MATHEMATICAL MODEL DEVELOPMENT FOR SALMONELLA TRANSFER

DURING WASHING AND SUBSEQUENT GROWTH IN FRESH CUT PRODUCE

By

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A Thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Master of Science

Graduate Program in Food Science

written under the direction of

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New Brunswick, New Jersey

[January 2014]

ABSTRACT OF THE THESIS

Mathematical Model Development For *Salmonella* Transfer During Washing And
Subsequent Growth In Fresh Cut Produce

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The causes of most fresh produce outbreaks in U.S. are unknown, but cross
contamination during washing or improper storage temperatures during retail storage,
distribution or home storage may play a role. The first objective of our research was
to integrate and compare published data, published models and data from the
ComBase database relevant to *Salmonella* growth in fresh and fresh-cut produce. The
second objective of our research was to develop a cross contamination model that
predicts the concentration of contaminated produce and the concentration of non-
contaminated produce after washing using literature data.

A literature research was conducted to find relevant data on the growth of *Salmonella*
on fresh cut produce. Data for *Salmonella* growth in a variety of fruit and vegetable
products was also extracted from ComBase. Calculated growth rates were converted
to square-root growth rates for comparative purposes and analyzed. Four published
Salmonella growth models (Koseki and Isobe on iceberg lettuce; Pan and Schaffner
on cut tomatoes; Li et al on cut melons; and Sant'Ana et al on lettuce) were compared
to the extracted data. The most conservative model (Koseki and Isobe, 2005) was
fail-safe for all but 5.5% (6/109) of the extracted data, predicting faster growth that
that actually observed.

A literature research was conducted to find relevant published data on the cross contamination rates between contaminated produce after wash, wash water and non-contaminated produce after wash. Data were converted to the same units, log transformed, used to create histograms and figures using Microsoft Excel. GInaFit and BestFit software were used to select suitable distributions. The software program @RISK was used to build a risk model. The simulation model predicted that when tomatoes were contaminated at 4 log CFU/tomato, after washing at 100 ppm chlorine, those same tomatoes contained ~1.0 log CFU/tomato, while contaminated cantaloupes contain ~2.8 log CFU/cantaloupe after washing at 0 ppm chlorine. The simulation model also predicted that uncontaminated tomatoes after washing at 0 ppm chlorine with contaminated tomatoes will contain ~ -0.59 log CFU/tomato (or 1 in 4 tomatoes containing $\geq$ 1 CFU), while uncontaminated cantaloupes after washing at 100 ppm chlorine with contaminated cantaloupes will contain ~ -2.83 log CFU/cantaloupe (or 1 in 676 tomatoes containing $\geq$ 1 CFU).

ACKNOWLEDGEMENT

TABLE OF CONTENTS

Chapter III –Mathematical model development for *Salmonella* transfer during washing fresh produce

LIST OF ILLUSTRATIONS

LIST OF TABLES

Chapter I – Introduction

I.1 Popularity of Fresh and Vegetables in US

Fresh fruits and vegetables are a growing part of the American diet, since these foods are low in fat and high in vitamins and minerals (*24*, *48*, *65*). The per capita consumption of fresh fruits and vegetables increased from 254.1 pounds in 1970 to 318.8 pounds in 1997 to 546.3 pounds in 2012 (*72*).

The fresh-cut segment of the market is growing especially fast, since such foods possess all the health benefits indicated above, and are more convenient, due to their fresh-cut nature. The US fresh-cut market had estimated annual sales of $6.8 billion in 2009 (*24*), and the volume of the fresh-cut lettuce market alone doubled from 1999 to 2004 (*36*). Due to US demand for fresh fruits and vegetables year-round, the import of fresh produce has also increased (*7*, *36*). USDA-ERS estimates that 13% vegetables and 32% of fruits consumed in the United States in 2007 were imported (*36*).

This increase in fresh and fresh-cut produce consumption has come with an increase in foodborne disease outbreaks associated with fresh and fresh cut produce (*11*, *37*). The Center for Science in the Public Interest (CSPI) concluded that produce has been linked to the greatest number of outbreaks and responsible for the greatest number of illnesses (*11*). During the 10-year period from 1996 to 2008, the US Food and Drug Administration (FDA) attributed 82 foodborne illness outbreaks to fresh produce (*31*). CSPI data indicate that *Salmonella* and Norovirus were responsible for more illnesses linked to fresh produce than any other pathogens or toxins (*11*).

I.2 Specific Produce

I.2a Tomato

Tomatoes are ranked third in U.S. per capita consumption of fresh vegetables, with an estimated 80 pounds consumed per person in 2012 (*73*). Fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts. About 90 percent of all tomatoes produced in 2008 were used to produce processed tomatoes (*73*). Tomatoes are an excellent source of antioxidants, dietary fiber, minerals and vitamins, but quantity and quality of any nutrients differs according to cultivar, ripeness and processing or cooking method (*73*).

Salmonella can survive on surface of tomatoes and can grow in the flesh of fresh-cut tomatoes (*46*). Asplund and Nurmi reported that the population of *S.* enteritidis, *S.* infantis and *S.* typhimurium in fresh cut tomatoes will increase 5 log CFU in 24 h at 22°C (*5*). Beuchat and Mann reported that the number of *Salmonella* in either low inoculum (0.88 to 0.99 log CFU/g) or high inoculum (2.88 to 2.99 log CFU/g) diced tomatoes stored at 21°C also increased during 10 days of storage (*8*). A mathematical model for the growth of *Salmonella* in fresh-cut tomatoes showed that the square root of the growth rate was linearly correlated with temperatures from 10 to 35 °C (*51*). The number of *Salmonella* outbreaks linked to tomatoes by is numerous. The US FDA reported 14 *Salmonella* outbreaks clearly linked to tomatoes from 1996 to 2008 in United States, with fresh-cut tomatoes implicated in at least 5 of the 14 outbreaks (*31*). Tomatoes can become contaminated by contact with un-composted manure fertilizers, irrigation water, infected wild or domestic animals, or infected workers during growing or harvesting and in the processing plant (*1, 2, 9, 10, 32, 37, 71*). For

many outbreaks, it has been assumed that *Salmonella* may have been transferred from the skin into the tomatoes flesh during cutting or slicing (*37, 47, 71*).

I.2b Cantaloupe

Cantaloupe is ranked fourth in total U.S. per capita consumption of fresh fruit, behind oranges, apples and bananas (*73*). The per capita consumption of cantaloupe increased from 2.5 kg in 1976 to 6.4 kg in 2002 (*72*). Cantaloupe is a good source of vitamin A, B6, C and potassium (*49*). A 1-cup serving (236g) of cantaloupe can provide the daily dietary requirement of vitamin A for adult males (*45*).

Cantaloupe is also a good growth environment for foodborne pathogens due to its low acidity (pH 5.2 to 6.7) and a water activity >0.97 (*37*). Golden et al reported that the *Salmonella* in cut cantaloupes can reach 7.3 log CFU/g from an initial population of 10^2 CFU/g after incubation at 25°C for 24 h (*35*). Ukuku and Sapers showed that *Salmonella* growth in fresh-cut cantaloupe cubes at 20°C reached 4 log CFU/g after 6 h (*71*). Recent research developed a mathematical model that predicts the growth rate of *Salmonella* on fresh-cut cantaloupe over a range of storage temperatures, and observed a linear correlation between the square root of *Salmonella* growth rate and temperature (*47*).

Climate and the growing environment influence the safety and quality of cantaloupes. Cantaloupes are easily damaged by chilling injury and they are cultivated in warm weather across the US (*30*). Harvest employees must pay attention to the presence of wildlife around production and harvest unit because feces can be a source of *Salmonella* or other pathogens. Also, cantaloupes can be grown in contact with the

soil, and heavy rains may increase cross contamination between soil and cantaloupes. Mature cantaloupes have an abscission scar where the vine attached to the fruit. Such scars provide a potential route for entry of human pathogens (*30*). Mechanical harvesting can also damage the fruit and provide an entry point for foodborne pathogens.

The sanitation of processing and packinghouse facilities, including the sanitizer concentration in wash water, the contact time of sanitizer, the quality of wash water and the way of cooling can affect the safety and quality of cantaloupes (*30*). In a fresh-cut processing unit, one of most important control points is to prevent microbial cross contamination from the surface of cantaloupes to the internal flesh during peeling and cutting. Storage time and temperature are the key control points during distribution. Some melons are sensitive to chilling injury but whole cantaloupes can be stored between 2.2 to 5°C without issue. FDA advises that all fresh-cut melons, including cantaloupes be stored between 0 and 5°C for safety (*30*).

I.3 Organism Used in This Study: *Salmonella* spp.

Salmonella is gram-negative, rod-shaped, motile and non-spore-forming bacterium. There are more than 2700 serotypes and Enteritidis is the main serotype causing human illness, followed by Typhimurium (*18, 27, 37, 63*).

Salmonella have been isolated from poultry, meat, eggs, milk, nuts and other dried foods, as well as fruits and vegetables (*4, 37, 50, 78*). *Salmonella* contamination can arise from many steps in the pre-harvest and postharvest continuum (*8, 56*).

Salmonellosis is the infection caused by *Salmonella*. In the United States, there are about forty thousand reports cases of salmonellosis reported every year (*18*). The actual number of infections many be much more because many cases are not

diagnosed, misdiagnosed or not reported. The milder syndromes of salmonellosis include diarrhea, fever and abdominal cramps, which typically occur 12 to 72 hours after infection and usually will last 4 to 7 days (*18*). Most persons infected with *Salmonella* develop milder syndromes and recover without treatment. However, in some cases, especially for immune compromised persons, young infants and the elderly, hospitalization may occur due to severe dehydration, high fever and the spread of infection to the bloodstream.

I.4 Outbreaks of *Salmonella* spp. in fresh produce.

Although most cases of salmonellosis were traditionally thought to arise from foods of animal origin, *Salmonella* outbreaks have been recently linked to contaminated produce (*4*, *36*). A wide variety of produce items have been linked to *Salmonella* outbreaks including lettuce, tomato, cantaloupe, honeydew, watermelon, mangos, peppers and sprouts (*12*).

A salmonellosis outbreak including at least 183 cases in 21 states of was traced back to tomatoes in 2006. Most of patients had fever and diarrhea and 12% of patients were hospitalized (*13*). A large, multi-state outbreak of *Salmonella* Saintpaul was associated with tomatoes and then jalapeno and Serrano peppers in 2008 with 1443 persons infected in 43 states (*19*). The outbreak strain was isolated from irrigation water collected on a Mexican farm, the samples of Serrano peppers collected on farm and the samples of jalapeno peppers collected in warehouse and patient's home (*19*). During 2009 to 2011, three salmonellosis outbreaks were traced back to alfalfa sprouts: A 2009 outbreak linked to *Salmonella* Saintpaul, a 2010 outbreak linked to *Salmonella* I4 and a 2011 outbreak linked to *Salmonella* Enteritidis (*15*, *16*, *51*). Two outbreaks associated with consumption of cantaloupes in the United States were

reported in 2011 and 2012 (*18*, *20*), with the 2011 outbreak linked to *Salmonella* Panama and the 2012 outbreak linked to *Salmonella* Typhimurium (*18,20,26*).

Short title (70 char): *Salmonella* Growth Models in Fresh Cut Fruits and Vegetables.

Title: Comparison of Four Growth Models in 16 Data Sets for *Salmonella* Growth in Fresh-cut Fruits and Vegetables

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Chapter II – *Salmonella* Growth Models in Fresh Cut Fruits and Vegetables

II.1 Abstract

Many mathematical models have been developed to predict the growth of *Salmonella* in fresh and fresh cut produce, but no systematic comparison of all relevant data and models has yet been published. The purpose of this study was to integrate and compare published data, published models and data from the ComBase database relevant to *Salmonella* growth in fresh and fresh-cut produce. A literature search was conducted to obtain relevant data and models on the growth of *Salmonella* in fresh cut produce. There were fifteen relevant datasets available from the ComBase database, eight published studies on *Salmonella* growth in cut tomatoes, two studies on *Salmonella* growth in melon and four published models for *Salmonella* growth in fruits and vegetables. Growth rates were converted to square-root growth rates for comparative purposes. Most of the collected data were fell in the areas between the most conservative model and the most liberal model.

Key words: *Salmonella*, Growth rate, Fresh cut, Model, Comparison,

II.2 Introduction

Fresh and fresh cut fruit and vegetable consumption is growing rapidly, and fresh cut produce alone has estimated annual sales of $6.8 billion (*24*, *48*). An increase in fresh and fresh cut produce consumption comes with an apparent increase in foodborne illness outbreaks associated with fresh and fresh cut produce (*11*). The Center for Science in the Public Interest (CSPI) concluded that over the past decade, produce was linked to the more outbreaks and illnesses than any other food type. During the 10-year period studied (2001 to 2010), CSPI data indicated that fresh produce caused a reported 696 foodborne disease outbreaks and 25,222 illnesses and *Salmonella* and *Norovirus* sickened more people than other identified pathogens and toxins (*11*).

A number of large *Salmonella* outbreaks have been linked to fresh produce. An outbreak of 183 cases of salmonellosis in 21 states was traced back to tomatoes in 2006 (*13*). A large, multi-state outbreak of *Salmonella* Saintpaul was associated first with tomatoes, then with jalapeno and Serrano peppers with 1443 persons infected in 43 states in 2008 (*14*). During 2009 to 2011, three salmonellosis outbreaks were traced back to alfalfa sprouts, with the 2009 outbreak linked to *Salmonella* Saintpaul, with the 2010 outbreak linked to *Salmonella* I4 and with the 2011 outbreak linked to *Salmonella* Enteritidis (*15*, *17*). Two outbreaks associated with consumption of cantaloupes in the United States were reported in 2011 and 2012 (*18*, *20*), with the 2011 outbreak linked to *Salmonella* Panama and the 2012 outbreak linked to *Salmonella* Typhimurium (*18,20,26*).

ComBase is a web-based resource for quantitative and predictive food microbiology. It has two main components: the ComBase database, which is a searchable and browsable database of microbial responses observation under a variety of food-related conditions and the ComBase Predictor, which is a collection of relevant predictive models (*23*). Modeling studies on *Salmonella* growth in foods include models for growth on cantaloupe (*46*), tomato (*51*) and lettuce (*25, 44, 62*). The differences found between published models are due to a variety of factors including using data obtained from culture media vs. foods, and well as differences in food characteristics (eg. pH and water activity). Microbial growth in culture media is often faster due to more readily available nutrients and the lack of a background microflora (*6, 23, 44, 57, 62*)

Many laboratory experiments have been performed on the growth of *Salmonella* in fresh and fresh cut produce. These studies include *Salmonella* growth on melon (*35, 74*), iceberg lettuce (*21*), fresh strawberries (*43*), sprouting alfalfa seeds (*22*), corn zein films (*38*), peeled fresh orange (*52*), sliced fresh fruit (*28*), rehydrated infant foods (*3, 40*), as well as orange (*64*) and tomato juices (*77*). Growth on fresh-cut tomatoes has been a popular research topic with eight different studies on the growth of *Salmonella* on fresh cut tomatoes (*5, 8, 51, 67, 76, 77, 79, 80*).

A literature search was conducted to obtain relevant data and models on the growth of *Salmonella* in fresh cut produce in this study. There were fifteen published literatures from ComBase database, eight tomato published studies, two melon published studies and four *Salmonella* published models. There were no studies identified that offered a comprehensive summary and analysis of published data and models for *Salmonella* growth in fresh and fresh-cut produce. The purpose of this study was to integrate and

compare published data, published models and data from the ComBase database

relevant to *Salmonella* growth in fresh and fresh-cut produce.

II.3 Methods - Literature Search

II.3.a ComBase Database

Microbial growth data was collected from the ComBase database for *Salmonella*, in

food types of vegetable or fruit in origin including infant foods and beverages. Heated,

dried, irradiated, sanitized and EDTA added products were excluded. Table 1

summarizes all the data points extracted from the ComBase Database.

Growth rates were converted to square-root growth rates for comparative purposes.

Square-root growth rates were fitted to the square root or Ratkowsky equation:

$$\sqrt{Growth\ Rate} = b\ (T\ -\ T_0)$$

which describes growth rate as a function of temperature, where b is the slope of the

regression line, T is the temperature and T_0 is the theoretical minimum temperature

for microbial growth (*59*).

Table 1: Summary of ComBase Database on Salmonella Growth Rates.

Food types	No of growth curves from ComBase Database	Reference
Cilantro broth	16	*(41)*
Iceberg lettuce	2	*(21)*
Fresh strawberries	3	*(43)*
Sprouting alfalfa seeds	2	*(22)*
Films of corn zein	2	*(38)*
Peeled fresh orange	3	*(52)*
Lettuce	5	*(44)*
Sliced fresh fruit	1	*(28)*
Green salad	8	*(41)*
Infant food hydrated with water	18	*(3)*

Infant food hydrated with milk	18	*(3)*
Infant food hydrated with milk	1	*(40)*
Infant food hydrated with apple juice	18	*(3)*
Orange juice	12	*(64)*
Tomato juice	8	*(77)*

II.3.b Tomato studies

Data on the growth of *Salmonella* on fresh cut tomatoes were obtained from the published literature (*5, 8, 51, 67, 76, 77, 79, 80*). Calculated growth rates were converted to square-root growth rates for comparative purposes and analyzed to access the relationship between square-root growth rate of *Salmonella* (log CFU/hour) and temperature (°C).

II.3.c Melon studies

Data on the growth of *Salmonella* on fresh cut cantaloupes, watermelons and honeydew were extracted from the published literature (*35, 71*). Calculated growth rates were converted to square-root growth rates for comparative purposes and analyzed to access the relationship between square-root growth rate of *Salmonella* (log CFU/hour) and temperature (°C).

II.3.d Published Models

Four published growth models for *Salmonella* were considered in this study. The model developed by Koseki and Isobe on iceberg lettuce, the model developed by Pan and Schaffner on cut tomatoes, the model developed by Li et al on cut melons and the model developed by Sant'Ana et al on lettuce (*44, 46, 51, 62*). The published growth models for *Salmonella* are based on the square root or Ratkowsky equation, described above.

II.4. Methods - Data Analysis

II.4.a Excel

Data were extracted directly from ComBase database results or published studies into Microsoft Excel (Redmond, WA) spreadsheets. Spreadsheet data included: produce type (tomato, cantaloupe, watermelon, honeydew), strains used, inoculation method, storage temperature, pH values, incubation time, initial and final concentrations, growth rates, and reference details. Data were plotted and trend lines added to describe the correlation between data and models.

II.4.b Linear Regression

Growth rates were converted to square-root growth rates for comparative purposes. Square-root growth rates were fitted in to the square root or Ratkowsky equation:

$$\sqrt{Growth\ Rate} = b\ (T - T_0)$$

which was used to describe *Salmonella* growth rate as a function of temperature, where b is the slope of the regression line, T is the temperature and T_0 is the conceptual minimum temperature for microbial growth (*59*).

Ratkowsky equations were also extracted from published models, which provide the values of b, T and T_0.

II.5 Results and Discussion

II.5.a ComBase Database Data

Figure 1shows the linear relationship between the square-root of growth rates reported in ComBase and growth temperatures (note that any symbols other than solid circle and solid square refer to ComBase data). The correlation between ComBase reported square root growth rates and temperatures is $R^2=0.661$ (regression line omitted). As

expected, no *Salmonella* growth on vegetables, fruits, infant food or juice was seen during refrigerated storage, as evidenced by the cluster of points in the lower left hand corner of Figure 1. Furthermore, no *Salmonella* growth on tomato, cantaloupe and peeled fresh orange was seen during storage temperature from 5°C to 8°C (see also Figure 1). No *Salmonella* growth on green salad was seen at a storage temperature at 12°C (black triangles in Figure 1). The higher the temperature, the wider the variability in square-root growth rates, likely due to variability in strains, methods and/or food types. Different food types provide different growth environments with differences in water activity, nutrient composition and pH. No *Salmonella* growth on fresh cut strawberries was seen during at 4°C storage (solid diamonds, Figure 1). The inability of *Salmonella* to multiply on cut strawberries is likely due to their naturally low pH (*43*) that ranges from 3.2 to 4.1(*42*). The pH of infant food hydrated with water used in this study ranged from 6.9 to 7.1, while the pH of infant food hydrated with apple juice ranged from 4.5 to 4.6 (*3*). Figure 1 shows the square root growth rates of infant food hydrated with apple juice (open diamond) at 15 or 25 °C is lower than the square root growth rates of infant food hydrated with water (open square).
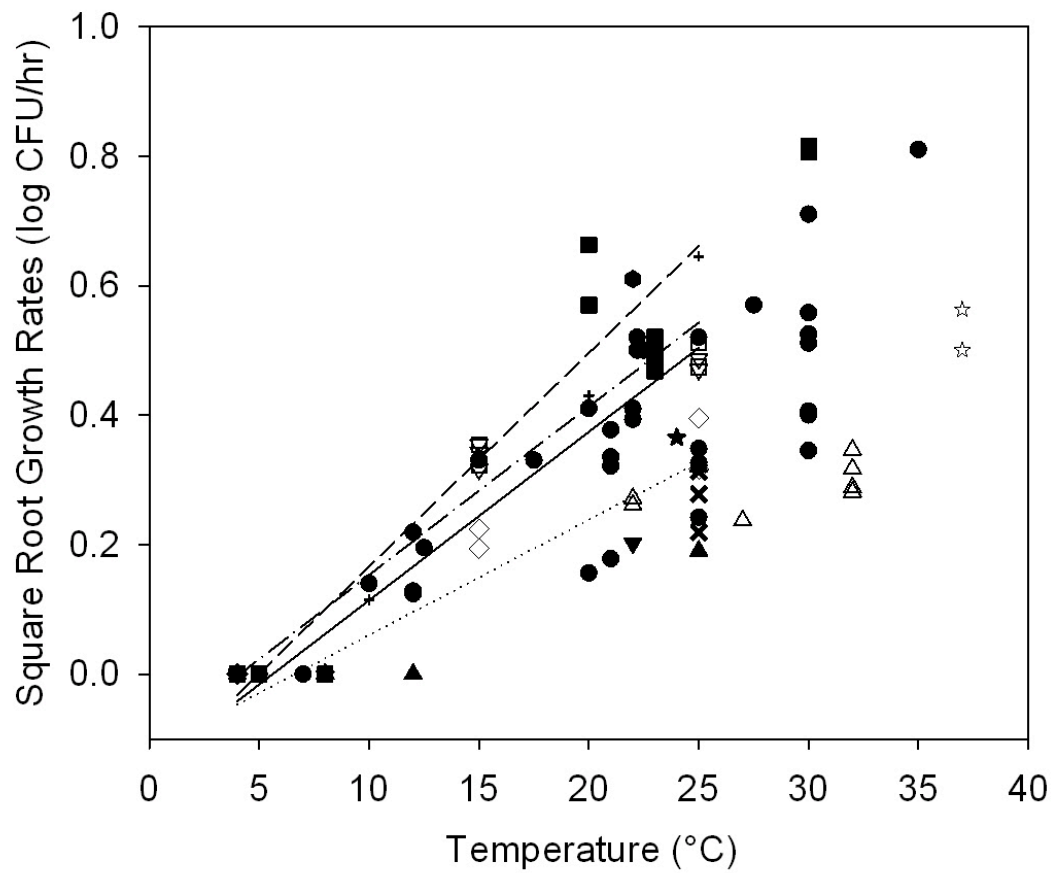
Figure 1. Summary of ComBase Data, Published data and Published Models on
***Salmonella* Square Root Growth Rates with Tomato (●), Cantaloupe (■),**
Cilantro Broth (△), Iceberg lettuce (▼), Fresh strawberry (u), Alfalfa seeds (×),
Films of corn zein (+), Peeled fresh orange (⋆), Lettuce (+), Sliced fresh fruit (⬢),
Green Salad (▲), Infant food with water (□), Infant food with milk (▽), Infant
food with apple juice (◇), Orange juice (○), Tomato juice (☆), Koseki and Isobe
(- -), Pan and Schaffner (-.-), Li (−), Sant'Ana (…).

II.5.b Model Comparison

Figure 1 also presents a comparison of linear regression among the growth rates

obtained from Koseki and Isobe, Sant'Ana et al, Li et al. and Pan and Schaffner (*44,*

46, 51, 62). The Koseki and Isobe model has the greatest slope among these models

(*44*), as can been seen in Fig. 1 (long dashed line). Figure 1 shows that the Li et al.

model (solid line) and Pan model (dash dot line) have similar slope but Pan model has

lower T_0 values (*44, 46, 51*). The Sant'Ana et al model (dotted line) shows the least

slope of all the models. Table 2 summarizes the square root equations of each

published growth models.

Table 2. Summary of Growth Models on Square Root Equations

Produce	Square root equation	Model Authors and Reference
Cantaloupe	$\sqrt{u}=0.026(T\text{-}5.61)$	Li et al. (*46*)
Tomato	$\sqrt{u}=0.026(T\text{-}4.12)$	Pan and Schaffner (*51*)
Lettuce	$\sqrt{u}=0.033(T\text{-}4.97)$	Koseki and Isobe (44)
Lettuce	$\sqrt{u}=0.018(T\text{-}6.63)$	Sant'Ana et al. [59]

II.5.c Model and Published Data Comparison

As can been seen in Figure 1, the square root growth rates of *Salmonella* on fresh cut

cantaloupe at 20°C in Ukuku and Sapers' study (*71*) is 0.57 (solid square), which is

much higher than the predicted in Li et al . (*46*) model, 0.37 (solid line). The square

root growth rates of *Salmonella* experiment data collected by Li et al . (*46*) from

watermelon, cantaloupe and honeydew at 20 to 25°C is in the range of 0.44 to 0.67

(black square). These data are well described by the model built in Li et al. (solid line)

(*35, 46, 71*).

Figure 1 shows the published data for square root of growth rates of *Salmonella* on

fresh cut tomato (solid circles), generally approximate the model from Pan and

Schaffner (dash dot line) (*51*). The square root growth rate of *Salmonella* on tomato

at 22°C in Pan's study is 0.47; while in FDA's study the value ranged from 0.5 to

0.52, and in Asplund and Nurmi's is 0.168. The square root growth rate of

Salmonella on tomato at 21°C in Beuchat and Mann's (*8*) study is 0.18, while in

Zhang et al. (*79*) it is 0.33. The square root growth rate of *Salmonella* on tomato at

30°C in Pan and Schaffner is 0.45; and Asplund and Nurmi (*5*) and Weissinger and

Beuchat (*77*) both report the similar value of 0.41. At temperature between 4°C to

12°C, the published growth rates of *Salmonella* on tomato (solid circle) are closely

matched by the model built by Pan and Schaffner study (dash dot line), as can been

seen in Figure 1 (*5*, *8*, *51*, *77*, *79*, *80*).

Most ComBase lettuce data match the model proposed by Koseki and Isobe (*44*),

butthe experiment data from Sant'Ana et al. are much lower (*44*, *62*). This could be

due to sample preparation. Koseki and Isobe's used 3-cm^2 pieces of iceberg lettuce,

while Sant'Ana et al used 2-cm^2 width strips. (*44*, *46*, *62*).

II.5.d Conclusion

Most of the published data and data from the ComBase Database are located between

the most conservative (fastest growth predictions) model (Koseki and Isobe), and the

most liberal (slowest growth predictions) model (Sant'Ana et al.). However, some of

the published data are located outside the area that the four models covered. For

example, the square root growth rates of *Salmonella* on fresh cut cantaloupes from

Ukuku and Sapers study (solid square) are higher than the data predicted in Koseki

and Isobe (*44*, *71*). The growth rates of *Salmonella* on fresh cut tomatoes in Zhuang

et al, Beuchat and Mann and Wei et al (solid circle) are lower than the data predicted

in Sant'Ana model (*8*, *76*, *80*). This study integrates available data and models for

Salmonella growth in fresh cut product and related foods into a single figure. The

square-root of growth rates of *Salmonella* on fresh cut fruits and vegetables increase

linearly as a function of temperature and are generally well described by the currently

available published models.

To be published in JFP

Running Head: *Salmonella* **Cross Contamination During Produce Washing**

Simulation modeling for *Salmonella* **Between Produce and Wash Water during Washing**

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Chapter III – Cross Contamination of *Salmonella* Between Produce and Wash Water during Washing

III.1 Introduction

Fruits and vegetables may become contaminated by manure-based fertilizers, irrigation or wash water, infected wild or domestic animals, or infected workers during growing, harvesting or during further processing (*1*, *2*, *9*, *10*, *32*, *37*, *71*). Farm-harvested produce may be processed through a series of washing steps after arriving at packinghouse or processing plant. Although washing produce can effectively remove soil, sand and other debris from fresh fruits and vegetables, it does not completely remove all microorganisms from produce (*1*, *9*, *54*, *56*, *63*, *68*, *79*), and fruits and vegetables commonly to have populations of 10^4 to 10^6 per gram naturally occurring non-pathogenic microorganisms at the packinghouse or processing plant before washing (*71*). Furthermore, inadequate postharvest washing can result in spread of pathogens, leading to serious cross-contamination and outbreaks (*53*). Several factors influence the efficiency of any postharvest washing system, especially the quality of wash water (*34*). When the wash water is recycled and not treated prior to reuse it can spread contamination to subsequent batches of washed produce (*54*).

Chlorine based sanitizers have been used for decades to sanitize produce surfaces within produce processing facilities and to reduce microorganisms in wash water during cleaning and packing operations (*54*). Numerous studies have been done which show that treatment concentration, treatment time, the presence of organic material and food type are all important factors that influence the effectiveness of chlorine in killing or removing bacterial pathogens on inoculated produce (*29*, *55*, *61*, *68*, *75*, *78*).

Other studies have characterized the effects of chlorine on reducing bacterial

pathogens present in wash water. Parnell et al. reported that 200 ppm chlorine in 1.5l

wash water can reduce *Salmonella* from 5.2 log CFU to less than 3.6 log CFU (*56*).

Pao et al. found that 5, 10 and 20 ppm ClO_2 reduced *S. enterica* populations from 7.1

log CFU/ml to the minimum detection level (10 CFU/ml) in wash water after about

10, 6, 4 s, respectively (*53*).

The mathematics of measuring and modeling cross contamination during washing can

be very complicated, but may be key to understanding and managing risk (*25*). The

purpose of this study is to create preliminary *Salmonella* cross contamination models

for whole fresh produce during washing based on the limited data available in the

published literature.

III.2 Materials and Methods

Data extraction from the published literature. A literature search was conducted

to obtain the relevant data on the behavior of *Salmonella* on two types of fresh whole

produce: tomato and cantaloupe. Data of three types were extracted from the

published literature: surface reduction on produce; transfer from produce to water and

transfer from water to produce. While the same list of publications was considered

for all three data types, not all types of data could be extracted from each paper. Data

on the effect of chlorine on contaminated produce were extracted from seven

published articles and analyzed to estimate *Salmonella* log reduction per cm^2 on

whole produce surfaces (*29*, *53*, *56*, *58*, *70*, *74*, *75*). Data were extracted from ten

published articles and analyzed to assess the transfer rates of *Salmonella* between

contaminated produce and wash water (*29*, *53*, *56*, *58*, *60*, *68-70*, *74*, *75*). Data on the

effect of chlorine on *Salmonella* transfer from wash water to previously un-

contaminated produce were extracted from five published articles (*53, 58, 60, 68, 69*).

III.3 Data Analysis

Data were converted to the same units (CFU/produce item, CFU/cm^2), log

transformed, used to create histograms using Microsoft Excel (Microsoft, Redmond,

WA). Those data were fit to the Weibull distribution using GInaFit (*33*). Distributions

were also fit using BestFit software (Palisade Corporation, Ithaca, NY). The program

@RISK (Palisade Corporation, Ithaca, NY) was used to build the risk model.

The source of concentration is defined as the sum of the amount on the surfaces of

produce after the transfer has taken place, such that:

Total source CFU   = CFU/all contaminated produce after wash

+ CFU/all wash water

+ CFU/all un-contaminated produce after wash

And when the source of contamination is the contaminated produce:

Transfer (%) = (CFU/all wash water)/(CFU/all contaminated produce)*100

When the source of contamination is the wash water:

Transfer (%)=(CFU/all un-contaminated produce)/(CFU/all wash water)*100

III.4 Results and Discussion

III.4.a Cantaloupe

Table 3 summarizes the overview of cross contamination simulation model variables

and parameters. The first column is the Excel cell number that contains the formula in

the value column. The second column, entitled variable, contains an English

description of the value. The third column is the value of variables, which can be a number, a formula or a @Risk formula. The fourth column contains the value units. The fifth column is the source of the information, which can be user input, calculated from other variables in this model, data or model developed in this study or a published literature.

Table 3. The overview of cross contamination simulation model variables and parameters

Cell	Variable	Value	Unit	Source
C1	Initial concentration on contaminated produce	-	Log CFU/produce	User input
C2	Cl treatment concentration	=RiskUniform(0,200)	ppm	This study
C3	Log reduction	=0.003*C1+0.8297	Log CFU/produce	This study
C4	Concentration on contaminated produce after washing	=RiskOutput()+C1-C3	Log CFU/produce	Calculated
C5	Transfer rate from contaminated produce to wash water	=(10^(-((C2/117.64)^0.81)))*(10^2.41)*100	%	This study
C6	Log transfer rate from contaminated produce to wash water	=2.41-((C2/117.64)^0.81)	No unit	Calculated
C7	Concentration in wash water	=LOG(10^C1*(C5/100))	Log CFU/ml	Calculated
C8	Transfer rate from wash water to uncontaminated produce at 0 ppm	=10^C9*100	%	This study
C9	Log transfer rate from wash water to uncontaminated produce at 0 ppm	=RiskExtvalueMin(-5.6755;0.6644)	No unit	Calculated
C10	Transfer rate from wash water to uncontaminanted produce at other concentrations	=10^C11*100	%	This study
C11	Log transfer rate from wash water to uncontaminated produce at other concentrations	-6.576	No unit	Calculated
C12	Concentration on noncontaminated produce after washing	=RiskOutput()+LOG(10^C7*(IF(C2=0;C8/100;C10/100)))	Log CFU/produce	This study

The first row in Table 3 contains the variable that represents the initial concentration of contaminated produce. Since our model is designed only to simulate cross contamination (rather than being a full "farm-to-fork" risk assessment) the initial concentration on the contaminated produce is designed to be user input. The second and third rows represent the chlorine concentration in wash water and the effect of chlorine on contaminated produce after washing. The chlorine concentrations in wash water collected from the published literature we analyzed ranged from 0 ppm to 200 ppm. We chose to represent this variable as a uniform distribution (using @RiskUniform). Our analysis of the published cantaloupe literature showed a linear relationship between the concentration of chlorine in wash water and the log

reduction on contaminated produce (log CFU/produce) after washing (R^2=0.92), as can been seen in Figure 2.
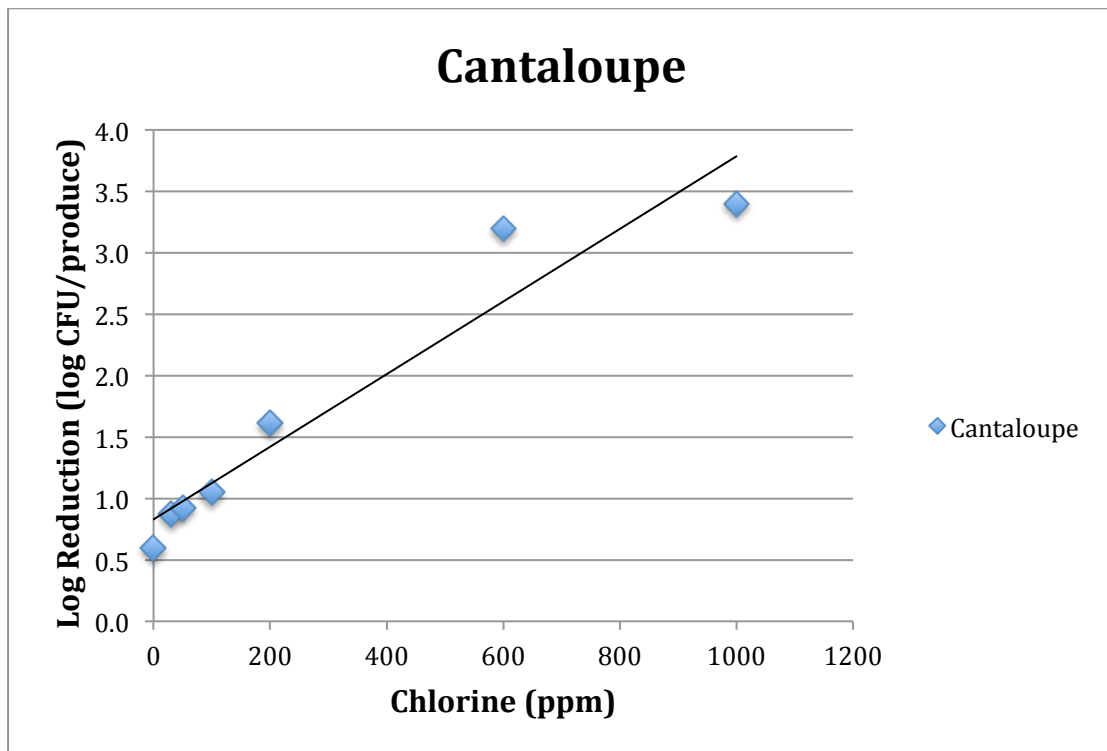
Figure 2. Published cantaloupe literature for the effect of chlorine washing on reduction of *Salmonella* on cantaloupe.

The third row in Table 3 calculates the expected log reduction on contaminated produce after washing as calculated by the linear relationship. The fourth row calculates the expected concentration on contaminated produce after washing as calculated by initial concentration on contaminated produce minus log reduction on contaminated produce after washing. The fifth row represents the transfer rate from contaminated produce to wash water. Our analysis of the published cantaloupe literature showed a Weibull distribution between the concentration of 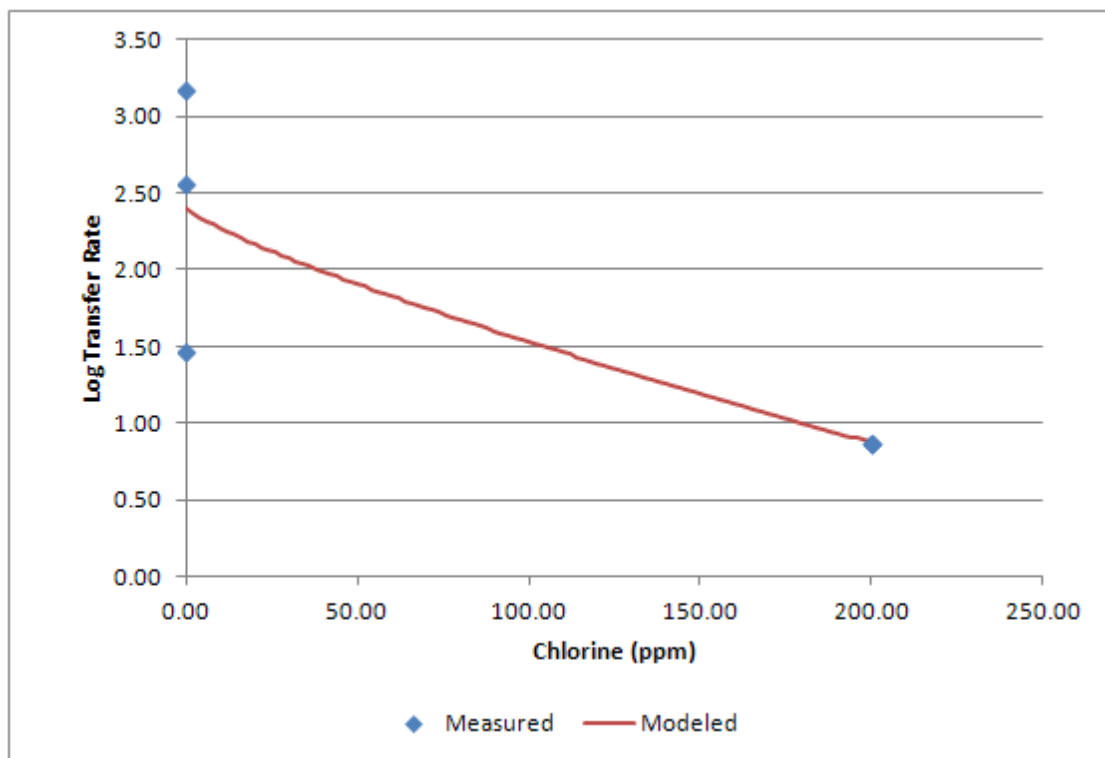chlorine in wash water and the log transfer rate from contaminated produce to wash water, as can been seen in Figure 3. Clearly the number of data points available for the creation of Figure 3 is quite limited, and other distributions are possible.

Figure 3. A Weibull distribution for published cantaloupe literature showed the relationship between the concentration of chlorine in wash water and the log transfer rate from contaminated produce to wash water

The fifth row in Table 3 calculates the expected transfer rate from contaminated produce to wash water as calculated by the Weibull distribution. The sixth row represents the log transfer rate from contaminated produce to wash water as calculated from the fifth row. The seventh row represents the concentration in wash water after washing in log scale. The seventh row calculates the expected concentration in wash water after washing as calculated by log of arithmetic of the initial concentration on contaminated produce multiply by the transfer rate from contaminated produce to wash water. The eighth and ninth rows represent the transfer rate and the log transfer rate from water at 0 ppm chlorine to uncontaminated produce. We chose to represent the log transfer rate from water at 0 ppm chlorine to

uncontaminated produce as an extvaluemin distribution (using @RiskExtvalueMin), based on @Risk BestFit, as can been seen in Figure 4.
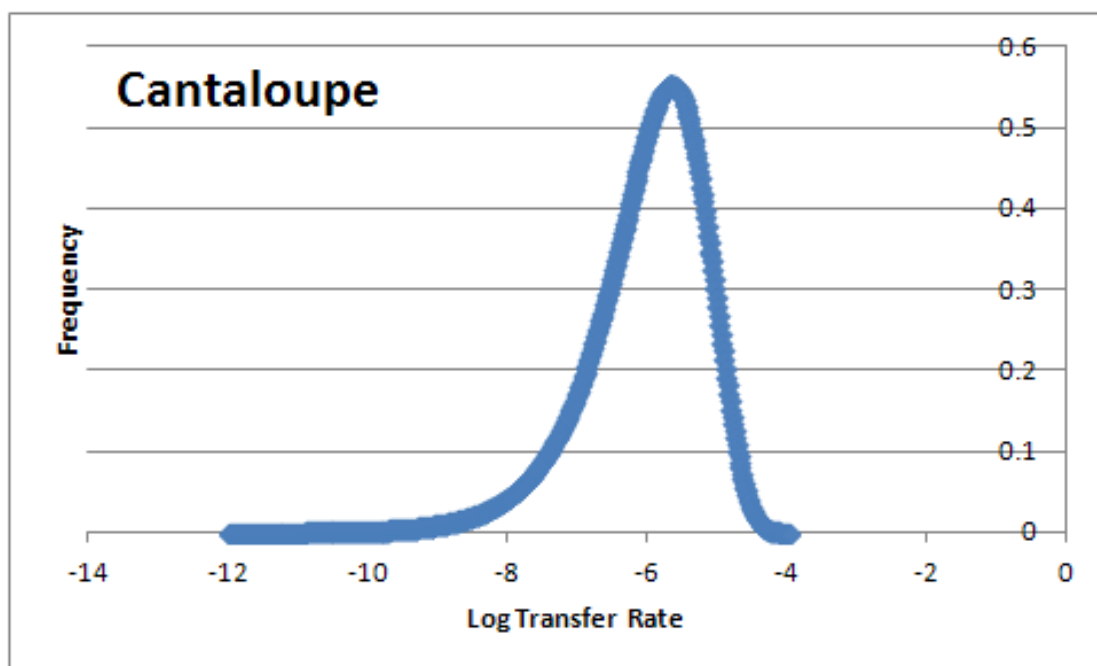
Figure 4. An extvaluemin distribution for published cantaloupe literature showed the log transfer rate from water at 0 ppm chlorine to uncontaminated cantaloupe.

The tenth and eleventh rows represent the transfer rate and the log transfer rate from water with chlorine (up to 200 ppm) to uncontaminated produce. The twelfth row represents the concentration on uncontaminated produce after washing. The twelfth row calculates the expected concentration on uncontaminated produce after washing as calculated by log of arithmetic of the concentration in wash water multiply by the transfer rate from wash water to uncontaminated produce. Two variables in this cross contamination risk model were added as @Risk outputs, which are the concentration on contaminated produce after washing and the concentration on uncontaminated produce after washing.

The results of the simulation model predicting are shown in Figure 5. When

cantaloupes are assumed to be contaminated at 4 log CFU/cantaloupe, after washing

in 100 ppm chlorine, those same cantaloupes contain ~2.9 log CFU/cantaloupe; after

washing at 0 ppm chlorine, those same cantaloupes contain ~3.2 log CFU/cantaloupe.

The simulation tornado plot shown in Figure 6 indicates the two major variables that

influence the shape of the lines describing the distribution of *Salmonella*

concentration on cantaloupe in Figure 5. Those two variables shown in Figure 5 are

the presence of chlorine in the wash water and the initial concentration on

contaminated produce.
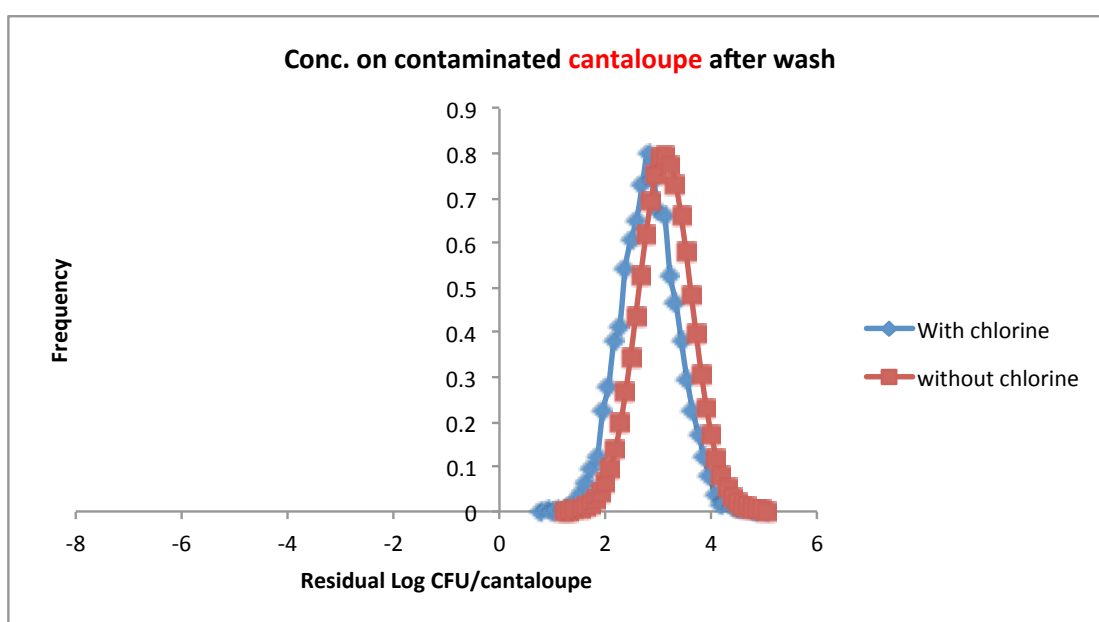
Figure 5. Simulation results predicting the concentration of *Salmonella* on

cantaloupe originally containing 4 log CFU/ cantaloupe after washing.

With chlorine (◆), without chlorine (■).
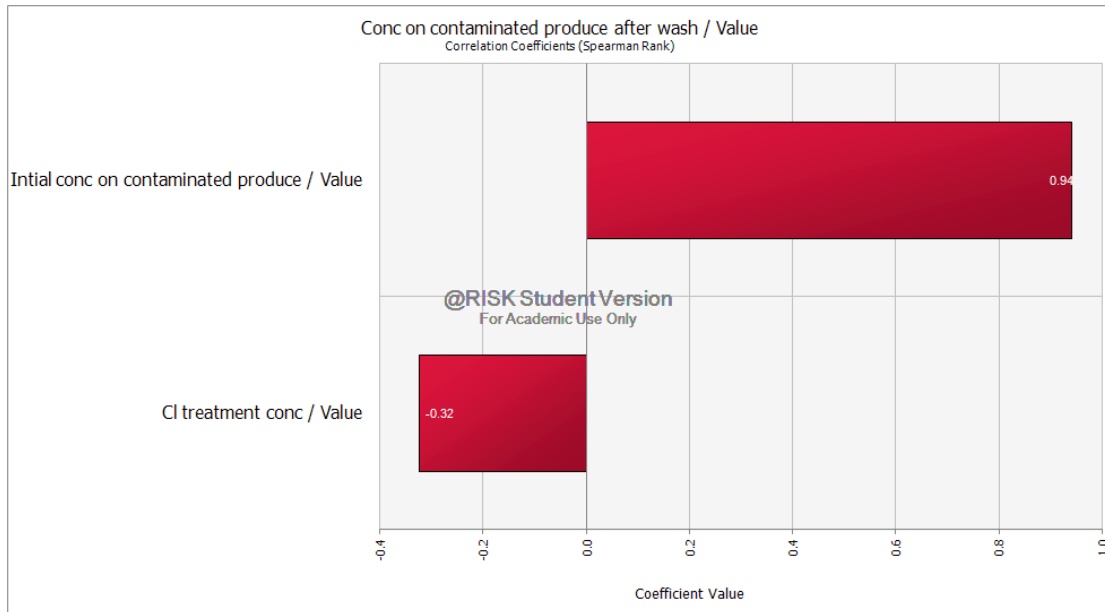
Figure 6. The tornado correlation coefficient graph for the concentration of *Salmonella* on cantaloupe containing 4 log CFU/cantaloupe after washing with chlorine.

Figure 7 shows the simulation model predictions for the *Salmonella* concentrations on previously uncontaminated cantaloupes after washing them with *Salmonella* contaminated cantaloupes. When the washing takes place in the presence of 100 ppm chlorine, the simulation predicts that previously uncontaminated cantaloupes will contain ~ -1.65 log CFU/cantaloupe (1 in 44 cantaloupes contaminated), but after washing in water containing 0 ppm chlorine, previously uncontaminated cantaloupes will contain ~ -1 log CFU/cantaloupe (1 in 10 cantaloupes contaminated).

Figure 7. Simulation results predicting the concentration of *Salmonella* on previously uncontaminated cantaloupe after washing. With chlorine (♦), without chlorine (■).

The simulation tornado plot in Figure 8 indicates the three major variables that influence the shape of the distribution for *Salmonella* on previously uncontaminated cantaloupe. Those variables are the presence of chlorine in the wash water, the initial concentration on contaminated produce and log transfer rates from was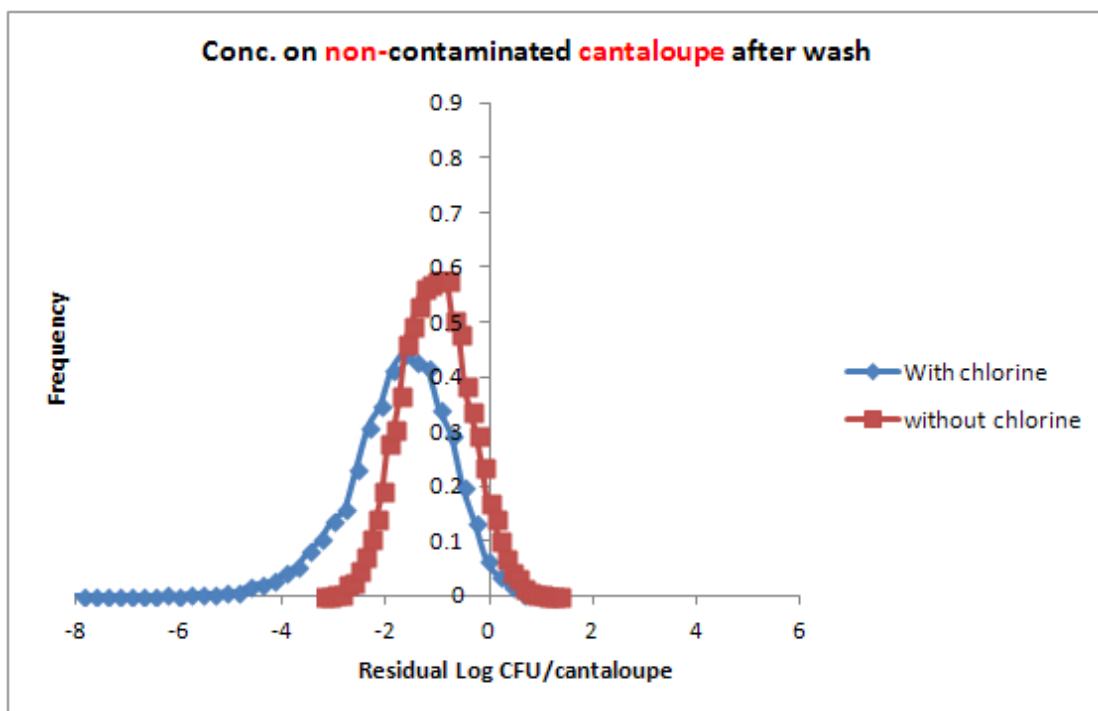h water to uncontaminated produce. Several points are clear from a comparison of the simulation models results in Figures 5 and 7. First, whether chlorine is used of not, the concentration on contaminated cantaloupe after washing is always higher than the concentration on uncontaminated cantaloupe after washing. Second, chlorine does not have a clear benefit, since the difference between the concentration of *Salmonella* on contaminated cantaloupe after washing with chlorine and the concentration of *Salmonella* on contaminated cantaloupe after washing without chlorine is 0.3 log

CFU/cantaloupe, less than 1 log CFU/cantaloupe and since the difference between the

concentration of *Salmonella* on uncontaminated cantaloupe after washing with

chlorine and the concentration of *Salmonella* on uncontaminated cantaloupe after

washing without chlorine is 0.65 log CFU/cantaloupe, less than 1 log CFU/cantaloupe

Finally, even using chlorine at 100 ppm is not sufficient to completely prevent the risk

of transfer to previously uncontaminated cantaloupe, at least based on the data used to

construct the simulation.
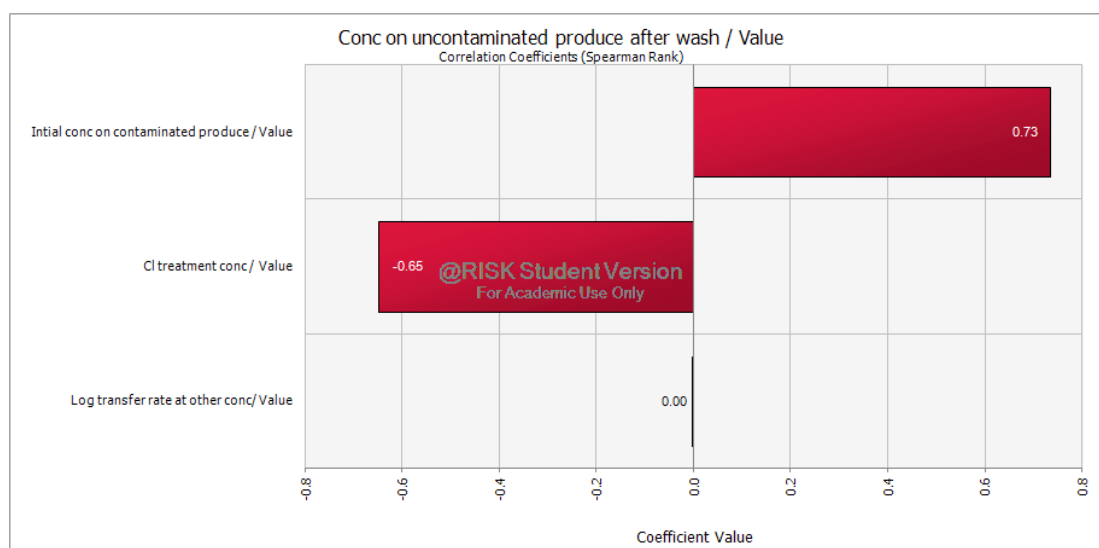
**Figure 8. The tornado correlation coefficient graph for the concentration of
Salmonella on previously uncontaminated cantaloupe after washing with
chlorine.**

III.4.b Tomato

Tomato. The framework of the cross contamination model for tomato is the same as

that used for cantaloupe, and only the values of some variables are different. A linear

relationship was observed between the concentration of chlorine in wash water and

the log reduction on contaminated produce (log CFU/produce) after washing (as with

cantaloupe). However, the slope of the linear equation for tomato is steeper than the slope of the linear equation for cantaloupe, but the correlation coefficient is less (R^2=0.51), as can been seen in Figure 9. These findings point out the need for more data on the effect of chlorine or other sanitizers on *Salmonella* concentration on tomatoes.
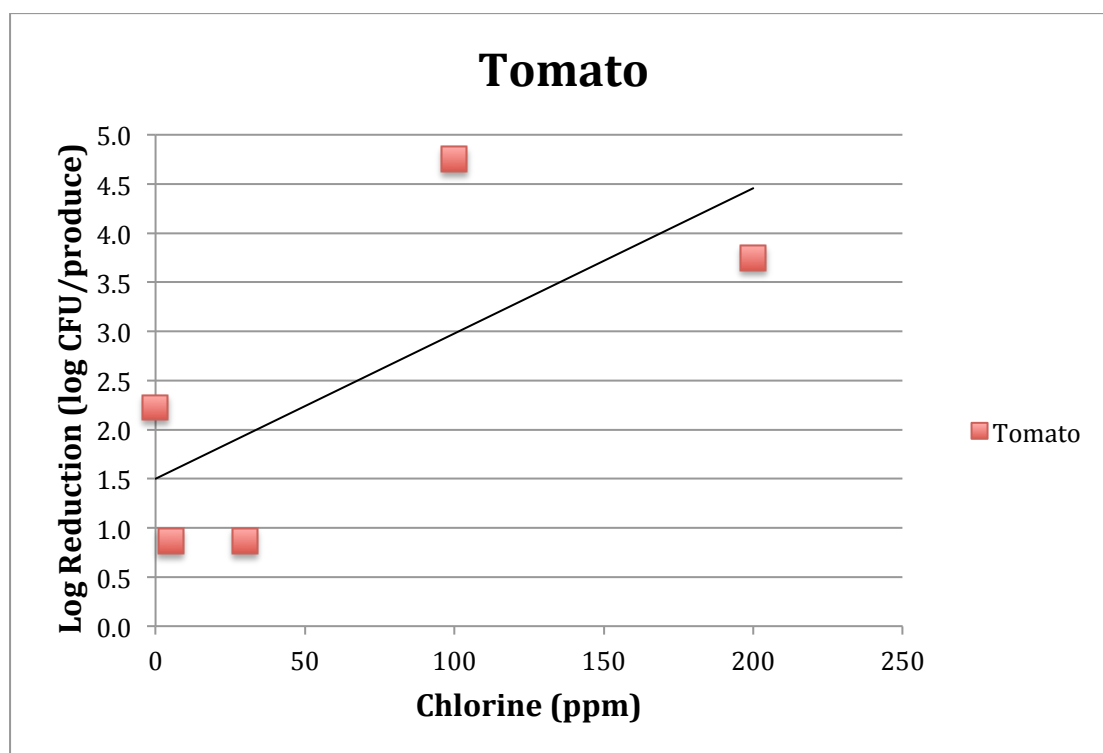
Figure 9. Published tomato literature for the effect of chlorine washing on reduction of *Salmonella* on tomato.

Our analysis of the publish tomato literature showed a Weibull distribution between the concentration of chlorine in wash water and the log transfer rate from contaminated produce to wash water, as can been seen in Figure 10.

Figure 11 shows the log transfer rate from wash water at 0 ppm to uncontaminated tomato as an extvaluemin distribution (using @RiskExtValueMin), based on @Risk Bestfit.
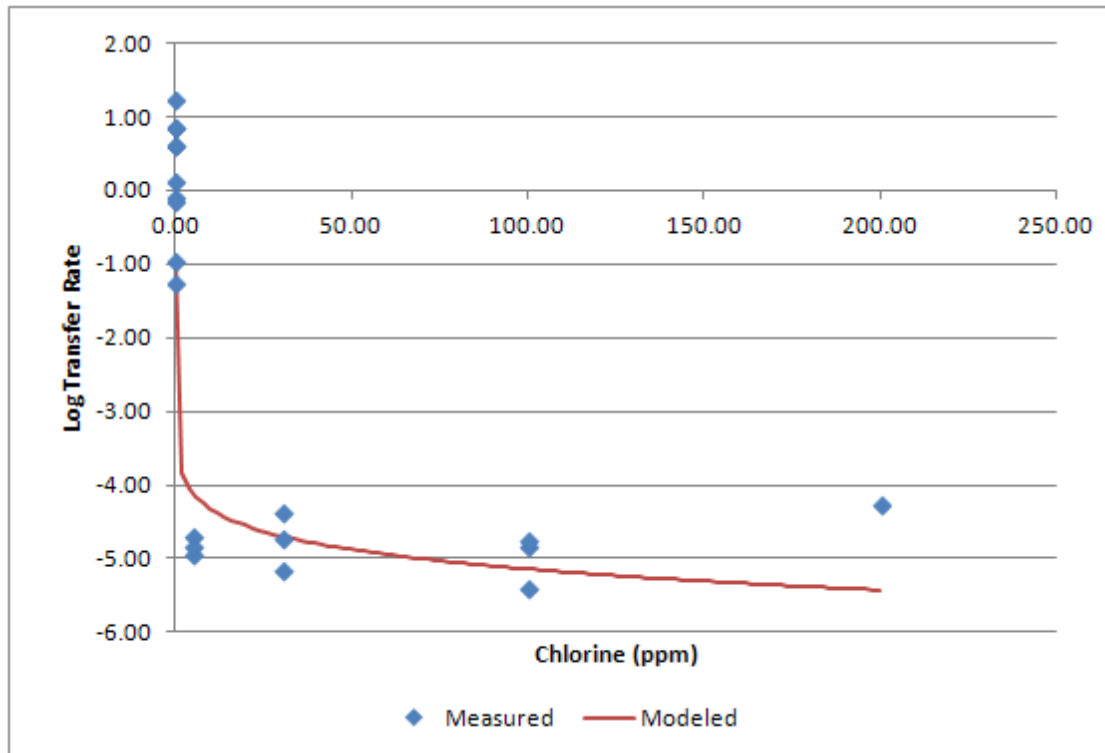
Figure 10. A Weibull distribution for published tomato literature showed the relationship between the concentration of chlorine in wash water and the log transfer rate from contaminated produce to wash water
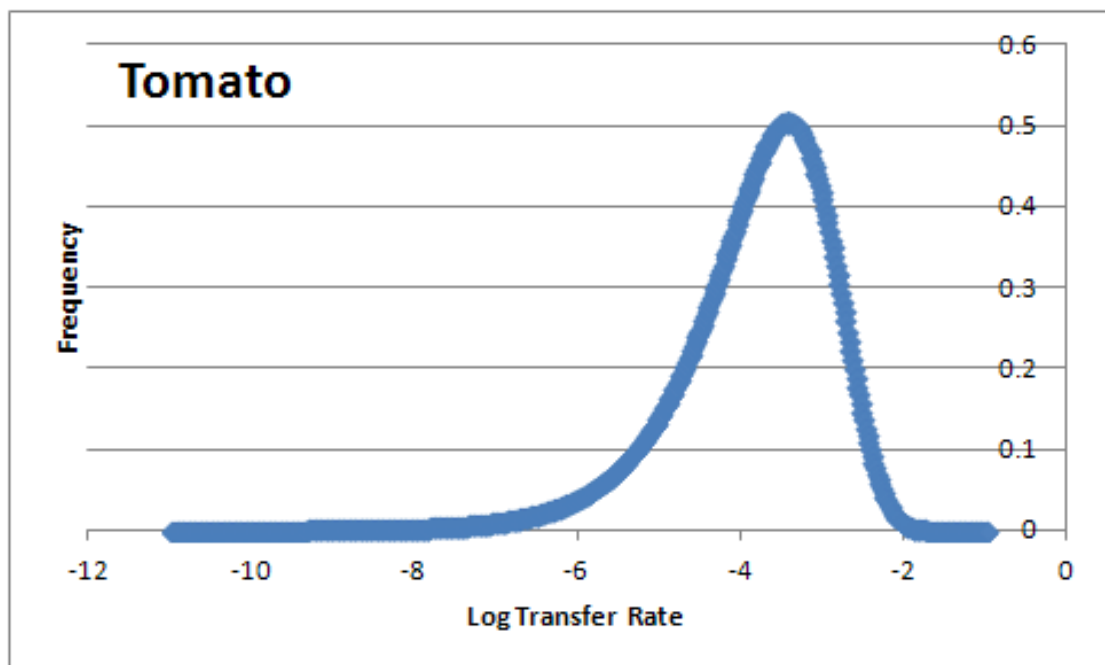
Figure 11. An extvaluemin distribution for published tomato literature showed the log transfer rate from water at 0 ppm chlorine to uncontaminated tomato

Figure 12 shows the simulation model predictions, showing that when tomatoes are contaminated at 4 log CFU/tomato, after washing at 100 ppm chlorine, those same tomatoes contain ~1.0 log CFU/tomato; after washing at 0 ppm chlorine, those same cantaloupes contain ~ 2.5 log CFU/tomato. Figure 13 shows the tornado plots for the two major variables that influence the shape of the distributions: the presence of chlorine in wash water and the initial concentration on contaminated produce.
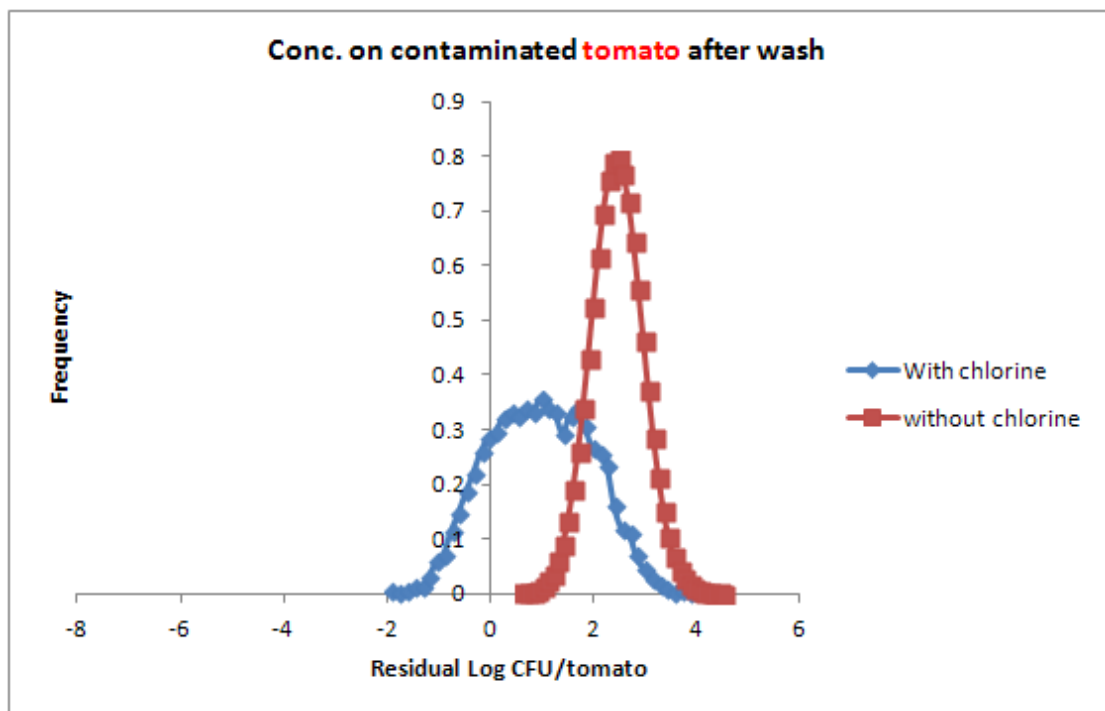
Figure 12. Simulation results predicting the concentration of *Salmonella* on tomato originally containing 4 log CFU/ tomato after washing. With chlorine (◆), without chlorine (■).
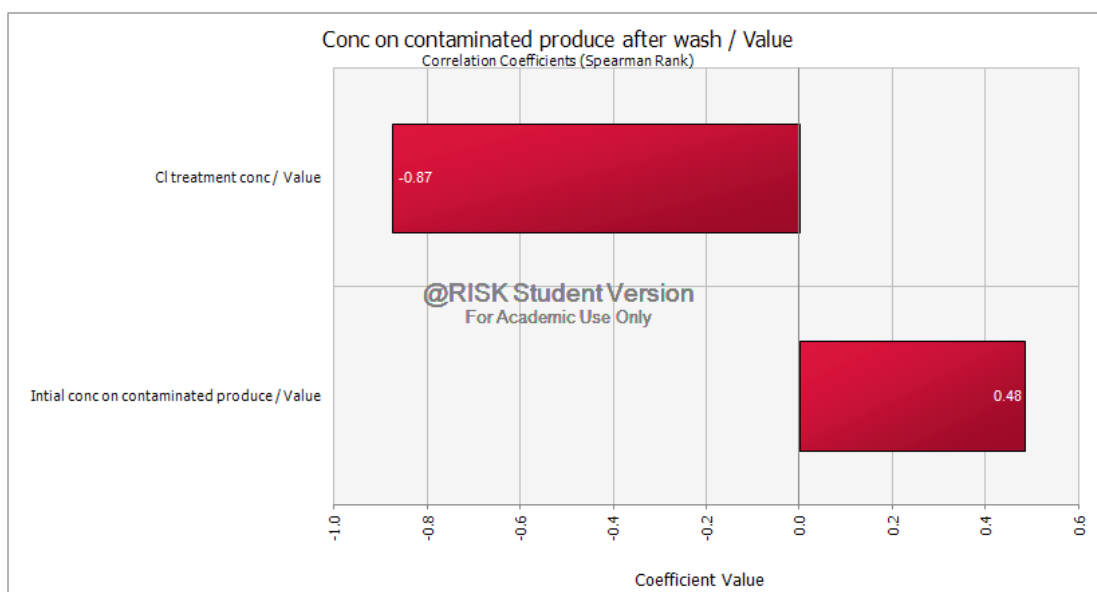
Figure 13. The tornado correlation coefficient graph for the concentration of

***Salmonella* on tomato containing 4 log CFU/tomato after washing with chlorine.**

Figure 14 shows the simulation model prediction for previously uncontaminated

tomatoes. After washing at 100 ppm chlorine in the presence of tomatoes

contaminated at a level of 4 log CFU/tomato, previously uncontaminated tomatoes are

predicted to contain ~ -2.83 log CFU/tomato (one in 676 tomatoes contaminated).

When the washing takes place in water with 0 ppm chlorine, the simulation predicts

that the previously uncontaminated tomatoes will contain ~ -0.59 log CFU/tomato (1

in ~4 tomatoes contaminated). The shape of the distributions in Figure 13 is

influenced by four variables, as shown in the simulation tornado plot in Figure 15.

Those variables are the initial concentration on contaminated produce, the presence of

chlorine, log transfer rate from wash water to uncontaminated produce and log

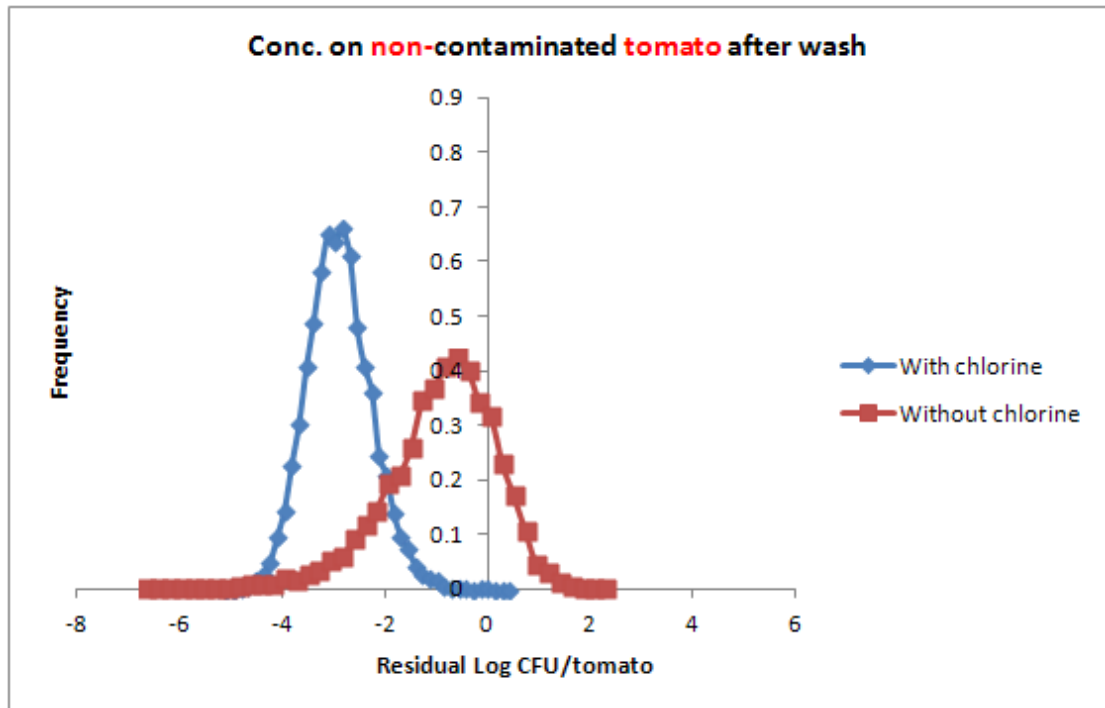transfer rate from wash water to uncontaminated produce.

Figure 14. Simulation results predicting the concentration of *Salmonella* on previously uncontaminated tomato after washing. With chlorine (♦), without chlorine (■).
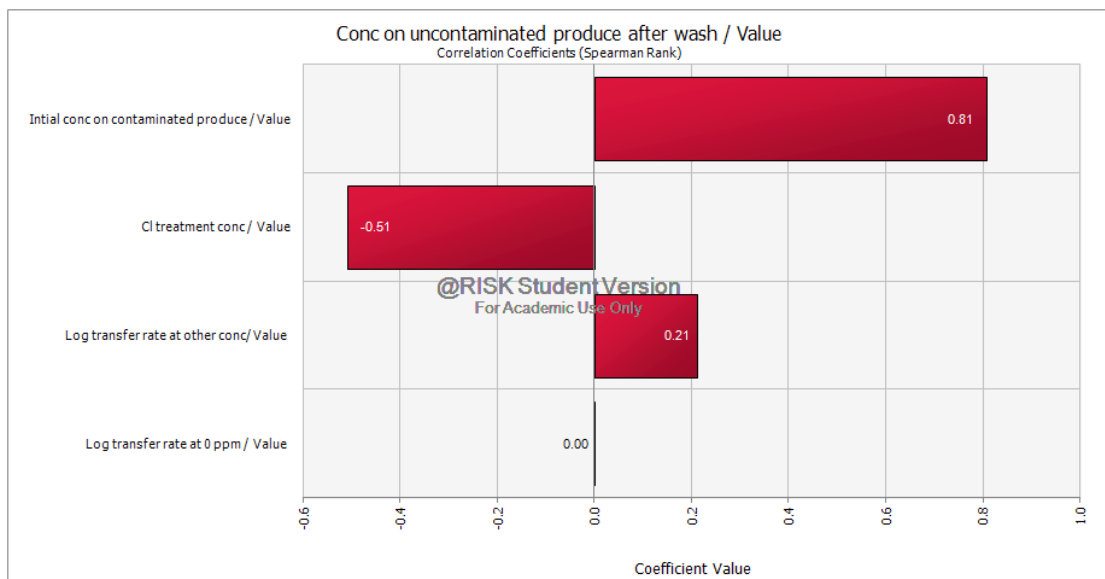
Figure 15. The tornado correlation coefficient graph for the concentration of Salmonella on previously uncontaminated tomato after washing with chlorine.

Several points are clear from a comparison of the simulation models results in Figures 12 and 14. First, whether chlorine is used of not, the concentration on contaminated tomato after washing is always higher than the concentration on uncontaminated tomato after washing. Second, chlorine does have a clear benefit, since the difference between the concentration of Salmonella on contaminated tomato after washing with chlorine and the concentration of Salmonella on contaminated tomato after washing without chlorine is 1.5 log CFU/tomato, and since the difference between the concentration of Salmonella on uncontaminated tomato after washing with chlorine and the concentration of Salmonella on uncontaminated tomato after washing without chlorine is 2.2 log CFU/tomato. Finally, even using chlorine at 100 ppm is not sufficient to completely prevent the risk of transfer to previously uncontaminated tomato, at least based on the data used to construct the simulation.

This study identified the variables and the parameters for cross contamination during washing. Also, this study shows that chlorine can reduce the cross contamination during washing whole produce and the log reduction on contaminated and uncontaminated produce after washing vary on the chlorine concentration in wash water. However, even used sufficient chlorine is not enough to completely prevent the risk of transfer *Salmonella* from contaminated produce to previously uncontaminated produce and to completely prevent the spread of *Salmonella* in wash water.

CONCLUSIONS

The main conclusions can be summarized as follows:

- Most of the published data and data from ComBase Database are located between the most conservative model (Koseki and Isobe), and the most liberal model (Sant'Ana et al.).

- The most conservative model (Koseki and Isobe, 2005) was fail-safe for all but 5.5 % (6/109) of the extracted data, predicting faster growth that that actually observed.

- The cross contamination simulation model predicted that when tomatoes were contaminated at 4 log CFU/tomato, after washing at 100 ppm chlorine, those same tomatoes contained ~1.0 log CFU/tomato, while contaminated cantaloupes contain ~2.8 log CFU/cantaloupe after washing at 0 ppm chlorine.

- The cross contamination simulation model also predicted that uncontaminated tomatoes after washing at 0 ppm chlorine with contaminated tomatoes will contain ~ -0.59 log CFU/tomato (or 1 in 4 tomatoes containing ≥ 1 CFU), while uncontaminated cantaloupes after washing at 100 ppm chlorine with contaminated cantaloupes will contain ~ -2.83 log CFU/cantaloupe (or 1 in 676 tomatoes containing ≥ 1 CFU).

- Whether chlorine is used of not, the concentration on contaminated produce after washing is always higher than the concentration on uncontaminated produce after washing.

- Chlorine does not have a clear benefit on cantaloupe, since the difference between the concentration of Salmonella on contaminated cantaloupe after washing with chlorine and the concentration of Salmonella on contaminated cantaloupe after washing without chlorine is 0.3 log CFU/cantaloupe, and

since the difference between the concentration of Salmonella on uncontaminated cantaloupe after washing with chlorine and the concentration of Salmonella on uncontaminated cantaloupe after washing without chlorine is 0.65 log CFU/cantaloupe, both less than 1 log CFU/cantaloupe.

- Chlorine does have a clear benefit on tomato, since the difference between the concentration of Salmonella on contaminated tomato after washing with chlorine and the concentration of Salmonella on contaminated tomato after washing without chlorine is 1.5 log CFU/tomato, and since the difference between the concentration of Salmonella on uncontaminated tomato after washing with chlorine and the concentration of Salmonella on uncontaminated tomato after washing without chlorine is 2.2 log CFU/tomato.

- Even using chlorine at 100 ppm is not sufficient to completely prevent the risk of transfer to previously uncontaminated fruit, at least based on the data used to construct the simulation.

- Initial concentration on contaminated produce, chlorine use or not during washing, chlorine concentration in wash water during washing can play important role in cross contamination during washing.

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