tomatoes (~5 log CFU/g) and ten 0.9 kg batches of uninoculated tomatoes. Each batch of diced

tomatoes was collected individually in Whirl-Pak[®] bags (Nasco, Fort Atkinson, WI), with 50 g of diced tomatoes/batch analyzed for *Salmonella*.



Figure 6.1: The mechanical dicer used for this study.

6.2.6 Flume tank washing of diced tomato

The pilot-scale size processing line assembled for washing diced tomatoes consisted of a 3.6-m flume tank (~130-liter capacity; Heinzen Manufacturing Inc., Gilroy, CA), shaker table, and water tank (~1,000-liter capacity) (Figure 6.2). The 890 L of sanitizer solution in the water tank was circulated through the flume tank by a centrifugal pump (model XB754FHA, Sterling Electric, Inc., Irvine, CA) connected to a hard plastic discharge hose (4.5 m × 0.1 m). A custom-made stainless steel screen attached to the end of flume tank was used to retain the tomatoes for longer washing. To avoid over-flow issues during washing, the centrifugal pump was shut off after the flume tank filled with wash water after 1 min. Followed by 2-min of washing, the pump was reactivated to push the diced tomatoes onto shaker table for subsequent collection.

6.2.7 Preparation of flume water containing an organic load

In order to mimic commercial flume water, 4 L of tomato juice extracted from 11 kg of Roma tomatoes using a juice maker (Horizontal Pressmaster, Beloit Corporation, Dalton,

Massachusetts, USA) was added to 700 L of tap water, giving a wash solution containing 0.05% total solid after 2 min of recirculation with the pump. Since preliminary trial showed that tomato pulp contained larger particles, which blocked the mesh screen of shaker table and stopped the processing, tomato juice was made to create water containing total solids. Thereafter, 80 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 80 ppm mixed peracid (Tsunami 200, Ecolab), or 80 ppm total chlorine (1.1 ppm free chlorine) (XY-12, Ecolab) adjusted to pH 6.0 with citric acid (CA, Sigma Aldrich, St. Louis, MO) was added to the 700 L of water, with sanitizer-free water serving as the control. The physicochemical parameters, including sanitizer concentration, ORP, pH, and temperature, were monitored before and after washing. The testing method was followed as described in chapter 2 and chapter 3.

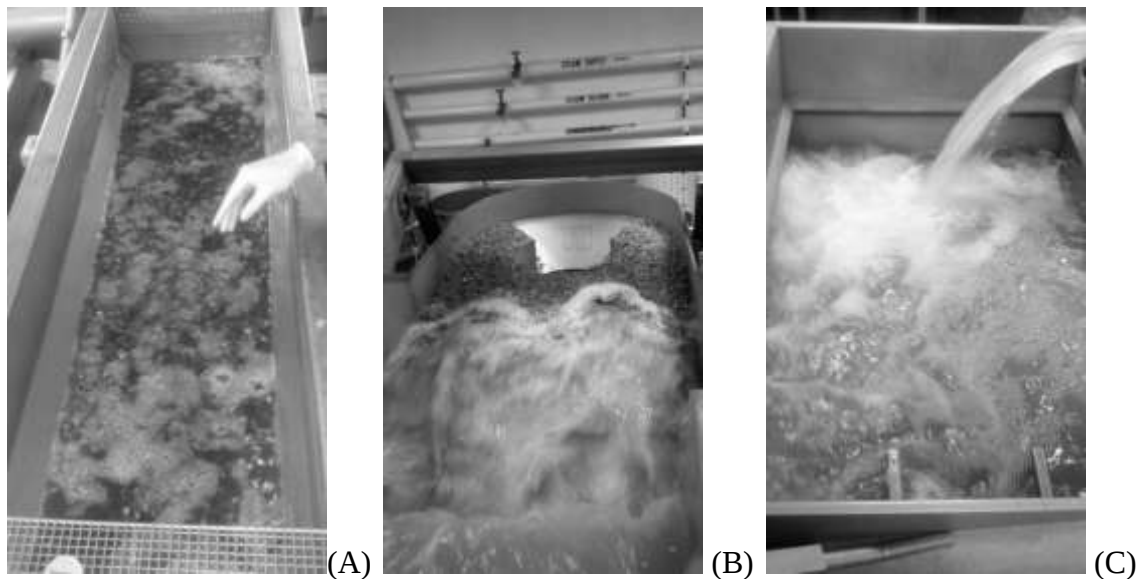


Figure 6.2: Pilot-scale tomato washing line: (A) flume tank, (B) shaker table, (C) water tank.

6.2.8 Preparation of diced tomatoes and sample collection

On the day of the experiment, 9.1 kg (20 lbs) of Roma tomatoes were inoculated to contain ~5 log CFU/g of *Salmonella* and diced, from which seven mesh bags (Pacon Inc.,

Baldwin Park, CA) were prepared to provide a quick sample collection during washing. Both ends of the mesh bag were tied and each bag contained 50 g of diced tomatoes. Thereafter, all diced tomatoes including the mesh bag samples were manually dumped into the flume tank. Every 20-s during 2 min of washing, tomato (1 mesh bag/time point) and water samples (30 ml) were collected in Whirl-Pak[®] bags containing sterile Difco Neutralizing Buffer (BD, Franklin Lakes, NJ). One tomato and one water sample were also collected from the shaker table and water tank. After washing, 1-ply composite tissues (171) were used to collect 4 water tank, 7 flume tank, and 6 shaker table samples (100 cm²/sample).

6.2.9 Conveyor belt inoculation

For conveyor belt inoculation, 4 kg of Roma tomatoes were diced, dip-inoculated to contain *Salmonella* Typhimurium LT2 at ~7 log CFU/g. Using a custom dual belt conveyor system (Figure 6.3), one 15 cm × 302 cm interlocking high-density polyethylene belt (Intralox, Harahan, LA) and one 15 cm × 302 cm smooth ThermoDrive rubber belt (Mol Industries, Grand Rapids, MI) were contaminated by passing through an inoculation tray that contained the 4 kg of inoculated diced tomatoes for 5 min at low speed. Thereafter, the inoculation tray was removed.

6.2.10 Conveyor belt sanitizer treatments and sample collection

Four sanitizers - 80 ppm peroxyacetic acid (Tsunami 100, Ecolab), 80 ppm mixed peracid (Tsunami 200, Ecolab), 80 ppm chlorine at pH 6.0 (XY-12, Ecolab), and 80 ppm chlorine in electrolyzed water at pH ~3.0 (PathoSans[®], Spraying Systems Co., Westfield, IN), along with a water control were evaluated by continuously spraying 30 L/h via a spray bar onto the running belts for 20 min. Two spray nozzles were installed under each belt, with ~60 PSI applied onto the pressurized tank. Before and after the sanitizer treatments, surface samples were collected in Whirl-pak bags[®] containing 50 ml of sterile neutralizing buffer from three different belt locations

using 1-ply composite tissues (171). Due to different dimension of belt piece, the sampling areas for the smooth and interlocking belts were 225 and 150 cm², respectively.

6.2.11 Microbiological analyses

Tomato samples collected in Whirl-pak[®] bags were homogenized by stomaching (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 300 rpm, and then surface-plated on trypticase soy agar (BD, Franklin Lakes, NJ) containing 0.6% yeast extract (BD), 0.05% ferric ammonium citrate (Sigma Aldrich, St. Louis, MO) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS) with or without 0.45 µm-membrane filtration (Milipore Corporation, Billerica, MA) to quantify *Salmonella* (limit of detection: 1 CFU/g). Water samples were either appropriately diluted in phosphate buffer and plated on TSAYE-FS or filtered through 0.45 µm-membranes to quantify *Salmonella* (limit of detection: 0.1 CFU/ml). Equipment and conveyor belt surface sample tissues were homogenized by stomaching for 1 min at 300 rpm and then similarly analyzed for salmonellae (limit of detection: 0.5, 0.29, and 0.33 CFU/100 cm² for water tank, flume tank, and shaker table, respectively; 0.76 and 1.13 CFU/100 cm² for smooth and interlocking belts, respectively). Plates were incubated at 37 °C for 24 h, after which all black colonies were counted as *S. Typhimurium* LT2. Selected colonies were confirmed as *Salmonella* using the Neogen Reveal[®] 2.0 kit (Neogen Corporation, Lansing, MI).

(A)



(B)



(C)



Figure 6.3: Dual belt conveyor system: (A) smooth (left) and interlocking (right) belt, (B) inoculation tray, (C) spray bar.

6.2.12 Statistical analysis

All experiments were performed in triplicate. *Salmonella* populations were converted to log CFU per g, per ml, or per 100 cm² for the tomato, water, and equipment surface samples, respectively. For the flume washing and conveyor studies, the analysis of variance and Tukey-Kramer HSD tests ($\alpha = 0.05$) were performed using JMP 11.0 (SAS Institute Inc., Cary, NC).

For the *Salmonella* transfer study, a two-parameter exponential decay model from a previous paper (140) was used to describe the *Salmonella* transfer pattern during dicing of tomatoes. The model used to fit the data is shown in Eq. (1):

$$Y = A \cdot \text{Exp} \left(\frac{X}{B} \right) \quad (1)$$

where Y (dependent variable) is the log CFU/g transferred and X (independent variable) is the weight of the uninoculated tomato that was diced. A and B are the transfer model parameters. The above equation was fitted using the *nlinfit* algorithm of MATLAB (R2012a, MathWorks, Natick, MA). The estimated parameters, root mean square error (RMSE) of the model, and asymptotic 95% confidence intervals of the parameters were then estimated.

6.3 RESULTS

6.3.1 *Salmonella* transfer during dicing

As shown in Figure 6.4 (original data in Appendix E, Table E1.1), all ten batches (0.9 kg/batch) of uninoculated tomatoes were cross-contaminated with *Salmonella* during dicing, with the numbers decreasing significantly ($P < 0.05$) from 3.3 to 1.1 log CFU/g with increased throughput. The exponential decay model fitted well to the *Salmonella* populations recovered from the previously uninoculated tomatoes, with the estimated values for parameters A and B being 3.4 log CFU/g and -8.31 kg, respectively. The asymptotic 95% confidence intervals for parameters A and B were 3.12 to 3.67 log CFU/g and -9.65 to -6.96, respectively. The root mean square error (RMSE), which indicates the goodness of fit for the model, was 0.25 log CFU/g.

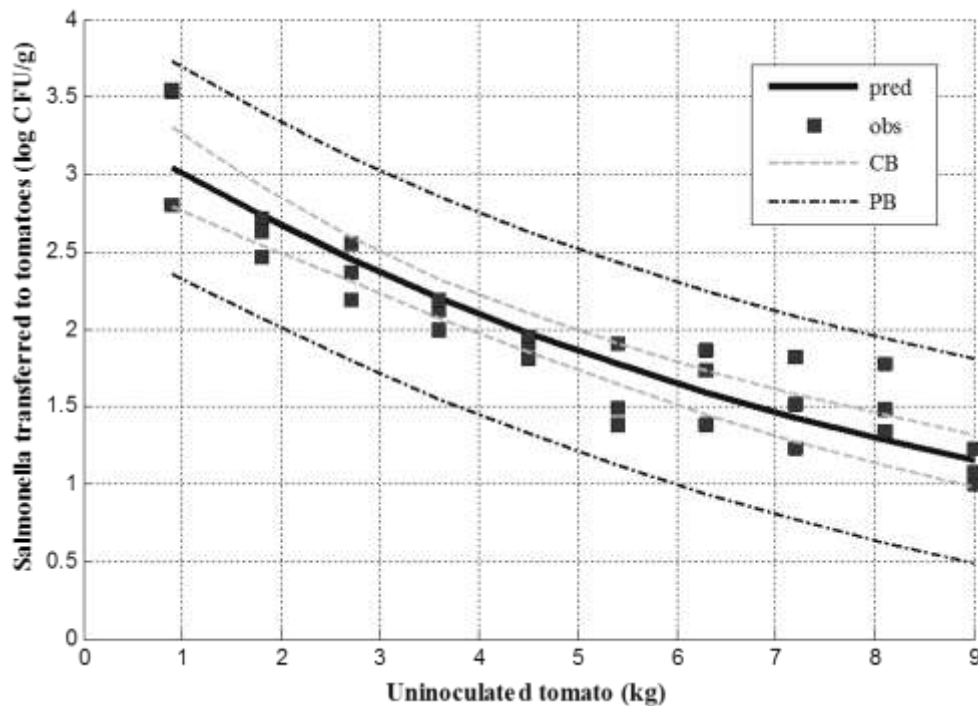


Figure 6.4: *Salmonella* transfer from one batch (0.9 kg) of inoculated tomato to 10 batches (9 kg) of uninoculated tomatoes through dicing (obs: observed value from experiment; pred: prediction value from modeling; CB: confidence bands for prediction line; PB: prediction bands for prediction line).

6.3.2 *Salmonella* populations on diced tomatoes during washing

As shown in Table 6.1, *Salmonella* populations on the inoculated tomatoes before washing were statistically similar for the water control and three sanitizer treatments ($P > 0.05$). During 2 min of washing, numbers of salmonellae on the diced tomato were significantly lower ($P \leq 0.05$) than on the initial inoculated tomato for all treatments. After processing, *Salmonella* populations on tomato samples collected from the shaker table ranged from 2.3 ± 0.3 to 3.3 ± 0.1 log CFU/g. All three sanitizer treatments were more effective ($P \leq 0.05$) in reducing *Salmonella* than the water control (1.3 ± 0.2 log CFU/g), with similar *Salmonella* log reductions of 2.3 ± 0.3 , 2.4 ± 0.4 , and 2.4 ± 0.1 log CFU/g for chlorine, mixed peracid, and peroxyacetic acid, respectively.

Table 6.1: Mean ($\pm$ SD) *Salmonella* populations (log CFU/g) on diced tomatoes during and after washing (n = 3).

	Water	Chlorine (80 ppm)	Mixed Peracid (80 ppm)	Peroxyacetic Acid (80 ppm)
Initial	a ^x 4.7 ± 0.2 A ^y	a 4.7 ± 0.1 A	a 4.7 ± 0.2 A	a 4.8 ± 0.3 A
20 s	b 3.4 ± 0.3	b 3.2 ± 0.1	b 3.3 ± 0.0	b 2.8 ± 0.2
40 s	b 3.6 ± 0.1	bc 2.9 ± 0.1	b 3.3 ± 0.1	b 3.0 ± 0.2
60 s	b 3.3 ± 0.2	bc 2.9 ± 0.1	bc 3.0 ± 0.2	b 2.9 ± 0.3
80 s	b 3.4 ± 0.1	b 3.0 ± 0.1	bc 3.1 ± 0.2	b 2.8 ± 0.2
100 s	b 3.2 ± 0.2	b 3.1 ± 0.1	bc 3.1 ± 0.1	b 2.6 ± 0.1
120 s	b 3.3 ± 0.2	bc 2.6 ± 0.1	bc 3.0 ± 0.3	b 2.9 ± 0.2
After process	b 3.3 ± 0.1	c 2.4 ± 0.0	c 2.4 ± 0.1	b 2.3 ± 0.3
Log reduction	1.3 ± 0.2 B	2.3 ± 0.3 A	2.4 ± 0.4 A	2.4 ± 0.1 A

^xMeans with the same letters in the same column are not significantly different ($P > 0.05$).

^yMeans with the same capital letters in the same row are not significantly different ($P > 0.05$)

6.3.3 *Salmonella* populations in wash water during washing

As shown in Table 6.2, for all four treatments, no significant differences ($P > 0.05$) in *Salmonella* population were seen for water samples collected at the different time points. The highest numbers of *Salmonella* were detected after 60, 80, 60, and 20 s of washing with water,

chlorine, mixed peracid, and peroxyacetic acid, respectively. After processing, *Salmonella* populations in the wash water were under the limit of detection ($-1.0 \log \text{CFU/ml}$) for all three sanitizers and were significantly lower ($P \leq 0.05$) than water alone ($1.5 \pm 0.2 \log \text{CFU/ml}$). For the physicochemical parameters of sanitizer treatments, after washing, the chlorine sanitizer significantly decreased from 81.3 ± 1.2 to 74.3 ± 2.1 ppm, with no significant difference observed for the rest of parameters (Appendix E, Table E1.2).

Table 6.2: Mean ($\pm$ SD) *Salmonella* populations ($\log \text{CFU/ml}$) in flume water during 2-min washing of diced tomatoes (n=3).

	Water	Chlorine	Mixed Peracid	Peroxyacetic Acid
20 s	a ^x 1.4 ± 0.3	a -0.8 ± 0.3	a -0.4 ± 0.3	a 0.0 ± 0.5
40 s	a 1.8 ± 0.3	a -0.8 ± 0.4	a -0.2 ± 0.1	a -0.3 ± 0.6
60 s	a 2.8 ± 0.6	a -0.9 ± 0.4	a 0.3 ± 0.6	a -0.4 ± 0.7
80 s	a 2.4 ± 0.5	a -0.2 ± 0.6	a -0.3 ± 0.4	a -0.5 ± 0.4
100 s	a 2.4 ± 0.6	a -0.9 ± 0.4	a -0.8 ± 0.4	a -0.7 ± 0.3
120 s	a 2.2 ± 0.5	a -1.0 ± 0.2	a -0.9 ± 0.2	a -1.1 ± 0.2
After process	a 1.5 ± 0.2 A ^y	a -1.3 ± 0.0 B	a -1.3 ± 0.0 B	a -1.3 ± 0.0 B

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

^y Means with the same capital letters in the same row are not significantly different ($P > 0.05$)

^z Limit of detection (LOD): $-1.0 \log \text{CFU/ml}$;

^z Half of LOD ($-1.3 \log \text{CFU/ml}$) was used when no *Salmonella* colony was recovered.

6.3.4 *Salmonella* populations on equipment surfaces after washing

After washing and draining the system, a film of water remained on all equipment surfaces, from which *Salmonella* was recovered. Regardless of the sanitizer treatment, similar numbers of salmonellae ($P > 0.05$) were recovered from the three different equipment surfaces. Using sanitizer-free water, the water tank, flume tank, and shaker table yielded *Salmonella* populations of -1.0 ± 0.7 , -0.8 ± 0.4 , and 0.2 ± 0.3 log CFU/100 cm², respectively. Within the same equipment surface, relatively higher numbers of *Salmonella* were recovered using water alone compared to the sanitizer, with *Salmonella* populations less than -1.5 log CFU/100 cm² for all three sanitizer treatments (Figure 6.5) (original data in Appendix E, Table E1.3).

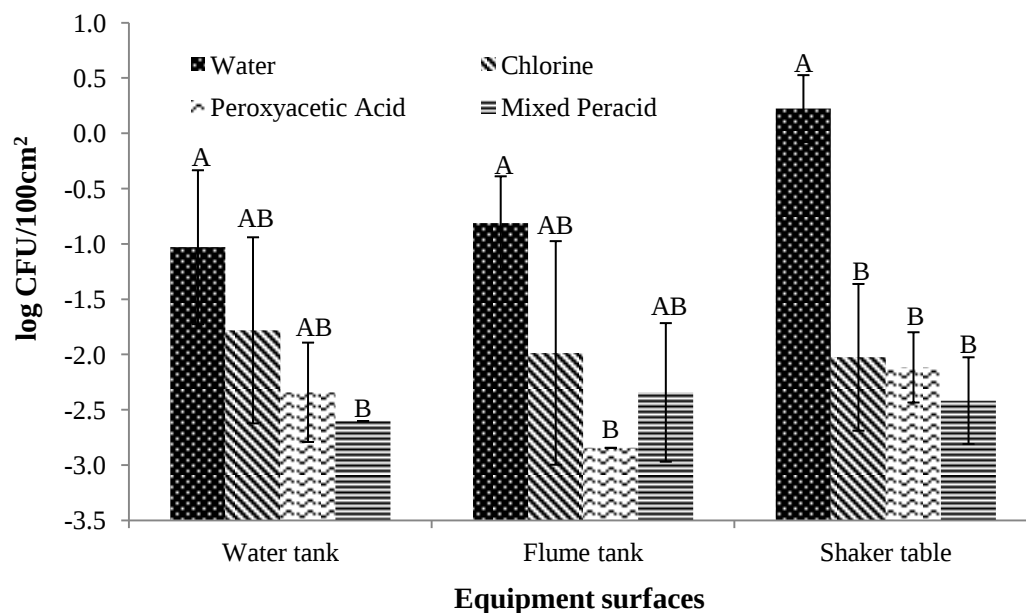


Figure 6.5: Mean ($\pm$ SD) *Salmonella* populations on equipment surfaces (Water tank; Flume tank; Shaker table) after washing of 9.1 kg of diced tomatoes ($n=3$). Means with the same letters within the same equipment surface are not significantly different ($P > 0.05$).

6.3.5 Sanitizer efficacy against *Salmonella* on conveyor belts during conveyance of diced tomato

As shown in Figure 6.6 (original data in Appendix E, Table E1.4), spraying the smooth and interlocking belts with sanitizer-free water decreased *Salmonella* populations only 1.5 logs. Compared to the water control, all four sanitizer treatments were more effective ($P \leq 0.05$) in reducing *Salmonella* on both conveyor belt surfaces. For the smooth and interlocking belts, *Salmonella* reductions were greater ($P \leq 0.05$) using mixed peracid (6.5 and 6.8 log) and peroxyacetic acid (5.9 and 6.1 log) as compared to chlorine (3.7 and 5.7 log) and electrolyzed water (3.5 and 4.5 log). Log reductions were similar for the smooth and interlocking belts ($P > 0.05$), except for chlorine, which was more efficacious for the interlocking belt.

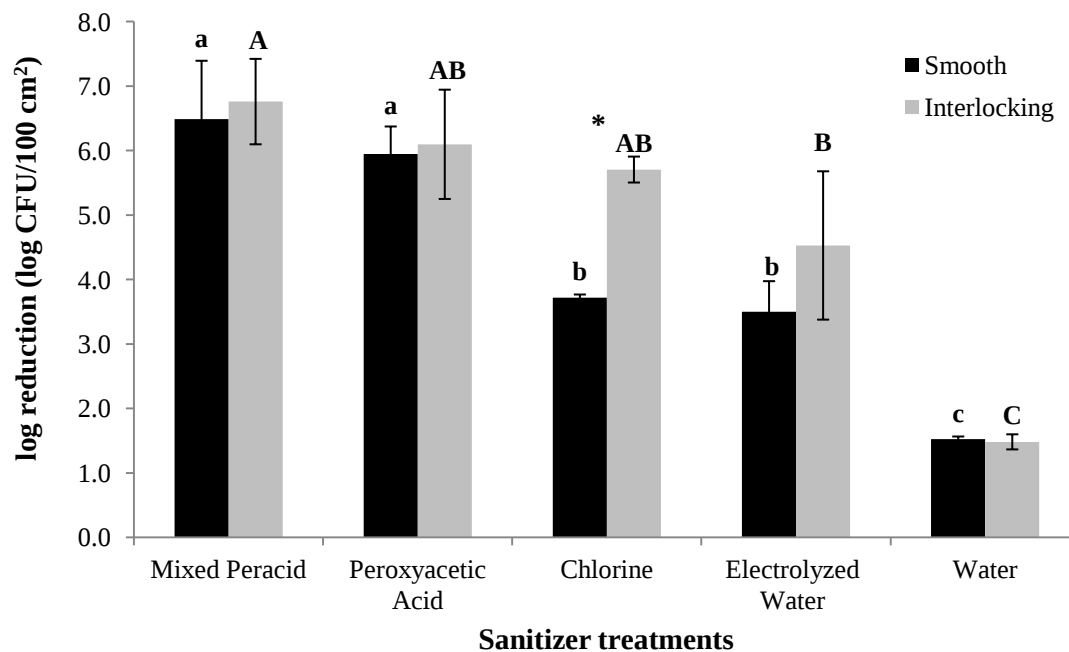


Figure 6.6: Mean ($\pm$ SD) *Salmonella* reductions against *Salmonella* contamination on conveyor belts after 20 min of 80 ppm sanitizer (mixed peracid, peroxyacetic acid, chlorine, electrolyzed water, or water) spray at speed of 30 L/h ($n=3$). Means with the same letters and capital letters are not significantly different for smooth and interlocking conveyor belts, respectively ($P > 0.05$). “*”: means are significantly different within the same sanitizer treatment ($P \leq 0.05$).

6.4 DISCUSSION

Most studies assessing bacterial transfer during slicing of delicatessen meats used the slice number (integer) and number of bacteria per slice (log CFU/slice) as the independent and dependent variables, respectively (1, 139, 140, 141). Shieh et al. (143) recently assessed the transfer of norovirus during slicing of tomatoes and fitted their quantitative data to a logarithmic decay model in Excel, using the sliced tomato number and norovirus population on the whole tomato (log MNV/tomato) as the independent and dependent variables, respectively. However, in the present work, the weight of the diced tomato (kg) and the numbers of salmonellae transferred to corresponding tomatoes (log CFU/g) were assigned as the independent and dependent variables, respectively, due to the large quantity of tomatoes that were processed. Although these fit the exponential decay transfer model well, increasing the amount of tomatoes processed in future work would lead to more accurate predictions of *Salmonella* transfer during mechanical dicing of tomatoes.

In a bench-top study conducted by Weissinger et al. (177) that evaluated the efficacy of a chlorine-based sanitizer against *Salmonella* Baildon in diced tomatoes, reductions of less than 1 log were observed after 40 s of immersion in water containing either 120 or 200 ppm free chlorine. In our pilot-scale study, the contact time between product and sanitizer solution was extended to 120 s and samples were collected at 20 s interval. In addition, a 0.05% total solid was added to the water containing sanitizer to better simulate commercial conditions. Although the chlorine level was lower than in the Weissinger study, greater *Salmonella* reductions in diced tomatoes were observed, which could be related to differences between *Salmonella* strains and different product /flume water ratios (1/10 and 1/15 for previous and current studies,

respectively). However, both peroxyacetic acid and mixed peracid showed similar efficacy against *Salmonella* and can potentially serve as alternatives to chlorine-based sanitizers.

Chemical sanitizers are far better able to decrease the microbial levels in flume water than on fresh produce during washing. Compared to the water control, all three sanitizer treatments decreased *Salmonella* populations in the water below the limit of detection after processing. These results differed from our previous sanitizer study with whole tomatoes, where chlorine was more effective than peroxyacetic acid and mixed peracid (data not shown). Similar results also observed by Davidson et al. (45) who assessed the efficacy of peroxyacetic acid, mixed peracid, and chlorine against *Escherichia coli* O157:H7 during processing of iceberg lettuce under pilot-scale conditions. Thus, the sanitizer must be matched to the particular product to be washed.

Unlike most of the bench top studies, in our study it is possible and critical to assess the *Salmonella* population on the equipment surfaces after the pilot-scale processing. While sanitizer application can clearly decrease the *Salmonella* population on equipment surfaces, no significant difference was observed between three different sanitizers used in this study. The information collected from equipment surface testing can be useful for the overall environmental testing and provide guidelines for post-processing sanitation practice.

Mechanical conveyors are extensively used in the food industry, with different types of conveyor belt materials having been developed based on the specific application. In this study, a sanitizer spray was simultaneously applied to an interlocking high-density polyethylene or smooth rubber belt. Irrespective of belt type, all four sanitizer spray treatments decreased *Salmonella* populations > 3 log compared to water (~ 1.5 log reduction). These findings differ from a previous study that mimicked *Listeria monocytogenes* contamination during conveying of

a turkey product (182). However, both studies showed that chlorine was no more effective than peroxyacetic acid or hydrogen peroxide based sanitizers. Considering the lower cost and ease of preparation, chlorine-based electrolyzed water provides an attractive alternative to traditional chlorine-based sanitizers for spray applications.

In conclusion, a pilot-plant scale study was conducted to mimic commercial production of diced tomatoes, which included dicing, washing, and conveying. An exponential decay model was fitted to the *Salmonella* transfer data collected during mechanical dicing. During flume tank washing, peroxyacetic acid, mixed peracid, and chlorine showed similar efficacy against *Salmonella* on diced tomatoes. While selection of the appropriate sanitizer is critical for effective washing, more attention should be given to monitoring the organic load that accumulates in flume water from diced tomatoes during extended processing. The application of spray sanitation technology on conveyor belts showed great potential to decrease cross-contamination and enhance the overall safety of diced tomatoes. These findings suggest some strategies to the produce industry and will be useful in future risk assessments.

CHAPTER 7:

***Salmonella* Attachment and Early-biofilm Formation on Tomatoes, High-Density Polyethylene and Stainless Steel as Impacted by Substrate, pH, and Temperature**

7.1 OBJECTIVE

Given the lack of data on industrial conditions that support attachment and biofilm formation, the objectives of this study were to 1) assess effect of temperature (4, 10, 23 °C) and pH (4.6 or 7.0) on changes in *Salmonella* viability, morphology, surface hydrophobicity, and surface charge and 2) evaluate the impact of temperature (4, 10, and 23 °C) and wash water quality (0 and 10% tomato organic load) on *Salmonella* attachment and biofilm formation on tomatoes as well as stainless steel and high density polyethylene surfaces found in packinghouses.

7.2 MATERIALS AND METHODS

7.2.1 *Salmonella* strains

The same *Salmonella* strain as described in 3.3.3 was used for this study.

7.2.2 Tomatoes and preparation of a 10% organic load in water

Red round tomatoes (*Solanum lycopersium* L.) were obtained from a local distributor (Mastronardi Produce Ltd., Livonia, MI), stored at 4 °C to keep fresh, and used within 7 d of delivery. An organic load of 10% (w/v) was achieved by homogenizing 100 g of tomatoes for 5 min in a household blender (Model BLC10650MB, Black & Decker, New Britain, CT) and then adding the homogenate to 900 ml of water.

7.2.3 Surface materials

In addition to tomatoes, two commonly used materials found in commercial packinghouses - stainless steel (Grade 304 with brush finish) and high density polyethylene (HDPE), were used for the attachment and biofilm studies. The stainless steel and HDPE were cut into 2 × 4 cm coupons and sterilized in an autoclave before use.

7.2.4 *Salmonella* viability and morphology

To measure *Salmonella* viability, *Salmonella* Typhimurium LT2 cells grew overnight in 900 ml of TSBYE broth was added into 4.1 liter of distilled water and incubated at 4, 10, and 23 °C, respectively. Since the same bacteria solution was used in our previous studies for tomato inoculation, it was chosen for viability study in this chapter. At day 0, 2, 4, and 8, 1-ml aliquots were withdrawn, serially diluted in Phosphate Buffer Solution (PBS), surface plated on TSAYE supplemented with 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate (TSAYE-FS) and incubated at 37 °C for 48 h to quantify *Salmonella*. At the same time, the morphological changes in *Salmonella*, including size and filament number, were quantified for the three

different incubation temperatures by calculating the percentages of cells that were <2, 2 to 4, and >4 times the typical cell length (2 μm) in three microscope fields of view (100 cells per field of view), as described by Mattick and et al. (98). Phase contrast was applied for filament imaging using a confocal laser scanning microscope (Olympus FluoViewTM FV1000, Olympus America, Inc., Centerville, PA, USA), with a PLN 40 $\times$ PH objective (NA: 0.65), 2.0 $\times$ zoom magnification, and excitation laser of 488 nm. The average bacterial cell length at the different temperatures was also calculated at day 8, using the FV10-ASW viewer software (Olympus FluoViewTM FV1000, Olympus America, Inc., Centerville, PA, USA).

7.2.5 Surface hydrophobicity of *Salmonella*

Surface hydrophobicity was determined as described by Li and McLandsborough (92). Briefly, the *Salmonella* culture containing ~ 8 log CFU/ml was centrifuged, washed three times with PBS solution, and re-suspended in sterile PBS. Half of the bacterial suspension was adjusted to pH 4.6 to mimic the high acid environment encountered during tomato dicing, with the other half maintained at pH 7.0. During 8 d of incubation at the three different temperatures, samples were collected at day 0, 2, 4, and 8. Each bacterial suspension was split into four 4-ml aliquots, after which 1 ml of xylene was added to 3 tubes, with the fourth tube without xylene serving as the control. All tubes were vortexed for 2 min and then incubated in a 37 $^{\circ}\text{C}$ water bath for 30 min. After incubation, the optical density of 2 ml liquid at the lower level was read at 600 nm with a spectrophotometer (Model SB-100XR, Spectronics Corporation, Westbury, NY), which was zeroed with PBS. The absorbency ratio between the sample tube (A_s) and control tube (A_c) was used to calculate the percent adhesion to xylene (%) = $(A_c - A_s) / A_c \times 100$.

7.2.6 Hydrophobicity of solid surfaces

Hydrophobicity of the three surfaces (tomato, stainless steel, and HDPE) was evaluated using a Goniometer in the College of Engineering at Michigan State University. The hydrophobicity was determined by measuring the surface contact angles from photographs of a 20 μ l drop of water on the surface, with a greater contact angle indicating greater hydrophobicity. The interaction between *Salmonella* and the tomato surface was also investigated by determining the extent of *Salmonella* attachment to tomatoes under different temperatures. Briefly, two types of *Salmonella* inoculum (~ 8 log CFU/ml) were prepared in water containing a 0 and 10% organic load (substrate), respectively. One tomato was immersed in a plastic package (IngeoTM, clear Lam Packaging Inc., Elk Grove Village, IL) containing 250 ml of inoculum and incubated at 4, 10, or 23 $^{\circ}$ C, respectively. After 24 h of incubation, the tomatoes were rinsed under running distilled water for 30 s, after which the center portion of each tomato was excised using a sterilize knife, placed in a Whirl-Pak[®] bag (Nasco, Fort Atkinson, WI) containing 20 ml of PBS, homogenized by stomaching and analyzed for numbers of *Salmonella* as previously described.

7.2.7 Surface charge of *Salmonella*

Salmonella surface charge was measured by determining the zeta potential (ZP) as described by Chia et al. (40). Briefly, the *Salmonella* culture was centrifuged, washed three times with PBS solution, and re-suspended in sterile PBS solution to contain ~ 8 log CFU/ml, followed by adjusting half of the solution to pH 4.6. These bacterial suspensions were then incubated at 4, 10, and 23 $^{\circ}$ C for 8 d. At day 0, 2, 4, and 8, 10 ml of the suspension was collected for zeta potential (ZP) measurement using a ZetaSizer (Nano-ZS; Malvern Instruments, Ltd. Worcestershire, UK) in the Department of Plant, Soil and Microbial Sciences at MSU.

7.2.8 *Salmonella* attachment and early-biofilm formation on tomatoes

Two different bacterial suspensions ($\sim 8 \log$ CFU/ml) were prepared to contain a 0 or 10% organic load as previously described. Thereafter, each of three 2.5 cm-diameter marked areas on the tomato surface was spot inoculated with 100 μ l of the culture. After 2 h of air drying, the tomatoes were incubated at 4, 10, and 23 $^{\circ}$ C for 6 d. On days 0, 2, and 6, the three previously inoculated circular areas on each tomato were excised using a sterile knife, placed in Whirl-Pak[®] bags containing 20 ml of PBS and analyzed for numbers of *Salmonella* as previously described. On day 6, the extent of biofilm formation on the tomato surface was also visually assessed using Confocal Scanning Laser Microscopy as described below. Since the specific biofilm composition was not analyzed and the detection methods applied were targeted in viable *Salmonella* cells, the “biofilm” formed in this study was likely still in the early stage. Therefore, the term of “early-biofilm” was used in this study to more accurately represent *Salmonella* development on different surfaces.

7.2.9 *Salmonella* attachment and early-biofilm formation on surface materials

As described by Dourou et al. (51), the sterile stainless steel and HDPE coupons were individually inserted upright into 50-ml centrifuge tubes containing 15 and 10 ml of aforementioned inoculums, respectively. The tubes were incubated for 6 d at 4, 10, or 23 $^{\circ}$ C. On days 0, 2, and 6, individual coupons were removed from the centrifuge tubes, rinsed with 20 ml of sterile deionized water (SDW) to remove unattached and loosely attached cells of *Salmonella*, and transferred to tubes containing 30 and 20 ml of maximum recovery diluent (MRD, 0.85% NaCl and 0.1% peptone) for stainless steel and HDPE respectively, along with 20 glass beads. The tubes were then vortexed for 2 min to detach adherent/attached cells and quantitatively

analyzed for *Salmonella*. Similarly, on day 6, biofilm formation on stainless steel and HDPE was visually assessed using Confocal Scanning Laser Microscopy as described below.

7.2.10 Confocal microscopy imaging

For confocal microscopy imaging, a Live/dead biofilm viability kit (FilmtracerTM, LIVE/DEAD[®] Biofilm Viability Kit, Life TechnologiesTM, Carlsbad, CA, USA) was used, with live and dead cells staining appearing green and red, respectively. On the day of imaging, the fluorescent stain was prepared by adding 3 µl of SYTO 9 stain and 3 µl of propidium iodide stain to 1 ml of filter-sterilized water. Then, 200 µl of staining solution was pipetted onto the imaging zone of tomato or equipment surface samples and placed in a staining dish, followed by 20-30 min of incubation in the dark at room temperature. After incubation, the samples were gently rinsed with filter-sterilized water and imaged using a confocal laser scanning microscope (Zeiss LSM5 Pascal, Zeiss Microimaging, Jena, Germany) equipped with a Plan-Apocromat 63×/1.4 oil DIC objective and an excitation laser set at 488 nm. BP 475-525, BP 505-530 and LP 545 emission filters were used for reflective, green and red imaging, respectively.

7.2.11 Microbial analysis

Similar microbial analysis was performed as described in 6.3.11.

7.2.12 Statistical analysis

Salmonella populations were converted to log CFU per ml, per gram, per tomato, or per coupon, respectively. Analysis of variance and the Tukey-Kramer HSD test ($\alpha = 0.05$) were performed using JMP 11.0 (SAS Institute Inc., Cary, NC). In addition, the effect of time, pH and temperature both alone and in combination on surface hydrophobicity and *Salmonella* surface charge were analyzed in JMP, using the “fit model” function. Similarly, the effect of time,

substrate, temperature, and their interactions on *Salmonella* attachment and biofilm formation on tomatoes, stainless steel, and HDPE were analyzed.

7.3 RESULTS

7.3.1 Bacteria viability

Regardless of temperature, the *Salmonella* population decreased from day 0 (8.9 ± 0.04 log CFU/ml) and then increased with final populations of 9.2 ± 0.01 , 9.3 ± 0.01 , and 8.9 ± 0.02 log CFU/ml after 8 d of incubation at 23, 10, and 4 °C, respectively (Figure 7.1). At day 2 and 4, *Salmonella* populations were higher ($P \leq 0.05$) at 23°C as opposed to 4 and 10°C. After 8 d, *Salmonella* populations were significantly higher at 23 and 10 °C as compared to 4 °C ($P \leq 0.05$) (original data in Appendix F, Table F1.1).

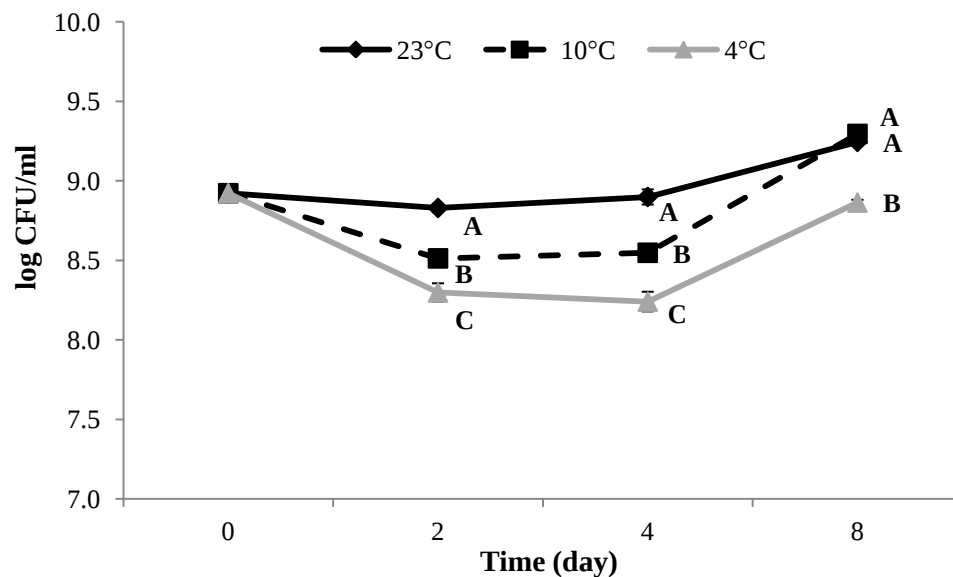


Figure 7.1: Mean ($\pm$ SE) viable *Salmonella* population during 8 days of incubation in the inoculums broth (mixture of 900 ml of TSBYE broth and 4.1 liter of distilled water) at 23, 10 and 4°C ($n = 3$). Means with the same capital letters on the same day are not significantly different ($P > 0.05$).

7.3.2 Bacterial morphology

Similar to previous studies (98, 163), some *Salmonella* cells elongated during prolonged incubation at 4°C as compared to 23 and 10 °C, under which no significant difference of cell morphology occurred. Incubation at lower temperatures resulted in higher percentages of *Salmonella* cells that were 2 times the length of typical cells (2 µm). At 10 and 4 °C, 61.3 ± 3.5 and 87.7 ± 0.7 % of the total cells were 2 – 4 times the length of typical cell (Table 7.1). However at 23 °C, most (88 ± 4.4 %) cells were less than $2 \times$ the length of typical cells. In addition, the cell length on day 8 was measured and the average length was calculated (Figure 7.2). The average cell length after 8 d of incubation was 5.80 ± 1.35 µm at 4 °C compared to 4.71 ± 1.67 and 3.16 ± 0.80 µm at 10 and 23 °C, respectively. Filamentous *Salmonella* cells were clearly observed after 4 d of incubation at 4 °C (Figure 7.3), with one elongated cell reaching a maximum length of 12.5 µm on day 8.

Table 7.1: Mean ($\pm$ SE) percentages of *Salmonella* cells of different lengths divided into three different categories after 8 d of incubation at 23, 10 and 4°C (n = 3).

Temperature	Percentage (%)		
	< 2 typical lengths ^a	2 - 4 typical lengths	> 4 typical lengths
23 °C	88 ± 4.4	12 ± 4.4	0
10 °C	36 ± 3.1	61.3 ± 3.5	2.7 ± 0.7
4 °C	6.3 ± 0.3	87.7 ± 0.7	6.0 ± 0.6

^aTypical length: 2 µm

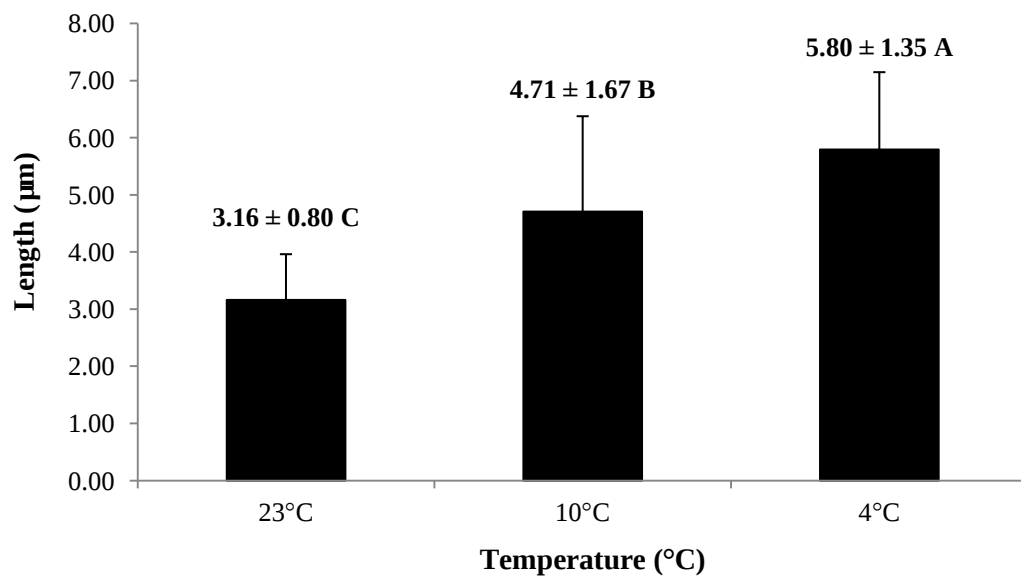
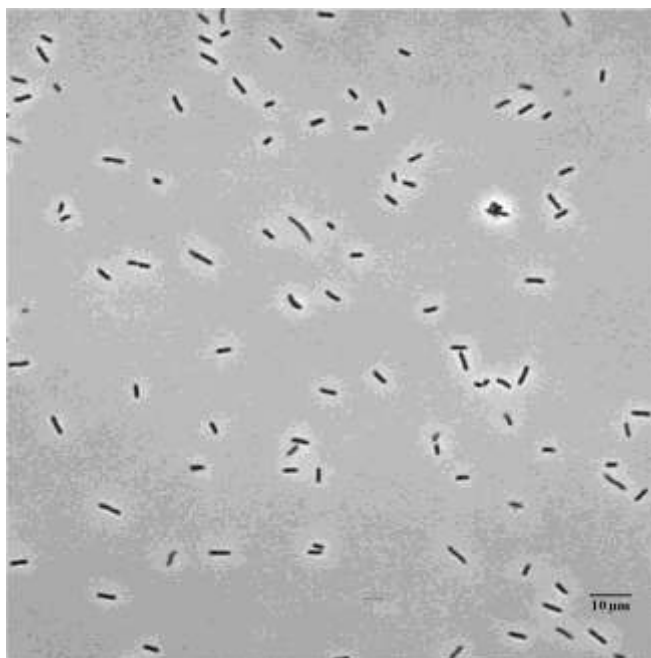


Figure 7.2: Mean ($\pm$ SD) *Salmonella* cell length after 8 d of incubation in the inoculums broth (mixture of 900 ml of TSBYE broth and 4.1 liter of distilled water) at 23, 10 and 4°C (n = 300). Means with the same letters are not significantly different ($P > 0.05$).

(A)



(B)

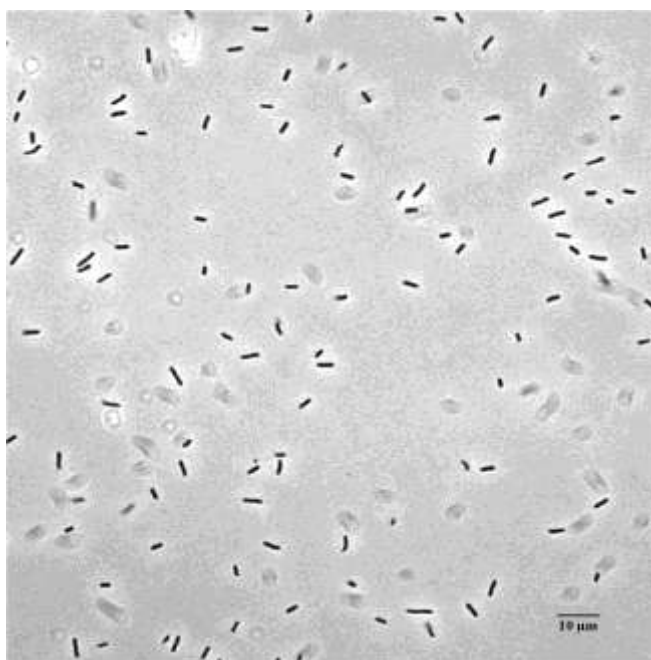
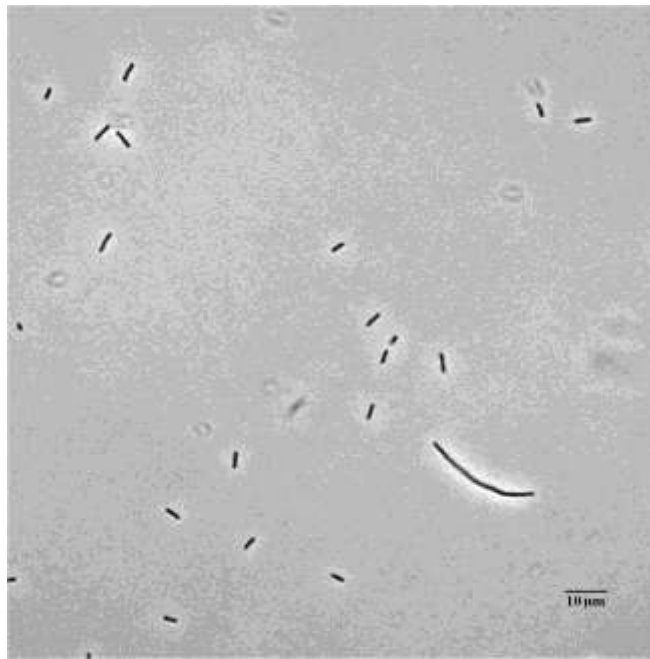


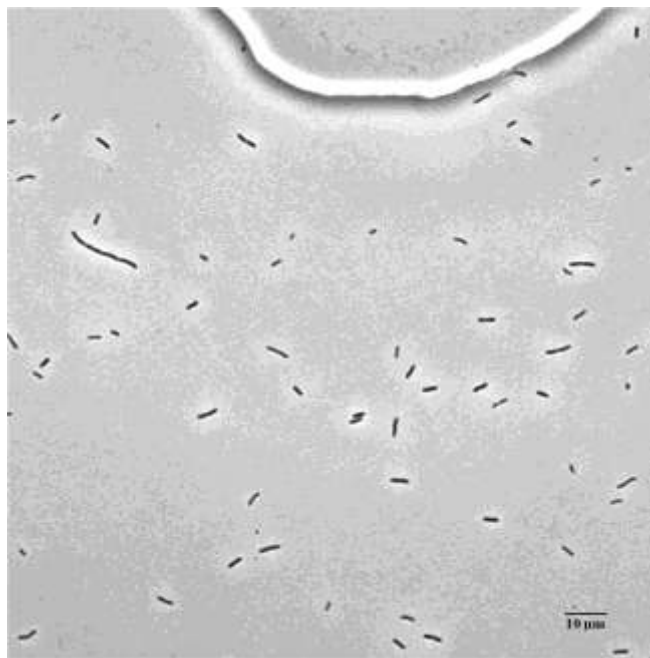
Figure 7.3: *Salmonella* morphology after 0 (A), 2 (B), 4 (C), and 8 (D) d of incubation at 4 °C.

Figure 7.3 (cont'd)

(C)



(D)



7.3.3 Surface hydrophobicity of *Salmonella*

The hydrophobicity of *Salmonella* cells at pH 4.6 decreased dramatically from day 0, with an increase from day 2 and day 4 for the cells stored at 10 °C and 4 °C, respectively (Figure 7.4). However, surface hydrophobicity steadily decreased from 62.61 ± 0.96 to $18.29 \pm 2.71\%$ during 8 d of incubation at 23 °C at pH 7.0, surface hydrophobicity remained relatively stable regardless of temperature. After 8 d, surface hydrophobicity was significantly higher ($P \leq 0.05$) for cells incubated at 10 and 4 °C under acid conditions compared to the other treatments (original data in Appendix F, Table F1.2). In addition to incubation time, both pH and temperature significantly impacted cell surface hydrophobicity, with P values of < 0.0001 and 0.0002 , respectively. When three factors interact with each other, all the interactions significantly affect the surface hydrophobicity, except the interaction between pH and temperature ($P = 0.1608$) (Table 7.2).

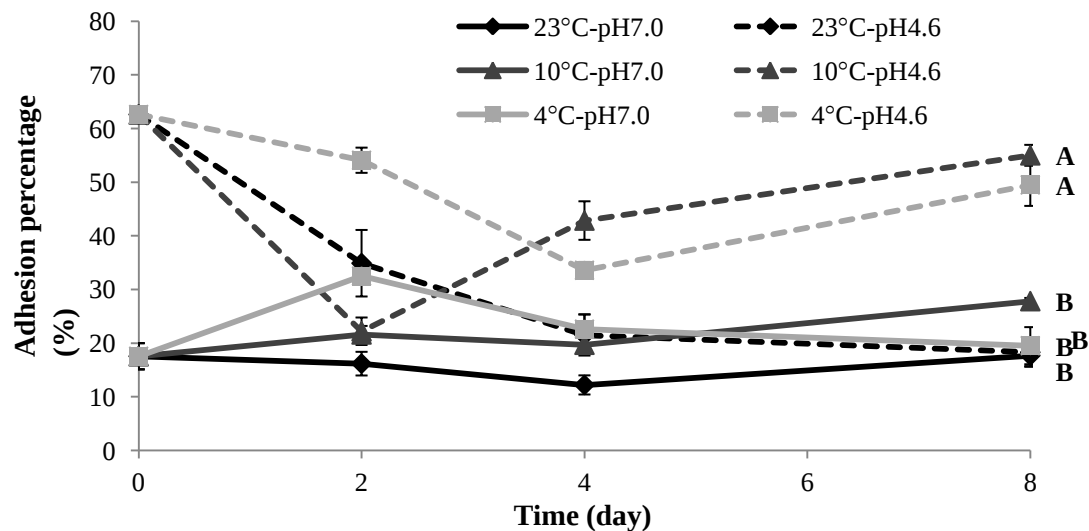


Figure 7.4: Mean ($\pm$ SE) *Salmonella* surface hydrophobicity (calculated as percent adhesion to xylene) during 8 d of incubation at pH 4.6 and 7.0 at 23, 10 and 4°C in PBS solution ($n = 3$). Means with the same letters on day 8 are not significantly different ($P > 0.05$).

Table 7.2: The effect of time, pH, temperature, and their interactions on *Salmonella* surface hydrophobicity and surface charge after 8 d of incubation.

Effect tests	Surface hydrophobicity		Surface charge	
	F ratio	Prob > F	F ratio	Prob > F
Time	4.8095	0.0319	53.461	<0.0001
pH	87.1375	<0.0001	22.4517	<0.0001
Temperature	15.1953	0.0002	0.235	0.6295
Time * pH	8.2866	0.0054	86.0341	<0.0001
Time * Temperature	4.2635	0.043	4.7616	0.0328
pH * Temperature	2.0134	0.1608	0.9419	0.3355
Time * pH * Temperature	4.2142	0.0442	0.9003	0.3463

7.3.4 Hydrophobicity of solid surfaces

Surface hydrophobicity of tomatoes, HDPE, and stainless steel was assessed by measuring the contact angle of a drop of liquid on the surface, with a greater contact angle representing higher hydrophobicity (Figure 7.5). As shown in Figures 7.6, all three tested surfaces were significantly different ($P \leq 0.05$) from each other, with contact angles of 100.02 ± 0.48 , 63.06 ± 1.83 , and $35.56 \pm 2.78^\circ$ for tomatoes, HDPE, and stainless steel, respectively.

(A)

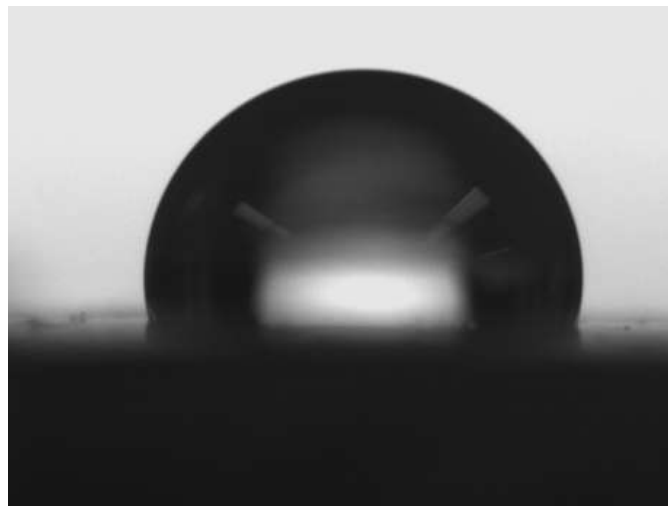
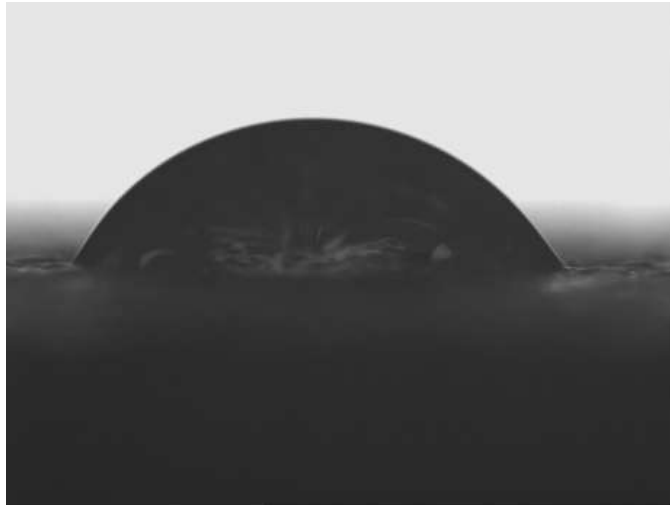


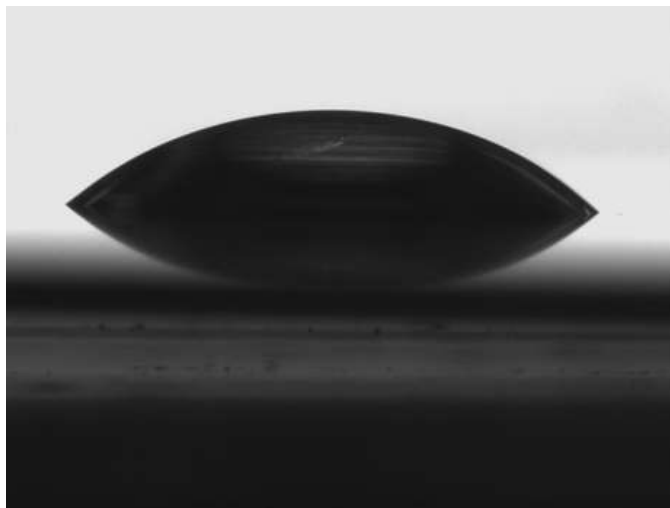
Figure 7.5: The image of contact angle images for A) tomato (100.02°); B) HDPE (63.06°), and C) stainless steel (35.56°).

Figure 7.5 (cont'd)

(B)



(C)



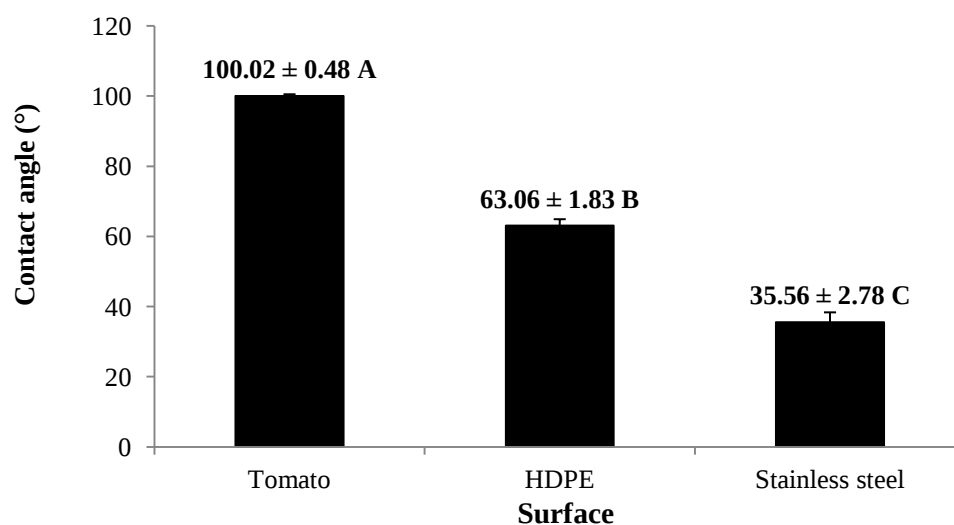


Figure 7.6: Mean ($\pm$ SE) contact angles for tomatoes, HDPE and stainless steel surfaces as evaluated using Goniometer ($n = 3$). Means with the same letters are not significantly different ($P > 0.05$).

7.3.5 *Salmonella* attachment to tomatoes at different temperatures

After 24 h of immersion in the inoculum, *Salmonella* remained attached to the tomato surface. At pH 4.6, significantly greater ($P \leq 0.05$) numbers of *Salmonella* attached to tomatoes at 4 °C (4.4 ± 0.2 log CFU/g) than 23 °C (2.8 ± 0.5 log CFU/g) and 10 °C (2.6 ± 0.4 log CFU/g). These findings supported the results from hydrophobicity testing of *Salmonella* cells, which showed higher hydrophobicity at 4 °C as compared to 23 and 10 °C. However, at pH 7.0, no statistically significant difference ($P > 0.05$) was observed at different temperatures (Figure 7.7).

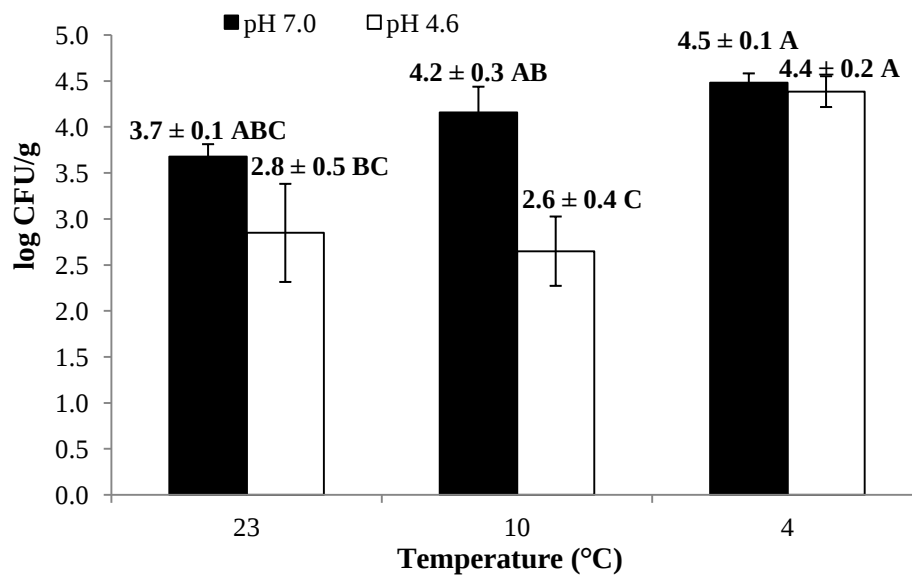


Figure 7.7: Mean ($\pm$ SE) *Salmonella* population attached to tomato surfaces after 24 h of immersion in inoculums (~ 8 log CFU/ml *Salmonella*) at pH 4.6 and 7.0 at 23, 10, and 4°C (n = 3). Means with the same letters are not significantly different ($P > 0.05$).

7.3.6 Surface charge of *Salmonella*

All *Salmonella* cells carried a negative charge, which was consistent with previous work (73). At pH 4.6, the surface charge increased from -16.58 ± 0.19 on day 0 to -10.35 ± 0.39 , -12.03 ± 0.18 , and -11.73 ± 0.45 on day 8 for cells incubated at 23, 10, and 4 °C, respectively. However, the surface charge remained unchanged at pH 7.0 regardless of incubation temperature. On day 8, the cell surface charge was significantly higher ($P \leq 0.05$) at pH 4.6 compared to 7.0 (Figure 7.8) (original data in Appendix F, Table F1.3). Time and pH very significantly impacted cell surface charge, with P value less than 0.0001 for both factors (Table 7.2). In addition, the interaction between time and pH, as well as time and temperature, significantly affected *Salmonella* surface charge during 8 d of incubation, with P value of < 0.0001 and 0.0328, respectively.

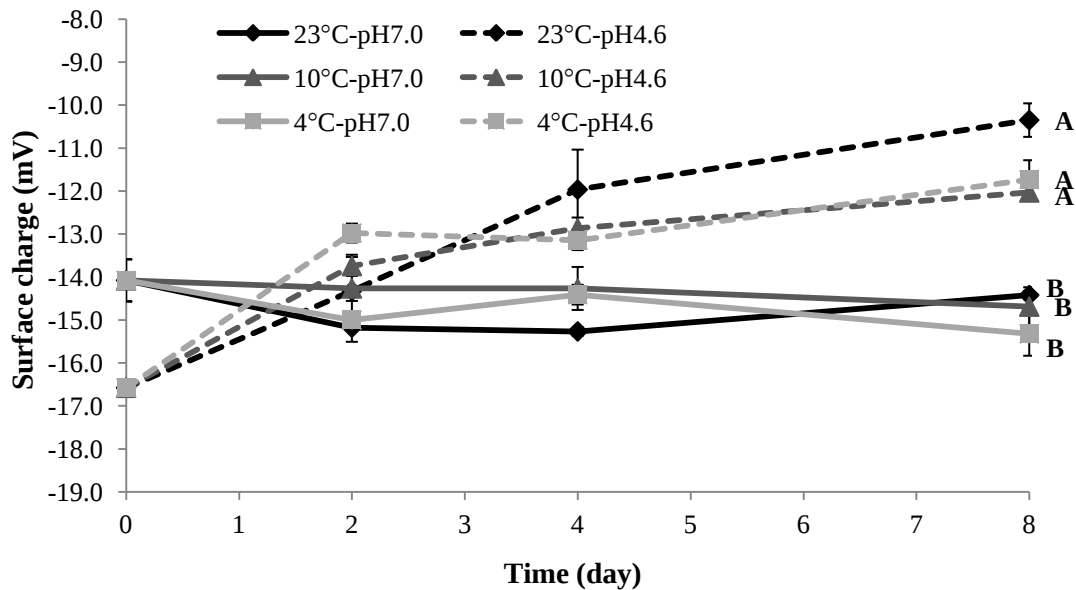


Figure 7.8: Mean ($\pm$ SE) *Salmonella* surface charge (measured as zeta potential using ZetaSizer) during 8 d of incubation in PBS solution at pH 4.6 and 7.0 at 23, 10 and 4°C (n = 3). Means with the same letters on day 8 are not significantly different ($P > 0.05$).

7.3.7 Correlation between surface hydrophobicity and surface charge

The correlation between surface hydrophobicity and surface charge of *Salmonella* was also analyzed. While the overall correlation between surface hydrophobicity and surface charge was poor ($R^2 = 0.016$), good correlations were observed for pH 7.0 at 10 °C and pH 4.6 at 23 °C, with R^2 values of 0.97 and 0.91, respectively (Table 7.3). The four remaining pH and temperature combinations were poorly correlated, with R^2 values less than 0.7. Thus, the correlation between surface hydrophobicity and surface charge might be pH and temperature dependent. More research is needed to illustrate a clear relationship between bacterial surface hydrophobicity and surface charge.

Table 7.3. Correlation between *Salmonella* surface hydrophobicity and surface charge.

Correlation between surface hydrophobicity and surface charge (R^2)		Temperature (°C)		
		23	10	4
pH	7.0	0.61	0.97	0.14
	4.6	0.91	0.11	0.33

7.3.8 *Salmonella* attachment and early-biofilm formation on tomatoes surface

Two types of substrates, water with and without 10% organic load, were used to mimic two different pH environments encountered in previous studies. As shown in Figure 7.9A, initial numbers of *Salmonella* on tomatoes were higher using a 10% (5.8 ± 0.1 log CFU/tomato) as compared to a 0% organic load (4.4 ± 0.2 log CFU/tomato). Thereafter, the population decreased and became relatively stable. Regardless of the organic load, greater biofilms ($P \leq 0.05$) formation was observed for tomatoes incubated at 23 as compared to 4 °C (original data in Appendix F, Table F1.4). When time, substrate, temperature, and their interactions were assessed, both time and temperature significantly affected biofilm formation on tomatoes, with P values of 0.0004 and <0.0001 , respectively. In addition, the interaction between time and temperature significantly affected *Salmonella* biofilm formation, with a P value of 0.0032 (Table 7.4).

7.3.9 *Salmonella* attachment and early-biofilm formation on stainless steel surfaces

Unlike tomatoes, biofilm formation on stainless steel was relatively consistent during 6 d of incubation. As shown in Figure 7.9B, among all six treatments, biofilm formation only increased slightly at 23 °C with the 0% organic load. On day 6, three 0% organic load treatments yielded stronger biofilm formation than the other three 10% organic load treatments, with *Salmonella* biofilm formation ranging from 3.4 ± 0.1 to 5.8 ± 0.5 log CFU/coupon (original data in Appendix F, Table F1.5). This result was also confirmed by the interaction test results, which showed that both time and substrate significantly affected *Salmonella* biofilm formation on

stainless steel. In addition, the interactions between time and substrate as well as substrate and temperature can significantly affect *Salmonella* biofilm formation, with *P* value of 0.0038 and 0.0279, respectively (Table 7.4).

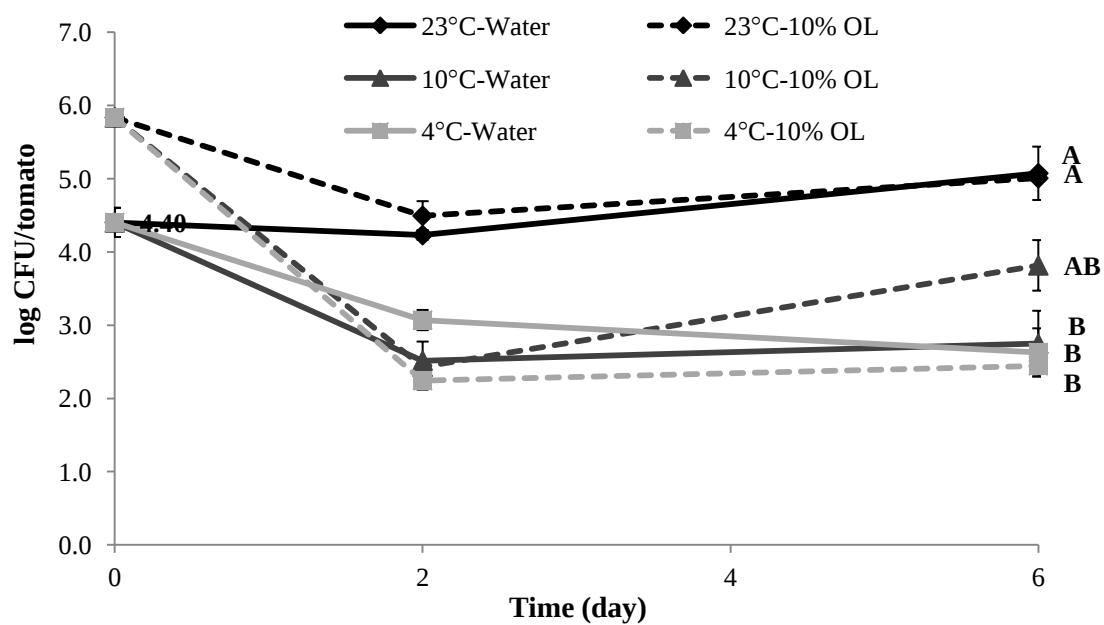
7.3.10 *Salmonella* attachment and early-biofilm formation on HDPE surfaces

Similar to stainless steel, *Salmonella* biofilm formation on HDPE was also relatively consistent during 6 d of incubation. As shown in Figure 7.9C, biofilm formation at 23 °C was significantly stronger ($P \leq 0.05$) using a 0 as compared to 10 % organic load, with no significant difference observed among the other treatments that ranged from 5.2 ± 0.1 to 5.5 ± 0.2 log CFU/coupon (original data in Appendix F, Table F1.6). Regarding the different environmental factors, “time” was the only factor that very significantly affected *Salmonella* biofilm formation on HDPE surface, with a *P* value less than 0.0001 (Table 7.4).

Table 7.4. The effect of time, substrate (water or 10% organic load), temperature (4, 10, 23 °C), and their interactions on *Salmonella* biofilm formation during 6 d of incubation.

Effect tests	Tomato		Stainless steel		HDPE	
	F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F
Time	14.9140	0.0004	8.1798	0.0063	21.0600	<0.0001
Substrate	3.7729	0.0582	16.9844	0.0002	3.0197	0.0889
Temperature	22.8091	<0.0001	0.8354	0.3655	0.4683	0.4972
Time * Substrate	2.0944	0.1546	9.2893	0.0038	1.0694	0.3065
Time * Temperature	9.6733	0.0032	0.1519	0.6985	0.0036	0.9524
Substrate * Temperature	0.2035	0.6540	5.1564	0.0279	1.9014	0.1746
Time * Substrate * Temperature	0.0719	0.7898	1.6634	0.2036	2.2163	0.1434

(A)



(B)

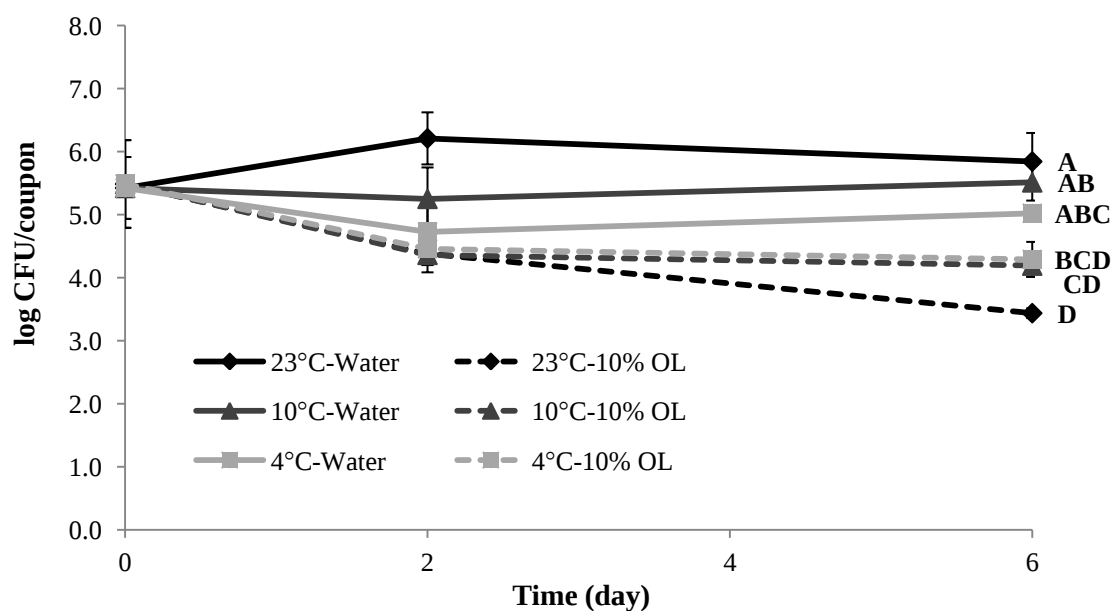
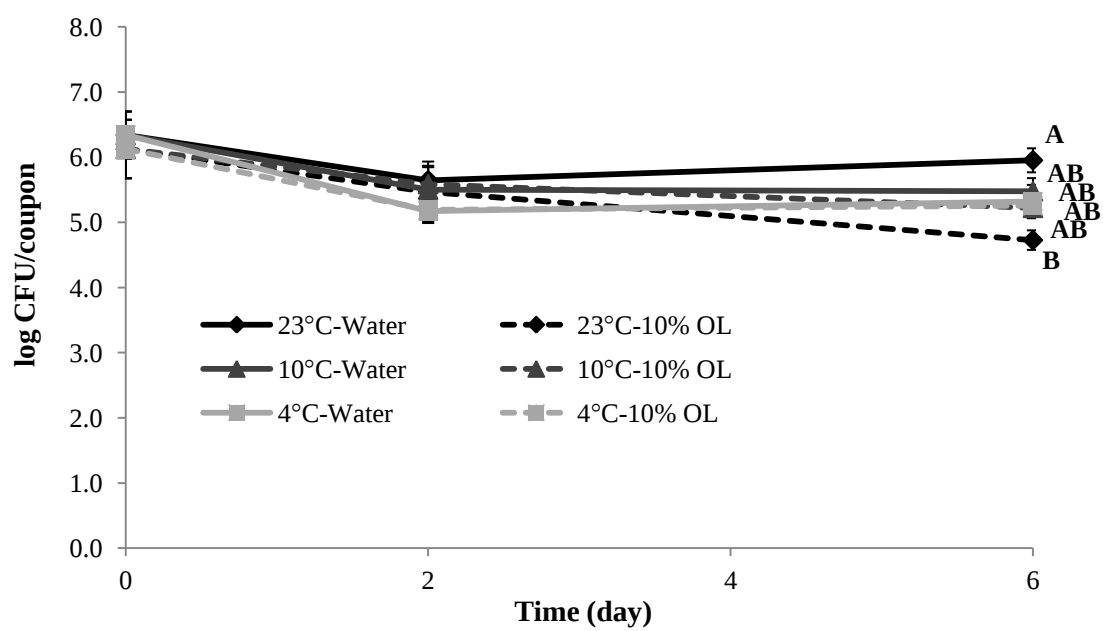


Figure 7.9: Mean ($\pm$ SE) *Salmonella* biofilm formation on A) tomatoes, B) stainless steel, and C) HDPE surfaces by inoculums prepared in water (solid line) or 10% organic load (dashed line) during 6 d of incubation at 23, 10, and 4°C ($n = 3$). Means with the same letters on day 6 are not significantly different ($P > 0.05$).

Figure 7.9 (cont'd)

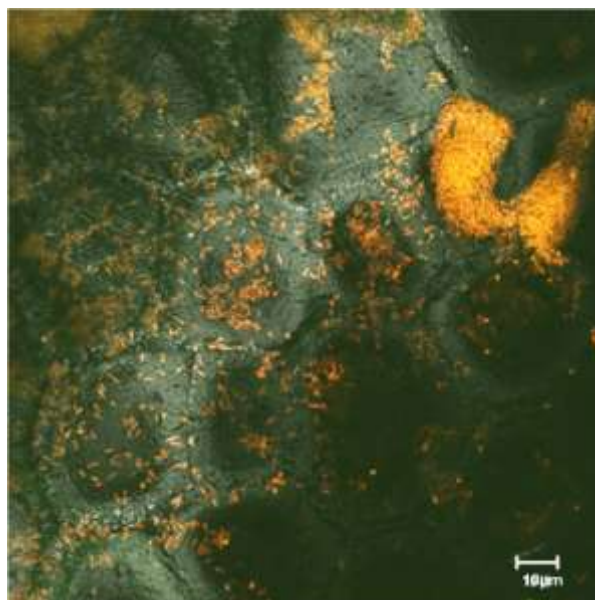
(C)



7.3.11 Confocal microscopy imaging

On day 6, *Salmonella* early-biofilm formation on three different surfaces was examined using confocal laser scanning microscopy (CLSM). As shown in Figures 7.10A & B, *Salmonella* aggregated and formed thick early-biofilms on portions of the tomato surface, especially in the “valleys” where bacteria tend to aggregate. In contrast, *Salmonella* more uniformly colonized the flat surface and “hill” portions of the tomato. Compared to the tomato surface, *Salmonella* and early-biofilms were distributed more uniformly on stainless steel (Figures 7.10 C & D), due to the flat nature of the stainless steel coupon. Although some clustering was observed on the surface, most salmonellae were present as single cells. Similar results were observed in a previous study that examined *Salmonella* biofilm formation on stainless steel during 7 d of incubation (175). Due to many crevices on the HDPE surface, *Salmonella* similarly aggregated closely in these areas (Figures 7.10 E & F) and was uniformly distributed on this relatively flat surface. Regardless of surface type, *Salmonella* biofilms showed more red/yellow fluorescence, indicated that most of the cells were dead or injured.

(A)



(B)

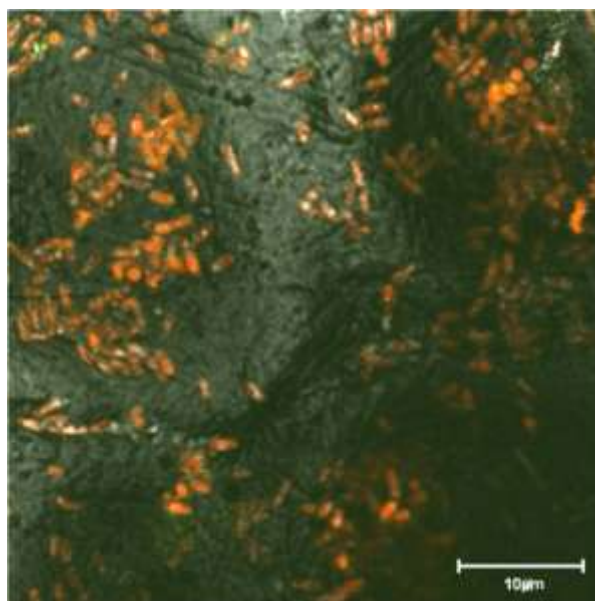
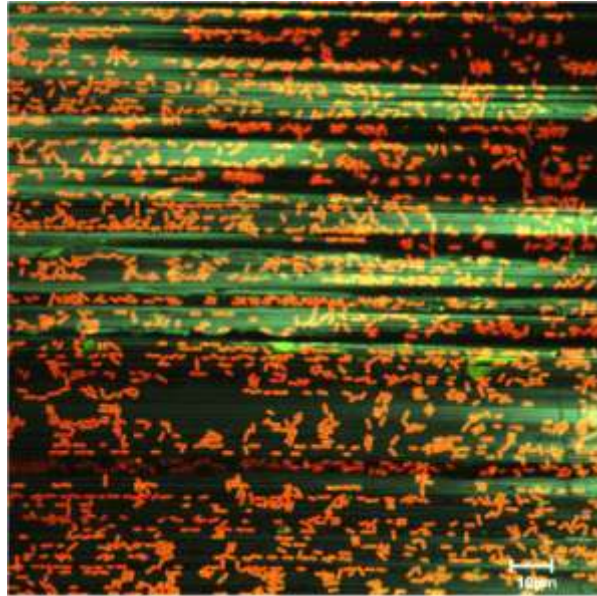


Figure 7.10: Selected CLSM images of *Salmonella* attachment and early-biofilms formed on tomato (A and B), stainless steel (C and D), and HDPE (E and F) surfaces after 6 d of incubation.

Figure 7.10 (cont'd)

(C)



(D)

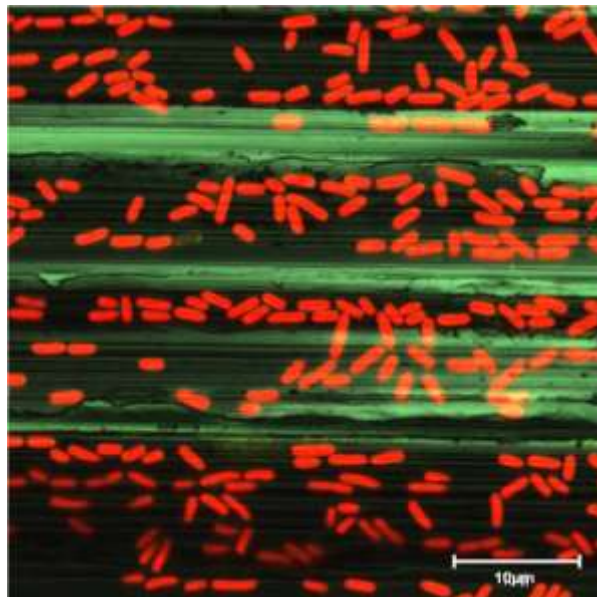
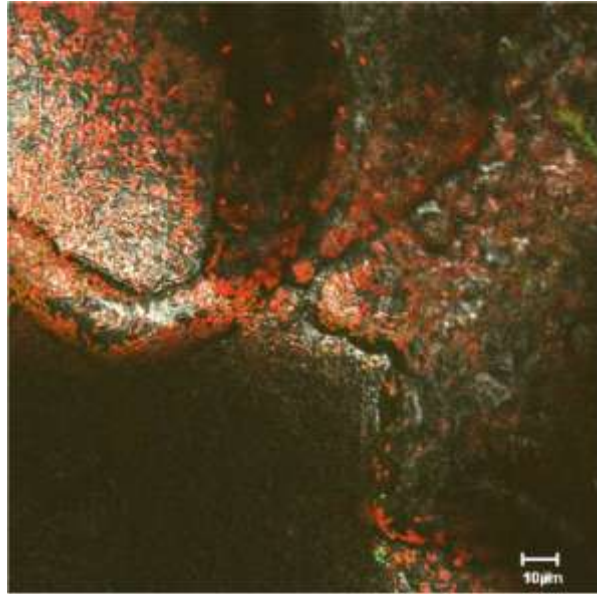
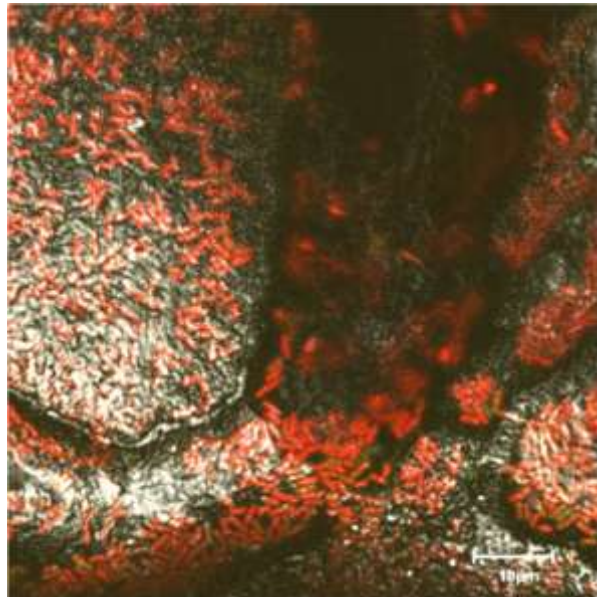


Figure 7.10 (cont'd)

(E)



(F)



7.4 DISCUSSION

This is the first study to look at the various conditions (temperature, pH, surface, and organic load) encountered in tomato processing environments. Three temperatures were selected, with 4 °C representing the temperature in industrial processing environments, 10 °C representing the optimal temperature for tomato storage, and 23 °C representing the typical temperature in commercial kitchens and other foodservice establishments. For the viability study, *Salmonella* populations at 10 and 4 °C decreased slightly from day 0 to day 4, followed by an increase after day 4, as has been observed for other foodborne pathogens including *Listeria monocytogenes* and *E. coli* O157:H7 (163, 167, 168). This bacterial response can be explained by cold adaptation, which is generally associated with a series of cold shock proteins (CspA - CspI) (137). Kinsella et al. (89) investigated the impact of attachment to beef surfaces on the survival, injury, and death of *Salmonella* cells, and showed that attachment initially prevented cell injury and death from hyperosmosis and low temperatures. However, improved survival in meat solutions was observed after 72 h and was considered to be related to the production of cold shock proteins. with the regulation of these proteins after cold shock being quite complex and not yet fully understood.

Similar to several previous studies (98, 129), elongation of *Salmonella* cells was also observed during extended low temperature incubation in this study, with significantly longer cells seen at 4 °C as opposed to 10 and 23 °C. In addition to *Salmonella*, both *E. coli* and *L. monocytogenes* have been investigated for their reactions to low temperature exposure with a similar filamentation response also having been observed (63, 163). Besides low temperature stress, many other studies have also shown that similar morphological changes can be induced by exposure to other environmental stresses such as starvation (172), osmotic stress (130), sublethal

alkaline (64), sublethal cinnamaldehyde (167), and reduced water activity (88, 147). Incomplete cell division leading to filament formation (elongation) is frequently observed in response to environmental stress (22, 86, 88). However, when exposed to ideal or less stressful environments, these filamentous cells can separate and rapidly divide into single cells (87, 98). Given the importance of fresh-cut tomatoes as a vehicle in salmonellosis outbreaks, a better understanding of the risk of these morphological changes is needed to prevent cell filamentation during cold processing and storage.

The ability of foodborne bacteria to adhere to surfaces and form biofilms has been investigated extensively. In addition to the nature of the solid surface, biofilm formation is also closely related to bacterial cell characteristics and environmental factors. Cell characteristics that normally play an important role in this process include flagella, surface appendages (fimbriae), and polysaccharides (164). Instead of focusing on these aspects, our study investigated the physicochemical characteristics that related to bacterial attachment, with surface hydrophobicity and surface charge as the points of interest. Both pH and temperature can significantly affect the surface hydrophobicity of *Salmonella* cells. Chia et al. (40) investigated the surface hydrophobicity of different *Salmonella* serotypes and classified them into three groups, with 0 to 35%, 36 to 70%, and 71 to 100% representing low, moderate, and high hydrophobic, respectively. The percent adhesion to xylene ranged from 22 to 53%, which is quite similar to the cell hydrophobicity of 17.7 to 55% on day 8 in our study. To our knowledge, this present study is the first to illustrate dynamic changes in *Salmonella* surface hydrophobicity during extended exposure to different pH and temperature conditions.

The various properties of surfaces, including roughness, cleanability, disinfectability, wettability, and durability, also play important roles in bacterial attachment and biofilm

formation (164). When the hydrophobicity (normally considered as an indicator of wettability) of tomatoes, HDPE, and stainless steel was assessed, the surface of tomatoes was more hydrophobic than HDPE or stainless steel. *Salmonella* cells bind better to hydrophobic than hydrophilic surfaces. One previous study also showed that *Staphylococcus epidermidis* strains adhered better to hydrophobic substrata (acrylic) than hydrophilic (glass) surfaces (37). Therefore, more attention should be given to the selection of materials for processing facility and equipment design, to lower the risk of bacterial attachment during processing. Instead of comparing attachment between bacteria cells and different surfaces, we assessed the attachment of *Salmonella* under different conditions to tomatoes and observed that attachment was stronger at lower temperatures, especially in an acidic environment. This attachment test confirmed the cell surface hydrophobicity test and will provide guidelines for environmental control during industrial processing of tomatoes.

The attachment between bacteria and surfaces can be described as a physicochemical process determined by Van der Waals, electrostatic and steric forces acting between the cells and the attachment surface (164). When a liquid medium containing bacteria contacts a solid surface, the bacterial surface charge becomes critical for overall bacterial attachment. The surface charge of *Salmonella* Typhimurium cells measured in this study was consistently negative, which is in agreement with results for *E. coli*, *Campylobacter*, and other *Salmonella* serovars (40, 92, 111). Li and McLandsborough (92) showed that the cellular surface charge of most *E. coli* O157:H7 strains were much less affected by changes in pH, ionic strength or concentration of surfactants in the suspending medium than was the surface charge of *E. coli* JM109 cells. The impact of temperature to surface charge change has not yet been studied. More importantly, no study has investigated the dynamic change of surface charge under industrial conditions. These

observations will enhance the current understanding of bacterial surface charge and also provide guidelines for minimizing cross-contamination.

According to our preliminary experiment, significant decay happened to tomatoes after 6 d of storage at 23 and 10 °C. Thus, only 6 d of storage was used for the biofilm study with samples collected on d 0, 2, and 6. Initially, a higher *Salmonella* population was maintained on the tomato surface when a 10% tomato organic load was applied. Thereafter, *Salmonella* populations decreased and stabilized after day 2. This decrease is related to the bacterial response to desiccation stress during drying and storage (120, 179). While other studies have shown that both pH and temperature can affect biofilm development (61, 104), only temperature significantly affected biofilm formation on tomatoes. A liquid-air interface was created to facilitate the biofilm formation on solid surfaces (62, 178). In addition to time, substrate significantly affected *Salmonella* biofilm formation on stainless steel but not HDPE. Overall, presence of a 10% organic load enhanced biofilm formation to a greater extent than did higher incubation temperatures. Dourou et al. (51) also showed that *E. coli* O157:H7 attachment and biofilm formation to beef-contact surfaces (stainless steel and HDPE) was influenced by the type of soiling and temperature. Therefore, good temperature control and effective sanitation become critical for biofilm prevention in processing facilities.

In this study, we first investigated the effect of temperature and pH on various changes in *Salmonella* including viability, morphology, surface hydrophobicity, and surface charge. Thereafter, the impact of temperature and substrate on *Salmonella* attachment and biofilm formation on different surfaces was evaluated, based on common conditions encountered in the tomato industry. While the microtiter plate method has been widely used to assess bacterial attachment/biofilm formation under standardized laboratory conditions (48, 93, 105, 126, 166),

limited research is currently available to illustrate bacterial attachment and biofilm formation under conditions that reflect real industry settings. The findings from the present study indicated that various environmental factors can affect the characteristics of *Salmonella* as well as the extent of attachment and biofilm formation on different surfaces encountered during tomato processing. Both temperature and pH served as critical factors for *Salmonella* attachment and biofilm formation. To our knowledge, this is the first study to investigate *Salmonella* attachment and biofilm formation based on real environmental parameters from tomato processing and will provide practical guidelines to the industry for the development of more effective sanitation programs and Good Manufacturing Practices (GMP).

CHAPTER 8:

Conclusions and Recommendations for Future Work

8.1 CONCLUSIONS OF THIS DISSERTATION

This presented dissertation includes six research chapters pertaining to post-harvest processing of tomatoes. Through a series of research activities including industry observation, bench-top evaluation, pilot-scale simulation, and further investigation, a systematic study was conducted to illustrate the overall transfer of *Salmonella* during post-harvest processing of tomatoes, provide more effective inactivation strategies for *Salmonella* control, and more importantly understand the mechanisms for some phenomenon observed during investigation.

The first research chapter – “Chapter 2: Microbial Cross-Contamination of Tomatoes during Washing with a Peroxyacetic Acid-Based Sanitizer in a Commercial Packinghouse”, demonstrated the findings observed from a commercial tomato packinghouse. In addition to the observation of very inconsistent sanitizer levels in the dump tank, the microbial population-change on tomato surfaces highlighted the risk of microbial transfer during contact with different equipment surfaces. Serving representations of “real-world” processing, these observations from chapter 2 provide critical information for the development of appropriate methodologies for further bench-top and pilot-scale investigations. For instance, pilot-scale studies were conducted in chapter 3 to simulate the commercial tomato processing to evaluate the efficacy of different sanitizer treatments (at consistent concentration of 40 ppm) against *Salmonella*. While the chlorine-based sanitizer treatments showed better efficacy than peroxyacetic/peracid-based treatments, it is proved previously that the efficacy of chlorine is more easily impacted when organic matter present. In addition, based on the finding from chapter 2 that roller conveyors play critical role in microbial cross-contamination during conveying of tomatoes after washing, a study was conducted in chapter 4 to investigate the impact of different roller conveyors on *Salmonella* transfer. It was concluded that brush roller is more suitable for tomato processing to

prevent cross-contamination. However, observations from chapter 1 emphasized the importance of proper post-process sanitation and maintenance of brush rollers on a regular basis.

Chapter 5 and 6 focused on the fresh-cut processing of tomatoes including slicing and dicing. Compared to whole-tomato processing, slicing is a more complex process, during which *Salmonella* transfer can be impacted by various variables. Among 6 variables tested, tomato surface wetness and tomato variety can significantly impact *Salmonella* transfer. Due to different transfer patterns detected for different processing temperatures, it was hypothesized that temperature plays a critical role in *Salmonella* attachment and biofilm formation. In chapter 6, various steps of tomato dicing including mechanical dicing, flume-tank washing, and conveying were investigated. Previous studies showed that, in addition to temperature, acidity of wash water can also affect *Salmonella* attachment and biofilm formation on different surfaces. Therefore, detailed investigation was conducted in chapter 7 to assess *Salmonella* attachment and biofilm formation based on practical industrial conditions. The results obtained from chapter 7 explained how decreasing viable *Salmonella* population by using lower temperature and relatively strong attachment of *Salmonella* to tomato surfaces can contribute to lower *Salmonella* transfer during tomato slicing. In addition, the cell surface charge for *Salmonella* cells increased as pH declined. Considering the low pH environment attributed to accumulation of organic matters in the flume tank, this change of cell surface charge may enhance *Salmonella* attachment and biofilm formation during dicing process. Therefore, when chlorine is used in the wash water for tomato dicing, the pH should be controlled at ~ 6.0 to ensure optimal efficacy, while reducing the risk of stronger *Salmonella* attachment to various surfaces.

8.2 IMPLICATIONS FOR TOMATO PROCESSING

Post-harvest processing of tomatoes on the bench-top has been investigated by previous researchers, with a major focus on whole tomato processing. Further processing of tomatoes such as slicing and dicing, has been under-investigated to the date. I focused on both transfer and inactivation of *Salmonella* during tomato processing and sought information that gives us a better understanding of *Salmonella* transfer to help generate practical recommendations and implications for tomato processors to enhance the safety of tomato products.

For the whole-tomato packing, it is of great importance to maintain a constant and effective sanitizer concentration in the wash water during washing in the dump tank. It was observed that the automatic sanitizer adjustment (based on ORP monitoring) might not maintain an ideal chlorine concentration. More frequent concentration monitoring by operators should be performed. Since the wash water is recycled in the dump tank, the accumulation of organic matter in the wash water is detrimental to the overall wash water quality. Proper filtration of wash water and replenish of fresh water can be used to maintain the overall water quality. In addition, it is recommended that brush roller is preferred over plastic and foam rollers to reduce the risk of *Salmonella* cross-contamination during conveying of tomatoes. However, it was also showed that, if not properly sanitized, the highly contaminated brush roller can lead to an increased risk of microbial transfer and contamination. Therefore, proper and thorough sanitation for the entire processing line should be implemented on a regular basis. Furthermore, the results from chapter 3 illustrated that chlorine based sanitizer with pH adjustment to 6.0 performed better than PAA based sanitizer to inactivate *Salmonella* on tomato surfaces and thus should be considered for tomato packing.

As for slicing and dicing of tomatoes, more factors than whole tomato processing need to be considered to reduce the overall risk of microbial contamination. Based on the findings from chapter 5, an electric slicer can reduce *Salmonella* cross-contamination relative to a manual slicer, while increasing operation efficiency. The slicing process should be conducted under lower temperature (4 °C) than ambient environment. Tomato varieties with tougher texture and lower water content are not recommended for slicing. Similarly, it was concluded that dry tomato surface and pre-wash with sanitizer significantly lower the overall *Salmonella* transfer during slicing. Therefore, after washing with a sanitizer solution, tomatoes should be handled to drain additional water on tomato surfaces before slicing.

Compared to slicing, tomato dicing involves more mechanical processing including dicing, flume-tank washing, and conveying. Multiple recommendations can be provided to processors based on the results obtained from chapter 6. For instance, proper sanitation should be applied to the mechanical dicer on a regular basis to minimize the spread of foodborne pathogen during dicing. In addition to chlorine-based sanitizer, PAA-based sanitizers should be considered for flume-tank washing due to their low reactivity with organic matter in wash water. Moreover, as an important step in dicing process, conveying of diced tomato can be improved by addition of sanitizer spray to reduce microbial load on conveyor belts thus lowering the risk of cross-contamination during conveyance. In this case, PAA-based sanitizer is preferred over chlorine-based sanitizer to obtain higher sanitizing efficacy. Furthermore, the design and material of conveyor belt should also be considered for an efficient and safe conveying.

8.3 RECOMMENDATIONS FOR FUTURE WORK

While the results from this study can provide valuable information, in the future, multiple tomato packinghouses should be evaluated to collect more data. Furthermore, packinghouses that use different sanitizers need to be included as well.

Since organic load build-up in wash water has become an important issue during continued processing, future research should include using various levels of organic load in the wash solution to better evaluate sanitizer efficacy under less ideal processing conditions. Furthermore, studies should be conducted to evaluate the effectiveness of various strategies, such as water filtration system, to lower the impact of organic load on sanitizer efficacy.

In this work, no waxing step was included for the whole tomato fruit handling (in chapter 4). A waxing step is normally included before conveying to the packing table. The layer of wax applied on tomato surface can potentially affect microbial levels, thus change the extent of bacteria transfer during conveyance. Therefore, future research may focus on assessing *Salmonella* transfer during conveying of waxed tomatoes, using different types of roller conveyors.

For chapter 5, the “transfer scenario” selected was transfer from an inoculated tomato to a clean blade and then to uninoculated tomatoes, without specifically assessing transfer from inoculated blade to uninoculated tomatoes. While the former transfer scenario represents a more practical condition, in the future research, the latter scenario should also be evaluated to clarify the role of the blade in *Salmonella* transfer.

For chapter 6, the conveyor belt was inoculated with *Salmonella* before proceed to sanitation in a discontinuous manner. However, in the real-world production, the “inoculation” and “sanitation” might occur simultaneously. Future research should focus on evaluating the

efficacy of spray sanitation on conveyor belts during continuous inoculation, with the newly developed conveyor belts containing antimicrobial agents also of interest.

Finally, additional future research should focus on investigating the impact of different parameters on *Salmonella* surface attachment, including flagella biology, surface appendages, and surface polysaccharides. Moreover, only 6 d of incubation was used in this study, due to the purpose of maintaining tomato quality. In the future, extended incubation times such as 10 d should be evaluated for the other equipment surfaces.

APPENDICES

APPENDIX A:

Microbial Cross-Contamination of Tomatoes during Washing with a Peroxyacetic Acid- Based Sanitizer in a Commercial Packinghouse

Table A1.1: Mean ($\pm$ SE) microbial (MAB: mesophilic aerobic bacteria; YM: yeast/mold) populations in the dump tank water during 3h of operation in a commercial tomato packinghouse (n = 3).

Time (h)	Microbial population (log CFU/ml)	
	MAB	YM
0	1.1 $\pm$ 1.0 a ^x	2.2 $\pm$ 0.5 A ^y
0.5	2.3 $\pm$ 0.6 a	2.9 $\pm$ 0.2 A
1	2.8 $\pm$ 0.8 a	3.0 $\pm$ 0.2 A
2	2.3 $\pm$ 0.5 a	3.2 $\pm$ 0.1 A
3	1.8 $\pm$ 0.3 a	2.9 $\pm$ 0.1 A

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

^y Means with the same capital letters in the same column are not significantly different ($P > 0.05$)

Table A1.2: Mean ($\pm$ SE) microbial population on equipment surfaces after 0 (at the beginning of operation), 2, and 4h of operation in a commercial tomato packinghouse (n = 3).

Time (h)	Microbial population (log CFU/100cm ²)	
	MAB	YM
0	3.1 $\pm$ 0.2 b ^x	2.5 $\pm$ 0.2 B ^y
2	4.5 $\pm$ 0.3 a	3.6 $\pm$ 0.2 A
4	4.3 $\pm$ 0.3 a	3.2 $\pm$ 0.2 AB

^x Means with the same letters in the same column for MAB (mesophilic aerobic bacteria) are not significantly different ($P > 0.05$)

^y Means with the same capital letters in the same column for YM (yeast/mold) are not significantly different ($P > 0.05$)

Table A1.3: Mean ($\pm$ SE) microbial population on brushes after 0 (at the beginning of operation), 2, and 4h of operation in a commercial tomato packinghouse (n = 3).

Time (h)	Microbial population (log CFU/bunch)	
	MAB	YM
0	3.9 $\pm$ 0.6 a ^x	2.6 $\pm$ 0.6 A ^y
2	4.2 $\pm$ 0.5 a	3.6 $\pm$ 0.4 A
4	4.1 $\pm$ 0.3 a	3.3 $\pm$ 0.4 A

^x Means with the same letters in the same column for MAB (mesophilic aerobic bacteria) are not significantly different ($P > 0.05$)

^y Means with the same capital letters in the same column for YM (yeast/mold) are not significantly different ($P > 0.05$)

APPENDIX B:

Efficacy of Various Sanitizers against *Salmonella* during Simulated Commercial Processing of Tomatoes

Table B1.1: Mean ($\pm$ SD) *Salmonella* populations on equipment surfaces (Dump tank: D1 to D10; Water tank: W1 to W4; Roller conveyor: R1 to R6) after washing 11.3 kg of inoculated tomatoes (~ 6 log CFU/g) with water alone (n=3).

Equipment surfaces	<i>Salmonella</i> population (log CFU/100cm ²)
Dump tank	D1
	D2
	D3
	D4
	D5
	D6
	D7
	D8
	D9
	D10
Water tank	W1
	W2
	W3
	W4
Roller conveyor	R1
	R2
	R3
	R4
	R5
	R6

^x Means with the same capital letters in the column are not significantly different ($P > 0.05$)

APPENDIX C:

***Salmonella* Transfer during Pilot-Plant Scale Washing and Roller Conveying of Tomatoes**

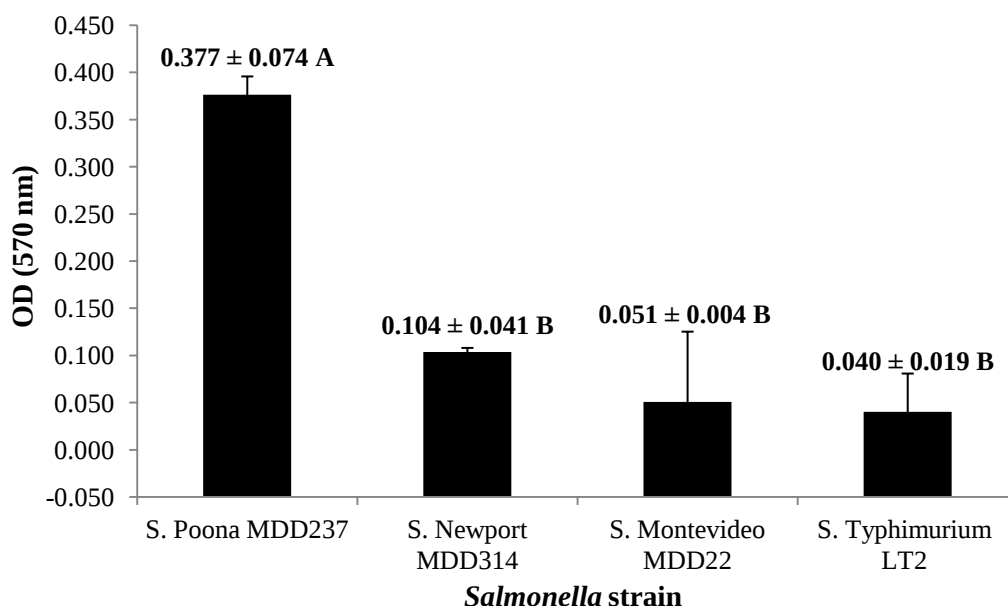


Figure C1.1: Mean ($\pm$ SD) OD (570 nm) values of avirulent *S. Typhimurium* LT2 and virulent *S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314 after 4 d of incubation at 23 °C in microtiter plate containing TSBYE broth (n = 3). The higher OD values represent higher biofilm formation ability. Columns with the same letters are not significantly different ($P > 0.05$)

Table C1.1: Mean ($\pm$ SD) *Salmonella* populations (log CFU/ml) of *S. Typhimurium* LT2, *S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314 after 0, 2, 4, 6, 8, 10, 12, 14, and 24 h of incubation without shaking in TSBYE broth at 37°C (n = 3). The generation time of each *Salmonella* strain was calculated based on the population increase during 12 h (exponential phase of growth) of incubation.

Incubation time (h)	<i>Salmonella</i> population (log CFU/ml)			
	<i>S. Typhimurium</i> LT2	<i>S. Montevideo</i> MDD22	<i>S. Poona</i> MDD237	<i>S. Newport</i> MDD314
0	2.2 $\pm$ 0.1	2.1 $\pm$ 0.1	2.2 $\pm$ 0.0	2.1 $\pm$ 0.0
2	2.8 $\pm$ 0.0	2.5 $\pm$ 0.1	2.5 $\pm$ 0.0	2.4 $\pm$ 0.1
4	3.9 $\pm$ 0.1	3.5 $\pm$ 0.1	3.7 $\pm$ 0.1	3.2 $\pm$ 0.1
6	5.1 $\pm$ 0.0	5.1 $\pm$ 0.0	5.2 $\pm$ 0.2	4.8 $\pm$ 0.1
8	6.7 $\pm$ 0.1	6.9 $\pm$ 0.0	7.0 $\pm$ 0.0	6.4 $\pm$ 0.0
10	8.3 $\pm$ 0.1	8.6 $\pm$ 0.1	8.6 $\pm$ 0.1	8.0 $\pm$ 0.1
12	9.1 $\pm$ 0.1	9.3 $\pm$ 0.0	9.2 $\pm$ 0.1	9.2 $\pm$ 0.1
14	9.1 $\pm$ 0.1	9.2 $\pm$ 0.1	9.2 $\pm$ 0.1	9.2 $\pm$ 0.0
24	9.3 $\pm$ 0.1	9.3 $\pm$ 0.1	9.2 $\pm$ 0.0	9.2 $\pm$ 0.0
Generation time (min)	104.9 ^x	100.8	102.8	101.3

^x Generation time (min) = 12 * 60 / (*Salmonella* population (12h) - *Salmonella* population (0h))

Table C1.2: Mean ($\pm$ SD) *Salmonella* populations (log CFU/g) of *S. Typhimurium* LT2 and virulent *Salmonella* cocktail (*S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314) on red round tomato surfaces after 2, 24, 48, 72, 96, 120, and 144 h of storage at 25°C (n = 3).

Storage time (h)	<i>Salmonella</i> population (log CFU/g)	
	<i>S. Typhimurium</i> LT2	Virulent cocktail
2	5.0 $\pm$ 0.0 A ^x	4.8 $\pm$ 0.0 A
24	5.2 $\pm$ 0.1 A	4.9 $\pm$ 0.3 A
48	5.1 $\pm$ 0.3 A	5.3 $\pm$ 0.5 A
72	5.2 $\pm$ 0.2 A	5.3 $\pm$ 0.1 A
96	5.3 $\pm$ 0.2 A	5.5 $\pm$ 0.2 A
120	5.4 $\pm$ 0.2 A	5.3 $\pm$ 0.9 A
144	4.8 $\pm$ 0.1 A	5.3 $\pm$ 0.2 A

^x Means with the same capital letters in the table are not significantly different ($P > 0.05$)

Table C1.3: Mean ($\pm$ SD) log reductions (log CFU/ml) of avirulent *S. Typhimurium* LT2 and virulent *Salmonella* cocktail of *S. Montevideo* MDD22, *S. Poona* MDD237, and *S. Newport* MDD314 after 1-min exposure of 1 ml of bacteria culture to 30 ml of peroxyacetic acid (60 ppm) and chlorine (50 ppm) (n = 3).

Sanitizer treatments	<i>Salmonella</i> log reduction (log CFU/ml)	
	<i>S. Typhimurium</i> LT2	Virulent cocktail
Peroxyacetic acid	5.7 $\pm$ 0.2 A ^x	5.9 $\pm$ 0.2 A
Chlorine	3.6 $\pm$ 0.2 A	3.5 $\pm$ 0.1 A

^x Means with the same capital letters in the same row are not significantly different ($P > 0.05$)

Table C1.4: Mean ($\pm$ SD) *Salmonella* populations (log CFU/g) of inoculated tomatoes before processing, after washing, and after conveying with three roller conveyors (foam, plastic, or brush) (n = 3).

Processing	<i>Salmonella</i> population (log CFU/g)		
	Foam roller	Plastic roller	Brush roller
Inoculated tomato	4.0 $\pm$ 0.1 A ^x	3.8 $\pm$ 0.1 AB	4.2 $\pm$ 0.2 A
After washing	3.3 $\pm$ 0.3 BC	3.0 $\pm$ 0.1 C	3.1 $\pm$ 0.3 C
After conveying	3.2 $\pm$ 0.3 C	3.1 $\pm$ 0.4 C	3.0 $\pm$ 0.1 C
Log reduction after washing	0.7 ^y	0.8	1.1

^x Means with the same capital letters in the table are not significantly different ($P > 0.05$)

^y Log reduction after washing = Inoculated tomato - After washing

Table C1.5: Mean ($\pm$ SE) *Salmonella* populations transferred to 25 uninoculated tomatoes after conveying through three different types (foam, plastic, and brush) of roller conveyor that contaminated with *Salmonella* from ~2 kg inoculated tomatoes (n = 3).

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Foam roller	Plastic roller	Brush roller
1	2.5 $\pm$ 0.1	1.2 $\pm$ 0.2	ND ^x
2	2.5 $\pm$ 0.0	0.8 $\pm$ 0.4	ND
3	2.2 $\pm$ 0.2	1.3 $\pm$ 0.1	ND
4	2.5 $\pm$ 0.2	1.1 $\pm$ 0.3	ND
5	2.2 $\pm$ 0.1	0.5 $\pm$ 0.3	ND
6	2.4 $\pm$ 0.0	1.4 $\pm$ 0.4	ND
7	2.5 $\pm$ 0.1	0.7 $\pm$ 0.2	ND
8	1.9 $\pm$ 0.5	1.4 $\pm$ 0.1	ND
9	2.4 $\pm$ 0.1	0.4 $\pm$ 0.3	ND
10	2.2 $\pm$ 0.1	0.4 $\pm$ 0.2	ND
11	2.2 $\pm$ 0.1	0.2 $\pm$ 0.2	0.4 $\pm$ 0.7
12	2.3 $\pm$ 0.0	0.7 $\pm$ 0.3	ND
13	2.3 $\pm$ 0.0	0.5 $\pm$ 0.2	ND
14	2.1 $\pm$ 0.2	0.2 $\pm$ 0.2	ND
15	2.4 $\pm$ 0.1	0.3 $\pm$ 0.2	ND
16	2.4 $\pm$ 0.1	0.3 $\pm$ 0.3	ND
17	2.1 $\pm$ 0.2	0.7 $\pm$ 0.4	ND
18	2.4 $\pm$ 0.1	0.3 $\pm$ 0.3	ND
19	2.2 $\pm$ 0.1	0.2 $\pm$ 0.2	ND
20	2.1 $\pm$ 0.1	0.2 $\pm$ 0.2	ND
21	2.2 $\pm$ 0.1	0.6 $\pm$ 0.3	ND
22	2.5 $\pm$ 0.1	0.3 $\pm$ 0.1	ND
23	2.6 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
24	2.1 $\pm$ 0.2	0.0 $\pm$ 0.0	ND
25	2.2 $\pm$ 0.2	0.6 $\pm$ 0.3	ND

^x ND: not detected (under the limit of detection of 0 log CFU/tomato)

Table C1.6: Mean ($\pm$ SE) *Salmonella* populations recovered from six plastic roller surface (R1 – R6) samples before and after conveying 25 uninoculated tomatoes (n = 3).

Surface samples	<i>Salmonella</i> population (log CFU/100 cm ²)	
	Before	After
R1	2.1 $\pm$ 0.3 A ^x	1.7 $\pm$ 0.1 A
R2	1.2 $\pm$ 0.6 ABC	1.2 $\pm$ 0.1 ABC
R3	1.3 $\pm$ 0.5 ABC	1.0 $\pm$ 0.3 ABC
R4	1.6 $\pm$ 0.3 AB	0.5 $\pm$ 0.4 BC
R5	1.7 $\pm$ 0.4 A	0.3 $\pm$ 0.3 C
R6	1.8 $\pm$ 0.4 A	0.5 $\pm$ 0.5 BC

^x Means with the same capital letters in the table are not significantly different ($P > 0.05$)

Table C1.7: Mean ($\pm$ SE) *Salmonella* populations recovered from three foam roller surface (R1 – R3) samples before and after conveying 25 uninoculated tomatoes (n = 3).

Surface samples	<i>Salmonella</i> population (log CFU/100 cm ²)	
	Before	After
R1	2.4 $\pm$ 0.1 A ^x	2.4 $\pm$ 0.1 A
R2	2.3 $\pm$ 0.3 A	2.1 $\pm$ 0.3 A
R3	2.1 $\pm$ 0.2 A	2.1 $\pm$ 0.4 A

^x Means with the same capital letters in the table are not significantly different ($P > 0.05$)

Table C1.8: Mean ($\pm$ SE) *Salmonella* populations transferred to 25 uninoculated tomatoes through three different types of roller conveyor that contaminated with *Salmonella* from ~2 kg inoculated tomatoes (~ 6 log CFU/g) that previously washed with 40 ppm peroxyacetic acid (n = 3).

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Foam roller	Plastic roller	Brush roller
1	0.3 $\pm$ 0.3	ND	ND
2	ND ^x	ND	ND
3	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
4	0.3 $\pm$ 0.1	0.2 $\pm$ 0.2	ND
5	ND	ND	0.1 $\pm$ 0.1
6	ND	ND	ND
7	ND	0.2 $\pm$ 0.2	ND
8	ND	0.3 $\pm$ 0.2	ND
9	ND	0.2 $\pm$ 0.2	ND
10	ND	ND	ND
11	ND	ND	ND
12	ND	ND	0.1 $\pm$ 0.1
13	ND	ND	ND
14	ND	0.2 $\pm$ 0.1	ND
15	ND	0.1 $\pm$ 0.1	ND
16	ND	ND	0.1 $\pm$ 0.1
17	ND	ND	ND
18	ND	ND	ND
19	ND	ND	ND
20	ND	ND	0.1 $\pm$ 0.1
21	ND	0.1 $\pm$ 0.1	ND
22	ND	ND	ND
23	ND	ND	ND
24	ND	ND	ND
25	0.7 $\pm$ 0.7	ND	0.3 $\pm$ 0.3

^x ND: not detected (under the limit of detection of 0 log CFU/tomato)

Table C1.9: Mean ($\pm$ SE) *Salmonella* populations transferred to 25 uninoculated tomatoes through three different types of roller conveyor that contaminated with *Salmonella* from ~2 kg inoculated tomatoes (~ 6 log CFU/g) that previously washed with 40 ppm chlorine + CA (n = 3).

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Foam roller	Plastic roller	Brush roller
1	0.7 $\pm$ 0.2	ND	ND
2	1.3 $\pm$ 0.2	ND	ND
3	0.9 $\pm$ 0.7	ND	ND
4	0.7 $\pm$ 0.7	0.1 $\pm$ 0.1	ND
5	0.5 $\pm$ 0.4	ND	0.2 $\pm$ 0.2
6	1.1 $\pm$ 0.6	ND	0.1 $\pm$ 0.1
7	0.5 $\pm$ 0.4	0.1 $\pm$ 0.1	ND
8	0.8 $\pm$ 0.5	ND	ND
9	1.0 $\pm$ 0.6	0.2 $\pm$ 0.2	ND
10	0.8 $\pm$ 0.4	0.1 $\pm$ 0.1	ND
11	ND ^x	ND	ND
12	0.8 $\pm$ 0.5	ND	0.1 $\pm$ 0.1
13	0.5 $\pm$ 0.4	ND	ND
14	0.8 $\pm$ 0.6	0.1 $\pm$ 0.1	ND
15	0.8 $\pm$ 0.4	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
16	0.3 $\pm$ 0.3	ND	ND
17	0.1 $\pm$ 0.1	ND	0.2 $\pm$ 0.2
18	0.2 $\pm$ 0.2	ND	ND
19	0.2 $\pm$ 0.2	ND	ND
20	0.2 $\pm$ 0.2	ND	ND
21	ND	ND	ND
22	0.8 $\pm$ 0.2	ND	ND
23	ND	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
24	ND	ND	ND
25	0.1 $\pm$ 0.1	ND	ND

^x ND: not detected (under the limit of detection of 0 log CFU/tomato)

APPENDIX D:

Transfer of *Salmonella* during Mechanical Slicing of Tomatoes as Impacted by Multiple Processing Variables

Table D1.1: Mean ($\pm$ SE) *Salmonella* distribution on nine tomato slices from inoculated and uninoculated tomatoes (1: top slice is the blossom end; 9: bottom slice is the stem end) after slicing with the manual slicer (n = 3).

Tomato slices	<i>Salmonella</i> population (log CFU/g)	
	Inoculated tomato	Uninoculated tomato
1 (blossom)	5.5 $\pm$ 0.3 A ^x	2.1 $\pm$ 0.3 a ^y
2	5.1 $\pm$ 0.2 AB	2.6 $\pm$ 0.5 a
3	4.9 $\pm$ 0.1 AB	2.6 $\pm$ 0.3 a
4	4.6 $\pm$ 0.1 B	2.8 $\pm$ 0.5 a
5	4.7 $\pm$ 0.1 B	3.2 $\pm$ 0.3 a
6	4.6 $\pm$ 0.2 B	3.2 $\pm$ 0.1 a
7	4.7 $\pm$ 0.0 B	2.6 $\pm$ 0.2 a
8	4.9 $\pm$ 0.1 AB	3.0 $\pm$ 0.3 a
9 (stem)	5.4 $\pm$ 0.2 A	3.3 $\pm$ 0.2 a

^x Means with the same capital letters for inoculated tomato slices are not significantly different ($P > 0.05$)

^y Means with the same letters for uninoculated tomato slices are not significantly different ($P > 0.05$)

Table D1.2: Mean ($\pm$ SE) *Salmonella* distribution on different components (blade, back plate, and bottom plate) of the manual slicer (contaminated by slicing one inoculated tomato) before and after slicing 20 uninoculated tomatoes (n = 3).

Slicer component	<i>Salmonella</i> population (log CFU/component)	
	Before slicing	After slicing
Blade	3.8 $\pm$ 0.3 A ^x	1.9 $\pm$ 0.8 a ^y
Back plate	3.3 $\pm$ 0.6 A	2.2 $\pm$ 0.1 a
Bottom plate	4.6 $\pm$ 0.4 A	2.3 $\pm$ 0.8 a

^x Means with the same capital letters for surface population before slicing are not significantly different ($P > 0.05$)

^y Means with the same letters for surface population after slicing are not significantly different ($P > 0.05$)

Table D1.3: Mean ($\pm$ SE) *Salmonella* distribution on different components (blade, pusher, and side plate) of the electrical slicer (contaminated by slicing one inoculated tomato) before and after slicing 20 uninoculated tomatoes (n = 3).

Slicer component	<i>Salmonella</i> population (log CFU/component)	
	Before slicing	After slicing
Blade	2.9 $\pm$ 0.5 A ^x	1.7 $\pm$ 0.1 a ^y
Pusher	2.2 $\pm$ 0.1 A	1.4 $\pm$ 0.8 a
Side plate	1.5 $\pm$ 0.2 A	1.8 $\pm$ 0.3 a

^x Means with the same capital letters for surface population before slicing are not significantly different ($P > 0.05$)

^y Means with the same letters for surface population after slicing are not significantly different ($P > 0.05$)

Table D1.4: *Salmonella* transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer. Rep 1, Rep 2, and Rep 3 are three replicates of the study.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6
2	4.5	4.7	4.4
3	4.3	4.8	3.3
4	4.3	3.1	3.7
5	3.0	4.4	3.4
6	3.7	3.5	2.8
7	3.6	3.2	2.9
8	3.2	2.9	2.5
9	2.5	3.1	2.7
10	2.9	2.7	2.2
11	2.9	2.9	2.5
12	2.1	+	+
13	2.9	2.1	+
14	2.3	3.2	2.2
15	2.8	2.2	+
16	2.2	+	+
17	2.1	+	+
18	2.9	- ^Y	+
19	+ ^X	+	-
20	2.1	2.1	2.2

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

^ZLOD (limit of detection): 2.2 log CFU/tomato

Table D1.5: Mean ($\pm$ SE) *Salmonella* population transferred from one inoculated tomato (~ 5 log CFU/g) to 20 uninoculated tomatoes through different parts (whole slicer, back & bottom plate, and blade) of manual slicer during slicing (n=3).

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Whole slicer	Back & bottom plate	Blade
1	4.9 $\pm$ 0.2	4.3 $\pm$ 0.4	2.6 $\pm$ 0.3
2	4.5 $\pm$ 0.1	4.0 $\pm$ 0.5	1.1 $\pm$ 0.6
3	4.1 $\pm$ 0.5	3.8 $\pm$ 0.0	0.5 $\pm$ 0.0
4	3.7 $\pm$ 0.3	3.2 $\pm$ 0.1	0.2 $\pm$ 0.2
5	3.6 $\pm$ 0.4	3.2 $\pm$ 0.1	2.3 $\pm$ 0.1
6	3.3 $\pm$ 0.3	3.1 $\pm$ 0.3	0.3 $\pm$ 0.2
7	3.2 $\pm$ 0.2	2.7 $\pm$ 0.1	1.6 $\pm$ 0.6
8	2.9 $\pm$ 0.2	2.8 $\pm$ 0.2	0.5 $\pm$ 0.0
9	2.8 $\pm$ 0.2	2.3 $\pm$ 0.1	ND ^x
10	2.6 $\pm$ 0.2	1.6 $\pm$ 0.6	ND
11	2.8 $\pm$ 0.1	1.6 $\pm$ 0.6	ND
12	1.0 $\pm$ 0.6	1.0 $\pm$ 0.6	0.1 $\pm$ 0.1
13	1.8 $\pm$ 0.7	1.1 $\pm$ 0.7	0.2 $\pm$ 0.2
14	2.5 $\pm$ 0.3	1.2 $\pm$ 0.7	ND
15	1.8 $\pm$ 0.7	1.2 $\pm$ 0.8	ND
16	1.0 $\pm$ 0.6	1.0 $\pm$ 0.8	0.2 $\pm$ 0.2
17	1.0 $\pm$ 0.6	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2
18	1.1 $\pm$ 0.9	0.2 $\pm$ 0.2	ND
19	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2	ND
20	2.1 $\pm$ 0.0	0.5 $\pm$ 0.0	ND

^x ND: not detected at the limit of detection of 0 log CFU/tomato

Table D1.6: Mean ($\pm$ SE) *Salmonella* population transferred from one inoculated tomato (~ 5 log CFU/g) to 20 uninoculated tomatoes through different parts (whole slicer, pusher & side plate, blade) of electrical slicer during slicing (n=3).

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)		
	Whole slicer	Pusher & side plate	Blade
1	4.5 $\pm$ 0.4	1.6 $\pm$ 0.6	3.0 $\pm$ 0.3
2	3.0 $\pm$ 0.5	0.2 $\pm$ 0.2	1.1 $\pm$ 0.7
3	2.9 $\pm$ 0.2	0.2 $\pm$ 0.2	0.9 $\pm$ 0.6
4	2.0 $\pm$ 1.0	ND	2.1 $\pm$ 1.2
5	1.6 $\pm$ 0.8	ND	0.2 $\pm$ 0.2
6	0.9 $\pm$ 0.7	ND	0.5 $\pm$ 0.0
7	0.2 $\pm$ 0.2	ND	0.9 $\pm$ 0.7
8	0.2 $\pm$ 0.2	ND	0.7 $\pm$ 0.7
9	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2	0.8 $\pm$ 0.8
10	0.3 $\pm$ 0.2	0.2 $\pm$ 0.2	ND
11	1.0 $\pm$ 0.8	0.1 $\pm$ 0.1	1.1 $\pm$ 1.1
12	ND	ND	0.5 $\pm$ 0.0
13	0.2 $\pm$ 0.2	ND	ND
14	0.2 $\pm$ 0.2	ND	0.1 $\pm$ 0.1
15	0.2 $\pm$ 0.2	ND	0.1 $\pm$ 0.1
16	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1
17	ND	ND	0.1 $\pm$ 0.1
18	0.7 $\pm$ 0.7	ND	ND
19	ND	ND	1.2 $\pm$ 1.2
20	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1

^x ND: not detected at the limit of detection of 0 log CFU/tomato

Table D1.7: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by different post-contamination hold time (after slicing one inoculated tomatoes, wait for 0 min or 30 min before slicing 20 uninoculated tomatoes). Rep 1, Rep 2, and Rep 3 are three replicates of each level.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)					
	Post-contamination hold time (0 min)			Post-contamination hold time (30 min)		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	5.4	4.8	4.8
2	4.5	4.7	4.4	4.8	4.2	4.9
3	4.3	4.8	3.3	3.9	4.1	5.0
4	4.3	3.1	3.7	3.8	3.6	4.4
5	3.0	4.4	3.4	2.9	3.4	4.5
6	3.7	3.5	2.8	2.9	3.6	4.4
7	3.6	3.2	2.9	2.7	3.4	4.3
8	3.2	2.9	2.5	3.3	+	4.0
9	2.5	3.1	2.7	2.7	3.4	3.8
10	2.9	2.7	2.2	2.2	2.5	3.7
11	2.9	2.9	2.5	3.1	2.2	3.7
12	2.1	+	+	+	+	3.3
13	2.9	2.1	+	2.8	+	3.3
14	2.3	3.2	2.2	+	2.2	3.2
15	2.8	2.2	+	+	+	3.5
16	2.2	+	+	2.2	+	3.1
17	2.1	+	+	+	+	2.7
18	2.9	- ^Y	+	2.6	+	3.1
19	+ ^X	+	-	2.2	+	3.1
20	2.1	2.1	2.2	+	2.2	3.4

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

Table D1.8: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by different tomato surface wetness (dry or wet tomato surfaces). Rep 1, Rep 2, and Rep 3 are three replicates of each treatment.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)					
	Tomato surface wetness (dry)			Tomato surface wetness (wet)		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	6.6	6.9	6.7
2	4.5	4.7	4.4	6.7	6.3	6.4
3	4.3	4.8	3.3	6.5	6.4	6.0
4	4.3	3.1	3.7	6.1	6.3	6.0
5	3.0	4.4	3.4	5.9	5.8	6.1
6	3.7	3.5	2.8	5.6	5.7	5.4
7	3.6	3.2	2.9	5.4	5.5	5.1
8	3.2	2.9	2.5	5.2	5.2	5.3
9	2.5	3.1	2.7	5.1	5.2	5.0
10	2.9	2.7	2.2	5.1	5.2	5.1
11	2.9	2.9	2.5	5.0	5.1	4.9
12	2.1	+	+	4.9	5.0	4.8
13	2.9	2.1	+	4.6	5.0	4.4
14	2.3	3.2	2.2	4.7	4.9	4.4
15	2.8	2.2	+	4.5	4.7	4.0
16	2.2	+	+	4.4	4.5	4.2
17	2.1	+	+	4.4	4.2	3.9
18	2.9	- ^Y	+	4.3	4.1	3.7
19	+ ^X	+	-	4.5	4.1	4.0
20	2.1	2.1	2.2	4.2	3.8	3.7

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

Table D1.9: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by different processing temperatures (23, 10, or 4 °C). Rep 1, Rep 2, and Rep 3 are three replicates of each treatment.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)								
	Temperature (23°C)			Temperature (10°C)			Temperature (4°C)		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	3.4	3.3	3.9	4.8	4.8	2.9
2	4.5	4.7	4.4	2.2	2.9	3.0	3.8	4.0	2.4
3	4.3	4.8	3.3	+	+	2.7	3.7	3.7	-
4	4.3	3.1	3.7	2.6	2.2	2.2	2.8	3.5	2.4
5	3.0	4.4	3.4	2.1	+	2.2	3.0	2.1	+
6	3.7	3.5	2.8	2.2	2.2	+	+	2.4	+
7	3.6	3.2	2.9	+	2.5	+	2.2	+	-
8	3.2	2.9	2.5	+	+	+	+	+	2.1
9	2.5	3.1	2.7	+	+	+	+	2.2	+
10	2.9	2.7	2.2	2.6	2.2	+	2.2	+	+
11	2.9	2.9	2.5	+	2.9	+	+	+	+
12	2.1	+	+	2.4	2.4	+	+	+	+
13	2.9	2.1	+	2.2	2.7	+	+	+	-
14	2.3	3.2	2.2	2.4	2.2	+	+	-	-
15	2.8	2.2	+	+	2.2	+	+	-	+
16	2.2	+	+	+	+	-	+	+	-
17	2.1	+	+	+	+	+	-	-	+
18	2.9	- ^Y	+	2.2	+	-	-	-	-
19	+ ^X	+	-	2.2	+	-	-	-	-
20	2.1	2.1	2.2	+	+	-	-	-	-

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

Table D1.10: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by different slice thickness (5.7, 4.8, or 9.5 mm). Rep 1, Rep 2, and Rep 3 are three replicates of each treatment.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)								
	Tomato slice thickness (5.7 mm)			Tomato slice thickness (4.8 mm)			Tomato slice thickness (9.5 mm)		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	4.4	4.9	4.9	4.5	4.3	3.2
2	4.5	4.7	4.4	4.6	5.0	4.4	3.9	4.1	-
3	4.3	4.8	3.3	4.0	4.0	4.0	3.6	2.7	+
4	4.3	3.1	3.7	4.2	3.8	4.1	3.2	+	2.9
5	3.0	4.4	3.4	3.8	3.8	4.1	2.2	+	2.5
6	3.7	3.5	2.8	4.1	3.7	3.7	2.9	+	3.6
7	3.6	3.2	2.9	3.9	3.9	3.5	3.0	+	3.2
8	3.2	2.9	2.5	3.9	3.4	3.8	2.7	2.9	+
9	2.5	3.1	2.7	3.4	3.0	3.3	+	0.5	+
10	2.9	2.7	2.2	3.5	2.9	3.1	2.2	2.5	-
11	2.9	2.9	2.5	3.5	2.9	3.3	3.5	2.6	-
12	2.1	+	+	3.5	2.9	2.6	2.2	2.2	+
13	2.9	2.1	+	3.5	3.0	2.7	2.5	+	+
14	2.3	3.2	2.2	3.0	2.6	+	+	0.5	+
15	2.8	2.2	+	3.0	2.8	2.3	3.0	-	-
16	2.2	+	+	2.5	2.9	2.5	+	0.5	+
17	2.1	+	+	3.1	2.6	2.5	+	0.5	+
18	2.9	- ^Y	+	2.9	2.7	2.8	-	2.6	-
19	+ ^X	+	-	2.2	2.3	+	+	+	+
20	2.1	2.1	2.2	2.7	2.2	2.2	+	-	+

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

Table D1.11: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by different tomato varieties (torero, rebelski, or bigdena). Rep 1, Rep 2, and Rep 3 are three replicates of each tomato variety.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)								
	Tomato variety			Tomato variety (rebelski)			Tomato variety (bigdena)		
	(torero)			Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	3.7	4.3	4.0	2.8	4.6	3.3
2	4.5	4.7	4.4	3.6	3.6	3.0	-	4.2	2.5
3	4.3	4.8	3.3	3.2	2.2	2.5	-	3.8	3.2
4	4.3	3.1	3.7	3.1	2.5	+	+	3.3	2.1
5	3.0	4.4	3.4	3.3	2.7	+	-	2.2	2.6
6	3.7	3.5	2.8	3.1	2.5	-	+	+	+
7	3.6	3.2	2.9	3.7	+	-	2.2	3.0	+
8	3.2	2.9	2.5	3.6	2.2	2.5	+	2.6	2.9
9	2.5	3.1	2.7	3.3	3.1	2.5	2.8	2.9	2.9
10	2.9	2.7	2.2	3.8	3.2	2.2	-	-	2.5
11	2.9	2.9	2.5	3.8	2.2	+	-	2.2	2.2
12	2.1	+	+	2.9	+	3.3	3.6	3.0	2.8
13	2.9	2.1	+	-	2.8	2.7	-	+	+
14	2.3	3.2	2.2	3.4	2.8	2.7	+	-	+
15	2.8	2.2	+	3.0	2.2	+	-	2.1	-
16	2.2	+	+	3.7	+	+	2.6	-	+
17	2.1	+	+	2.7	2.9	+	-	2.4	+
18	2.9	- ^Y	+	3.3	2.2	+	2.5	-	2.1
19	+ ^X	+	-	+	2.5	+	-	-	+
20	2.1	2.1	2.2	3.0	2.8	2.2	+	-	+

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

Table D1.12: *Salmonella* (log CFU/tomato) transfer from one inoculated tomato (~ 5 log CFU/g) to twenty uninoculated tomatoes via the manual slicer as impacted by wash treatments (no wash, tap water wash, or 100 ppm chlorine wash before conveying). Rep 1, Rep 2, and Rep 3 are three replicates of each wash treatment.

Uninoculated tomatoes	<i>Salmonella</i> population (log CFU/tomato)								
	Wash treatment (no wash)			Wash treatment (tap water wash)			Wash treatment (100 ppm chlorine)		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.2	4.9	4.6	4.6	5.1	4.1	-	-	-
2	4.5	4.7	4.4	4.3	4.6	3.5	-	-	-
3	4.3	4.8	3.3	3.9	4.3	3.4	-	-	-
4	4.3	3.1	3.7	3.2	2.9	2.5	-	-	-
5	3.0	4.4	3.4	3.0	3.0	2.5	-	-	-
6	3.7	3.5	2.8	2.7	3.8	2.6	-	-	-
7	3.6	3.2	2.9	2.5	3.7	2.6	-	-	-
8	3.2	2.9	2.5	2.9	3.1	2.1	-	-	-
9	2.5	3.1	2.7	2.5	3.4	2.1	-	-	-
10	2.9	2.7	2.2	+	2.8	2.1	-	-	-
11	2.9	2.9	2.5	2.2	3.3	-	-	-	-
12	2.1	+	+	+	3.0	-	-	-	-
13	2.9	2.1	+	+	2.5	-	-	-	-
14	2.3	3.2	2.2	-	2.8	+	-	-	-
15	2.8	2.2	+	-	+	-	-	-	-
16	2.2	+	+	-	+	-	-	-	-
17	2.1	+	+	+	2.2	-	-	-	-
18	2.9	- ^Y	+	+	2.2	-	-	-	-
19	+ ^X	+	-	+	+	+	-	-	-
20	2.1	2.1	2.2	+	+	-	-	-	-

^X“+”: tomato sample was positive for *Salmonella* after enrichment

^Y“-”: tomato sample was negative for *Salmonella* after enrichment

APPENDIX E:

Transfer and Sanitizer Inactivation of *Salmonella* during Simulated Commercial Dicing and Conveyance of Tomatoes

Table E1.1: *Salmonella* transfer from one batch (0.9 kg) of inoculated tomato (~ 5 log CFU/g) to 10 batches (9 kg) of uninoculated tomatoes through dicing. Rep 1, Rep 2, and Rep 3 are three replicates of this study.

Uninoculated tomatoes (kg)	<i>Salmonella</i> population (log CFU/g)		
	Rep 1	Rep 2	Rep 3
0.9	2.8	3.5	3.6
1.8	2.7	2.5	2.6
2.7	2.2	2.6	2.4
3.6	2.2	2.0	2.1
4.5	1.9	2.0	1.8
5.4	1.9	1.5	1.4
6.3	1.9	1.7	1.4
7.2	1.8	1.2	1.5
8.1	1.8	1.5	1.3
9	1.0	1.2	1.1

Table E1.2: Mean ($\pm$ SD) physicochemical parameters (sanitizer concentration, ORP, pH, and temperature) of sanitizer treatments (peroxyacetic acid, mixed peracid, chlorine, and water control) before and after 2-min washing of diced tomatoes (n=3).

Physicochemical parameters	Sanitizer treatments	Before processing	After processing
Sanitizer concentration (ppm)	Water	--	--
	Peroxyacetic acid	80.7 $\pm$ 0.6 A ^x	79.0 $\pm$ 2.0 A
	Mixed peracid	80.3 $\pm$ 0.6 A	76.0 $\pm$ 3.5 A
	Chlorine	81.3 $\pm$ 1.2 A	74.3 $\pm$ 2.1 B
ORP (mV)	Water	344.3 $\pm$ 38.7 A	336.7 $\pm$ 33.2 A
	Peroxyacetic acid	360.0 $\pm$ 7.9 A	359.7 $\pm$ 8.0 A
	Mixed peracid	480.0 $\pm$ 23.8 A	471.7 $\pm$ 14.6 A
	Chlorine	889.0 $\pm$ 16.5 A	907.0 $\pm$ 14.7 A
pH	Water	7.5 $\pm$ 0.1 A	7.4 $\pm$ 0.1 A
	Peroxyacetic acid	7.4 $\pm$ 0.1 A	7.3 $\pm$ 0.1 A
	Mixed peracid	5.9 $\pm$ 0.4 A	5.7 $\pm$ 0.4 A
	Chlorine	6.0 $\pm$ 0.1 A	5.9 $\pm$ 0.1 A
Temperature (°C)	Water	17.9 $\pm$ 0.3 A	17.8 $\pm$ 0.3 A
	Peroxyacetic acid	19.1 $\pm$ 0.8 A	17.8 $\pm$ 1.3 A
	Mixed peracid	19.2 $\pm$ 0.5 A	17.6 $\pm$ 0.4 A
	Chlorine	15.8 $\pm$ 1.0 A	16.3 $\pm$ 0.3 A

^x Means with the same letters in the same row are not significantly different ($P > 0.05$)

Table E1.3: Mean ($\pm$ SD) *Salmonella* populations on equipment surfaces (Water tank; Flume tank; Shaker table) after washing of 9.1 kg of inoculated diced tomatoes containing ~ 5 log CFU/g of *Salmonella* (n=3).

Equipment surfaces	<i>Salmonella</i> population (log CFU/100 cm ²)			
	Water	Chlorine	Peroxyacetic Acid	Mixed Peracid
Water tank	-1.0 $\pm$ 0.7 A ^x	-1.8 $\pm$ 0.8 AB	-2.3 $\pm$ 0.4 AB	-2.6 $\pm$ 0.0 B
Flume tank	-0.8 $\pm$ 0.4 A	-2.0 $\pm$ 1.0 AB	-2.8 $\pm$ 0.0 B	-2.3 $\pm$ 0.6 AB
Shaker table	0.2 $\pm$ 0.3 A	-2.0 $\pm$ 0.7 B	-2.1 $\pm$ 0.3 B	-2.4 $\pm$ 0.4 B

^x Means with the same letters in the same row are not significantly different ($P > 0.05$)

Table E1.4: Mean ($\pm$ SD) *Salmonella* reductions against *Salmonella* contamination on conveyor belts after 20 min of 80 ppm sanitizer (mixed peracid, peroxyacetic acid, chlorine, electrolyzed water, or water) spray at speed of 30 L/h (n=3).

Sanitizer treatments	<i>Salmonella</i> reduction (log CFU/100 cm ²)	
	Smooth	Interlocking
Mixed Peracid	a ^x 6.5 $\pm$ 0.9 A ^y	a 6.8 $\pm$ 0.7 A
Peroxyacetic Acid	a 5.9 $\pm$ 0.4 A	a 6.1 $\pm$ 0.8 AB
Chlorine	a 3.7 $\pm$ 0.0 B	b 5.7 $\pm$ 0.2 AB
Electrolyzed Water	a 3.5 $\pm$ 0.5 B	a 4.5 $\pm$ 1.2 B
Water	a 1.5 $\pm$ 0.0 C	a 1.5 $\pm$ 0.1 C

^x Means with the same letters in the same row are not significantly different ($P > 0.05$)

^y Means with the same capital letters in the same column are not significantly different ($P > 0.05$)

APPENDIX F:

***Salmonella* Attachment and Early-biofilm Formation on Tomatoes, High-Density Polyethylene and Stainless Steel as Impacted by Substrate, pH, and Temperature**

Table F1.1: Mean ($\pm$ SE) viable *Salmonella* population during 8 days of incubation in the inoculums broth (mixture of 900 ml of TSBYE broth and 4.1 liter of distilled water) at 23, 10 and 4°C (n = 3).

Growth time (day)	<i>Salmonella</i> population (log CFU/ml)		
	23 °C	10 °C	4 °C
0	8.9 $\pm$ 0.04 A	8.9 $\pm$ 0.04 A	8.9 $\pm$ 0.04 A
2	8.8 $\pm$ 0.03 A	8.5 $\pm$ 0.04 B	8.3 $\pm$ 0.06 C
4	8.9 $\pm$ 0.05 A	8.5 $\pm$ 0.02 B	8.2 $\pm$ 0.06 C
8	9.2 $\pm$ 0.01 A	9.3 $\pm$ 0.01 A	8.9 $\pm$ 0.02 B

^x Means with the same letters in the same row are not significantly different ($P > 0.05$)

Table F1.2: Mean ($\pm$ SE) *Salmonella* surface hydrophobicity (as calculated as percent adhesion to xylene) during 8 d of incubation at pH 4.6 and 7.0 at 23, 10 and 4°C in PBS solution (n = 3).

Incubation conditions			Adhesion percentage (%)			
			Day 0	Day 2	Day 4	Day 8
pH	4.6	4 °C	62.61 $\pm$ 0.96	54.08 $\pm$ 2.35	33.57 $\pm$ 1.55	49.48 $\pm$ 3.91 A ^x
		10 °C	62.61 $\pm$ 0.96	22.21 $\pm$ 2.56	42.84 $\pm$ 3.59	54.99 $\pm$ 1.95 A
		23 °C	62.61 $\pm$ 0.96	34.88 $\pm$ 6.2	21.50 $\pm$ 3.83	18.29 $\pm$ 2.71 B
	7.0	4 °C	17.54 $\pm$ 2.44	32.50 $\pm$ 1.57	22.59 $\pm$ 2.76	19.51 $\pm$ 3.46 B
		10 °C	17.54 $\pm$ 2.44	21.61 $\pm$ 1.58	19.69 $\pm$ 1.16	27.82 $\pm$ 0.6 B
		23 °C	17.54 $\pm$ 2.44	16.16 $\pm$ 2.2	12.20 $\pm$ 1.78	17.67 $\pm$ 1.97 B

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

Table F1.3: Mean ($\pm$ SE) *Salmonella* surface charge (measured as zeta potential using ZetaSizer) during 8 d of incubation in PBS solution at pH 4.6 and 7.0 at 23, 10 and 4°C (n=3).

Incubation conditions			Zeta potential (mV)			
			Day 0	Day 2	Day 4	Day 8
pH	4.6	4 °C	-16.58 $\pm$ 0.19	-12.98 $\pm$ 0.22	-13.14 $\pm$ 0.23	-11.73 $\pm$ 0.45 A
		10 °C	-16.58 $\pm$ 0.19	-13.74 $\pm$ 0.21	-12.87 $\pm$ 0.25	-12.03 $\pm$ 0.18 A
		23 °C	-16.58 $\pm$ 0.19	-14.32 $\pm$ 0.84	-11.97 $\pm$ 0.93	-10.35 $\pm$ 0.39 A
	7.0	4 °C	-14.08 $\pm$ 0.49	-15.00 $\pm$ 0.17	-14.41 $\pm$ 0.23	-15.32 $\pm$ 0.51 B
		10 °C	-14.08 $\pm$ 0.49	-14.27 $\pm$ 0.29	-14.27 $\pm$ 0.5	-14.69 $\pm$ 0.45 B
		23 °C	-14.08 $\pm$ 0.49	-15.18 $\pm$ 0.33	-15.27 $\pm$ 0.1	-14.42 $\pm$ 0.1 B

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

Table F1.4: Mean ($\pm$ SE) *Salmonella* biofilm formation on tomatoes surfaces by inoculums prepared in water or 10% tomato organic load solution during 6 d of incubation at 23, 10, and 4°C (n=3).

Incubation conditions			<i>Salmonella</i> population (log CFU/tomato)		
			Day 0	Day 2	Day 6
Inoculum	0% organic load (water)	4 °C	4.4 $\pm$ 0.2	3.1 $\pm$ 0.1	2.6 $\pm$ 0.3 B
		10 °C	4.4 $\pm$ 0.2	2.5 $\pm$ 0.3	2.7 $\pm$ 0.4 B
		23 °C	4.4 $\pm$ 0.2	4.2 $\pm$ 0.1	5.1 $\pm$ 0.4 A
	10% organic load	4 °C	5.8 $\pm$ 0.1	2.2 $\pm$ 0.1	2.4 $\pm$ 0.1 B
		10 °C	5.8 $\pm$ 0.1	2.4 $\pm$ 0.1	3.8 $\pm$ 0.3 AB
		23 °C	5.8 $\pm$ 0.1	4.5 $\pm$ 0.2	5.0 $\pm$ 0.0 A

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

Table F1.5: Mean ($\pm$ SE) *Salmonella* biofilm formation on stainless steel surfaces by inoculums prepared in water or 10% tomato organic load solution during 6 d of incubation at 23, 10, and 4°C (n=3).

Incubation conditions			<i>Salmonella</i> population (log CFU/tomato)		
			Day 0	Day 2	Day 6
Inoculum	0% organic load (water)	4 °C	5.4 $\pm$ 0.5	4.7 $\pm$ 0.4	5.0 $\pm$ 0.1 ABC
		10 °C	5.4 $\pm$ 0.5	5.3 $\pm$ 0.5	5.5 $\pm$ 0.3 AB
		23 °C	5.4 $\pm$ 0.5	6.2 $\pm$ 0.4	5.8 $\pm$ 0.5 A
	10% organic load	4 °C	5.5 $\pm$ 0.7	4.5 $\pm$ 0.3	4.3 $\pm$ 0.3 BCD
		10 °C	5.5 $\pm$ 0.7	4.4 $\pm$ 0.3	4.2 $\pm$ 0.2 CD
		23 °C	5.5 $\pm$ 0.7	4.4 $\pm$ 0.2	3.4 $\pm$ 0.1 D

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

Table F1.6: Mean ($\pm$ SE) *Salmonella* biofilm formation on HDPE surfaces by inoculums prepared in water or 10% tomato organic load solution during 6 d of incubation at 23, 10, and 4°C (n=3).

Incubation conditions			<i>Salmonella</i> population (log CFU/tomato)		
			Day 0	Day 2	Day 6
Inoculum	0% organic load (water)	4 °C	6.3 $\pm$ 0.4	5.2 $\pm$ 0.2	5.3 $\pm$ 0.3 AB
		10 °C	6.3 $\pm$ 0.4	5.5 $\pm$ 0.1	5.5 $\pm$ 0.2 AB
		23 °C	6.3 $\pm$ 0.4	5.6 $\pm$ 0.3	6.0 $\pm$ 0.2 A
	10% organic load	4 °C	6.1 $\pm$ 0.4	5.2 $\pm$ 0.2	5.3 $\pm$ 0.1 AB
		10 °C	6.1 $\pm$ 0.4	5.6 $\pm$ 0.3	5.2 $\pm$ 0.1 AB
		23 °C	6.1 $\pm$ 0.4	5.5 $\pm$ 0.4	4.7 $\pm$ 0.2 B

^x Means with the same letters in the same column are not significantly different ($P > 0.05$)

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