EFFECTS OF SANITIZER TIME AND CONCENTRATION IN PRODUCE WASHING
WATER ON CROSS-CONTAMINATION BY *Salmonella* AND *Escherichia coli* O157:H7

by

JACOB A. POLSKY

(Under the Direction of Mark A. Harrison)

ABSTRACT

Sanitizer strength and exposure time effects on cross-contamination of produce in wash waters were determined. Tomatoes and cantaloupes inoculated with *Salmonella* and lettuce inoculated with *E. coli* were added to a wash tank with non-inoculated produce. Tank conditions mimicked conditions in commercial wash tanks. At time intervals, non-inoculated produce was removed from the tank and sampled for the pathogens. For all produce, cross-contamination occurred in the absence of sanitizer. With 50 ppm chlorine, 11-16% of tomatoes were positive at all sampling times, while 100 ppm chlorine prevented cross-contamination. Tomatoes (~11%) exposed to 80 ppm peroxyacetic acid were *Salmonella* contaminated after 2 min but after 4 min none were positive. Peroxyacetic acid prevented *Salmonella* cantaloupe cross-contamination, while chlorine was less effective. For lettuce, chlorine exposure was more effective than peroxyacetic acid in preventing *E. coli* O157:H7 cross-contamination. This data could be used when evaluating process control points.

INDEX WORDS: *Salmonella, E. coli*, tomatoes, lettuce, cantaloupes, cross-contamination
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by

JACOB A. POLSKY

B.S., Georgia Southern University, 2005

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2008
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by

JACOB A. POLSKY

Major Professor:  Mark A. Harrison
Committee:  Mark Berrang
  William C. Hurst

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2008
DEDICATION

This project is dedicated to my parents and grandmother who have patiently put up with me throughout my education.
ACKNOWLEDGEMENTS

I would like to express my gratitude to all my professors at the University of Georgia who made my education possible. I would also like to thank Mark Harrison, Ruth Ann Morrow, Karen Simmons, and all those who worked with me to help me get through my research. The project was funded through a grant from the National Integrated Food Safety Initiative (Grant no. 2002-51110-01982) of the Cooperative State Research, Education, and Extension Services, U.S. Department of Agriculture and by the Georgia Agricultural Experiment Stations.
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Chapter 1

Introduction

Because there is some debate over whether it is a safer practice to wash or field pack produce, one step along the lines of processing fresh produce was analyzed as a source of cross-contamination; more specifically the wash, or dump-tank step. Western states such as California often choose to field package produce rather than transport it to a packing shed facility for a washing treatment as done in many eastern states such as Georgia (38). The issue here is that in field packed produce, if a pathogen is present on an individual piece of produce, then without a washing step, there is no way to remove the pathogen. On the other hand, if there are fecal pathogens present, a washing step could serve as a stage for cross-contamination through the processing water. Though visible fecal contamination may not be as often an issue with fresh produce being washed prior to packaging, monitoring wash water pH, chlorine (or other chemical disinfectant used) concentration, water temperature, oxidation reduction potential (ORP), testing the temperature of the produce, and also occasional testing the bacterial contamination level of the water can be used in a preventative plan to reduce the likelihood of cross-contamination.

Experiments done with immersion chilling for poultry found that while immersion chilling does reduce the bacterial numbers on carcasses and did not significantly affect the numbers of *Escherichia coli*, the prevalence of *Salmonella* on previously uncontaminated carcasses was increased up to 25% after chilling as a result of cross-contamination (37). For poultry immersion chillers, the U. S. Department of Agriculture/Food Safety and Inspection
Service (USDA/FSIS) mandates a zero tolerance for visible fecal contamination on carcasses going into the chillers (37).

Water was shown to be a vehicle for cross-contamination in another study on air-chilled poultry. It was concluded that air-chilling is a means of spreading *Salmonella* and the use of water spray increased the incidence of cross-contamination (32). Other researchers looked at poultry chilling to determine a probability model of cross-contamination for *Salmonella* and *Campylobacter jejuni*. Using 0 and 50 ppm chlorine, cross-contamination occurred for both bacterial types during the immersion-chilling step (40).

For the current study, six strains of *Salmonella*, each associated with illness outbreaks involving tomatoes and cantaloupes were chosen: *Salmonella enterica* serotypes Montevideo, Hadar, Agona, Javiana, and Typhimurium 654 and 848. Also, six strains of *Escherichia coli* were chosen for trials with lettuce: *Escherichia coli* O157:H7 932 (human isolate), ATCC 8677, ATCC 11775 (O1:K:H7), ATCC 25922 (clinical isolate, vero-toxin), K-12 LM1010, and MC4100. The experiment was designed to determine the probability of cross-contamination of produce in a dump/wash tank and to determine what effect sanitizers used in wash water have on cross-contamination potential. In the experiment, inoculated produce was placed in a tank along with non-inoculated produce, to mimic conditions that could be found in a produce packing operation. *Salmonella* spp. were used in experiments with tomatoes and cantaloupes, while *E. coli* was used in experiments with lettuce.
With the ever increasing demand for fresh fruits and vegetables, food safety related to produce has become an important topic. Because of the lack of processing done to fresh produce, Good Agricultural Practices (36) play a very important role in protecting the public from health concerns such as contamination with human pathogens. Contamination can occur anywhere along the production line from growing to market. Helping to reduce the likelihood of cross-contamination of produce during washing is one small but important aspect in protecting the food supply.

Foodborne illnesses continue to be a public health risk even with all the safety precautions implemented today in U.S. agriculture. Though many measures are taken to prevent these illnesses, the large quantities and broad spectrum of food produced make it impossible to monitor every piece of food produced. It is not surprising that illness such as salmonellosis are so prevalent, because it is most often associated with Salmonella enterica serovar Enteritidis, a bacterium which can be present in hens which seem healthy but are producing contaminated eggs (34). The Center for Disease Control and Prevention (CDC) estimates that each year there are approximately 76 million cases of foodborne illness in the U.S., with 325,000 resulting in hospitalization, and 5,000 resulting in death (6).

The “Five a Day for Better Health” program was implemented in 1991 by the National Cancer Institute to encourage the consumption of fresh fruits and vegetables (31). The increasing consumption of fresh fruits and vegetables which accompanies a trend for a healthier
American diet is a possible reason for recent notable outbreaks of foodborne pathogens (31). This campaign helped to spark the growing increase in fresh produce consumption. Produce related outbreaks between 1987 and 1992 remained fairly constant, while from 1993 to 1997, produce related outbreaks increased five-fold (31). From 1970 to 1994, there was a 27% increase in fresh fruit and vegetable consumption in the average American diet (24). Lettuce production more than tripled between the years 1960 and 2004 (33). Along with the increasing consumption rates, the number of foodborne illnesses linked to fresh produce has grown. For example, multiple, multistate outbreaks of salmonellosis have been tied to tomatoes, cantaloupes and lettuce since 1989. This is likely due to the dispersal of produce with a low level contamination over a broad geographical area (24).

**Produce Related Foodborne Illness**

*Salmonella* and *Escherichia coli* O157:H7 are thought to have arisen from a common ancestor. The gastrointestinal tract of animals and also animal feeds are important sources for *Salmonella* (26). Soil and water, plant and plant products, food handlers, as well as the gastrointestinal tract are important sources for *Escherichia* O157:H7 (26). Recently, there have been several incidences of illnesses associated with *Salmonella* and *E. coli* occurring in the United States associated with fresh produce (1). Moreover, produce such as tomatoes, cantaloupes, and lettuce, which are grown on or near the ground, have the increased chance to become contaminated with human pathogens at multiple steps through the production chain from growing, harvesting, washing and packing (1).

In February of 2007 there was a large multi-state outbreak linking *S. Tennessee* to Great Value and Peter Pan peanut butters. These products were processed in Sylvester, GA at a ConAgra processing plant, and after testing, the Food and Drug Administration (FDA) found
Salmonella in the environment, suggesting that the product was contaminated prior to the product reaching consumers (9, 18). During June and July of 2007, on-going investigations linked Robert’s American Gourmet brand Veggie Booty, a puffed rice and corn snack with a vegetable coating, to an outbreak due to S. Wadsworth. The FDA and local health department investigators suspect the seasoning to be the source of contamination. Also associated with this same product, S. Typhimurium was found in a bag collected at the same time as the bags containing S. Wadsworth. Using Pulsenet, the CDC identified 10 people ill from S. Typhimurium during the same time frame of the S. Wadsworth outbreak, 8 of which were interviewed to reveal that all had consumed the Veggie Booty (5). In October 2006, another multi-state outbreak involved a strain of S. Typhimurium. The CDC confirmed previous assumptions that contaminated tomatoes at various restaurants were responsible for the outbreak (7).

Another fruit to have been associated with Salmonella contamination is cantaloupe. Castle Produce announced a recall of fresh cantaloupes after discovering that some cantaloupes tested positive for Salmonella after being delivered in February of 2007. However, there were no reported illnesses in the weeks that followed (17). Between 1990 and 2000, cantaloupes have been linked to more than 800 cases of salmonellosis in the United States and Canada alone (1). Numerous cases were reported in 2001 and 2002, which resulted in two deaths in the U.S. from consumption of Mexican cantaloupes contaminated with S. Poona (1). The FDA conducted a recent survey which 2.4% of 115 cantaloupes sampled tested positive for Salmonella (1). More recently there have been two foodborne illness outbreaks of salmonellosis involving Honduran cantaloupes and tomatoes. The FDA monitored cantaloupe recalls from the Agropecuaria Montelibano Company in Honduras after an ongoing investigation in the United States which
began in January 2008. As a follow-up, the FDA worked with the company to ensure that the corrective measures implemented were adequate to prevent the risk of contamination (20, 23). The bacterium linked to this outbreak was *Salmonella* Litchfield. As of March 22, 2008, there have been 50 known illnesses in the United States and 9 illnesses in Canada, with 14 total hospitalizations (20, 23).

In 2006, there was a multi-state outbreak of *E. coli* O157:H7 with fresh spinach processed by Natural Selection Foods being determined the source (8). Chopped green onions, used in Taco Bell restaurants, were determined to be the source of an *E. coli* 0157:H7 outbreak in some northeastern states in November and December of 2006 (3, 4).

A current outbreak (April-June 2008) involving tomatoes appears to be due to *Salmonella* Saintpaul, an uncommon strain. Since the outbreaks began in April 2008 through early July, there have been over 100 hospitalizations and 900 cases reported. The FDA used traceback analysis to compose lists of states, countries, and areas where tomatoes were grown and harvested which were not related to the outbreak. At the time of preparing this literature review, the source of this outbreak was still in question. These outbreaks stress the importance of using proper preventative measures in the growing, harvesting, and packing of produce (12).

**Packing Houses**

Packing houses can harbor pathogenic microorganisms which in turn increase the risk of cross-contamination (12). In one study microbial levels on produce, tested at different points through a packing house, showed that microbial levels increased from field through packing for produce such as cilantro, parsley, and cantaloupe (29). The FDA developed the “Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables” in 1998 to provide a document which offers safety guidelines for critical steps along the production line. This
document, as well as others, are known more commonly as Good Agricultural Practices, GAPs (15). In 1990 and 1993, using laboratory-based surveillance of isolates obtained by health departments in four states, S. Javiana and S. Montivideo, were identified as the pathogens responsible for multi-state outbreaks. The outbreaks were traced back to a single packing house in South Carolina. The investigations revealed that contamination most likely occurred in the packing house where field grown tomatoes were washed in a common water bath (24). The quality of the wash water dictates its potential for bacterial contamination (12). Research involving E. coli O157:H7 and S. Typhimurium showed survival characteristics of both organisms at 4°C and 22°C, with a 1 log CFU/g reduction after 22 days in water (10). Other researchers showed that washing cabbage leaves with distilled water yielded a 1 log CFU/g reduction, while using a chlorine treatment (acidified sodium chlorite) yielded a 3 log CFU/g reduction (41). Laboratory experiments have shown that tomatoes and other fresh fruits and vegetables, though not commonly thought of as a vehicle for Salmonella, can in fact support growth of enteric bacterial pathogens, including Salmonella, E. coli O157:H7, and Shigella (24). Additional tests financed by the tomato industry for South Carolina and Florida have shown that when tomatoes are placed into water baths in which the water is cooler than the tomato, the pulp can absorb water, and in the process pull in or internalize the bacteria (24). Furthermore, this research illustrates that the Salmonella surviving on the skin can survive and even multiply in high numbers on cut or sliced tomatoes (24).

**Chlorine Effectiveness** (For the extent of this paper, when referring to chlorine, it will be in the hypochlorous acid (HOCL) form unless otherwise stated.)

Chlorine and other disinfectants are added to wash water to help prevent cross-contamination from free-floating microbes, but are not considered an effective means of
sanitizing the surface of produce (36). Though washing has been shown to significantly reduce the mean population on produce such as conventionally grown spring salad mix (36), fresh cut produce such as sliced melons, lettuce, and others, should be expected to have a normal non-pathogenic microflora associated with them (15). One study compared chlorine at 70-80 ppm to electrolyzed oxidizing water, which has approximately the same chlorine concentration, and determined that the chlorine alone was able to bring about the same reduction as electrolyzed oxidizing water in a simple aqueous solution. However, it was noted that the effectiveness of chlorine in the presence of organic material decreases, citing a study in which treatment with chlorine levels at 200 ppm yielded less than 2 log CFU/g reduction of *L. monocytogenes* on fresh produce (39). A readily reactive chemical, chlorine can combine with almost any oxidizable substrate to form secondary compounds (22). Oxidation can be described as the addition of oxygen or the loss of hydrogen, and reduction as the loss of oxygen and the gain of hydrogen (25, 27).

Chlorine in the hypochlorous acid form is the most effective. This is also the form most often used in chlorine wash waters. In this form, chlorine is readily transferred across the cell membrane. Chlorine is a very good antimicrobial in water, killing most bacteria at concentrations as low as 1-2 ppm in less than one minute (22, 28). However, under some circumstances even at high levels (320 ppm), it may not be possible to eliminate contamination from *Salmonella*, or other pathogens depending on pH and organic material present (24).

Chlorine usage on produce, recommended at a concentration between 50 - 200 ppm for post-harvest treatments of fresh produce, is a subject of study with mixed results (2, 14, 16, 22). This may be because the effectiveness of chlorine is dependent on many variables such as pH, temperature, and organic matter which accumulate in the dump tank.
For chlorine to be effective and safe, pH values should be between 6.0 and 7.5. When the pH is above 7.5, very little chlorine is available as hypochlorous acid, but rather as inactive hypochlorite ($\text{OCl}^-$). When the pH is below 6.0, chlorine gas may be formed, which poses a serious health hazard (2, 14).

pH is not the only method of monitoring chlorine’s effectiveness. Measuring the oxidation reduction potential, (ORP), is being widely adopted as a means to measure water quality when chlorine or ozone is used as a sanitizer, especially when it comes to fresh produce and fresh-cut produce. ORP offers real-time monitoring, in millivolts (mV), and gives the operator a single value rather than multiple values to be referenced on a chart, which might be needed when using titrations or other kits which read chlorine and pH separately. More sophisticated systems use pH sensors and ORP meters to continually monitor water, and inject acid, typically citric acid, or hypochlorite on a “demand-basis”. It has been shown that ORP readings of 650-700 mV are sufficient to rapidly kill pathogenic bacteria such as *Salmonella* and *E. coli*. One problem with continuous ORP monitoring, would be build up of organic and inorganic matter because this could result in over-injection of chlorine or other disinfectants. This could cause damage to the produce, or even result in production of hazardous by-products such as chlorine gas. Maintenance is another issue because equipment routinely needs cleaning and calibration. A producer should always have back up methods available to monitor the situation such as hand-held ORP monitoring devices or kits to test ppm of chlorine and acidity (22). A reading of at least 650 mV is recommended to provide sufficient hypochlorous acid (15). Readings above 800 mV are unnecessary in most processing waters (15, 22).

Because of findings in previously mentioned studies, temperature is a critical control point for tomatoes when they are dumped into a water bath. It is recommended that the water be
at least 10°C warmer than the produce introduced into it, to prevent a temperature-generated pressure differential resulting in microbial uptake and potential internalization by the produce (12, 14). In 1995, Zhuang found that when tomatoes were placed in water which was 15°C cooler than the produce, tomatoes took up greater numbers of *Salmonella* spp. from the aqueous environment (13).

Organic material, as mentioned before, affects chlorine activity. Organic material can include food deposits or residues, petroleum deposits, from lubrication oils, grease, or other lubrication products, and non-petroleum deposits such as animal fats and vegetable oils (21). Some compounds which can leak out of tissues of cut or damaged produce can neutralize some of the chlorine before it reaches the bacterial cell, voiding the action of the chlorine (14, 15).

Chlorination alone is not sufficient to eliminate bacteria from contaminated produce. It has been shown that *S. Montevideo* inoculated in cracks on the skin of mature green tomatoes survived treatment with 100 ppm chlorine (14). Because of all the variables which can alter chlorine effectiveness, it is very important to establish good monitoring practices for using a dump tank step in produce washing.

**Other Potential Food Washing Agents**

Chlorine (sodium hypochlorite) is not the only product considered for produce washing practices. Other products include ozone (O₃), chlorine dioxide (ClO₂), electrolyzed oxidizing water, hydrogen peroxide (H₂O₂), peroxyacetic acid containing products (i.e., Tsunami 100), and others (28). For the purposes of this paper, we will only look at Tsunami 100.

Tsunami 100, a registered product with the Environmental Protection Agency (EPA), was developed by Ecolab®. Recommended use is at 80 ppm peroxyacetic acid with a contact time of 45 seconds (2). This product is the only EPA registered antimicrobial product to make a claim to
reduce 99.9% of pathogens, including *E. coli* and *Salmonella*, in processing waters used to wash produce. Also, it is used to control spoilage bacteria which are present on fresh and fresh-cut produce thus, extending shelf-life of the produce. Tsunami 100, unlike chlorine (sodium hypochlorite), has a low reactivity with organics and soils which may be present in wash waters (2). Furthermore, Tsunami 100 does not require constant pH testing because it is effective across a wide pH range of acid to slightly alkaline (11). Tsunami 100 can be used at multiple stages along the processing line including multi-stage flumes, chill tanks, coolers, and wash waters (2). The active ingredient in Tsunami 100 is peroxyacetic acid, with the main by-products being acetic acid, oxygen, and water (2, 11). When tested in wash waters for Braeburn apples, Tsunami 100 was the most effective at reducing bacteria. When comparing products to sanitize retail lettuce, 80 ppm peroxyacetic acid (Tsunami 100) was more effective at lowering bacteria than sodium hypochlorite (28). A major benefit of Tsunami is that no final rinse is required, which can cut down on processing costs (11).

Clearly foodborne illnesses pose a potential problem here in the U. S., and in all parts of the world. Legislation to instill Good Manufacturing Practices and GAPs should be at the forefront of reducing the likelihood of illness. However, scientific research in many different areas of the food supply is needed to help develop legislation, especially within the fresh produce industry.
Chapter 3

Materials and Methods

Bacterial Strain Selection and Inoculum Preparation. The following *Salmonella enterica* serotypes were used from our stock collection: Typhimurium 654 and 848, Montevideo, Hadar, Agona, and Javiana. In addition, *E. coli* O157:H7 932 (human isolate), *E. coli* ATCC 8677, 11775, and 25922 from our stock cultures, and *E. coli* K-12 LMM 1010 and *E. coli* K-12 MC 4100 from Dr. Sadhana Ravishankar (National Center for Food Safety and Technology, Illinois Institute of Technology, Summit-Argo, IL) were used. Both *Salmonella* and *E. coli* strains were initially sensitive to a level of 50 µg/ml nalidixic acid (Sigma Chemical Co., St. Louis, MO). The strains were adapted to be nalidixic acid resistant by subculturing through increasing concentrations of nalidixic acid from 2.5 to 50 µg/ml for 7 days (30). Cultures were grown at 37ºC for 24 h in tryptic soy broth (Becton, Dickinson and Co., Sparks, MD) supplemented with each concentration of sterile filtered (0.22 µm) nalidixic acid (TSBN). Cultures grown in TSBN were surface plated on tryptic soy agar supplemented with 50 µg of nalidixic acid/ml (TSAN) and incubated at 37ºC for 24 h to generate stock cultures. Colonies picked from TSAN were transferred into cryovials containing a cryopreservative (Microbank™, PRO-LAB Diagnostics, Austin, TX) and stored at -80ºC until used.

Prior to use, the individual frozen *Salmonella* strains were reactivated in 10 ml of TSBN for 24 h at 37ºC, while frozen *E. coli* strains were reactivated under similar conditions in 10 ml of lactose broth (Becton, Dickinson and Co.) supplemented with 50 µg of nalidixic acid/ml. Cultures were harvested by centrifugation at 9,000 x g for 10 min and the pellets were washed.
twice with sterile deionized water (SDW) and resuspended in phosphate buffer saline (PBS). After the final wash, the 6 strains of *Salmonella* were pooled to form a multistrain cocktail inoculum to be used to inoculate tomatoes and cantaloupes. The 6 strains of *E. coli* were likewise pooled for the inoculum for lettuce.

**Selection of Produce and Inoculation.** Tomatoes, cantaloupes, and iceberg lettuce were purchased from a local Athens, GA grocery store. Fresh tomatoes which had been washed but not waxed were used. All produce purchased was kept at 4°C until used, no longer than two weeks. Produce showing deterioration was not used in the experiments.

Tomatoes and cantaloupes were used as whole produce, while the lettuce was aseptically cut into 25 cm² pieces before inoculation. Three hundred µl of *Salmonella* (for tomatoes and cantaloupes), or *E. coli* O157:H7 (for lettuce) cocktail was spotted over a 25 cm² surface area on each piece of produce which resulted in approximately 3.0 x 10⁵ cfu/ml. The 25 cm² area was marked with a black ‘permanent laboratory marker’ to distinguish inoculated pieces from non-inoculated. After inoculation, the produce was allowed to dry for 12-14 h in a biological safety cabinet at ambient temperature.

**Preparation of Water in Dumptank.** Municipal water, which also served as a control, was used to prepare all water samples. Approximately 60 L of water was measured in the tank (61 x 46 x 61 cm) for tomatoes and cantaloupes, while approximately 16 liters of water was used in a smaller tank (31 x 31 x 31 cm) for the lettuce sampling. For chlorinated water treatments, chlorine concentration was adjusted using common household bleach, to concentrations of 50 or 100 ppm. Chlorine concentration was measured using chlorine strips (Quantab®, Hach Company, Loveland, CO). Because chlorine is most effective between pH 6.0 and 7.5, 0.1N citric acid (T. J. Baker Mallinekrodt, Baker, Inc., Phillipsburg, NJ) was added until a value in this range was
achieved. The pH was measured with a pH meter (Accumet AB15, Fisher Scientific, Suwanee, GA). The oxidation reduction potential (ORP) was measured with a hand-held ORP meter (Orion Research, Inc., Beverly, MA).

Peroxyacetic acid (Tsunami 100, Ecolab®, St. Paul, MN) was also used at a concentration of 80 ppm according to the manufacture’s instructions. The concentration of peroxyacetic acid was tested with a titration kit provided. Though not tested in agricultural use, ORP was tested the same as with chlorinated water concentrations for comparative purpose.

Water for all treatments was used at an average temperature of 39°C to ensure a minimum of 10°C higher temperature than the produce.

Produce Washing. After inoculation of the produce and preparation of the water, fresh uninoculated and inoculated produce were introduced into the dump tank using aseptic procedures to avoid cross-contamination before the experiments. The dump tank process was simulated by using a sterile rod to circulate the water, moving the produce and causing agitation between individual fruits (likely conditions in an actual tank). For each type of produce, different concentrations of either chlorine or peroxyacetic acid were compared to a control of no added sanitizer.

For tomatoes, the following concentrations were tested in triplicate for chlorine: 0, 50, or 100 ppm. Also peroxyacetic acid was tested at a concentration of 80 ppm, as recommended by the manufacturer. Four inoculated tomatoes were added to a tank with 24 non-inoculated ones for each wash treatment. Non-inoculated tomatoes were aseptically removed from the tank, 6 at a time, at 4 different time intervals: 2, 4, 6, and 8 min. These time intervals were chosen to be somewhat representative of how long the produce actually spends in a wash tank. After removal, the produce was thoroughly massaged in bags containing 20 ml of DE neutralizing broth.
Two hundred ml of lactose broth (Becton, Dickinson and Company) was added to each bag as an enrichment broth, and after its addition, the tomatoes were incubated for 24 h at 37°C. The inoculated tomatoes were immediately removed from the tank following the removal of the last non-inoculated fruit and incubated in a similar manner.

For cantaloupes, the chlorine and peroxyacetic acid concentrations used were the same as those used for the tomato treatments. Two inoculated cantaloupes were added to a tank with 12 non-inoculated cantaloupes for each washing treatment. Non-inoculated cantaloupes were aseptically removed 6 at a time, at 2 different time intervals, 4 and 8 min, while the inoculated melons were immediately removed from the tank following the removal of the last non-inoculated fruit. After removal, the produce was then put into bags containing 20 ml of DE neutralizing broth plus 200 ml lactose broth, thoroughly massaged, and incubated as described above. The cantaloupes were removed and discarded from the bags before the bags were incubated.

For lettuce, the chlorine and peroxyacetic acid concentrations used were the same as described above. Four inoculated pieces of lettuce were added to a tank containing 55-65 non-inoculated 25 cm² pieces of lettuce for each variation. Non-inoculated pieces were aseptically removed 6 at a time, after 45 sec in the tank. After removal, the lettuce was put into bags containing 20 ml of DE neutralizing broth and 200 ml modified EC broth (Becton, Dickinson and Company) and thoroughly massaged before enrichment incubation. The inoculated lettuce was immediately removed following the removal of the last non-inoculated lettuce sampling. The bags containing the individual pieces of lettuce were then incubated for 24 h at 37°C.

After incubation, bags containing tomatoes and bags which contained cantaloupes were treated with the same procedure as follows. First, two-1 ml samples were drawn from each bag.
One ml was put into a 9 ml tube containing Rappaport Vassiliadis broth (Becton, Dickinson and Company) and incubated for 24 h at 42°C. The other 1 ml portion was put into a tube containing 9 ml of tetrathionate broth (Becton, Dickinson and Company) for incubation for 24 h at 37°C. Following incubation, both tubes were streak-plated onto plates of tryptic soy agar (Becton, Dickinson and Company) + nalidixic acid (50 ug/ml) + sodium pyruvate ((Becton, Dickinson and Company) (TSANP), as well as plates of XLT-4 (Becton, Dickinson and Company) + nalidixic acid (50 ug/ml) + 0.1% sodium pyruvate (XLT-4NP) agar and all plates were incubated at 37°C for 24 h.

Because XLT-4NP is a more selective agar for *Salmonella* spp., presumptive positive colonies were selected first from this media. If there were no presumptive positive *Salmonella* colonies on the XLT-4NP plate, then a presumptive positive, if present, was taken from the TSANP plate. After selecting presumptive positives colonies, tubes containing triple sugar iron agar (TSI; Becton, Dickinson and Company) and lysine iron agar (LIA; Becton, Dickinson and Company) were inoculated and incubated at 37°C for 24 h. After incubation, the tubes were examined for typical reactions indicative of *Salmonella*. If tubes gave a positive indication of *Salmonella*, a latex agglutination test was used as a final indicator.

For lettuce samples incubated in modified EC broth, samples were streaked onto sorbitol MacConkey agar (Oxiod LTD., Basingstoke Hampshire, England) + nalidixic acid (50 µg/ml) (SMACNP) and TSANP. If these plates gave a presumptive positive reading for *E. coli* O157, Dry Spot coagulation tests (Oxiod LTD., Basingstoke Hampshire, England) were performed on the samples for a confirmation of the presence of *E. coli* serotype O157.

**Water Sampling:** For each trial, a 200 ml sample of water was drawn and filtered through a 0.45 µm mixed cellulose esters filter (Millipore Corp., Billerica, MA). The filter was then stomached
with 100 ml of 0.1% peptone. To enumerate the *Salmonella* in the water, 100 ml of 2X lactose broth was added to the stomached filter and peptone. To enumerate the *E. coli* O157:H7, 100 ml of 2X modified EC broth was used in place of lactose broth. Dilutions were plated onto the same media as described above (XLT-4NP for *Salmonella*, or SMACNP and TSANP for *E. coli*) and incubated as described above. These plates were used to enumerate pathogens in the water.

**Data Analysis:** Three replications of each treatment were done. The average number of colony forming units and percentage of *Salmonella* or *E. coli* O157:H7 positive samples were calculated.
Tomatoes: When no disinfectant was used in the wash water, almost all of the uninoculated tomatoes became contaminated with *Salmonella* when exposed to contaminated tomatoes (Table 1). By 2 min, most tomatoes without sanitizer became contaminated with *Salmonella* and by 4 min all were positive. The addition of either chlorine or peroxyacetic acid to the wash water greatly reduced the number of cross-contaminated tomatoes with 100 ppm of chlorine being the most effective. When exposed to 50 ppm chlorine, 11-16% of tomatoes were positive at all sampling times, while exposure to 100 ppm chlorine prevented cross-contamination of any tomatoes. Approximately 11% of tomatoes exposed to 80 ppm peroxyacetic acid were contaminated with *Salmonella* after 2 min but after 4 min, none were positive. Chlorine and peroxyacetic acid treatments resulted in the inactivation of *Salmonella* on many of the inoculated tomatoes but did not completely eliminate *Salmonella* on the inoculated tomatoes. There was little difference in the *Salmonella* recovery rates between using Rappaport Vassiliadis or tetrathionate broths. Chlorine at 50 and 100 ppm completely eliminated *Salmonella* free-floating in the water samples. The peroxyacetic acid was not as effective in reducing *Salmonella* in the water.

Cantaloupe: For cantaloupes without sanitizer, all melons were positive for *Salmonella* after 4 min exposure to wash water containing contaminated cantaloupes (Table 2). As with the tomatoes, there was also a trend with the cantaloupes showing somewhat of a relationship between increasing the concentration of chlorine and decreasing pathogen cross-contamination.
However, the peroxyacetic acid was more efficient at reducing cross-contamination between cantaloupes than either of the chlorine concentrations. Chlorine and peroxyacetic acid were effective at reducing free-floating *Salmonella* in the water. Cross-contamination of cantaloupes did not occur with the peroxyacetic acid treatment, while both chlorine treatments were less effective (28-78% positive).

**Lettuce:** Lettuce was washed for 45 seconds (shorter time because typical lettuce washing practices are much quicker than the washing of tomatoes or cantaloupes) in water with concentrations of 0, 50, and 100 ppm chlorine, and also 80 ppm peroxyacetic acid. For lettuce, exposure to chlorine at either concentration for 45 sec was effective in preventing *E. coli* O157:H7 cross-contamination. Peroxyacetic acid was not as effective with at least 77% of the samples being positive. Both the chlorine and the peroxyacetic acid treatments eliminated free-floating *E. coli* O157:H7 from the water.
Chapter 5
Discussion

As the results of this experiment show, it is possible for cross-contamination to occur in the wash/dump-tank even if a sanitizer is used. Other studies demonstrated that cross-contamination can occur through processing fresh produce, particularly in the packing shed (14, 22, 36). Though the results are not startling, it does show that steps could be taken to help reduce the likelihood of such an event.

In comparison to the tomatoes and lettuce, the higher contamination rates of the cantaloupe after exposure to disinfectants could be due to the rough surface of the fruit which may offer a good place for bacteria to adhere, as well as protection to the bacteria from the sanitizer. Even though non-waxed tomatoes were used, the nature of the tomato has a waxy surface which does not allow the bacteria to easily adhere to the fruit or offer any protection from the sanitizer. The presence of organic material could also be responsible for reducing the effectiveness of the chlorine (12).

The lettuce showed 100 percent contamination at 0 ppm chlorine in just 45 sec, while 0% contamination after exposure to 50 or 100 ppm chlorine. The peroxycetic acid did not seem to be an effective sanitizer in the lettuce wash. This could be due to the short amount of time that lettuce is actually washed. Chlorine has been shown to kill pathogens free-floating in water in as little as 5 seconds (12, 29). Peroxyacetic acid may need a longer contact time to be as effective as the chlorine.
In any case, these experiments showed that cross-contamination is possible during the washing step. Currently most facilities that do not field pack wash produce by loading them into a tank to be moved by water flow. The problem with this is that the bacteria are moving in the same direction as the produce. Other researchers have employed techniques with chicken carcasses in which the birds were moving in an opposite direction as the movement of water. The result is that the bird exiting was hit by a clean spray of water. If the water were coming from behind, the dirty birds could possibly contaminate the birds about to exit (37).

Employing the same principal (counter-current washing), the suggestion for a design by which screens could move the produce in a direction opposite of the flow of water is shown in Figure 1. The result is that the produce exiting is exposed to the cleanest water immediately before exiting the tank. The diagram depicts screens being pulled in a direction opposite the flow of water. The side which the fruit would enter has a ramp so that the screen can slide on the bottom so that no produce is missed. The water entering in the opposite direction would be chlorinated as well as having the temperature and pH adjusted before entering. It should be noted that this is just a conceptual idea and that engineers would be needed to come up with an actual plan.

Adding sanitizers to wash waters is primarily intended to prevent cross-contamination. Complete disinfection of the produce is a less obtainable target. The results of this study show that though aqueous sanitizers are more beneficial at reducing cross-contamination than water alone, cross-contamination can occur even with these sanitizers. GAPs should always be followed to help reduce the likelihood of contamination from natural factors such as animals, insects, humans, soil and other sources. Even with GAPs in place, there is still need for more effective ways to clean produce. Other researchers are looking into different strategies. Some
studies show that gaseous CLO$_2$ application is more lethal to microorganisms than aqueous chlorine. Research to help ensure the safety of the food supply should continue to be at the top of our nation’s agenda.
References


7. Center for Disease Control and Prevention. 3 November 2006. Salmonellosis - Outbreak Investigation, October 2006. Available at:


Figure 1. Proposed counter current produce wash tank design.
Table 1. Number of *Salmonella* positive tomatoes due to cross-contamination after varying exposure times to contaminated tomatoes during washing. In addition, the numbers of inoculated tomatoes remaining positive after washing are presented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 min Exposure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 min Exposure</th>
<th>6 min Exposure</th>
<th>8 min Exposure</th>
<th>Inoculated Tomatoes</th>
<th>Wash Water Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TT</td>
<td>RV</td>
<td>TT</td>
<td>RV</td>
<td>TT</td>
</tr>
<tr>
<td>0 ppm Chlorine or peroxycetic acid</td>
<td>6/6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.67/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(94.4)</td>
<td>(100.0)</td>
<td>(100.0)</td>
<td>(100.0)</td>
<td>(100.0)</td>
</tr>
<tr>
<td>50 ppm Chlorine</td>
<td>1/6</td>
<td>1/6</td>
<td>0.67/6</td>
<td>0.67/6</td>
<td>0.67/6</td>
<td>0.67/6</td>
</tr>
<tr>
<td></td>
<td>(16.7)</td>
<td>(16.7)</td>
<td>(11.1)</td>
<td>(11.1)</td>
<td>(11.1)</td>
<td>(11.1)</td>
</tr>
<tr>
<td>100 ppm Chlorine</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>80 ppm Peroxyacetic acid</td>
<td>0.67/6</td>
<td>0.67/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>(11.1)</td>
<td>(11.1)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Unionculated tomatoes were mixed with *Salmonella* contaminated tomatoes for the designated times

<sup>b</sup> RV: Rappaport Vassiliadis broth; TT: tetrathionate broth

<sup>c</sup> Average # of *Salmonella* positive tomatoes per replication/# of tomatoes sampled per replication; Values within parenthesis are percentages of the average number of *Salmonella* positives per replication (n=6). 3 replications of each treatment were done.
Table 2. Number of *Salmonella* positive cantaloupes due to cross-contamination after varying exposure times to contaminated cantaloupes during washing. In addition, the numbers of inoculated cantaloupes remaining positive after washing are presented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 min Exposure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>8 min Exposure</th>
<th>Inoculated Cantaloupes</th>
<th>Wash Water Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TT</td>
<td>RV</td>
<td>TT</td>
</tr>
<tr>
<td>0 ppm Chlorine or peroxyacetic acid</td>
<td>6/6&lt;sup&gt;c&lt;/sup&gt; (100.0)</td>
<td>6/6 (100.0)</td>
<td>6/6 (100.0)</td>
<td>6/6 (100.0)</td>
</tr>
<tr>
<td>50 ppm Chlorine</td>
<td>4.67/6 (77.8)</td>
<td>4.33/6 (72.2)</td>
<td>3/6 (50.0)</td>
<td>4/6 (66.7)</td>
</tr>
<tr>
<td>100 ppm Chlorine</td>
<td>2.67/6 (44.4)</td>
<td>3.67/6 (61.1)</td>
<td>2/6 (33.3)</td>
<td>1.67/6 (27.8)</td>
</tr>
<tr>
<td>80 ppm Peroxy-acetic acid</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Unionculated cantaloupes were mixed with *Salmonella* contaminated cantaloupes for the designated times

<sup>b</sup> RV: Rappaport Vassiliadis broth; TT: tetrahionate broth

<sup>c</sup> Average # of *Salmonella* positive cantaloupes per replication/# of cantaloupes sampled per replication; Values within parenthesis are percentages of the average number of *Salmonella* positives per replication (n=6). 3 replications of each treatment were done.
Table 3. Number of *E. coli* O157:H7 positive lettuce samples due to cross-contamination after varying exposure times to contaminated lettuce during washing. In addition, the numbers of inoculated lettuce samples remaining positive after washing are presented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>45 sec Exposure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Inoculated Lettuce</th>
<th>Wash Water Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SMAC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TSA-NP</td>
<td>SMAC</td>
</tr>
<tr>
<td>0 ppm Chlorine or peroxoacetic acid</td>
<td>6/6&lt;sup&gt;c&lt;/sup&gt; (100.0)</td>
<td>6/6 (100.0)</td>
<td>4/4 (100.0)</td>
</tr>
<tr>
<td>50 ppm Chlorine</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
<td>4/4 (100.0)</td>
</tr>
<tr>
<td>100 ppm Chlorine</td>
<td>0/6 (0.0)</td>
<td>0/6 (0.0)</td>
<td>4/4 (100.0)</td>
</tr>
<tr>
<td>80 ppm Peroxyacetic acid</td>
<td>4.67/6 (77.83)</td>
<td>5.33/6 (88.8)</td>
<td>4/4 (100.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Unionculated lettuce were mixed with *E. coli* contaminated lettuce for the designated times

<sup>b</sup> SMAC: sorbitol MacConkey agar + nalidixic acid (50 µg/ml); TSANP: tryptic soy agar + nalidixic acid (50 µg/ml) + sodium pyruvate

<sup>c</sup> Average # of *E. coli* O157:H7 positive lettuce samples per replication/# of lettuce samples sampled per replication; Values within parenthesis are percentages of the average number of *E. coli* O157:H7 positives per replication (n=6). 3 replications of each treatment were done.