LISTERIA MONOCYTOGENES TRANSFER DURING SLICING AS IMPACTED BY INTRINSIC CHARACTERISTICS OF FRESH PRODUCE

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Food Science – Doctor of Philosophy

2018

ABSTRACT

LISTERIA MONOCYTOGENES TRANSFER DURING SLICING AS IMPACTED BY INTRINSIC CHARACTERISTICS OF FRESH PRODUCE

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Listeria monocytogenes outbreaks and recalls associated with fresh-cut produce are a major public health concern. Several studies have investigated the extent of microbial cross-contamination during slicing of fresh-cut produce. However, few have examined how product characteristics influence pathogen transfer. In response, a series of studies were conducted to assess the impact of inherent product characteristics on *Listeria* transfer during mechanical slicing.

Using cucumbers and zucchini squash as model products based on their inherent compositional differences, the transfer of *L. monocytogenes* from inoculated cucumbers and zucchini to various surfaces of rotating and stationary slicers was assessed. After slicing one inoculated product followed by fifteen uninoculated ones, *Listeria* populations on different parts of the stationary slicer decreased significantly ($P \leq 0.05$). When the spread of *Listeria* was assessed during slicing of zucchini and cucumbers at different slicing speeds, both high and low speed resulted in statistically similar ($P > 0.05$). Another objective of this study was to evaluate the effect of water content on transfer of *Listeria* during slicing. Floral foam, to which different amounts of water were added, was used as a model system in order to obtain different percent moisture levels of 95.1, 96.7 and, 97.6% under the same conditions. The decay rate observed at all three percent moisture conditions were statistically similar ($P > 0.05$).

The next study focused on quantifying the impact of various physicochemical characteristics (water content, pH, cutting force, soluble solids content, surface hydrophobicity,

and surface roughness) of produce (pears, onions, radishes, tomatoes, potatoes, carrots, zucchini, cantaloupe, apple, cucumber, gray zucchini and, sweet potatoes) on *L. monocytogenes* transfer during slicing. To evaluate the effect of pear firmness on bacterial transfer, three pear firmness categories were determined; firm (10-15 N), medium (6 - 9 N), and soft (< 6 N). For pear slicing, one pear was dip-inoculated with an avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) as well as a 3-strain *Salmonella* cocktail (Montevideo, Poona, Newport) at ~7.5 log CFU/pear and air-dried in a bio-safety cabinet for 1 h before slicing. The inoculated product was sliced using a NEMCO slicer # 59155491, followed by 15 uninoculated pears, all of which yielded quantifiable numbers of bacteria after slicing. Statistically similar ($P > 0.05$) decay rates were observed for firm, medium, and soft pears, respectively.

Finally, onions, radishes, tomatoes, potatoes, carrots, zucchini, cantaloupe, apple, sweet potato, gray zucchini, and cucumber were assessed for *Listeria* transfer, after which a two-parameter exponential decay model was fit to the *Listeria* populations obtained during subsequent slicing of 15 uninoculated samples of the same product type. The decay rate (parameter B) ranged from 0.008 ± 0.002 to 0.09 ± 0.01 for cucumbers and radish, respectively. The root mean square error (RMSE) ranged from 0.25 to 0.68 log CFU/product across the different types of produce, indicating a relatively good fit. When the inherent physicochemical characteristics were fitted into a generalized linear model to describe their impact on the decay rate during slicing, the model was heavily dependent on the product type with a statistical significance ($P \leq 0.05$).

To my parents, Abdulaziz Alnughaymishi and Huda Alshalan, and my wife Balqes Albarrak

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Dr. Elliot Ryser for his endless support, guidance, and trust throughout my Ph.D. study, for which I will be forever grateful. Since I joined his lab on August 2012, I've been learning so much from him and also established great relationships that will last forever. No matter what time I visited him in his office, he's always there to offer his support.

I would also like to thank my committee members Dr. John Linz, Dr. Bradley Marks, Dr. Randy Beaudry and Dr. Leslie Bourquin. They have contributed tremendously to improve my research and study. Despite the huge number of interactions between us through committee meetings and one on one meetings, they provided me with extraordinary support and challenged me and my ideas throughout my Ph.D. which contributed to my evolution as a researcher.

I want to thank all the previous lab mates – Gordon Davidson, Haiqiang Wang, Lin Ren, Andy Scollon, Victor Jayeola, Ryann Gustafson, Haley Smolinski, Nurul Hawa Ahmad, and Gayathri Gunathilaka for their support and the beautiful memories they helped me create during my stay at MSU. Special thanks to Ian Hildebrandt, Beatriz Mazon, Francisco Garces-Vega, and Daewoo Pak for the countless meetings we had to help me better understand my data.

Lastly, I would like to thank all my family members and friends for their continuous support and love specially my parents Abdulaziz Alnughaymishi and Huda Alshalan, who always believed in me. I want to thank my wife Balqes Albarrak for all her love and support. She is not only my wife, she is my best friend, partner, my companion and my inspiration. I love you!

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KEY TO SYMBOLS AND ABBREVIATIONS

AFM	Atomic Force Microscopy
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit(s)
CAC	Codex Alimentarius Commission
CT	Computed tomographic
CLSM	Confocal Laser Scanning Microscopy
d	day(s)
DI	deionized water
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSMA	Food Safety Modernization Act
g	grams
GHP	good hygiene practices
GMP	good manufacturing practices
h	hour(s)
HU	Hounsfield unit
Kg	kilogram
L	liters
lb	pounds
LOD	limit of detection
min	minutes(s)

ml	milliliter(s)
MRA	microbial risk assessment
NRC	National Research Council
PBS	Phosphate Buffered Saline
ppm	parts per million
RMSE	root mean square error
s	second(s)
SD	standard deviation
SE	standard error
SCC	soluble solids content
TSAYE	Trypticase Soy Agar with 0.6 % Yeast Extract
TSB	Tryptic Soy Broth
TSBYE	Trypticase Soy Broth with 0.6 % Yeast Extract
US	United States of America
USDA	United States Department of Agriculture
WHO	World Health Organization
W	weight
Y	response variable
R_a	roughness
μm	micron(s)
x1, x2, x3	independent variables of the linear model
β1, β2, β3	model parameters

INTRODUCTION

Since the early 1970s, a significant increase in the consumption of fresh produce has been observed in the United States, presumably due to active promotion of fruits and vegetables as an important part of a healthy diet. According to the U.S. Department of Agriculture's fruit yearbook report, per capita consumption of fresh vegetables increased from 51.2kg in 1983 to 77kg in 2013, while per capita consumption of fresh fruits increased from 40kg to 50.1kg for the same years (Thornsbury and Jerardo 2017). With this tremendous increase in consumption and production of fruits and vegetables, the incidence of foodborne outbreaks associated with them has also increased. The Centers for Disease Control and Prevention identified about 600 leafy vegetable-associated outbreaks between 1973 and 2012 which, included 20,003 associated illnesses, 1,030 hospitalizations, and 19 deaths(CDC 2012).

Listeria monocytogenes has been isolated from a wide range of fresh fruits and vegetables, including potatoes, cucumbers, tomatoes, cabbages, radishes, apples, cantaloupe, and leafy greens (Heisick et al. 1989). *Salmonella* has been associated with all major food groups, including fresh produce, which has become the leading contributor to foodborne illness, with outbreaks involving grapes, cabbage, lettuce, sprouts, herbs, leafy green salads, and coleslaw(Todd 2014). The CDC reported more than 1,974 confirmed cases of illness associated with fresh- cut produce from 2010 to 2018(CDC 2018a).

From farm to fork, fresh-cut produce can become contaminated with pathogens. Due to the nature of post-harvest processes, such as cutting, slicing, shredding and storing, cross-contamination could ultimately lead to outbreaks and/or recalls. Several studies investigated the extent of pathogen transfer during processing of fresh-cut produce (Van Asselt et al. 2008; Brar and Danyluk 2013; Y. Chen et al. 2001; Luo et al. 2011; Ukuku and Fett 2002). Such studies

generally have been conducted to understand bacterial attachment and growth. In contrast, very few studies (Mazon 2017; Wang and Ryser 2016) have analyzed bacterial transfer in terms of fundamental physical variables, such as contact pressure, surface roughness, contact time, and surface hydrophobicity.

Water content has been shown to facilitate bacterial transfer (Wang and Ryser 2016; Miranda and Schaffner 2016; Jensen et al. 2013). Wang and Ryser (2016) assessed bacterial transfer during slicing of different tomato varieties. Significantly lower transfer decay rates and *Salmonella* transfer percentages were observed for Rebelski and Bigdena as compared to Torero tomatoes. Further analysis of the three tomato varieties (Torero, Rebelski and Bigdena) indicated that Torero tomatoes, which yielded greater extended transfer of *Salmonella* during slicing, had a tougher texture and lower water content compared to the other two varieties. The free liquid released during slicing can potentially "wash off" attached bacteria from the blade, resulting in less bacterial transfer to subsequent tomatoes. The impact of contact time between bacteria and surfaces on transfer have also been assessed . In one recent study to quantify cross-contamination between various foods and common kitchen surfaces (Miranda and Schaffner 2016), more bacteria were transferred to watermelon (~ 0.2 to 97%) than to any other food examined, regardless of the contact time, which may be due to watermelon's moisture, which was significantly higher (0.99 ± 0.01) than other foods tested. Therefore, it is of interest to evaluate bacterial transfer during slicing as impacted by the type of slicer and water content.

Modeling bacterial transfer during slicing of fruits and vegetables can be used to determine exposure to foodborne pathogens. In some studies (Buchholz et al. 2012; Rodríguez et al. 2011; Scollon et al 2016; Wang and Ryser 2016), several mathematical models were developed from experimental transfer data to describe bacterial spread during processing, which

can be used as a guide to help estimate the amount of product that may have become cross-contaminated during processing and would need to be recalled. However, none of these studies attempted to quantify the impact of physicochemical properties (water content, pH, cutting force, soluble solids content, surface hydrophobicity and surface roughness) across a wide range of fresh produce on bacterial transfer during slicing.

It is hypothesized that: 1) *Listeria* transfer is impacted by the type of slicer, slicing speed and product moisture content**;** 2) different firmness levels of pears will affect the transfer rate of *Listeria* during slicing; 3) different types of produce will yield different transfer decay rates during slicing due to differences in physicochemical properties; and 4) produce transfer decay rate during slicing can be predicted using a mathematical model based on the physicochemical properties of the produce

The ultimate goal of this research was to collect quantitative data on *Listeria* transfer during slicing of fresh produce and collect quantitative data of physicochemical properties of fresh cut produce (water content, pH, cutting force, soluble solids content, surface hydrophobicity and surface roughness) for subsequent model development to enhance the current understanding of interaction between these properties and transfer during slicing. Thus, this dissertation includes five primary objectives: 1) assess the impact of slicer type and speed on the transfer of *Listeria* during slicing; 2) evaluate the impact of water content on the transfer of *Listeria* during slicing; 3) determine the effect of pear firmness on transfer of *Listeria* during mechanical slicing; 4) quantify *Listeria* transfer during slicing across different types of fresh produce; and 5) develop a model to describe *Listeria* transfer during slicing of fresh produce based on physicochemical properties of the product (water content, pH, cutting force, soluble solids content, surface hydrophobicity and surface roughness).

CHAPTER 1:

Review of Pertinent Literature

1.1 FRESH-CUT PRODUCE

Fresh-cut produce is defined by the FDA as “fresh fruits and vegetables for human consumption that have been minimally processed and altered in form by peeling, slicing, chopping, shredding, coring, or trimming, with or without washing, prior to being packaged for use by the consumer or a retail establishment (e.g., pre-cut, packaged, ready-to-eat salad mixes)”. These products have an estimated consumer market value of about $27 billion (Cook 2014). Since the early 1970s, a significant increase in the consumption of fresh produce has been observed in the United States, presumably due, in part, to active promotion of fruits and vegetables as an important part of a healthy diet. Moreover, bioactive compounds present in fruits and vegetables have been repeatedly linked to a lower risk of cardiovascular disease, stroke, cancer, and type 2 diabetes (K Jordan et al. 2014), which has increased the consumption of produce.

According to the U.S. Department of Agriculture’s fruit yearbook report, per capita consumption of fresh fruit in 2016 was 52.4 kg, up 3% from 51.kg in 2015 with a total of about 58.5 billion kg of vegetables produced commercially. With this increase in consumption and production of fruits and vegetables, the incidence of foodborne outbreaks associated with them has also increased. The Centers for Disease Control and Prevention identified about 600 leafy vegetable-associated outbreaks between 1973 and 2012 which included , 20,003 associated illnesses, 1,030 hospitalizations, and 19 deaths. (Herman, Hall, and Gould 2015).

Fresh-cut processing of produce involves various steps such as peeling, trimming, deseeding, slicing, and dicing to a specific size (Figure 1) with each of these steps potentially impacting quality and safety (James and Ngarmsak 2011).

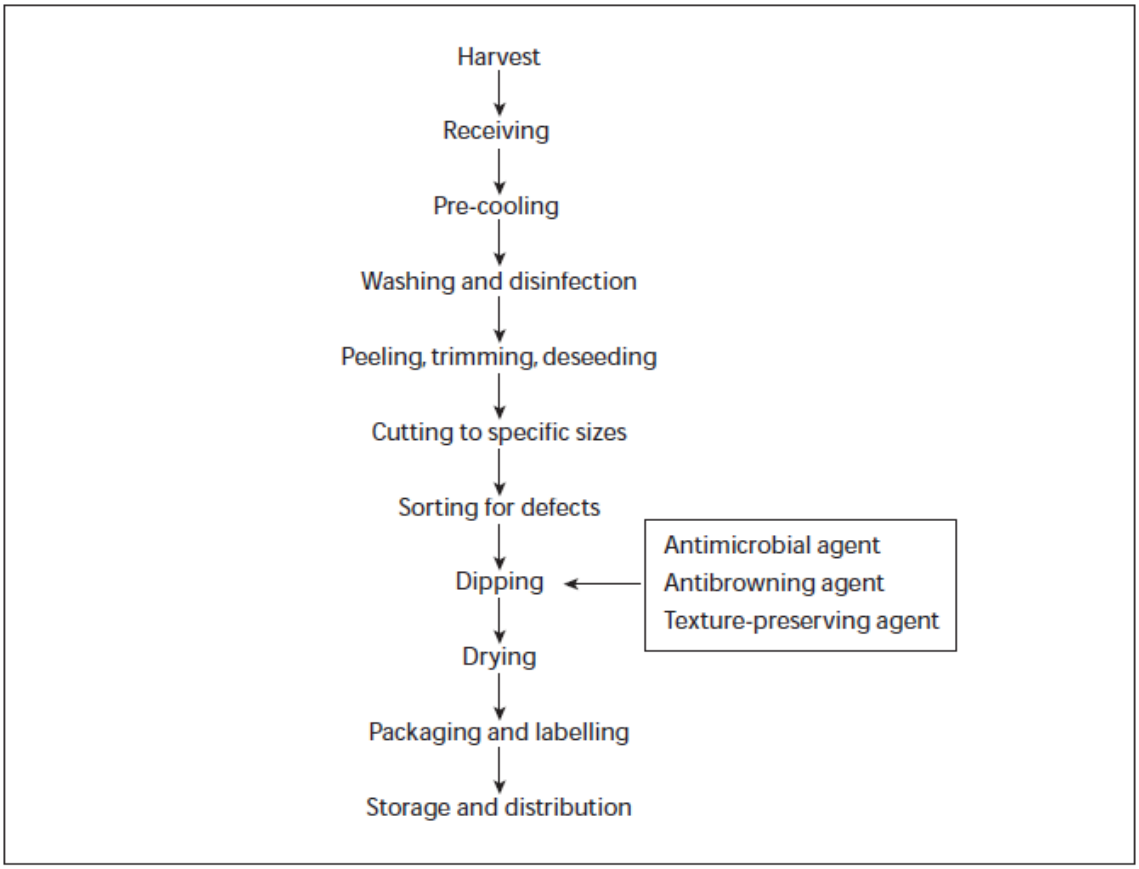


Figure 1-1-1: Typical fresh-cut process flow chart for fruits, vegetables, and root crops

The quality of fresh-cut produce includes a combination of characteristics that determine the value of produce to the consumer. Characteristics such as appearance, cutting force, flavor, and nutritional quality are essential for both producers and consumers. Pre- and post-harvest conditions can affect the quality of fresh-cut produce. For instance, pre-harvest quality of produce is influenced by the cultivars, genotypes and rootstocks, climate, cultural practices, maturity and ripening process (Garrett 2002). Post-harvest conditions such as handling practices and management of both relative humidity and temperature can negatively affect both the internal (physiological processes) and external (microbiological, chemical, environmental and mechanical) quality of harvested produce.

The safety of fresh-cut produce is one component of quality. In fact, many experts believe that safety is the most important component of quality, since unsafe food can result in serious illness or death in some cases. Physical, chemical or microbial hazards can pose a threat to consumers throughout the produce production process. Several measures are taken in fresh-cut facilities to maintain the microbial safety of produce. Washing in water containing a sanitizer in order to minimize cross-contamination during processing is standard practice for many types of fresh produce. Chlorine is currently the most commonly used sanitizer in washing operations. Chlorine has been successfully used at concentrations of 50 to 200 parts per million (ppm) to wash fresh-cut produce. However, excess amounts of free chlorine may react with organic compounds in produce wash water to generate carcinogenic halogenated disinfection by-products (DBPs), such as trihalomethanes (THMs).

To maintain high food safety standards and minimize foodborne disease outbreaks, various guidelines have been published such as the "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" by The U.S. Food and Drug Administration (FDA 2008), "Safety and Quality of Fresh Fruit and Vegetables: Manual for Trainers" by the United Nations (Lineback 2002) and "Guidance on Environmental Monitoring and Control of *Listeria* for the Fresh Produce Industry" by the United Fresh Produce Association (Bierschwale N.D). With the passage and implementation of the Food Safety Modernization Act (FSMA), the FDA has recently published the final rule for produce safety, which sets standards related to agricultural water, worker training and health and hygiene, and equipment, tools and buildings, among other processes that impact food safety (FDA 2018).

1.2 FOODBORNE DISEASE RELATED TO FRESH PRODUCE

Foodborne disease is an important public health problem worldwide, which can negatively impact travel, trade, and development. The World Health Organization (WHO) estimated that 31 foodborne hazards caused 600 million foodborne illnesses and 420,000 deaths worldwide in 2010 (Havelaar et al. 2015). Five different categories of foodborne disease are recognized: infections, intoxications, metabolic food disorders, allergies, and idiosyncratic illnesses. The CDC has described more than 250 different foodborne diseases, most of which are infections caused by bacteria, viruses and parasites, or noninfectious chemicals and toxins. Many of these agents commonly cause diarrhea, vomiting and in some cases death but there is no single clinical syndrome for all foodborne diseases. Every year about one in six Americans or 48 million people become ill, 128,000 are hospitalized, and 3,000 die of foodborne illness (CDC 2017).

The burden of foodborne disease and associated economic cost have been estimated based on (1) the annual number of illnesses caused by a particular pathogen, (2) attributions of foodborne disease to particular foods, (3) acute illness severity and outcome, and (4) chronic complications (Jakob and Tritscher 2014). Despite several attempts to estimate the costs and burden of foodborne disease (Table 1), such studies do not reflect the magnitude of foodborne illness because most foodborne illnesses are under-diagnosed or under-reported (Figure 2).

Table 1-1: Sample of estimated costs and burden of foodborne disease (Jakob and Tritscher 2014)

Method	(year of study)	Foodborne disease	Estimated costs	Country
COI the cost-of-illness	1997	Six bacteria, one parasite	$6.5–34.9 billion	US
COI	2000	STEC O157:H7 outbreak	$16,7 million	UK
DALY The disability-adjusted life year	2000	*Campylobacter* sp.	1400 DALY/case	Netherlands
COI	2000	All foodborne disease	$55.1 million	New Zealand
COI	2001	Foodborne disease	$123 million	Sweden
COI	2002	STEC O157:H7 outbreak	$779,728	Japan
COI	2005	STEC O157 (all sources)	$344 million	US
COI	2006	All foodborne disease	$989 million	Australia

Table 1-1 (cont'd)

Method	(year of study)	Foodborne disease	Estimated costs	Country
WTP Willingness-to-pay (WTP)	2007	All foodborne disease	$1.4 trillion	US
DALY/COI	2009	Select foodborne diseases and irritable bowel syndrome	$ 81.3 million	Netherlands

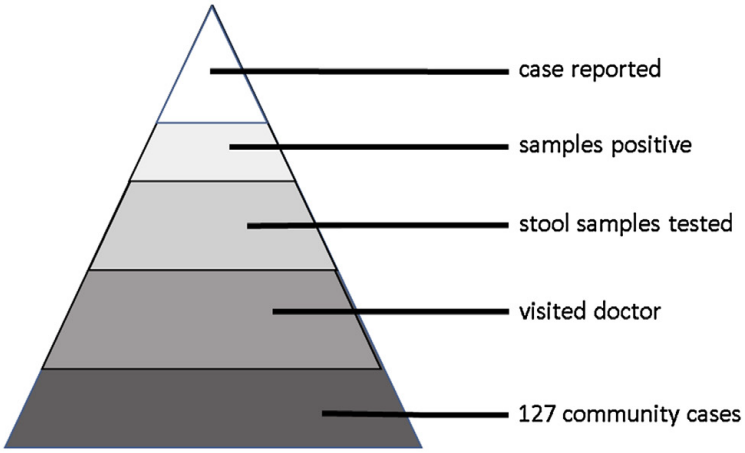


Figure 1-1-2: Surveillance pyramid Hoffmann and Scallan (2017)

During the last few years, the CDC has published annual reports of Foodborne Disease Outbreaks. In 2012, more than 800 foodborne disease outbreaks were reported, resulting in 14,972 illnesses, 794 hospitalizations, and 23 deaths. Vegetable row crops and fruits respectively

accounted for 12% and 21% of the total illnesses reported that year. In 2013, 818 foodborne disease outbreaks were reported, resulting in 13,360 illnesses (including 11 and 4% from fruits and vegetables), 1,062 hospitalizations, and 16 deaths. During 2014, 864 foodborne disease outbreaks were reported, resulting in 13,246 illnesses, 712 hospitalizations, and 21 deaths. The highest number of outbreak- associated illnesses were from seeded vegetables (e. g. cucumbers or tomatoes; 428 illnesses, 16%). Foodborne disease outbreaks and outbreak-associated illnesses, by food category from 2012 to 2014 are summarized in (Table 2).

Table 1-2: Reported foodborne disease outbreaks and outbreak-associated illnesses, by food category —Foodborne Disease Outbreak Surveillance System, United States, 2012, 2013 and 2014

Year 2012				
Food type	**No. Outbreaks**		**No. Illnesses**	
	Total	**%**	**Total**	**%**
Oils and sugars	1	1	7	0
Fungi	5	3	15	0
Sprouts	2	1	25	1
Root and other underground vegetables	5	3	34	1
Seeded vegetables	3	2	206	5
Herbs	0	0	0	0
Vegetable row crops	23	12	377	9
Fruits	16	8	858	21
Grains and beans	8	4	190	5
Nuts and seeds	1	1	42	1

Table 1-2 (cont'd)

Total	63	33	1754	42
Year 2013				
Oils and sugars	1	0	7	0
Sprouts	1	0	3	0
Root and other underground vegetables	2	1	69	2
Seeded vegetables	8	4	305	8
Herbs	1	0	38	1
Vegetable row crops	9	4	207	5
Fruits	15	7	422	11
Grains and beans	8	4	61	2
Nuts and seeds	2	1	25	1
Total	47	22	1137	29
Year 2014				
Oils and sugars	1	0	2	0
Fungi	5	2	11	0
Sprouts	4	2	141	5
Root and other underground vegetables	2	1	31	1
Seeded vegetables	7	3	428	16
Herbs	1	0	7	0
Vegetable row crops	13	6	174	6
Fruits	10	5	139	5

Table 1-2 (cont'd)

Grains and beans	9	4	104	4
Nuts and seeds	3	1	55	2
Total	55	27	1092	40

1.3 PRE-HARVEST VS POST-HARVEST CONTAMINATION

The normal microflora on fruits and vegetables is usually nonpathogenic to humans. However, microorganisms from many sources, whether animal, environmental or human, have the potential to contaminate fruits and vegetables during field production, harvesting, further processing and transportation (FDA, 2008). Sources of contamination can be divided into two main categories: pre-harvest and post-harvest. The former includes irrigation water, green or inadequately composted manure, air (dust), wild and domestic animals, human handling, and water used for other purposes (for example, pesticides, foliar treatments, growth hormones). The latter category includes human handling (workers, consumers), harvesting equipment, transport containers (field to packing shed), wash and rinse water, sorting, packing, slicing/dicing and further-processing equipment, transport vehicles, improper storage (temperature, physical environment), improper packaging (including new packaging technologies), cross-contamination in food storage, preparation and display areas, and improper handling after wholesale or retail purchase (FDA 2015).

Although most bacterial contamination occurs during pre-harvest, contaminants can spread quickly during post-harvest processing. This is due to the nature of post-harvest processes such as slicing, shredding and storing which could ultimately lead to outbreaks or recalls (Beuchat and Ryu 1997). In a survey of fresh and minimally-processed fruit and vegetables conducted by Abadias et al. (2008), *L. monocytogenes* was present in 0.7% of 300 samples.

Although the incidence of *Listeria* was low, fresh-cut packaged vegetables that support growth of the pathogen could represent a risk to consumers.

Several studies investigated the extent of pathogen transfer during processing of fresh-cut produce (Van Asselt *et al*. 2008; Brar and Danyluk 2013; Y. Chen *et al*. 2001; Luo *et al*. 2011; Ukuku and Fett 2002). In a large-scale experiment, Buchholz (Buchholz et al. 2012b) demonstrated that *E. coli* O157:H7 transferred from inoculated lettuce to both the shredder and conveyor belt. The study found that processing lettuce inoculated with 10^6 or 10^4 CFU/g of *E. coli* before shredding 90.8 kg uninoculated lettuce was sufficient to contaminate the entire product lot. Another experiment by Ukuku and Fett (2002) showed that *L. monocytogenes* transferred from the inoculated rind of cantaloupe to the interior flesh during cutting. Carrots, watermelon, celery and lettuce were also examined by Jensen et al.(2013) for the transfer of pathogens between produce and common kitchen surfaces. It was determined that more than 90% of bacteria transferred to the fresh-cut produce in almost all of the scenarios studied.

1.4 *LISTERIA MONOCYTOGENES* AND FRESH-CUT PRODUCE

The official discovery of *Listeria* dates back to 1924, when Murray, Webb, and Swann isolated *L. monocytogenes* as the etiological agent of a septicemic disease affecting rabbits and guinea pigs in their laboratory at Cambridge in England. This strain was named *Bacterium monocytogenes*, as it was observed to infect monocytes of the blood. Although the first cases of human listeriosis were reported in 1929 in Denmark, *L. monocytogenes* was not recognized as a foodborne pathogen until the 1980's when an outbreak in humans was directly linked to the consumption of contaminated coleslaw salad in Canada(Magalhaes *et al*. 2014).

Listeria monocytogenes is a member of the genus *Listeria*, a group of Gram-positive bacteria closely related to *Bacillus* and *Staphylococcus*. The genus *Listeria* includes 15 different species, two of which are considered pathogenic. *L. monocytogenes* is pathogenic to humans, causing listeriosis, and *L. ivanovii* is mainly pathogenic to animals, although a few cases of human infection have been reported (Jordan *et al*. 2015). *Listeria monocytogenes* strains van be divided into serotypes based on somatic (O) and flagellar (H) antigens.

Listeria monocytogenes is catalase-positive, oxidase-negative, regular short rod with a diameter of approximately 0.5 µm and a length of 0.5–2.0 µm (Figure 3). The organism is a facultative anaerobe that does not form a capsule or spores, and is motile by peritrichous flagella when cultured at 20–25 ^{o}C and non-motile at 37 ^{o}C. *L. monocytogenes* has the ability to hydrolyze esculin and sodium hippurate but not urea, gelatin, or casein (Motarjemi *et al*, .2014).

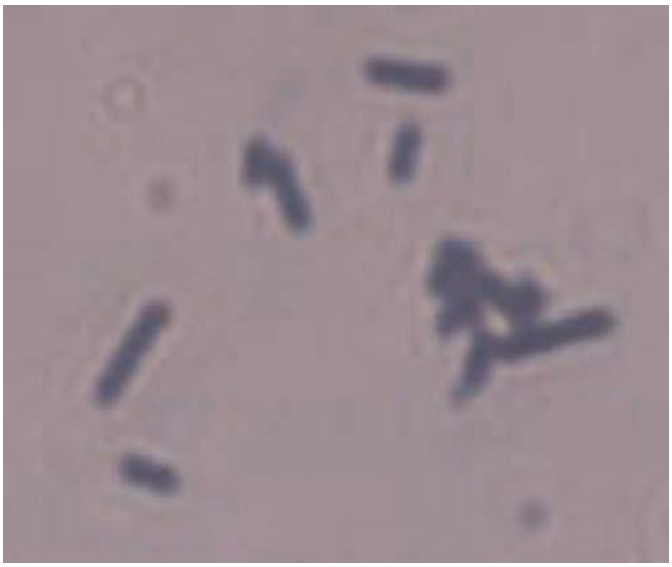


Figure 1-1-3: Gram staining of *Listeria monocytogenes*

The organism's ability to grow and reproduce under harsh conditions makes it a foodborne pathogen of great concern. *Listeria monocytogenes* can grow at 1.5 to 45 ^{o}C, with optimum growth between 30 and 37 ^{o}C. It also can grow over a wide range of pH values (4.3 to 9.6) with

optimum growth between pH 6.0 and 8.0 (Uyttendaele et al. 2014). Unlike most human pathogens, *L. monocytogenes* can grow at refrigeration temperatures and has been associated with sporadic outbreaks.

Listeria monocytogenes is capable of causing serious invasive illness (listeriosis) with a fatality rate of about 20%, especially in older adults, pregnant women, newborns, and adults with weakened immune systems. While the infectious dose of *Listeria* is unknown, data from previous outbreaks suggests that levels of *L. monocytogenes* in foods identified as being responsible for outbreaks or sporadic cases are often greater than 100 CFU/g (Rees and Doyle 2017). Consequently, the presence of *L. monocytogenes* in food at levels less than 100 CFU/g is thought to have a very low probability of causing disease and that less than 1000 CFU is of no concern to healthy adults. However, the FDA has a "zero tolerance" policy in which the presence of *L. monocytogenes* in any cooked, RTE food is a violation (The analytical method that FDA uses can detect 1 CFU of *L. monocytogenes* per 25 g of food to determine whether *L. monocytogenes* is present in the food (i.e., 0.04 CFU/g)).

After ingesting food contaminated with *Listeria*, the organism passes through the stomach and crosses the intestinal barrier via M-cells. It is then transported by the lymph or blood to the mesenteric lymph nodes, spleen, and liver. The fact that *L. monocytogenes* is a facultative intracellular pathogen allows it to replicate in macrophages and a variety of non-phagocytic cells, such as epithelial and endothelial cells. After entering the cell, *Listeria* escapes early from the phagocytic vacuole, multiplies in the host cell cytosol, and then moves through the cell by induction of actin polymerization. The bacteria then protrudes into cytoplasmic evaginations, and these pseudopod-like structures are phagocytized by the neighboring cells (Simjee 2007). *L. monocytogenes* virulence factors are involved in the cell-to-cell spread, which helps the pathogen

avoid the extracellular environment and immune system during its spread in the host. These virulence genes form a 9-kb gene cluster known as the *Listeria* pathogenicity island 1 (LIPI-1).

Despite extensive research, *L. monocytogenes* has remained one of the most problematic pathogens in the food industry. *Listeria* typically contaminates food from direct contact with equipment and the general environment after foods have been processed. Moreover, *L. monocytogenes* can persist in food processing facilities for years with the same strains having been isolated from both the food-processing environment (e.g., drains, equipment, etc.), and food-contact surfaces (e.g., slicing machines), rather than raw materials (Motarjemi *et al.* , 2014). The finding that persistent strains are often recovered from the environment and equipment after cleaning and sanitizing, emphasizes the risk of growth and establishment of *L. monocytogenes*, particularly in sites difficult to access leading to ongoing food product contamination.

Listeria can exist in the environment either as planktonic cells or as communities in biofilms, where they are attached to a surface and enclosed in a matrix predominantly made up of polysaccharide material (Gandhi and Chikindas 2007). Microbial biofilms demonstrate an enhanced resistance to sanitizers, disinfectants and antimicrobial agents and can form on a wide range of surfaces in food processing facilities, and industrial equipment. Biofilms in food processing environments occur on product contact surfaces or areas where food is stored or on food processing surfaces such as conveyer (Gandhi and Chikindas 2007). Biofilms of *Listeria* are of particular concern, since they are more resistant to disinfectants and sanitizing agents compared to planktonic cells and this makes their elimination from food processing facilities a

big challenge. Although biofilm formation is more studied in terms of bacterial attachment, it could contribute directly or indirectly to bacterial transfer.

Listeria monocytogenes has been isolated from a wide range of fresh fruits and vegetables including potatoes, cucumbers, tomatoes, cabbages, and radishes (Heisick *et al*. 1989). Crépet *et al*. (2007) calculated the probabilities of fresh unprocessed and minimally processed vegetables being contaminated, based on data from 165 prevalence studies of *L. monocytogenes* in fresh vegetables (25,078 samples). Their results showed that the probabilities of contamination with populations higher than 10, 100 or 1000 viable *L. monocytogenes* organisms/g were 1.44, 0.63 and 0.17% respectively, indicating that there is approximately a 1.44% chance of fresh produce being contaminated with 10 cells/g. Moreover, based on product type, the mean log concentrations of *L. monocytogenes* on leafy salads, sprouts and other vegetables was 3.36, 3.09 and 3.43 log CFU/g, respectively.

Although listeriosis is responsible for only 0.02% of gastroenteritis, it accounts for about 25% of deaths as a result of gastroenteritis (Ross 2000). Several studies have attempted to evaluate the cost of listeriosis case in the USA (Mead et al. 1999; Scallan et al. 2011). These costs include human cost, litigation, industry cost, product recalls and regulatory costs (Jordan *et al*. 2014). For example, in 2008, 57 cases of listeriosis and 24 deaths in Canada were linked to contaminated delicatessen meat from one meat processing plant. It was estimated that the costs for this outbreak reach $2.2 million (Thomas et al. 2015). In south Africa earlier this year, the World Health Organization (WHO) reported the largest *Listeria* outbreak ever recorded with more than 1000 confirmed cases and more than 67 deaths. Although not confirmed by health officials, food is the suspected source of the outbreak (WHO 2018). In 2015, a multistate outbreak of listeriosis was linked to commercially produced, prepackaged caramel apples resulting in 7 deaths and 34

hospitalizations according to the CDC (CDC 2015). More recently, an outbreak of listeriosis linked to packaged Dole Food Company salads resulted in 19 hospitalizations and one death, across nine U.S. states. The company stopped all production at the processing facility and recalled all packaged salads on the market CDC (CDC 2016). Table 3 summarizes the fresh cut outbreaks and recalled reported by CDC since 2011.

Table 1-3: *Listeria* outbreaks associated with fresh produce:

Year	Produce	Cases	Death	Hospitalizations	Number of states	Recall
2016	Frozen vegetables	9	3	9	4	Yes
2016	Packaged Salads	19	1	19	9	Yes
2014	Caramel Apples	35	7	34	12	Yes
2014	Bean Sprouts	5	2	5	2	Yes
2011	Cantaloupes	147	33	143	28	Yes

1.5 *SALMONELLA* AND FRESH-CUT PRODUCE

Salmonella has been recognized for over 100 years as a cause of illnesses ranging from mild to severe food poisoning (gastroenteritis), and even more severe typhoid (enteric fever), paratyphoid, bacteremia, septicemia and a variety of associated longer-term conditions

(sequelae). Some of these severe conditions can result in high rates of mortality and can occur in outbreaks involving large numbers of people, particularly in relation to typhoid outbreaks and septicemic conditions (Blackburn 2009).

The history of *Salmonella* (Blackburn 2009) dates back to the late 1800s when an American bacteriologist D.E. Salmon characterized the hog cholera bacillus causing 'swine plague' which, at that time, was named *Bacterium suipestifer* but later renamed as the type species of the genus named after him, *Salmonella cholerae-suis*. It was not until the 1960s, however, that the name *Salmonella* became the widely accepted for this genus of the family Enterobacteriaceae.

Salmonella spp. (Figure 4) are facultatively anaerobic, Gram-negative, straight, small (0.7-1.5 x 2.0-5.0 μm) rods, which are usually motile by peritrichous flagella. Being Gram-negative, *Salmonella* is more resistant to antibiotics and sanitizers than Gram-positive bacteria. This is primarily due to their thin peptidoglycan layer, which is located between two thin membranes. The thin outer membrane surrounding the peptidoglycan layer is impermeable and resists toxic materials that could damage the cell (Mitchell 2015). *Salmonella* is an infectious organism that multiplies in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin and causing an inflammatory reaction and diarrhea. Moreover, the organism can enter the blood stream and/or the lymphatic system and cause more severe illnesses (Blackburn 2009).

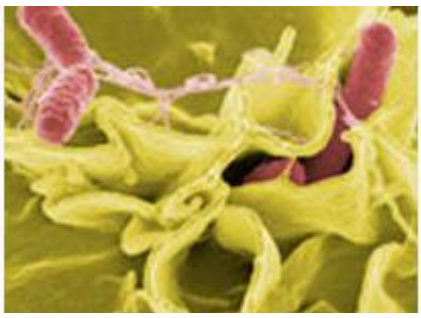


Figure 1-1-4: *Salmonella* CDC (2014)

Every year approximately 1.2 million illnesses and 450 deaths occur due to non-typhoidal *Salmonella* in the United States according to the CDC (2018). The most common serotypes of *Salmonella* that causes human infection are Enteritidis, Typhimurium, Newport, and Javiana. These *Salmonella* serotypes account for about half of culture-confirmed *Salmonella* isolates reported by the CDC (2018). The symptoms of *Salmonella* infection include sudden onset of diarrhea (which may be bloody), abdominal cramps, fever (almost always present), nausea, vomiting and less frequently, headaches.

Salmonella has been associated with all major food groups including fresh produce, which has become the leading contributor to this foodborne illness with outbreaks involving grapes, cabbage, lettuce, sprouts, herbs, leafy green salads, and coleslaw. The CDC has reported more than 1974 confirmed cases of illness associated with fresh cut produce from 2010 until 2018 (Table 4). Tomatoes have been most commonly associated with *Salmonella* with 5,324 cases of illness in the U.S. and 35 outbreaks between 1990 and 2012 according to the Center for Science in the Public Interest (2013).

Table 1-4: *Salmonella* outbreaks associated with fresh produce (CDC 2018b)

Year	Produce	Cases	Deaths	Hospitalizations	# of states	Serotype	Recall
2018	Raw Sprouts	8	0	0	3	Montevideo	No
2017	Maradol Papayas	220	1	68	23	Thompson, Kiambu, Agona, and Gaminara	Yes
2017	Maradol Papayas	20	1	5	3	Anatum	Yes
2016	Alfalfa Sprouts	36	0	7	9	Abony	Yes
2016	Alfalfa Sprouts	26	0	8	12	Muenchen and Kentucky	No
2015	Cucumbers	907	6	204	40	Poona	Yes
2014	Cucumbers	275	1	101	29	Newport	

Table 1-4 (cont'd)

Year	Produce	Cases	Deaths	Hospitalizations	# of states	Serotype	Recall
2014	Bean sprouts	115	0	28	12	Enteritidis	No
2013	Cucumber	84	0	17	18	Saintpaul	No
2012	Mangoes	127	0	33	15	Braenderup	Yes
2012	Cantaloupe	261	3	94	24	Typhimurium and Newport	Yes
2011	Whole, Fresh Imported Papayas	106	0	10	25	Agona	Yes
2011	Cantaloupe	20	0	3	10	Panama	No
2010	Alfalfa Sprouts	44	0	7	11	Newport	Yes

1.6 BACTERIAL TRANSFER DURING SLICING AND DICING

A series of previous studies investigated bacterial transfer during slicing of delicatessen meats (Sheen and Hwang 2008; Vorst *et al*. 2006) ,(Lin *et al.* 2006), (Chen et al. 2014), shredding of leafy greens (Beuchat and Doyle 1995) ,(Buchholz et al. 2012c), (Nou and Luo 2010) and slicing/dicing of tomatoes (Wang and Ryser 2014, 2016). These studies demonstrated that the

likelihood for cross-contamination during slicing is high. However, due to the nature of bacterial transfer, large variations within replicates were observed for most of the transfer studies, particularly at lower initial inoculation levels.

After a series of recalls in 2012 involving diced yellow onions contaminated with *L. monocytogenes* from one manufacturer, Scollon *et al*. (2016) conducted a study to quantify the extent of *L. monocytogenes* transfer during mechanical slicing of onions. After slicing 20 onions, *L. monocytogenes* was quantifiable on both the pusher plates and blades of the slicer, allowing for further transfer. Their research clearly showed the potential for cross-contamination from inoculated to uninoculated onions during sequential slicing. Similarly, Kaminski *et al*. (2014) investigated the transfer of *L. monocytogenes* to previously uncontaminated product during mechanical dicing of celery and found *Listeria* present throughout 15 uninoculated batches.

In an attempt to better understand bacterial transfer, Wang and Ryser (2016) assessed bacterial transfer during slicing of different tomato varieties. Significantly lower transfer decay rates and *Salmonella* transfer percentages were observed for Rebelski and Bigdena as compared to Torero tomatoes. Further analysis of the three tomato varieties (Torero, Rebelski and Bigdena) indicated that Torero tomatoes, which yielded greater transfer, had a tougher texture and lower water content compared to the other two varieties. This free liquid released during slicing can potentially "wash off" attached bacteria from the blade, resulting in less bacterial transfer to subsequent tomatoes (Wang *et al*. 2016). Preliminary work in our lab also has shown that different types of produce with varying characteristics (water content, pH, cutting force, soluble solids content, surface hydrophobicity and surface roughness) have different transfer rates. This difference in transfer rate could be attributed to any of the above factors. Hence, more research is

needed to better understand bacterial transfer during processing with this information leading to improved sanitation programs and risk assessments.

1.7 FACTORS EFFECTING TRANSFER DURING SLICING

The rate at which pathogens transfer during slicing depends on a number of factors including the chemical and physical properties of the food, the equipment surface and materials, and the microorganism(s) involved in addition to environmental and operational conditions. Sheen and Hwang (2010) summarized the factors affecting microbial transfer which include (1) food composition (moisture, fat contents, formulation), (2) food texture (homogeneity, hydrophobicity, roughness), (3) the blade for slicing (blade speed (rpm), slicing speed (i.e., slices per minute), blade size, blade sharpness and the material of the blade), (4) bacterial factors (age, strain, inoculation level, stress response, attachment to surfaces), and (5) the environmental conditions (e.g., temperature). Identifying which of these factors has the most significant impact on bacterial transfer provides critical quantitative data for mathematical modeling that will be useful in refining current risk assessments.

Surface characteristics of fresh-cut produce play an important role in the way bacteria are able to attach, transfer and proliferate. Several factors including produce type, maturity, variety, and growing condition can change the surface characteristics of fresh-cut produce by altering surface hydrophobicity, surface constitutional characteristics, and surface topography (Wang et al. 2009). The influence of the fruits' roughness and hydrophobicity on bacterial adhesion was evaluated by Fernandes et al. (2014) who found that the average roughness (Ra) of mangoes (4.54 ± 1.95 mm) was significantly different ($P \leq 0.05$) compared to tomatoes (2.88 ± 2.15 mm). However, the numbers of bacteria on both fruit surfaces were similar ($p > 0.05$), reaching $5.95 \pm$

0.36 log CFU cm2 and 5.81 ± 0.39 log CFU cm2 on mangoes and tomatoes, respectively suggesting that bacterial adhesion is a multifactorial process.

In another study, Adhikari *et al.* (2015) examined the effectiveness of UV-C inactivation of pathogens on different products including apples, pears, strawberries, red raspberries and cantaloupes. These researchers reported greater pathogen on products having smooth rather than rough surfaces with rough surface products presumably providing greater shelter for pathogens from UV-C. Similarly *Syamaladevi et al.* , (2013) looked at the influence of surface characteristics of pears on the kinetics of UV-C inactivation of *E. coli* and concluded that the physical and morphological characteristics (i.e. surface roughness) of pears influenced the ability of UV-C to achieve specific levels of reduction in *E. coli* population.

Using Confocal Laser Scanning Microscopy (CLSM) to quantify produce surface roughness, Wang *et al*. (2009) showed a positive linear correlation between average surface roughness (***Ra***) and the adhesion rate of *E. coli* O157:H7 for Golden Delicious apples (1.43 ± 0.13 μm), navel oranges (10.94 ± .07 μm), avocadoes (9.58 ± 0.27 μm) and cantaloupe (14.18 ± 0.25 μm), while surface hydrophobicity for the same produce was 77.27 ± 4.57, 78.23 ± 8.37, 56.33 ± 5.16 and 47.20 ± 18.52 θ, respectively. The populations of *E. coli* O157:H7 on fresh fruit surfaces after a 5-min washing treatment were 2.61 ± 0.20, 3.99 ± 0.33, 5.19 ± 0.19 and 6.03 ± 0.29 log CFU/cm^2, respectively. Another study conducted by the same group examined the relationship between surface roughness of apples, avocadoes, and cantaloupes and the removal of pathogens during washing (H. Wang et al. 2007). Produce with lowest surface roughness (apples 1.43 μm) had the highest pathogen reduction rate during washing, whereas produce with the highest roughness (cantaloupe 14.18 μm) had the lowest reduction rate. It is likely that the rougher the produce surface, the more protection is provided to the *E. coli* O157:H7.

Adhesion of *Salmonella* Enteritidis to lettuce leaves was evaluated in the context of leaf roughness by Lima et al. (2013). Lettuce grown hydroponically had a significantly rougher surface (mean *Ra* of1211 ± 171 nm) compared with conventional cultivation (293 ± 59 nm). The number of adherent *S.* Enteritidis cells was 0.64 and 0.14%, respectively, for hydroponic and conventional systems. Adherence may be facilitated by increased contact area between the microorganisms and the surface.

Other factors that may affect bacterial transfer during slicing of produce such as produce firmness and juiciness have not been extensively studied. Firmness of fruits and vegetables is dependent upon cell morphology, cell size, shape, packing, wall thickness and strength, extent of cell-to-cell adhesion, and turgor status as described by Toivonen and Brummell (2008a). Usually these factors are interrelated. For instance, a tissue with small cells would have more cell wall material which means a greater area of cell-to-cell contact and fewer intercellular air spaces, leading to a firmer and less juicy tissue (Toivonen and Brummell 2008c). Juiciness, however, is determined by tissue breakdown during mechanical action such as chewing, biting or slicing. This breakdown occurs when cell walls are split open releasing juice, or when cell separation occurs along the middle lamellae causing the tissue to split with minimum cell rupture. More specifically, during slicing, tissue failure involves cell separation, cell breakage, or a combination of both. If the cell walls are stronger than the forces holding cells together, separation will occur and the tissue in this case is usually firm such as in unripe fruit. Alternatively, if forces attaching cells together are stronger than the cell walls themselves, then failure will occur releasing juice from the cell (Waldron, et al. 2003). Other than the tomato work done by Wang and Ryser (2016), no other studies have assessed effect of firmness and juiciness on bacterial transfer. However, the effect of fruit ripeness on the survival and growth of *L.*

monocytogenes on fresh-cut conference pear slices was studied by Colas-Meda *et al*. (2015). Pears of four different ripeness stages - mature-green(54-60 N), partially ripe (43-53 N), ripe (31-42 N) and overripe (> 42 N)), were dip-inoculated in a 10^5 CFU/ml *Listeria* suspension and stored for 8 day at 5, 10 and 20°C. *L. monocytogenes* grew under all experimental conditions, showing an increase of approximately 2 log CFU/g at 5 °C. No significant differences in *L. monocytogenes* populations were seen between the different ripeness stages after 8 days of storage at 5 ^{o}C. This study, however, did not look at the effect of pear ripeness on *Listeria* transfer or attachment. Understanding the impact of firmness on bacterial transfer during slicing could potentially improve our ability to better predict the extent of transfer.

The effect of contact time between pathogens and the surface needs to be more closely examined to better understand the dynamics of bacterial transfer. In a recent study to quantify cross-contamination between various foods and common kitchen surfaces at different contact times (Miranda and Schaffner 2016), more bacteria transferred to watermelon (~ 0.2 to 97%) than to any other food examined, regardless of the contact time, which may be due to watermelon's moisture which was significantly higher (0.99 ± 0.01) than the other food tested. However, Rodriguez *et al.* (2008) examined the impact of contact time on attachment of *Listeria* biofilms to stainless steel surfaces using atomic force microscopy (AFM) and concluded that contact time did not affect the microorganism's ability to attach at the cellular level, indicating that transfer is likely to be more effected by physicochemical rather than cellular factors. When Jensen *et a*l. (2013) determined the cross-contamination rates between a variety of fresh-cut produce including mini-peeled carrots, celery, watermelon, and romaine lettuce and common kitchen surfaces, they found that bacterial transfer depended on produce type, surface moisture, and drying time. Freshly inoculated celery or lettuce transferred more bacteria (2 to 25% of the

inoculum) compared to freshly inoculated carrots and watermelon (1 to 8%). However, the study did not measure the physicochemical characteristics of the fresh-cut produce used that could have helped explain the differences in transfer rates.

Several blade characteristics including thickness, roughness and sharpness affect the extent of bacterial transfer during slicing of meats, fruits, and vegetables. Wang and Ryser (2016) studied the impact of slice thickness on *Salmonella* transfer during slicing tomatoes using slices that were 3/16″ (0.48 cm), ¼″ (0.64 cm) or 3/8″ (0.95 cm) thick, and found no significant effect on transfer.

These and many other studies yielded contradicting conclusions, since the experimental procedures for these studies varied. The different inoculation methods, contact times and organisms used are likely responsible for the variations seen.. Thus, further investigations into such scenarios are essential to a better understanding of the dynamics of bacterial transfer and how transfer relates to the physicochemical characteristics of fresh produce, which will result in better practices to limit *Listeria* spread.

1.8 MODELING OF BACTERIAL TRANSFER DURING SLICING

Predictive microbiology is a relatively new scientific branch of food microbiology that uses mathematical models to quantitatively assess microbial behavior in foods. These models help food microbiologists describe different microbial processes, including kinetic processes such as microbial death and growth, or physical processes such as bacterial transfer (Perez-Rodriguez and Valero 2013).

Three types of models are recognized – primary, secondary and tertiary. Primary models aim to describe the kinetics of a process using as few parameters as possible while still being able to accurately define microbial growth and inactivation. Secondary models describe the

effect of environmental conditions (i.e., physicochemical and biological factors) on the parameters of the primary model. (c) Tertiary models based on computer software programs provide an interface between the underlying mathematics and the user, allowing model inputs to be entered and the estimates to be observed through simplified graphical outputs (Whiting and and Buchanan 1993).

There exists a specific need to understand bacterial transfer during the slicing and cutting process since many studies have shown that this process is a major source for contamination. Based on previous studies, bacterial transfer during slicing follows a logarithmic decline, and hence, exponential decay models have been successfully applied to describe bacterial transfer (Perez-Rodriguez and Valero 2013) . Wang and Ryser (2016) modeled the transfer of *Salmonella* during slicing of tomatoes using an exponential model (y = a*e(-x/b)), where Y (dependent variable) is the log CFU/tomato transferred and X (independent variable) is the order number for the specific uninoculated tomato that was sliced. The model fit the data from different test conditions and was suitable for predicting *Salmonella* transfer during slicing of tomatoes with a root mean square error (RMSE) < 0.5 under all test conditions. Similarly, Scollon *et al*.(2016) investigated the transfer of *L. monocytogenes* during slicing of onions and observed a logarithmic decrease from initial inoculum levels of 8.6, 6.8, and 5.9 log CFU/onion, respectively, with 20 slices being obtained. When fit to the transfer data, an exponential decay model yielded good fits, with RMSE values < 0.3 for all three inoculation levels.

Bacterial transfer can occur at different stages across the food chain. Foods can be re-contaminated after an inactivation process, during food transportation/preparation, or at retail or the time of consumption. Cross-contamination, however, refers to indirect and direct transfer of microorganisms from a contaminated food surface to other recipient food surfaces in food-

related environments (Perez-Rodriguez and Valero 2013). Cross-contamination in household settings also has been subjected to modeling. For example, Zilelidou *et al*. (2014) investigated the different *E. coli* O157:H7 and *L. monocytogenes* transfer rates (Tr) between cutting knives and lettuce leaves. The quantitative data regarding the extent of *E. coli* O157:H7 and *L. monocytogenes* transfer from contaminated lettuce to kitchen knives and subsequent transmission to fresh lettuce were used to develop a semi-mechanistic model describing bacterial transfer. The model sufficiently described the transfer rates with RMSE values of 0.426 - 0.613 and 0.531 - 0.908 for *L. monocytogenes* and *E. coli* O157:H7, respectively. However, the model underestimated bacterial transfer during extrapolation experiments.

Although the current models seem to well-represent the observed transfer rates on an empirical basis, bacterial transfer models are still in their early stages. Filling current knowledge gaps of how environmental and intrinsic factors influence the transfer phenomenon will improve how we approach public health.

1.9 QUANTITAVE MICROBIOLOGICAL RISK ASSESSMENT

During the late 1960's and early 1970's scientists were trying to categorize substances like commercial chemicals and environmental pollutants as carcinogens. In response, the National Research Council (NRC) formed the Institutional Means for Assessments of Risks to Public Health committee which aimed to define ways to "strengthen the reliability and objectivity of scientific assessment that forms the basis for federal regulatory policies applicable to carcinogens and other public health hazards" (Simjee 2007). The results of the committee's work were summarized and published in 1983 as an NRC report entitled Risk Assessment in the Federal Government: Managing the Process. This report, also known as "The Red Book" (based

on the color of its cover), marks the beginning of a formalized concept of risk assessment (Simjee 2007).

Risk assessment consists of four major domains: "(1) hazard identification (the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods); (2) exposure assessment (the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant); (3) hazard characterization/ dose–response (the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard); and (4) risk characterization (the integration of the hazard identification, hazard characterization, and exposure assessment determinations to provide qualitative or quantitative estimates of the likelihood and severity of the adverse effects which could occur in a given population)" (Simjee 2007).

Use of risk analysis to develop food standards was proposed by the Expert Consultation from the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Codex Alimentarius Commission (CAC). Risk assessment is a crucial tool in the development of food safety policies and procedures as well as validating some of the existing safety programs.

There are two types of microbial risk assessment (MRA): qualitative and quantitative. Qualitative risk assessment describes the likelihood of illness (high vs low), whereas quantitative risk assessment predicts the number of illnesses, provides numerical expressions of risk, and indicates the attendant uncertainties. Quantitative Microbial Risk Assessment (QMRA) modeling, a relatively new approach in the field of microbial risk, uses probability models to

evaluate the likelihood of adverse human health effects from exposure to pathogenic microorganisms (Simjee 2007).

Modeling bacterial transfer during slicing of fruits and vegetables is an essential tool for determining exposure to foodborne pathogens. In some of the aforementioned transfer studies, several mathematical models were developed from the experimental transfer data to describe bacterial spread during processing, which can be used as a guide to help estimate the amount of product that may have become cross-contaminated during processing and would need to be recalled. Further research is needed to derive more reliable mathematical models that take into account environmental and intrinsic factors that can influence bacterial transfer phenomenon for different types of fresh-cut produce. A model covering a wide range of fresh-cut produce based on product characteristics would be very useful for estimating the extent of transfer of other types of produce without the need to quantify the transfer for the many dozens of product types.

CHAPTER 2:

Microbial Cross-Contamination of Cucumber, Zucchini, and Floral Foam During Slicing as Impacted by Mechanical Slicer Type, Slicing Speed and Water Content

2.1 OBJECTIVE

The objective of this study was to assess the transfer of *L. monocytogenes* from inoculated cucumber and zucchini to various surfaces of rotating and stationary slicers, as well as to subsequently uninoculated products during slicing at different speeds. Another aim was to assess the relationship between water content and bacterial transfer during slicing.

2.2 MATERIALS AND METHODS

2.2.1 Cucumber and zucchini

several lots of cucumber (Cucumis sativus) and zucchini (Cucurbita pepo) were purchased from a local supplier (Stan Setas Produce Company, Lansing, MI) over a period of four months with each lot stored at 4 ^{o}C for no more than 7 d before use. The root and sprout ends of each product were removed using a sterile kitchen knife. Products were tempered to room temperature (23 ^{o}C ± 2 ^{o}C) and dimensions were recorded prior to slicing.

2.2.2 Bacterial strains

Three avirulent *L. monocytogenes* strains - M3 serotype 1/2a (Hly^{-}, parent strain Mackaness), J22F serotype 4b (Hly^{+}, purB mariner-based mutant of H7550- Cd^{S}, parent strain NCTC 10527), and J29H serotype 4b (Hly^{-}, parent strain NCTC 10527) (obtained from Dr. Sophia Karthariou, North Carolina State University, Raleigh, NC) were used in all slicing experiments. All strains were stored at -80 ^{o}C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. Each strain was initially streaked for isolation to plates of trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, Becton, Dickinson and Company) and incubated for 24 h at 35^{o}C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35^{o}C transfers in TSBYE. When used as cocktails, the three avirulent strains were combined in equal volumes and appropriately diluted to obtain populations of ~ 6.0 log CFU/ml for produce inoculation, with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI). cucumber and zucchini were dip-inoculated to contain ~ 7.5 log CFU/product *Listeria*.

2.2.3 Identification of contact areas between rotating slicer and product:

To identify the product contact areas of a manual rotating slicer (NEMCO slicer, Model #N55200AN, Nemco Food Equipment Inc., Hicksville, OH), and stationary slicer (NEMCO model # 59155491, Nemco Food Equipment Inc.), Glo Germ reagent (Glo-Germ Company, Moab, UT) was used as reported previously (Buchholz *et al*. 2012a , Vorst *et al*., 2006). One cucumber or zucchini was immersed in 0.5% (w/v) Glo-Germ solubilized in 5% ethanol, dried for 90 min, and then manually sliced, after which the components of the slicer were viewed under UV light (352 nm, Sankyo Denki Co., Ltd, Tokyo, Japan). Three product contact areas: the blade plate, pusher plate, and bottom plate were identified for subsequent sampling (Figure 1). The same procedure was used for a stationary NEMCO slicer (Model # 59155491) that had a set of fixed blades. As shown in Figure 2, two contact areas were identified: the blades and pusher.

2.2.4 *Listeria* distribution on individual slices

One cucumber or zucchini (15 cm in length) was dip-inoculated for two minutes in the 3-strain avirulent *L. monocytogenes* cocktail to contain ~ 7.5 log CFU/cucumber, air-dried for 1h and sliced using the rotating slicer. From each intact cucumber and zucchini, 6 slices were generated (1^{st}, 2^{nd}, 15^{th} ,16^{th}, 29^{th} and last slice), each individual slice (0.5 cm in width) was quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on MOX. After slicing the inoculated produce, one uninoculated produce of the same product type was sliced, after which the same slice order (1^{st}, 2^{nd}, 15^{th} ,16^{th}, 29^{th} and last slice), was sampled and quantitatively analyzed for *Listeria*. This study was done in triplicate and analysis of variance and the Tukey-Kramer HSD test were performed using JMP 12.0 (SAS Institute Inc., Cary, N.C.).

Figure 2-1: Components of the NEMCO model #N55200AN rotating slicer: (A) blade plate, (B) pusher plate, and (C) bottom plate.

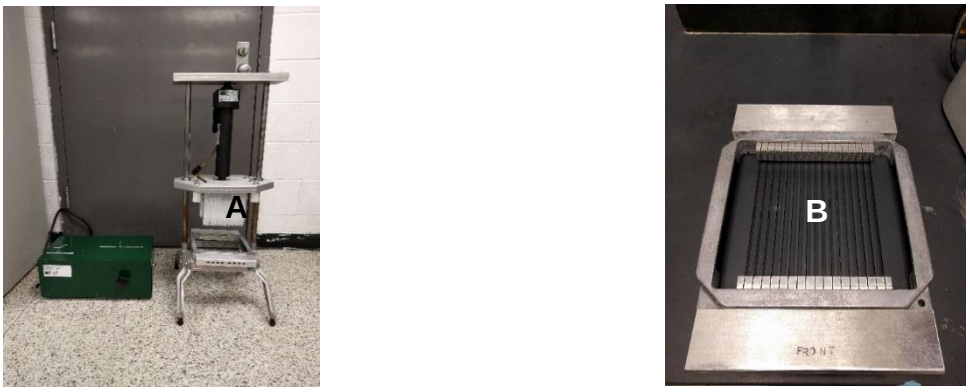


Figure 2-2: Components of NEMCO model # 59155491 stationary slicer: (A) pusher, and (B) blade

2.2.5 *Listeria* transfer from inoculated cucumbers and zucchini to a rotating and stationary hand slicer

Transfer of *L. monocytogenes* to the previously identified contact areas of a rotating and stationary slicer, which yielded 0.5 and 0.8 cm-thick slices respectively, was assessed after slicing one inoculated cucumber and zucchini squash. Similarly, after slicing one inoculated followed by 15 uninoculated samples, the same areas of the slicer were sampled to quantify *Listeria.* For the sampling protocol, one dip-inoculated product was sliced to contaminate the

slicer. Using one-ply composite tissues moistened with 1 ml of sterile phosphate buffer, samples were taken and tested for *Listeria* (Vorst *et al*. 2004).

2.2.6 Cleaning and decontaminating the slicer

After use, the slicer was completely disassembled, and the slicer pusher and blade were brushed under running water for 2 min. Slicer parts were disinfected with the 10% acidified bleach(vol/vol), and all components were rinsed with deionized water, spread with 70% ethanol (vol/vol) and dried under uv light for 20 min before use. Follow-up sampling Using one-ply composite tissues moistened with 1 ml of sterile phosphate buffer and tested for *Listeria* indicated that the slicer was free of *Listeria*.

2.2.7 *Listeria* transfer from surface-inoculated cucumber and zucchini to the cut surface using a rotating and stationary hand slicer

Transfer of *L. monocytogenes* from the outer skin/rind of cucumber and zucchini squash to the cut surface (flesh) was assessed using both the manual rotating and stationary slicer. One cucumber and zucchini were dip-inoculated to contain ~7.5 log CFU/product, air-dried for 1 h, and then sliced using the rotating or stationary slicer. A total of 5 slices from the middle, each 5.0 ± 0.2 cm in diameter, was generated from each cucumber and zucchini using both slicers. After aseptically removing the skin/rind, all samples were collected and quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on MOX.

2.2.8 *Listeria* transfer from inoculated to uninoculated cucumbers and zucchini during sequential slicing using a rotating and stationary slicer

In these experiments, one cucumber or zucchini squash was dip-inoculated in the avirulent 3-strain *L. monocytogenes* cocktail to contain ~ 7.5 log CFU/product, air-dried for 1 h, and sliced using the rotating hand slicer or stationary slicer to contaminate the slicer. Thereafter, *Listeria* transfer from the slicer to 15 uninoculated product of the same product type was assessed by sampling each one of the15 uninoculated product. For the rotating slicer, the 1st and every fifth slice from each of the 15 uninoculated product (total of 6 slices/product) were collected and quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on MOX. for the stationary slicer, the first, middle and last slice from each of the 15 uninoculated product were composited and examined for numbers of *Listeria*.

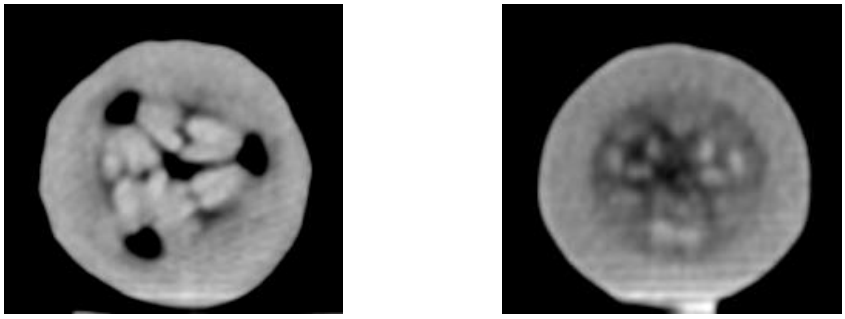
2.2.9 Impact of cutting speed on *L. monocytogenes* transfer during slicing

One cucumber or zucchini squash was dip-inoculated in the 3-strain avirulent *L. monocytogenes* cocktail to contain ~ 7.5 log CFU/product, air-dried for 1 hour, and sliced using the rotating slicer at a constant speed. The slicer shown in Figure 2 was modified by the MSU Biosystems and Agricultural Engineering Department by attaching electric powered hydraulics to the slicing blades in order to maintain a constant cutting speed during slicing. After contaminating the slicer by slicing one inoculated sample, 15 uninoculated zucchini or cucumbers were sliced at either high (3.3 cm/sec) or low speed (2.0 cm/sec). The first, middle and last slice from each of 15 uninoculated products were collected and analyzed for *Listeria* by surface-plating on MOX.

2.2.10 Density, cutting force, and water content of cucumbers and zucchini

Computed tomographic (CT) scans were performed on three locally obtained cucumbers and zucchini squash using a GE BrightSpeed™ Elite CT Scanner (General Electrics Healthcare, Buckinghamshire, United Kingdom). Two-dimensional CT images were acquired every 0.625 mm, at a voltage and current of 120keV and 240mA, respectively (Figure 3). The overall mean bulk density for both products (n = 3) was indirectly calculated from all of the 2D CT images (number varied depending on product size) using MATLAB V2012a (Table 1). The Hounsfield unit (HU), known as the CT number, is a quantitative scale for describing radiodensity. Once the mean HU was obtained from the images, the bulk density was calculated by using the following equation (Orsi and Anderson 1999) :

$$\textbf{Density} = ((0.689 \div 1000) \times HU) + 0.997$$



a b

Figure 2-3: Computed tomographic (CT) images for (a) cucumber and (b) zucchini

Alongside the CT imaging, standard water displacement method was used to measure the density of cucumber and zucchini. briefly, water was added in a graduated cylinder in which

cucumber and zucchini was placed. The amount of displaced water (in milliliters) was recorded as the volume of the product. density was calculated as: p=m/v

Texture analyses were based on the force required to shear the sample using a texture analyzer TA-XT2i (Texture Technologies Corp, Scarsdale, New York) with a custom stainless-steel knife blade (length 22.5 cm, width 17.5 cm) made specifically to simulate a knife cut. Each produce sample (n = 3) was cut five times at a cutting blade speed of 40 mm/sec with the blade traveling a total distance of 85 mm. Peak positive force was measured and expressed in N.

Moisture content was calculated based weight loss after drying at 100 -105°C for 12 - 24 h. Samples of locally obtained cucumbers and zucchini squash (5 - 7 g each) were placed in aluminum pans, weighed to 4 decimal points, dried overnight in a a forced-air oven at 100°C, removed, and placed into desiccators until completely cooled. Thereafter, the dried product samples were weighed and the moisture content was calculated using the following equation:

$$\text{Moisture}(\%) = \frac{[(\text{wt wet sample} + \text{wt pan}) - (\text{wt dried sample} + \text{wt pan})]}{(\text{Wt wet sample})} \times 100$$

In addition, the amount of free liquid released during slicing was quantified based on the weight loss of the sample before and after slicing.

2.2.11 Impacted of water content on bacterial transfer using floral foam as a model

The effect of water content on transfer of *Listeria* during slicing was also evaluated using a floral foam (OASIS® Floral Products, Kent, OH). Floral foam to which different amounts of water were added, was used as a model system in order to obtain different moisture contents under the same conditions. The stationary slicer was first contaminated by slicing one inoculated

cucumber. Ten uninoculated pieces of floral foam (length 15 cm, width 2 cm and height 2 cm) were used to which sterile water was added (150, 100 and, 75 ml) to achieve percent moisture of 97.6, 96.7 and, 95.1% respectively . The floral foam was then sliced and samples were collected and quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on MOX. In addition, an uninoculated control experiment was conducted by slicing an uninoculated cucumber to ensure cleanness of the slicer. The experimental design for this objective is illustrated in Figure 4.

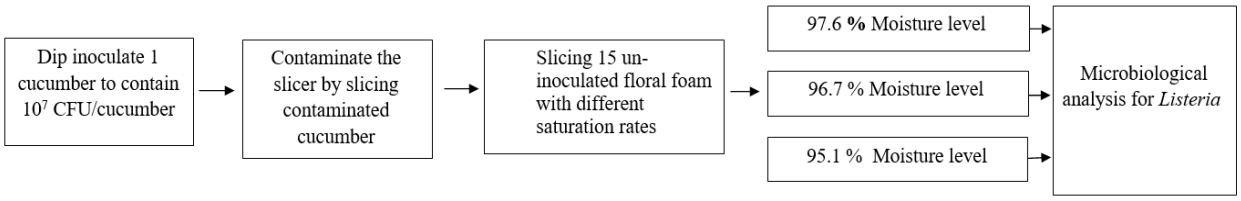


Figure 2-4 Experimental design for the floral foam experiment

2.2.12 Microbiological analysis:

All produce samples were homogenized by stomaching in 50 ml of phosphate buffered saline (PBS) in a Whirl-pak bag® for 1 min and then quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on Modified Oxford Agar (MOX). All colonies resembling *Listeria* were counted after 48 h of incubation at 35 ºC.

2.2.13 Statistical analysis

All experiments were performed in triplicate. *Listeria* populations were converted to log CFU/cm^2 or CFU/product and subjected to ANOVA using JMP 12.0 (SAS Institute Inc., Cary,

N.C.). Statistical significance was set at $P \leq 0.05$. The Tukey-Kramer HSD test was performed using JMP.

In order to describe the sequential transfer of *Listeria* during slicing, the *Listeria* transfer curve data (log CFU/product) were fitted to a two-parameter exponential decay model Eq [1]

$$Y = A \cdot \exp(-B \cdot X)$$

where Y (dependent variable) is the log CFU/produce transferred and X (independent variable) is the order number for the specific uninoculated produce that was sliced. A (*Listeria* population transferred to the first product) and B (decay rate) are two model parameters. The parameter ± standard error for the aggregate of replications and the root mean square error (RMSE) of the model were obtained using JMP 12.0. In addition, the percentage of the *Listeria* population transferred from one inoculated to 15 uninoculated samples was calculated as follows:

$$= \frac{Bacteria\ population\ transferred\ to\ 15\ uninoculated\ produce}{Bacteria\ population\ on\ the\ original\ inoculated\ produce} \quad X\ 100$$

2.3 RESULTS

2.3.1 *Listeria* distribution on individual slices

After slicing one inoculated cucumber (7.6 ± 0.1 log CFU/cucumber), populations on the six cucumber slices ranged from 4.2 ± 0.0 to 4.8 ± 0.1 log CFU/cm^2 with significantly fewer ($P \leq 0.05$) *Listeria* transferred to slices 2 through 29 compared to the first and last slice (Figure 5). However, when one inoculated cucumber followed by one uninoculated cucumber was sliced, a statistically similar ($P > 0.05$), *Listeria* populations on the uninoculated cucumber ranged from 3.4 ± 0.1 to 3.8 ± 0.0 log CFU/cm^2, were observed. When inoculated zucchini (5.8 ± 0.0 log CFU/cm^2) was sliced, a similar trend was observed, whereby populations of *Listeria* on the six slices ranged from 4.1 ± 0.1 to 5.3 ± 0.1 log CFU/cm^2 with significantly fewer ($P \leq 0.05$) *Listeria* transferred to slices 2 through 29 compared to the first and last slice. After slicing one inoculated followed by 15 uninoculated zucchinis, *Listeria* populations ranged from 2.4 ± 0.2 to 3.0 ± 0.1 log CFU/cm^2 with no significant difference between any of the slices. Since the inoculated sample was dip-inoculated, high *Listeria* populations were expected on the first and last slices due to the larger surface area for first and last compared to the middle slices. Based on statistical similarity ($P > 0.05$), the 1st, every 5th slice of cucumber and zucchini were used for sampling whenever the rotating slicer was used.

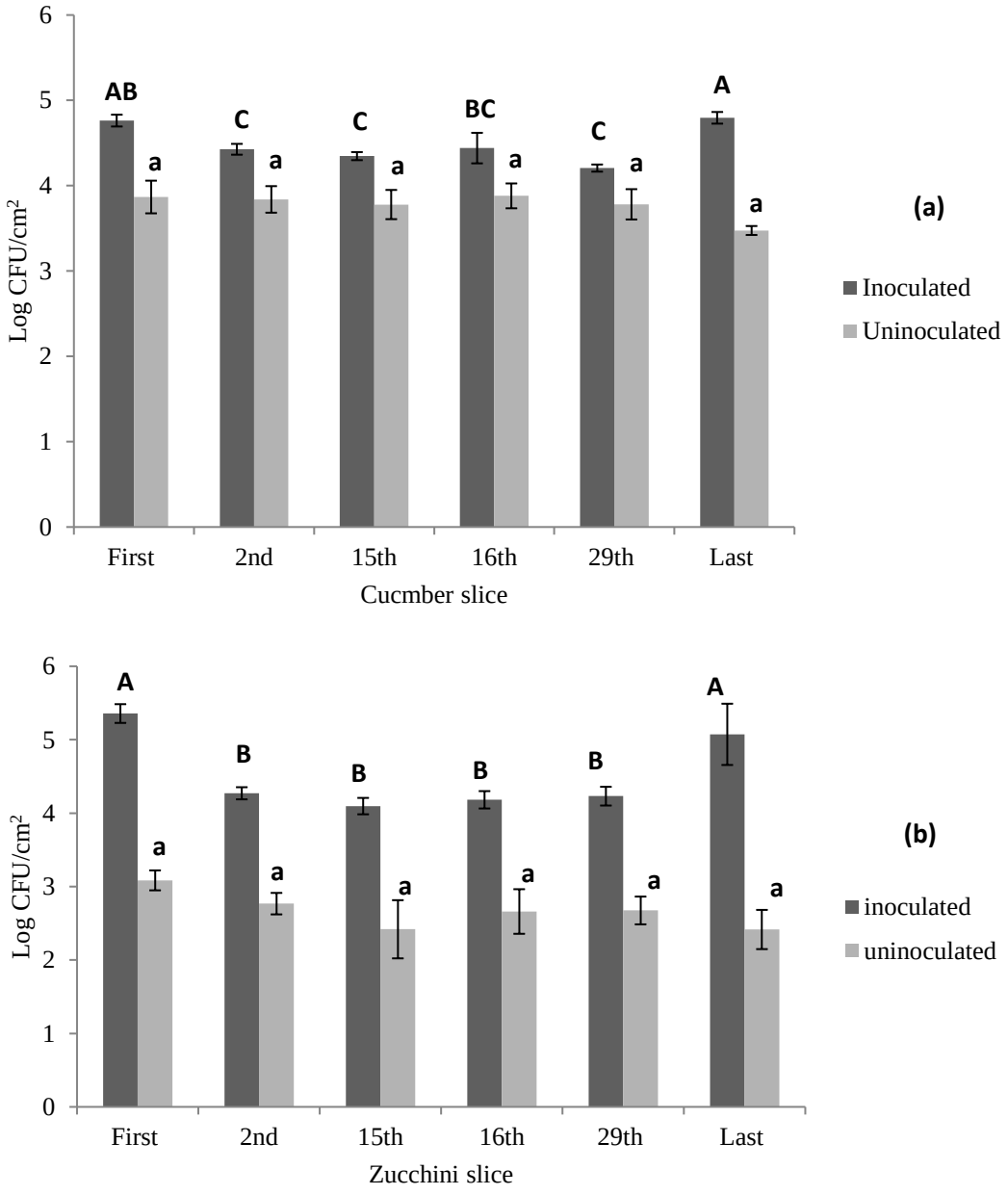


Figure 2-5: Mean (± SE) *L. monocytogenes* distribution on slices from inoculated and uninoculated cucumber (a) and zucchini (b) after slicing with a rotating slicer. Means with different capital letters for inoculated slices are significantly different ($P \leq 0.05$). Means with different letters for uninoculated slices are significantly different ($P \leq 0.05$).

2.3.2 *Listeria* transfer from inoculated produce to a rotating and stationary hand slicer

When one inoculated cucumber was sliced using the rotating and stationary slicers, the percentage of *Listeria* cells transferred to the slicers were statistically similar ($P > 0.05$), 0.4±0.1

and 0.7 ± 0.3, respectively. Similarly, when one inoculated zucchini was sliced using the rotating and stationary slicers, the percentage of *Listeria* cells transferred to the slicers were statistically similar ($P > 0.05$), 1.4±0.2 and 0.9 ± 0.2, respectively.

After slicing one inoculated zucchini squash on the rotating slicer, the slicing plate, bottom plate, and pusher plate yielded *Listeria* populations of 4.2 ± 0.1, 3.4 ± 0.4, and 4.5 ± 0.1 log CFU/component, respectively (Figure 6A). After slicing 15 uninoculated zucchinis, *Listeria* populations for the same slicer component decreased to 3.3 ± 0.3, 3.5 ± 0.1 and 2.7 ± 0.7 log CFU/component and were not statistically different from the same component after only slicing one zucchini *($P > 0.05$).*

Similarly, for cucumbers no significant differences in *Listeria* populations *($P > 0.05$)* were observed for any of the three components either before or after slicing 15 uninoculated cucumbers. However, when the stationary slicer was used to slice inoculated zucchini, the blade and pusher yielded *Listeria* populations of 3.9 ± 0.2 and 4.8 ± 0.2 log CFU/component, respectively. These numbers decreased significantly *($P \leq 0.05$)* for the same slicer component after slicing 15 uninoculated zucchinis to 3.1 ± 0.1 and 2.8 ± 0.1 log CFU/component (Figure 6B). Similar trends were also seen for the same components when one inoculated cucumber was sliced followed by 15 uninoculated cucumbers. *Listeria* populations on the same slicer components decreased significantly *($P < 0.05$)* after slicing 15 uninoculated cucumbers.

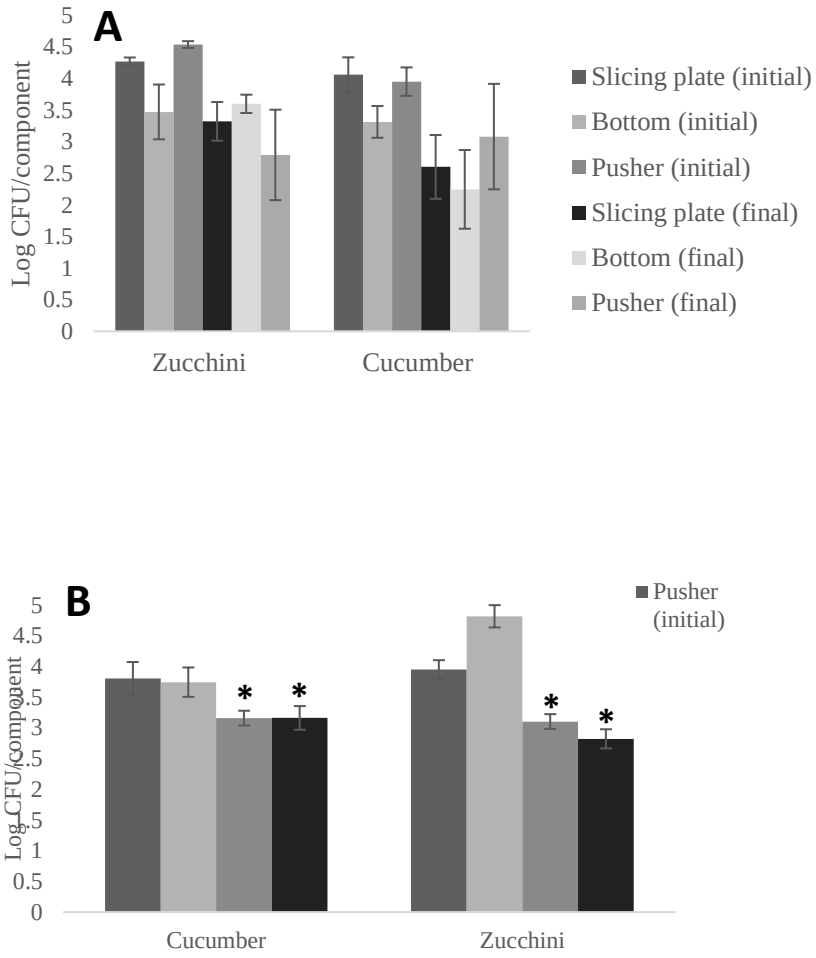


Figure 2-6: *Listeria* distribution (mean ± SE) on different components of the rotating slicer (A) and stationary slicer (B) before and after slicing 15 uninoculated zucchini and cucumbers. Columns with asterisks are significantly different *(P* ≤ 0.05*)* from the corresponding component

2.3.3 *Listeria* transfer from surface-inoculated cucumber and zucchini to the cut surface using a rotating and stationary hand slicer

Cucumber slices yielded statistically similar *Listeria* populations of 4.7 ± 0.1, 4.7 ± 0.1 log CFU/cm^2 after using the rotating and stationary slicer, respectively, with *Listeria* populations in the flesh of 1.7 ± 0.1, 1.4 ± 0.1 log CFU/cm^2 also statistically similar for both slicers (Figure 7A). When zucchini was sliced, the skin yielded *Listeria* populations of 5.3 ± 0.05, 5.4 ± 0.03

log CFU/cm^2, respectively, which were statistically similar ($P > 0.05$). However, unlike cucumber, statistically higher *Listeria* populations were seen in zucchini flesh using the rotating (2.4 ± 0.1 log CFU/cm^2) as opposed to the stationary slicer (1.7 ± 0.08 log CFU/cm^2) (Figure 7B).

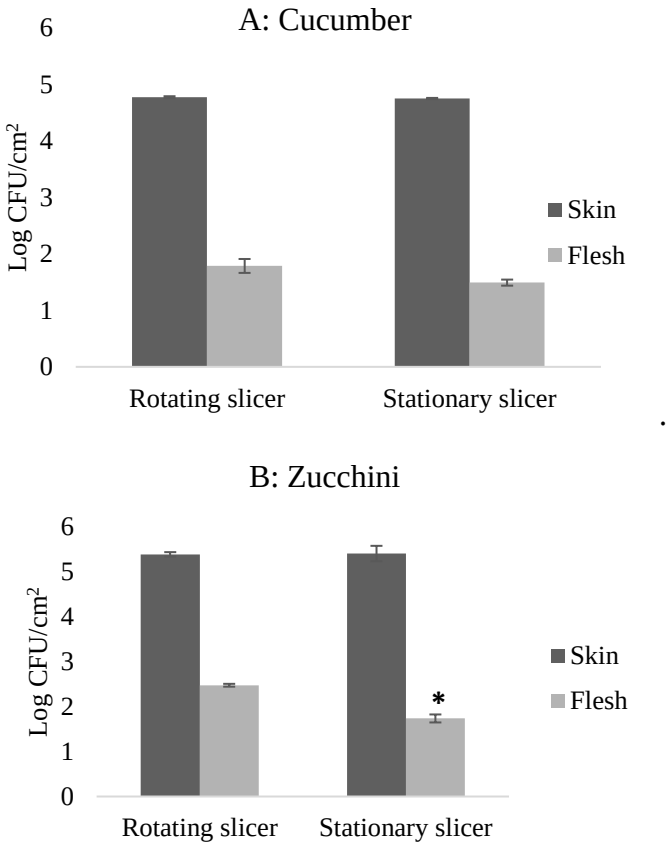


Figure 2-7: *Listeria* populations (mean ± SE) on different locations of a cucumber (A) and zucchini slice (B). Columns with asterisks are significantly different ($P \leq 0.05$) from the corresponding location.

2.3.4 *Listeria* transfer from inoculated to uninoculated cucumbers and zucchini during sequential slicing using a rotating and stationary slicer

After slicing one inoculated zucchini or cucumber (~ 7 log CFU/product) followed by 15 uninoculated samples of the same product type, *Listeria* was detectable in all 15 samples using

either the stationary or rotating slicer (Figures 8 and 9). These results were then fitted into a two-parameter exponential decay model to compare the decay rates (Table1). When the rotating slicer was used to slice cucumber, a significantly higher ($P \leq 0.05$) transfer decay rate (0.02±0.005) was observed compared to zucchini (0.01±0.002). However, when using the stationary slicer, a statistically similar ($P > 0.05$) decay rate was seen for cucumber (0.008±0.002) as opposed to zucchini (0.01 ± 0.005). The RMSE for all processing variables ranged from 0.33 to 0.65 log CFU/produce, which supported the exponential decay model. The original transfer data for each variable is presented in Appendix A.

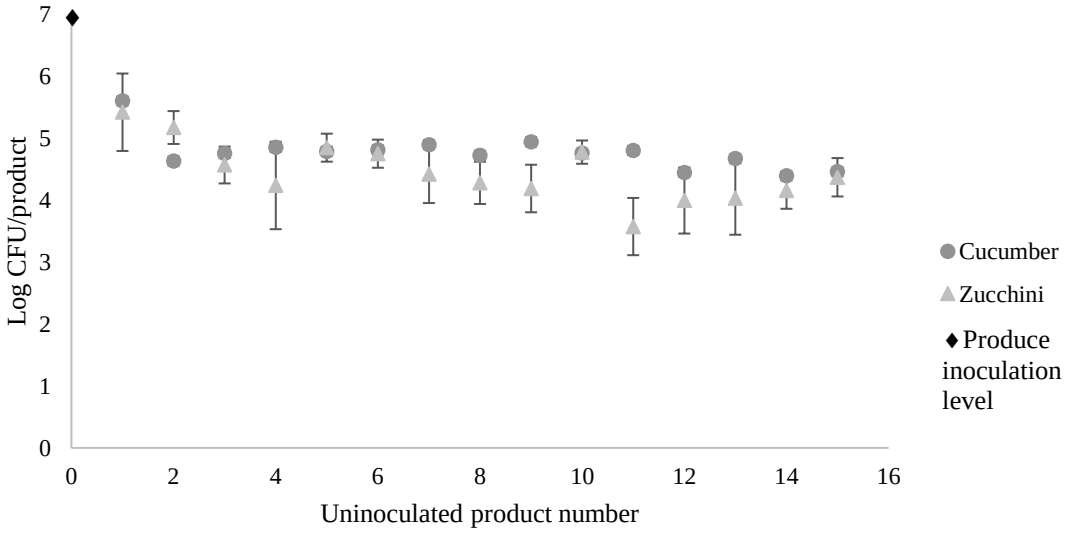


Figure 2-8: *Listeria* transfer from an inoculated product (~ 7 log CFU/product) to 15 inoculated zucchini and cucumber using a stationary slicer

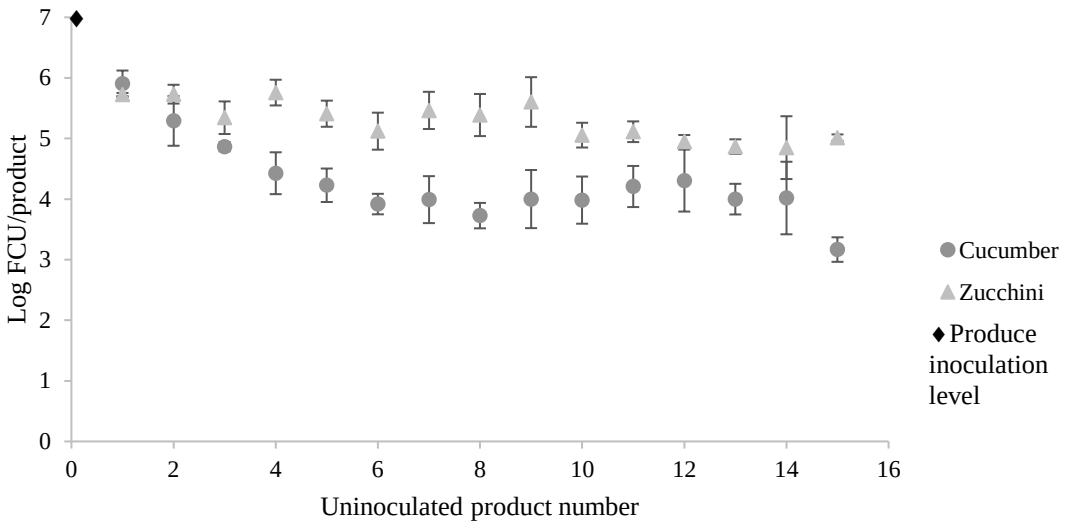


Figure 2-9: *Listeria* transfer from an inoculated product (~ 7 log CFU/product) to 15 inoculated zucchini and cucumber using a rotating slicer

Table 2-1: Transfer model parameters (A and B) for *Listeria* from inoculated zucchini and cucumber to the stationary and stationary slicer during sequential slicing and percent transfer (n = 3)

Product	Slicer type	A ± SE (Log CFU/Product	B ± SE	RMSE[1] (log CFU/Product
Cucumber	Rotating	5.3±0.2	0.02±0.005[a2]	0.65
	Stationary	5.1±0.1	0.008±0.002[b]	0.32
Zucchini	Rotating	5.7±0.1	0.01±0.002[a]	0.41
	Stationary	5.1±0.2	0.01±0.005[a]	0.65

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

[2]Means with the same letters for the slicer type are not significantly different ($P > 0.05$)

2.3.5 Impact of cutting speed on *L. monocytogenes* transfer during slicing

After zucchini slicing, *L. monocytogenes* was quantifiable in all samples examined as shown in Figure 10. Overall, the 1st, 7th, and 15th uninoculated zucchinis yielded average *Listeria*

populations of 5.5 ± 0.2, 5.2 ± 0.2 and 4.8 ± 0.3 log CFU/zucchini, respectively, when sliced at high speed. Sequentially slicing the 1st, 7th, and 15th zucchini at low speed resulted in average *Listeria* populations of 5.4 ± 0.3, 4.9 ± 0.5 and 4.5 ± 0.1 log CFU/zucchini. A similar trend was observed during slicing cucumber at different speeds with the 1st, 7th, and 15th uninoculated cucumber yielding average *Listeria* populations of 5.5 ± 0.2, 4.8 ± 0.1 and 4.4 ± 0.1, and 5.3 ± 0.1, 5.4 ±0.3 and 4.8 ± 0.1 log CFU/cucumber at high and low speed, respectively (Figure 10). After fitting these data in the previous exponential decay model, the decay rates for both zucchini and cucumber (Table 2) were statistically similar ($P > 0.05$)

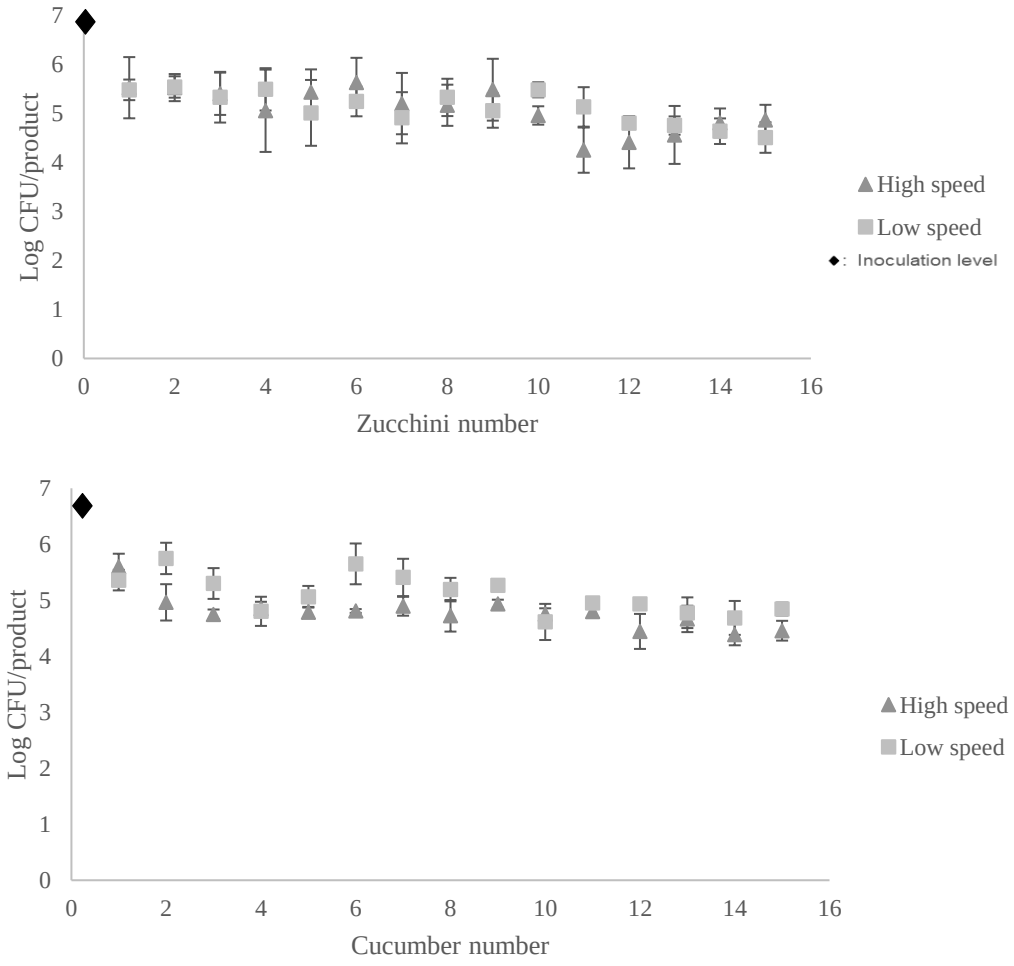


Figure 2-10: *L. monocytogenes* transfer from inoculated to uninoculated cucumber and zucchini during slicing at high (3.3 cm/sec) and low speed (2.0 cm/sec)

Table 2-2: Transfer model parameters (A and B) for *Listeria* from inoculated zucchini and cucumber to the slicer during sequential slicing at high and low speed (n = 3)

Product	Slicing Speed	A ± SE (Log CFU/produce	B ± SE	RMSE (log CFU/produce)
Zucchini	High (3.3 cm/sec)	5.3±0.2	0.01±0.004	0.65
	Low (2.0 cm/sec)	5.6 ± 0.1	0.01 ± 0.003	0.56
Cucumber	High (3.3 cm/sec)	5.1±0.1	0.01±0.003	0.33
	Low (2.0 cm/sec)	5.5± 0.1	0.01 ± 0.002	0.43

2.3.6 Produce density, cutting force and water content

A significantly *($P \leq 0.05$)* greater force was required to cut through cucumber (35.3 ± 0.3 N) compared to zucchini (10.8 ± 0.6 N) (Table 3) since the density of cucumber was significantly higher. Although the water content was similar for both cucumber and zucchini *($P>0.05$)*, the amount of liquid released during slicing varied. When cucumber was sliced using the rotating and stationary slicer, the amount of free liquid was 7.5 ± 0.5 and 1.9 ± 0.2 g, respectively, which was significantly higher than for zucchini using the rotating (2.1±0.1 g) and stationary slicer (0.3 ± 0.04 g).

Table 2-3: Mean (± SE) peak positive force, density, and water content

Product	Mean peak positive force (N)	Density (g/cm^3) using water displacement	Density (g/cm^3) using CT scan	Water content (%)	Free liquid when sliced with rotating slicer (g)	Free liquid when sliced with stationary slicer (g)
Zucchini	10.8^a ± 0.6	0.94^a±0.02	0.4910^a ±0.08	95.6^a±0.03	$2.1±0.1^a$	$0.3±0.04^a$
Cucumber	35.3^b ± 0.3	0.98^b±0.04	0.6567^b±0.02	94.1^a±0.03	$7.5±0.5^b$	$1.9±0.2^b$

Means with different letters for different produce are significantly different ($P \leq 0.05$).
Means with different capital letters for different slicers are significantly different ($P \leq 0.05$).

2.3.7 Impact of water content on *Listeria* transfer using floral foam as a model

At all three floral foam percent moisture levels (95.1, 96.7 and, 97.6%), both the pusher and the blades yielded statistically ($P \leq 0.05$) similar *Listeria* populations after slicing one inoculated cucumber followed by 10 uninoculated pieces of floral foam as compared to *Listeria* populations recovered after slicing one inoculated cucumber (Figure 11). When the transfer data (Figure 12) were fitted to the exponential decay model to determine decay rates, (Table 4), all three percent moisture levels (95.1, 96.7 and, 97.6%) resulted a statistically similar decay rates.

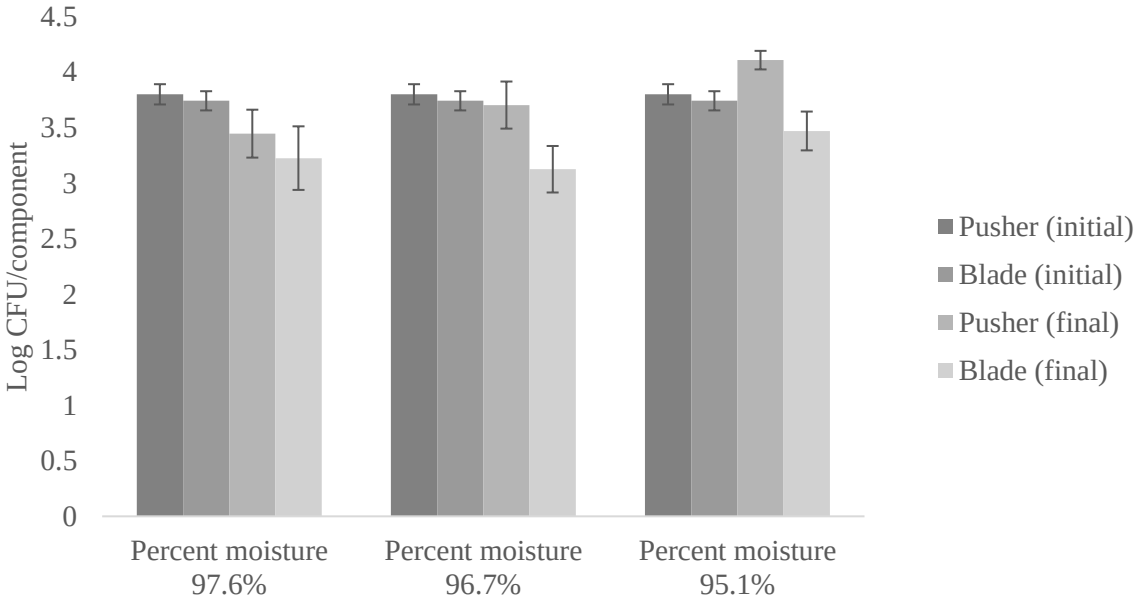


Figure 2-11: *Listeria* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated pieces of floral foam at percent moisture levels of 95.1, 96.7 and, 97.6%

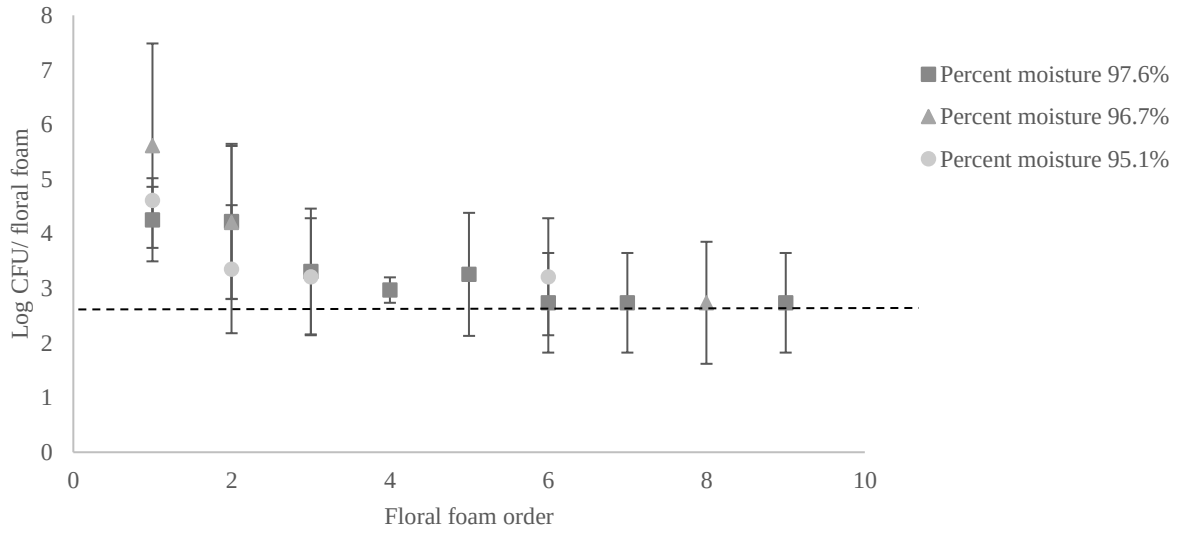


Figure 2-12: Sequential transfer of *Listeria* during slicing of floral foam at percent moisture levels of 95.1, 96.7 and, 97.6%

Table 2-4: Model parameters (A and B) for transfer of *Listeria* from inoculated cucumber to 15 uninoculated pieces of floral foam at percent moisture levels of 95.1, 96.7 and, 97.6% (n = 3).

Percent moisture levels	A ± SE (Log CFU/produce	B ± SE	RMSE (log CFU/produce)
97.6 %	4.5 ± 0.4	0.07 ± 0.02	0.67
96.7 %	5.8 ± 0.5	0.1 ± 0.04	0.64
95.1 %	4.6 ± 0.5	0.08 ± 0.05	0.75

2.4 DISCUSSION:

The overall objective of this study was to assess the effect of slicer type and compositional differences between cucumber and zucchini on the transfer of *L. monocytogenes*. In this study, after slicing one inoculated sample followed by fifteen uninoculated samples, *Listeria* populations on different parts of the stationary slicer decreased significantly ($P \leq 0.05$). However, the different parts of the rotating slicer were able to retain a greater proportion of the initial *Listeria* population transferred to the same part. This may be partially explained by the larger contact area between the product and slicer, which allowed more *Listeria* to be transferred initially. In addition, the direction of the force applied during slicing in the rotating slicer could explain the larger transfer of *Listeria* to the rind. Observations using the stationary slicer are consistent with a previous cross-contamination study by Scollon *et al.* (2016) where numbers of *Listeria* before and after slicing 20 onions decreased significantly using a similar stationary slicing mechanism.

The sequential transfer of *Salmonella* during tomato slicing using two different types of slicers (electric vs. manual) was previously investigated by Wang and Ryser (2016). Similar transfer trends were seen for both slicers except for the blades of an electric slicer that yielded greater *Salmonella* transfer compared to the blades of the manual slicer. However, both slicers used in the Wang and Ryser (2016) experiment are similar to the stationary slicer used in this study.

The decay rate and percent recovery for *Listeria* after one zucchini or cucumber inoculated at ~ 7 log CFU followed by 15 uninoculated product samples varied greatly. When slicing 15 zucchini or cucumber with the rotating slicer, the blades contacted the product approximately 450 times compared to only 15 times for the stationary slicer due to the different

slicing mechanism. This could partially explain the similar decay rate for *Listeria* transfer when the stationary slicer was used, while the higher contact times between product and rotating slicer allowed more of a washing effect, which resulted in a higher decay rate for cucumber compared to zucchini. Another difference between the stationary and rotating slicing mechanism is the direction of force applied during slicing which might have resulted in greater transfer with the rotating slicer since the added force applied could increase *Listeria* transfer. Meanwhile, the force applied from above resulted in less transfer when the stationary slicer was used. Application of pressure has been shown to effect bacterial transfer. For example, *Vorst et al.* (2006) reported significantly greater ($P \leq 0.05$) transfer of *Listeria* to the table and back plate of a mechanical delicatessen slicer when a force of 4.5 kg as opposed to 0 was applied against the product during slicing. Bower *et al*. (1996) suggest that in bacterial adhesion, the increase in pressure pushes surfaces closer, avoiding the initial repulsion forces and enabling binding forces.

Tomatoes of varying texture have yielded different transfer rates during slicing as reported by Wang and Ryser (2016). They were able to show that tomato varieties with tougher texture and lower water content transferred more *Salmonella* during slicing compared to softer, moister varieties. Vorst *et al.* (2006) reported less *L. monocytogenes* transfer during mechanical slicing of delicatessen turkey breast compared to salami with similar findings also reported for delicatessen hams containing different levels of water (unpublished data). This could be explained by the "washing effect" of bacterial cells from blades, thus decreasing the number of cells available for transfer. Therefore, it is recommended that when using a rotating slicer, cucumber should be sliced first to minimize cross-contamination during fresh-cut processing.

Decay rates after sequential transfer of *Listeria* during slicing of zucchini and cucumber at different speeds were statistically similar ($P > 0.05$). This observation contradicts work done

by Mazon (2017) (unpublished data) who found that bacterial transfer via dynamic contact from a stainless steel plate to a potato increased as sliding speed increased. However, the transfer process dynamics of these two studies varied greatly, which may partially explain the different results obtained as well as the difference between high and low speed slicing in our experiment, which might have been insufficient to impact bacterial transfer. The use of floral foam to investigate the effect of water content on bacterial transfer during slicing has not been previously attempted. The water saturation percentages were chosen to represent the varying water content of fresh produce. Although the low numbers of *Listeria* recovered after slicing negatively affected the model parameter resulting in high RMSE values, decay rate were statistically similar in all saturation level. These findings contradict those from other studies (Jensen et al. 2013; Miranda and Schaffner 2016; Wang and Ryser 2016) who showed that high moisture products facilitate greater bacterial transfer which is likely due to low *Listeria* recovery during slicing of floral foam as well as the relatively small difference in water saturation levels used in this experiment.

In summary, this study clearly shows that the product and type of slicer both influence the numbers of *Listeria* transferred. Therefore, the order in which different types of fresh produce are sliced, along with type of slicer used, are important considerations when attempting to minimize potential cross-contamination during slicing. To further investigate these claims, the effect of various intrinsic parameters of fresh produce, including firmness and surface texture, were assessed in the following chapter. Such practical research should be of interest to the fresh-cut produce industry, since this work serves to lay the foundation for the development of more reliable science-based transfer models for risk assessments.

CHAPTER 3:

Quantify *Listeria* and *Salmonella* transfer during slicing of different fresh cut produces as impacted by produce firmness and other physiological characteristics

3.1 OBJECTIVE

The objective of this study is to quantify the impact of pear firmness and other product characteristics (water content, pH, cutting force, soluble solids content, surface hydrophobicity and surface roughness) on the transfer of *Listeria* and *Salmonella* during slicing of different types of fresh produce (onions, radishes, tomatoes, potatoes, carrots, zucchini, cantaloupe, apple, sweet potato, gray zucchini and cucumber).

3.2 MATERIALS AND METHODS:

3.2.1 Microbial cross-contamination of pears during slicing as impacted by pear firmness

The effect of pear firmness on the transfer of *Listeria* and *Salmonella* was assessed in this study. Three categories of pear firmness based on ripeness were assessed for slicing: firm (10-15 N), medium (6 - 9 N) and soft (< 6 N). 'Bartlett' pears (*Pyrus communis* 'Williams pear') were obtained from a local shipper and stored at 4 °C until use. After reaching the desired firmness, one pear was dip-inoculated with the avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) as well as a 3-strain cocktail of *Salmonella* (Montevideo, Poona, Newport) at ~7 log CFU/pear and air-dried in a bio-safety cabinet for 1 h before slicing using a NEMCO slicer # 59155491(Nemco Food Equipment Inc., Hicksville, OH). After slicing the inoculated pear to contaminate the slicer, 15 uninoculated pears of the same firmness category were sliced to assess the extent of bacterial transfer during slicing. The slicer was modified by the MSU Biosystems and Agricultural Engineering Department to allow control of the cutting speed during slicing. In addition, every trial had an uninoculated control whereby one uninoculated product was sliced before the inoculated to ensure that the slicer was disinfected. Furthermore, the blades and pusher were sampled for *Listeria* and *Salmonella* before and after slicing the 15 uninoculated samples to account for all bacteria transferred. Surface roughness, hydrophobicity and physiological characteristics of the pears in all firmness categories were also measured.

3.2.2 Pears firmness categories

As pears ripen, the firmness changes. As described by Colás-Medà *et al*. (2015), pears were ripened at 20 °C for a maximum of 72 h until the desired firmness was achieved. Pear firmness was quantitatively assessed by measuring the force in N required to penetrate the fruit

using a texture analyzer TA-XT2i (Texture Technologies Corp, Scarsdale, New York) equipped with a 8 mm diameter probe. Pears subsequently categorized as firm (10-15 N), medium (6 - 9 N) and soft (< 6 N) were then held overnight at 4 °C before slicing.

3.2.3 Produce selection and slicing

In the second objective, different types of produce were selected for slicing based on their physicochemical characteristics (water content, surface roughness, and firmness.). These fruits and vegetables include Spanish yellow onions (*Allium cepa*), Red round tomatoes *(Solanum lycopersicum L.*), Radish (*Raphanus sativus*), Cucumber (*Cucumis sativus*), Potato (*Solanum tuberosum*), Carrot (*Daucus carota*), Sweet potato (*Ipomoea batatas*), Apple (*Pyrus malus*), Cantaloupe (*Cucumis melo var. cantaloupensis*), Zucchini, (*Cucurbita pepo*), Gray zucchini (*Cucurbita pepo*), 'Bartlett' pears (*Pyrus communis*) purchased from a local supplier (Stan Setas Produce Company, Lansing, MI). All products were sliced using a NEMCO # 59155491(Nemco Food Equipment Inc., Hicksville, OH) stationary slicer that was modified to allow slicing at a fixed speed.

3.2.4 Bacterial strain and produce inoculation

Three avirulent *L. monocytogenes* strains - M3 serotype 1/2a (Hly^-, parent strain Mackaness), J22F serotype 4b (Hly^+, purB mariner-based mutant of H7550- Cd^S, parent strain NCTC 10527), and J29H serotype 4b (Hly^-, parent strain NCTC 10527) (obtained from Dr. Sophia Kathariou, North Carolina State University, Raleigh, NC) were used for inoculating the different products. All strains were stored at -80°C in trypticase soy broth containing 0.6% yeast extract (TSBYE) and 10% glycerol, and subjected to two successive transfers (24 h at 37°C) in TSBYE before inoculation. The three avirulent strains were combined in equal volumes and

appropriately diluted to obtain populations of ~ 6.0 log CFU/ml for produce inoculation, with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI). Produce was dip-inoculated in the *L. monocytogenes* cocktail and air-dried in a bio-safety cabinet for 1 hour before processing. similarly, a 3-strain *Salmonella* cocktail including *Salmonella* Montevideo MDD22 (tomato outbreak, human isolate), *Salmonella* Poona MDD237 (cantaloupe outbreak, human isolate), and *Salmonella* Newport MDD314 (tomato outbreak, environmental isolate) (Dr. Lawrence Goodridge, Colorado State University, Fort Collins, CO) were used to inoculate the pears of different firmness categories.

3.2.5 Quantify *Listeria* transfer during slicing of different fresh cut produces

One inoculated sample was sliced with a mechanical slicer to contaminate the slicer after which 15 uninoculated samples of the same produce type were sliced to assess the extent of *L. monocytogenes* transfer. Moreover, the transfer of *L. monocytogenes* to different parts of a stationary hand slicer, which yielded 0.5 cm-thick slices, was similarly assessed by slicing one inoculated sample followed by 15 uninoculated samples of the same produce type, after which the various slicer parts were sampled using the one-ply composite tissue method of Vorst *et al*. (2004) to quantify *Listeria*.

3.2.6 Physicochemical characteristics measurements of produce

Before slicing, water content, the pH and the soluble solids content (SCC) were measured. A forced air drying oven was used to measure water content. The pH was measured by using a HANNA® HI 221 pH meter with a penetration electrode. After the pH reading, products were squeezed, and the soluble solids content (SSC) was determined by using a handheld optical refractometer Fisher Scientific® refractometer at 20 ^{o}C.

3.2.7 Surface roughness determination

Surface roughness measurements for the internal (flesh) and external surface (skin, rind) were obtained for all products as described by Wang *et al.* (2009). Briefly, a 1 cm^2 produce section cut from the interior was placed on microscope slide. Confocal Laser Scanning Microscopy (CSLM) was conducted at The Center for Advanced Microscopy (Michigan State University) using a Nikon C2 Confocal Microscope which allowed 2-D images (100 × 100 μm) to be obtained by optically slicing the sample surface. Separation between the observation planes was set at 0.05 μm for all products. Surface profile information was expressed by parameter (*Ra*), which is the arithmetic average of the absolute values of the surface height deviations measured from the mean plane calculated using ImageJ software.

3.2.8 Surface hydrophobicity assay

Surface hydrophobicity of the produce was measured using a goniometer**.** Briefly, small drops of deionized (DI) water 2 to 4 mm in diameter were created using a microsyringe. A side view photograph of the drop at a magnification of approximately 7.6 times was obtained with an inspection microscope and mirror a greater contact angle between the drop and produce surface indicate greater hydrophobicity

3.2.9 Microbiological analysis

All produce samples were homogenized by stomaching in 50 ml of phosphate buffered saline (PBS) in a Whirl-pak bag® for 1 min and then quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on Modified Oxford Agar (MOX). All colonies resembling

Listeria were counted after 48 h of incubation at 35 ºC. *Salmonella* was enumerated by surface-plating appropriate dilutions on trypticase soy agar (BD) containing 0.6% yeast extract (BD), 0.05% ferric ammonium citrate (Sigma) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS). Plates were incubated at 37°C for 24 h, after which all black colonies were counted as *Salmonella*.

3.2.10 Statistical analysis

All transfer experiments were performed in triplicate. *Listeria* and *Salmonella* populations were converted to CFU per cm^2 and/or CFU per unit. The percentage of the *Listeria* population transferred from one inoculated to 15 uninoculated samples was calculated as follows:

$$= \frac{Bacteria\ population\ transferred\ to\ 15\ uninoculated\ produce}{Bacteria\ population\ on\ the\ original\ inoculated\ produce} \quad \text{X } 100$$

Also, the percentage the *Listeria* population recovered from an inoculated slicer after slicing 15 uninoculated product was calculated by adding the total CFU on inoculated product after slicing (CFU) and *Listeria* population transferred to 15 uninoculated product (CFU) and total *Listeria* population left on slicer (CFU) divided by the total CFU on inoculated product before slicing (CFU) multiply by 100.

Analysis of variance and the Tukey-Kramer HSD test were performed using JMP to compare percent transfer and recovery. For the multiple comparison of the decay parameter for the different types of produce, the t-test was used after adjusting the *p*-value using the Bonferroni method. Statistical significance was set at $P \leq 0.05$.

3.2.11 A primary exponential decay model

Listeria transfer curve data (log CFU/product) were fitted to a two-parameter exponential decay Eq:

$$Y = A \cdot \exp(-B \cdot X)$$

where Y (dependent variable) is the log CFU/product transferred and X (independent variable) is the order number for the specific uninoculated produce that was sliced. A (*Listeria* population transferred to the first product) and B (decay rate) are two model parameters. The parameter ± standard error for the aggregate of replications and the root mean square error (RMSE) of the model were obtained using JMP 12.0. Data below LOD were not included in the model.

3.2.12 A secondary multiple linear model

The following linear model was used to describe the effect of physicochemical characteristics, such as water content, pH, firmness and surface roughness on the bacterial decay rate during slicing:

Eq:

$$B = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta k x k + \varepsilon$$

where β_0 is an intercept, and B (dependent variable) is the decay rate after slicing 15 un-inoculated products. The linear model has six independent variables: x_1 (pH), x_2 (water content (%)), x_3 (cutting force (N)), x_4 (soluble solids content (^{o}Brix)), x_5 (surface hydrophobicity(^{o})), x_6 (surface roughness(μm)), and xk (product type) with ε as the random error. the model were obtained using JMP 12.0.

3.3 RESULTS:

3.3.1 Microbial cross-contamination of pears during slicing as impacted by pear's firmness:

At all firmness categories, both pusher and blades yielded significantly ($P \leq 0.05$) lower *Salmonella* populations after slicing one inoculated pear followed by 15 uninoculated pears as compared to after slicing one inoculated pear (Figure 1). Moreover, across all firmness categories, the reduction in *Salmonella* populations on the slicer (Figure 2) before and after slicing 15 uninoculated pears was statistically similar ($P > 0.05$). After slicing one inoculated followed by 15 uninoculated pears, all firmness categories yielded detectable levels of *Salmonella* in one or more replicates (Figure 3). For the high firmness category, pears 1, 9 and 15 respectively yielded average *Salmonella* populations of 4.7 ± 0.1, 3.1 ± 0.4, and 2.7 ± 0.00 log CFU/pear, which were statistically similar ($P > 0.05$) to both medium and soft pears.

Similar trends were observed when *Listeria* was used to contaminate the slicer. At all firmness categories, both the pusher and blades yielded significantly ($P \leq 0.05$) lower *Listeria* populations after slicing one inoculated pear followed by 15 uninoculated pears as compared to after slicing one inoculated pear (Figure 4). The reduction in *Listeria* on the slicer (Figure 5) before and after slicing 15 uninoculated was also statistically similar ($P > 0.05$) across the three firmness categories. Finally, after slicing one inoculated followed by 15 uninoculated pears, *Listeria* was sporadically detected in one or more replicates, regardless of pear firmness (Figure 6). For the high firmness category, pears 1, 9 and 15 respectively yielded average *Listeria* populations of 2.8 ± 0.4, 1.3 ± 0.3, and 1.2 ± 0.5 log CFU/pear, which were statistically similar ($P > 0.05$) to both medium and soft pears.

When these results were fitted to a previously published two-parameter exponential decay model (Scollon, et al (2016); Wang and Ryser (2016)), estimated *Salmonella* populations were 4.6, 5.0, and 4.9 log CFU/pear for the firm, medium, and soft pear trials after slicing one inoculated pear, respectively. Similar transfer ($P > 0.05$) decay rates of 0.04, 0.03, and 0.03, were observed for the firm, medium, and soft pear trials, respectively, with a RMSE less than 0.75 log CFU/pear, indicating a relatively good fit (Figure7). Meanwhile, *Listeria* estimates were 4.4, 5.0, and 4.9 log CFU/pear for the firm, medium, and soft pear trials after slicing one inoculated pear. The transfer decay rates of 0.02, 0.04, and -0.03, observed for the firm, medium, and soft pear trials, respectively, were statistically similar with a RMSE less than 0.58 log CFU/pear, indicating a relatively good fit(Figure8).

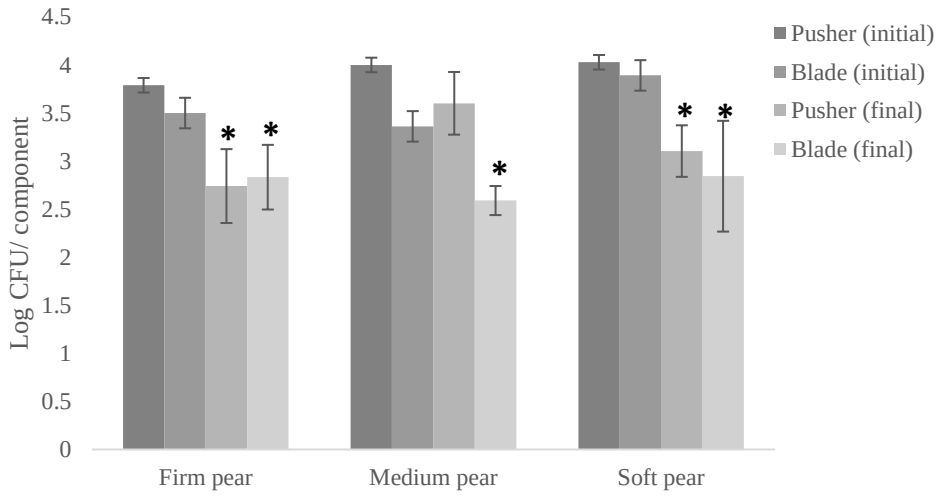


Figure 3-1: *Salmonella* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated firm, medium and soft pear. Columns with asterisks are significantly different ($P \leq 0.05$) from the corresponding component.

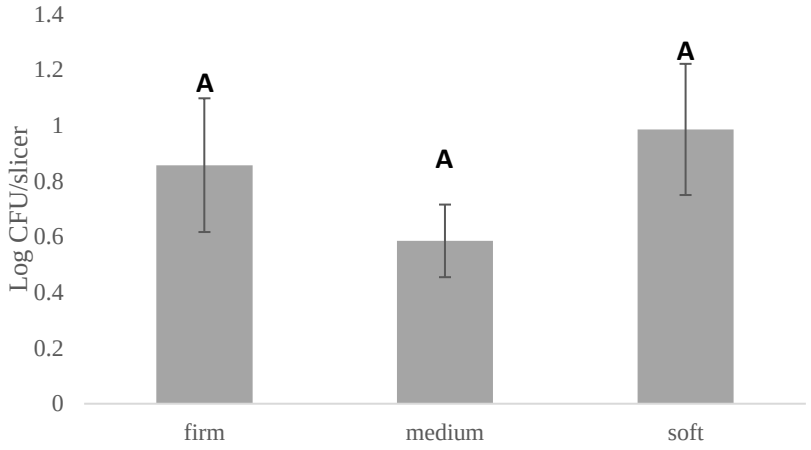


Figure 3-2: Reduction in *Salmonella* populations on the before and after slicing 15 uninoculated firm, medium, and soft pears.

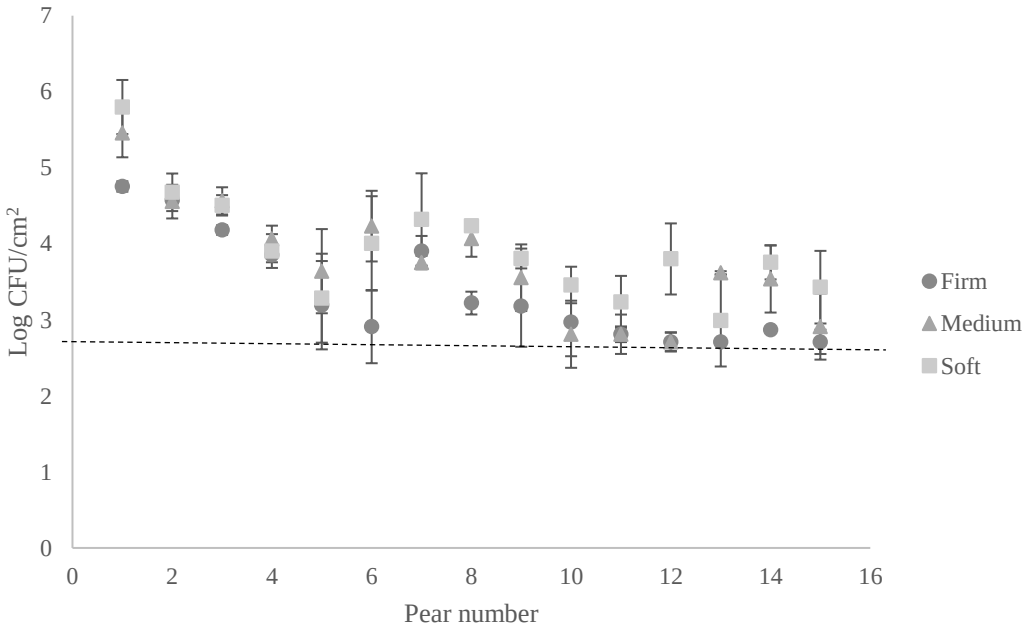


Figure 3-3: Sequential *Salmonella* transfer during slicing of pears

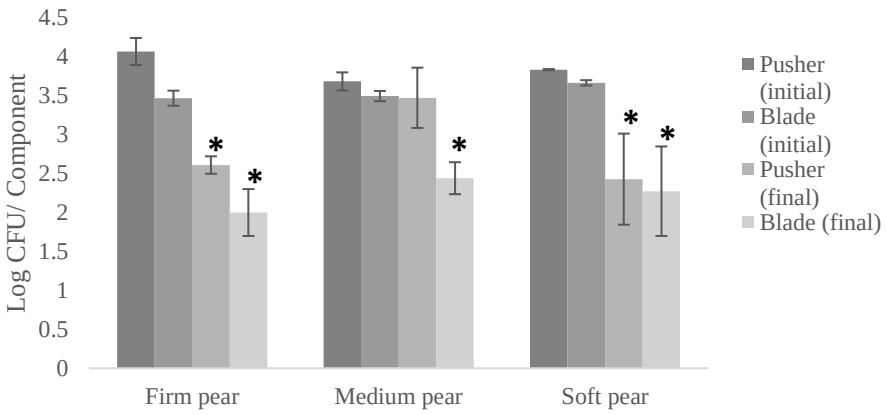


Figure 3-4: *Listeria* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated firm, medium, and soft pears.

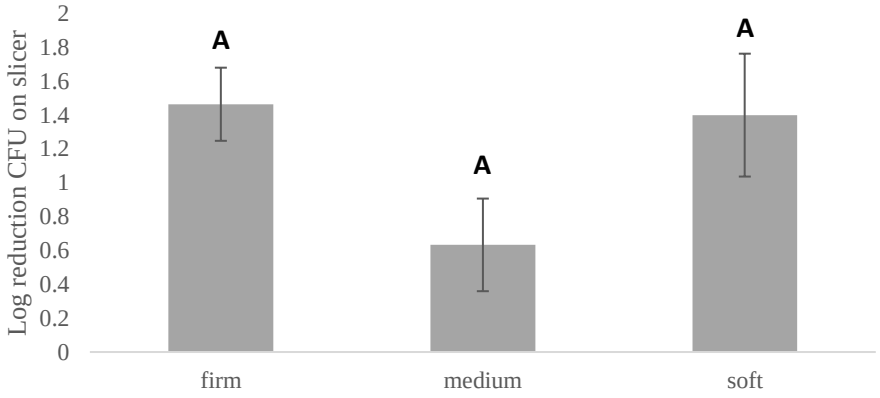


Figure 3-5: Reduction of *Listeria* populations on the slicer (mean ± SE) before and after slicing 15 uninoculated firm, medium and soft pears.

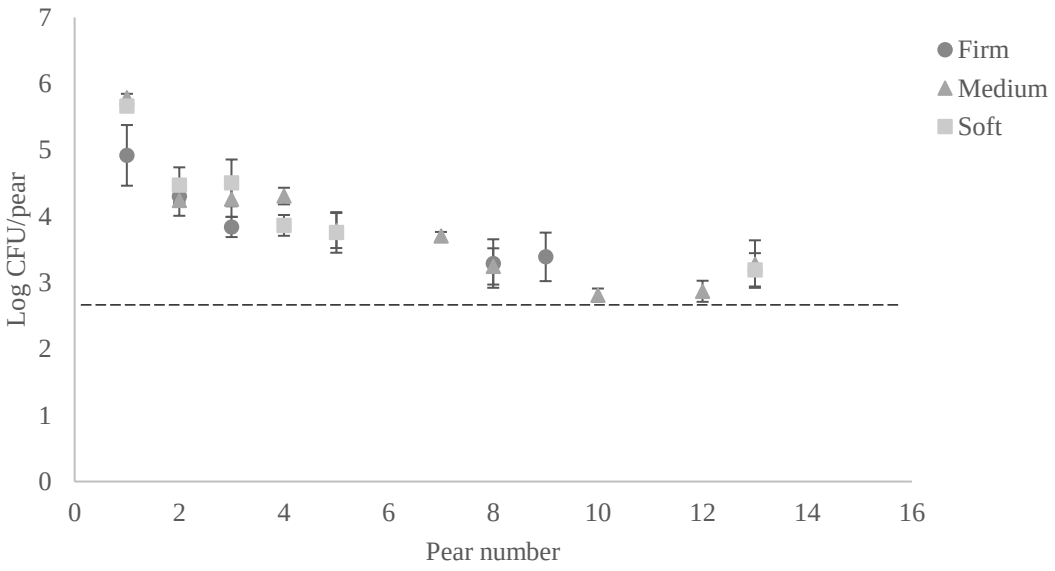


Figure 3-6: Sequential *Listeria* transfer during slicing of pears

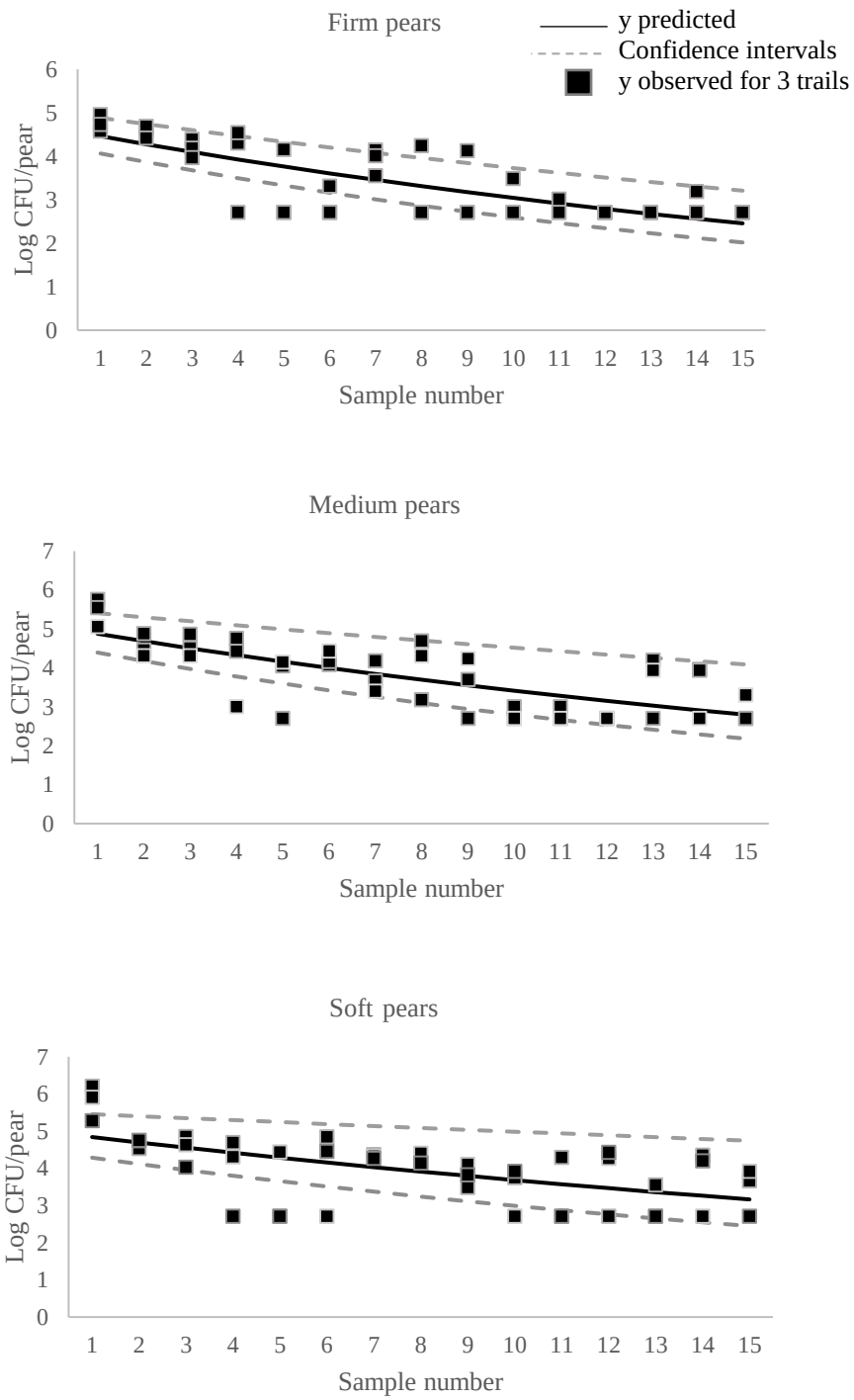


Figure 3-7: Predicted *Salmonella* transfer from one inoculate pear (firm, medium, and soft) to 15 uninoculated sample. y predicted is the line of prediction; y observed is the observed line for 3 trails; Confidence intervals is the confidence intervals for the line of prediction.

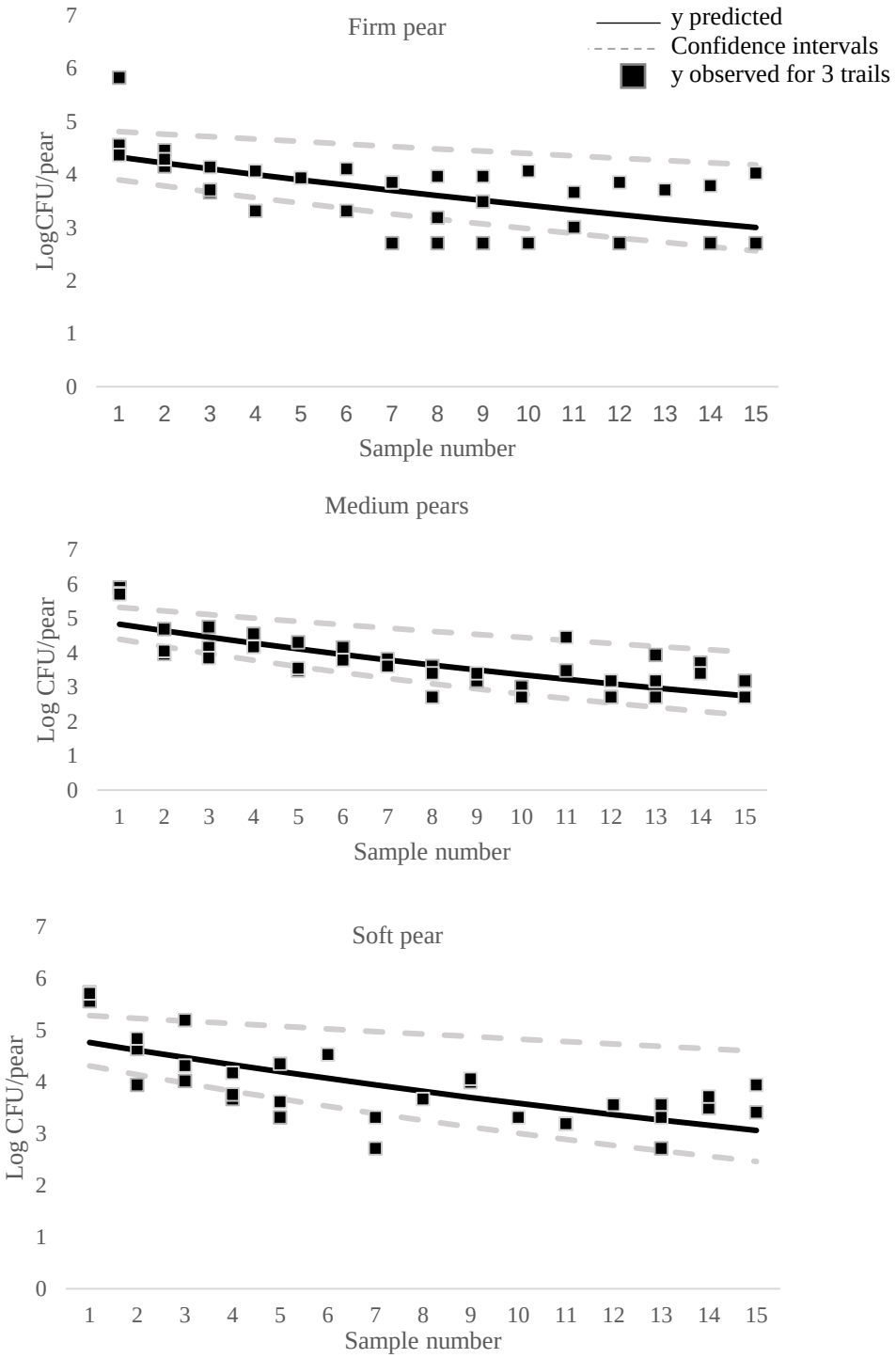


Figure 3-8: Predicted *Listeria* transfer from one inoculate pear (Firm, medium, and soft) to 15 uninoculated sample. y predicted is the line of prediction; y observed is the observed line for 3 trails; Confidence intervals is the confidence intervals for the line of prediction.

3.3.2 Quantify *Listeria* transfer during slicing of different fresh cut produce:

For all products sliced, both the pusher and blades yielded significantly ($P \leq 0.05$) lower *Listeria* populations after slicing one inoculated product followed by 15 uninoculated products as compared to *Listeria* populations recovered after slicing one inoculated product (Figure 9). However, the reduction in *Listeria* population varied across products sliced, with significantly ($P \leq 0.05$) less reduction for tomatoes (0.79 ± 0.1 log CFU/slicer) and potatoes (0.83 ± 0.3 log CFU/slicer) as compared to zucchini (1.9 ± 0.1 log CFU/slicer) (Figure 10).

Inoculation level for product after dip-inoculating in ~ 6 log cfu/ml, initial transfer from inoculated product (~5.5 Log CFU/cm^2) to slicer, the percentage of the *Listeria* population transferred from one inoculated to 15 uninoculated samples, and the percent recovery of *Listeria* is summarized in table 1. Percent transfer during sequential slicing of one inoculated product to 15 uninoculated samples was affected by product type. Transfer was significantly ($P \leq 0.05$) higher for cantaloupe (41.1 ± 14.4%) and tomato (41.1 ± 16.1%) compared to potatoes (2.3 ± 0.8%), onions (2.2± 0.4%), radishes (1.4 ± 0.9%) and pears (0.49 ± 0.2%). *Listeria* was not detectable during slicing of carrots. Although affected by slicer sampling method, the percent recovery for all product varied greatly with significant higher recovery for tomato (2002.41 ± 607%). as opposed to radish (16.2 ± 11.4%).

For each of the nine products assessed for *Listeria* transfer, a previously described two-parameter exponential decay model was fitted to the *Listeria* populations obtained during the slicing of 15 uninoculated samples. Model parameters and RMSE are shown in Table 2, along the line of prediction, the observed line for 3 trails and, the confidence intervals for the line of

prediction are shown in (Figure 11). The decay rate (parameter B) ranged from 0.008±0.002 for cucumbers to 0.09±0.4 for radishes. The multiple comparisons for the decay rate across all products are (summarized in Table 3) shows that decay rates are significantly different between products ($P \leq 0.05$). The RMSE ranged from 0.25 for gray zucchini to 0.68 log CFU/produce for onion, indicating a relatively good fit.

The physicochemical measurements including water content, cutting force, pH, surface, soluble solids content, surface hydrophobicity, surface roughness, are summarized in Table 4. When these physiological characteristics including the products were fitted into a generalized linear model to describe their impact on the decay rate during slicing, the model was heavily dependent on the product type with a statistical significant ($P \leq 0.05$). However, pH, cutting force, and surface hydrophobicity had were more statically relevant as oppose to the other physicochemical characteristics. The predicted decay rates for all the products is summarized in table 2. The individual component of the regression model is shown in figure 12.

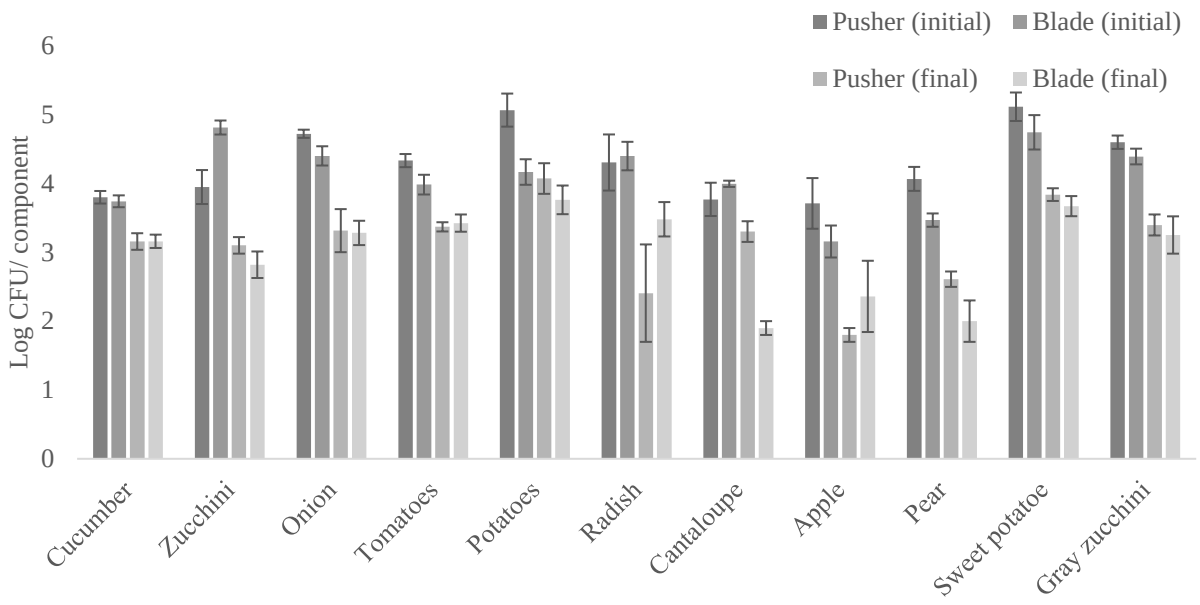


Figure 3-9: *Listeria* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated product samples.

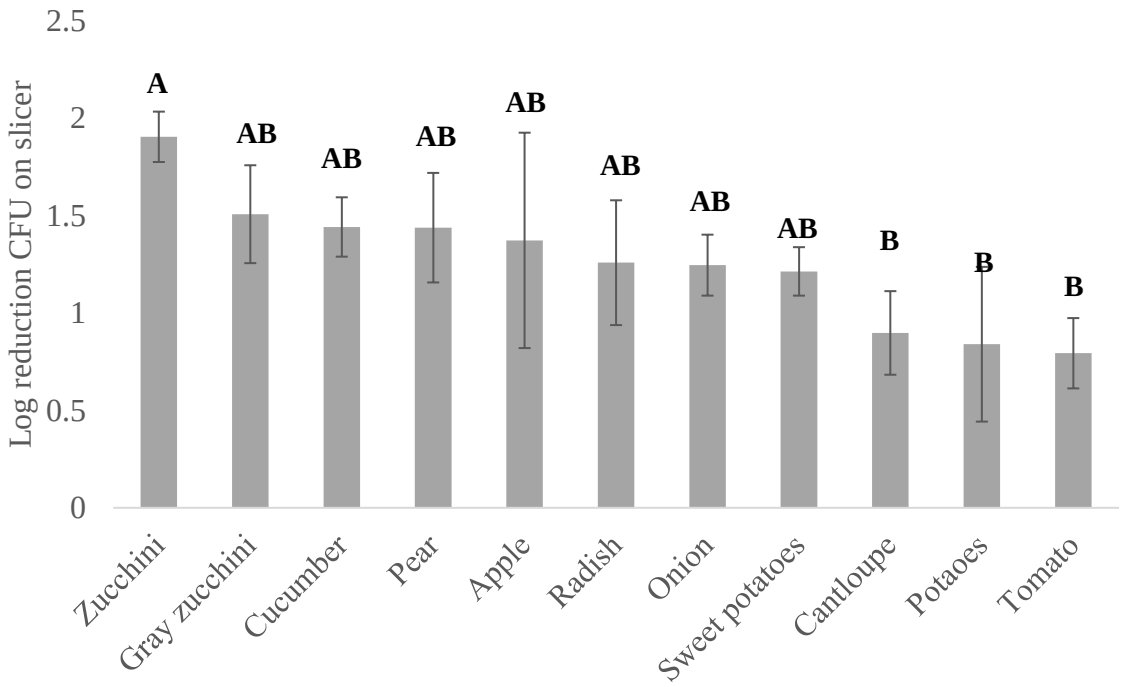


Figure 3-10: Reduction of *Listeria* populations on the slicer (mean ± SE) before and after slicing 15 uninoculated product samples. Means with different letters for produce are significantly different ($P \leq 0.05$).

Table 3-1: Inoculation level for product after dip-inoculating in ~6 log cfu/ml, initial transfer from inoculated product (~7.5 Log CFU/product) to slicer, the percentage of the *Listeria* population transferred from one inoculated to 15 uninoculated samples, and the percent recovery of *Listeria*

Product	Inoculation level (Log CFU/product)	Initial transfer (%)	% Transfer	Recovery (%)
Apple	7.7±0.1 ^{bcd}	1.3±0.6^{a}	14.4±14.0^{b}	0.9±0.1 ^{cd}
Cantaloupe	7.9±0.05 ^{bc}	0.16±0.06 ^{a}	41.1±14.4^{a}	0.2±0.1 ^{d}
Cucumber	7.6±0.1 ^{bcd}	0.2±0.03 ^{a}	5.2±0.6^{b}	11.9±2.8 ^{abcd}
Gray zucchini	7.7±0.1^{bcd}	0.2±0.1 ^{a}	6.1±2.7^{b}	17.3±2.8 ^{ab}
Onion	7.5±0.1^{d}	1.2±0.6 ^{a}	2.25±0.4^{b}	13.3±6.8 ^{abcd}
Pear	7.9±0.06 ^{b}	0.03±0.01 ^{a}	0.4±0.2^{b}	20.3±8.5 ^{a}
Potato	8.4±0.1 ^{a}	0.1±0.06 ^{a}	2.3±0.8^{b}	15.8±4.2^{abc}
Radish	7.5±0.08 ^{d}	12.5±3.2 ^{b}	1.4±0.9^{b}	0.8±0.1 ^{cd}
Sweet potatoes	8.3±0.1 ^{a}	0.2±0.1 ^{a}	1.6±0.4^{b}	13.4±5.1 ^{abcd}
Tomato	7.6±0.1 ^{cd}	1.8±1.0 ^{a}	41.0±16.1^{a}	4.0± 2.0 ^{bcd}
Zucchini	7.6±0.05 ^{bcd}	0.3±0.1 ^{a}	4.0±2.4^{b}	25.1±10.2 ^{a}

Table 3-2: Transfer model parameters (A and B) and predicted decay rate during transfer of *Listeria* from inoculated produce to the slicer during sequential slicing (n = 3)

PRODUCE	A ± SE (LOG CFU/PRODUCE	B ± SE	RMSE (LOG CFU/PRODUCE)	PREDICTED DECAY RATE B
RADISH	3.1 ± 0.1	0.09 ± 0.01	0.41	0.09
TOMATO	5.5 ± 0.1	0.04 ± 0.002	0.33	0.03
CANTALOUPE	2.9 ± 0.14	0.04 ± 0.006	0.39	0.03
ONION	4.7 ± 0.2	0.03 ± 0.006	0.68	0.03
SWEET POTATO	5.5 ± 0.1	0.03 ± 0.003	0.40	0.03
APPLE	4.1 ± 0.2	0.02 ± 0.01	0.56	0.05
PEAR	4.4 ± 0.2	0.02 ± 0.006	0.57	0.03
POTATOES	5.8 ± 0.1	0.02 ± 0.002	0.34	0.02
ZUCCHINI	5.1 ± 0.2	0.01 ± 0.005	0.65	0.01
GRAY ZUCCHINI	5.6 ± 0.08	0.01 ± 0.001	0.25	0.02
CUCUMBER	5.1 ± 0.1	0.008 ± 0.002	0.32	0.006

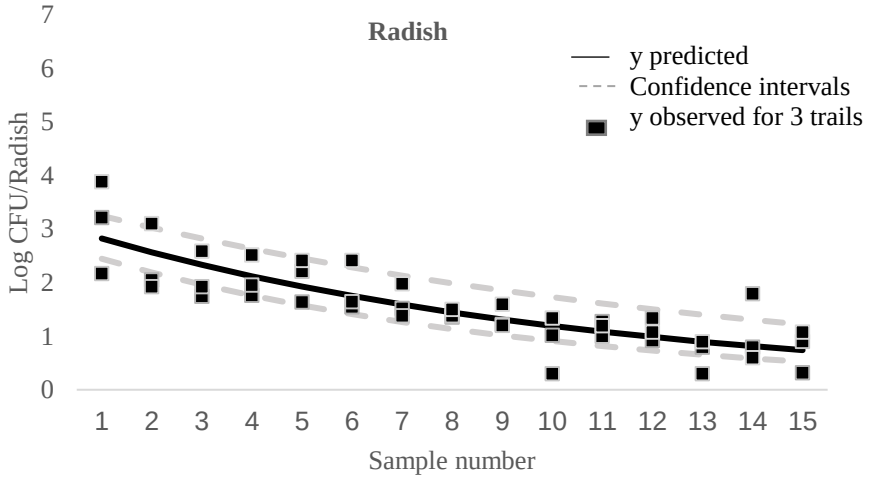
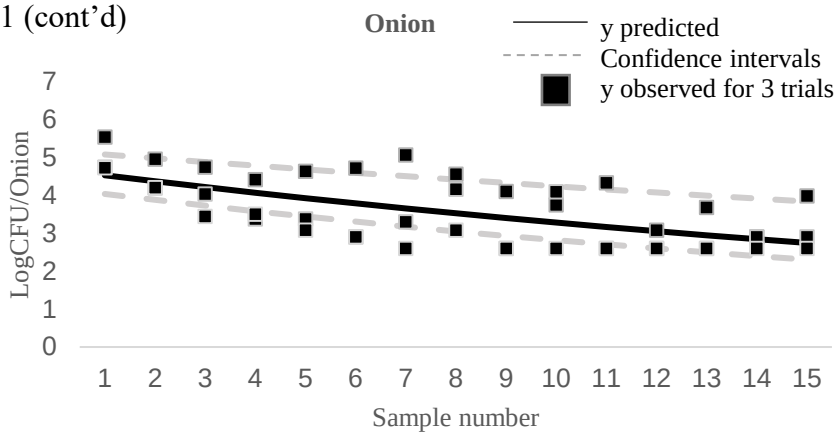
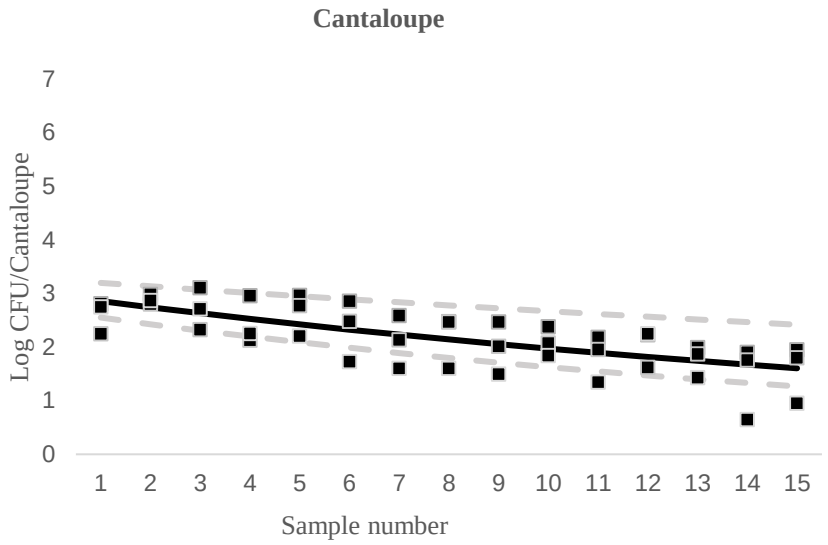
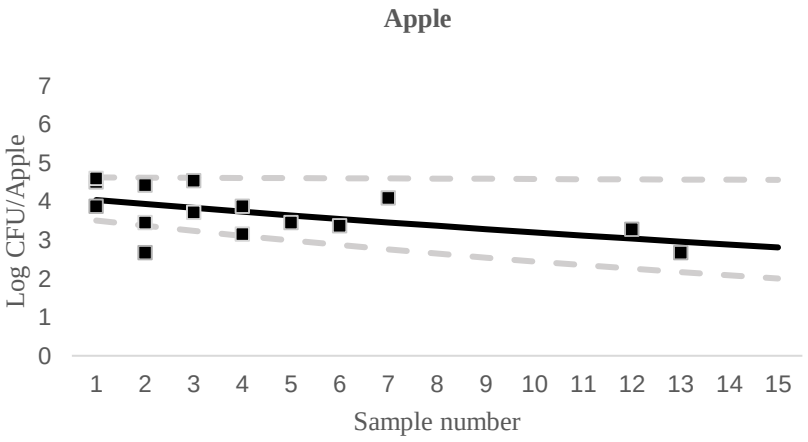


Figure 3-11 Predicted *L. monocytogenes* transfer from one inoculate (Radish, Onion, Cantaloupe, Apple, Cucumber, Pear, Tomato, Potato, Zucchini, Gray zucchini, and sweet potato) to 15 uninoculated sample. y predicted is the line of prediction; y observed is the observed line for 3 trails; Confidence intervals is the confidence intervals for the line of prediction.

Figure 3-11 (cont'd)







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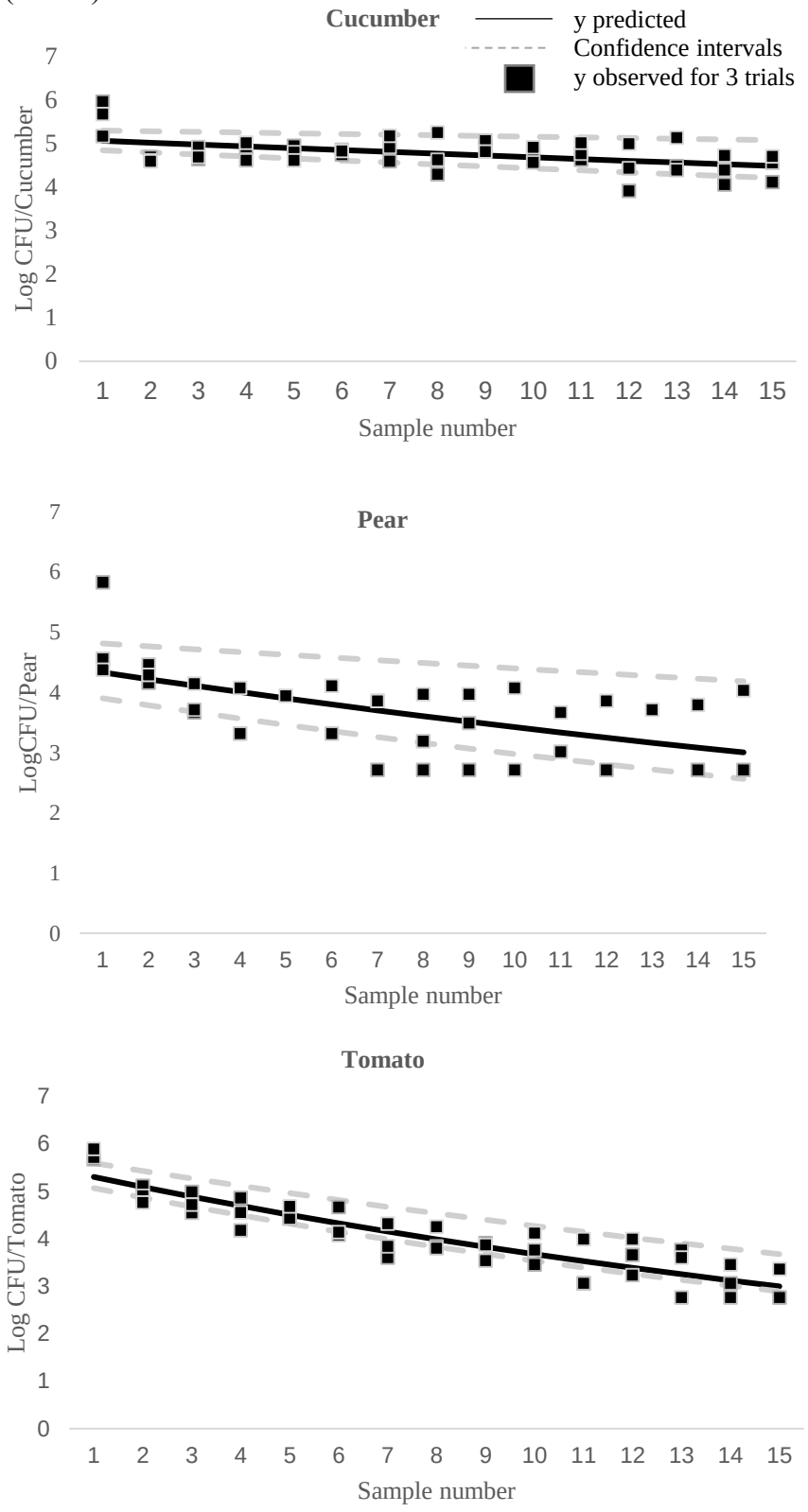

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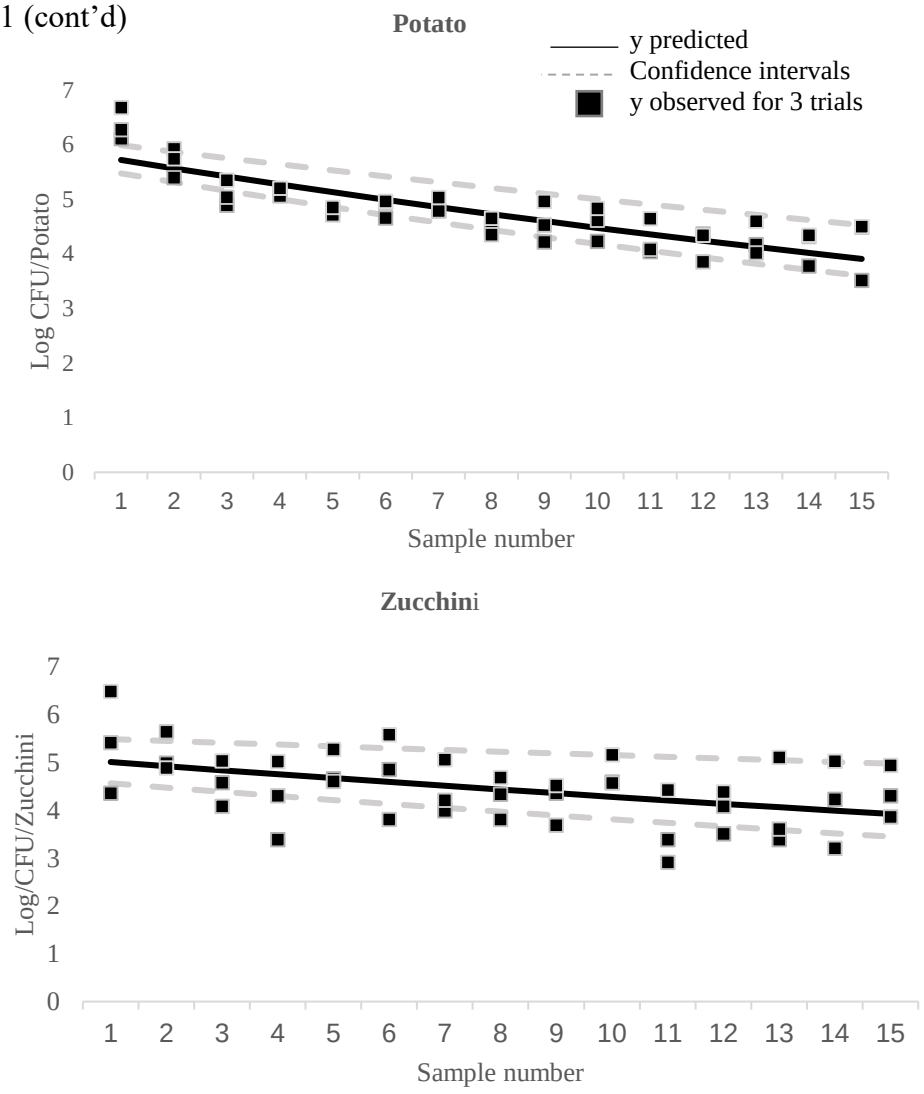

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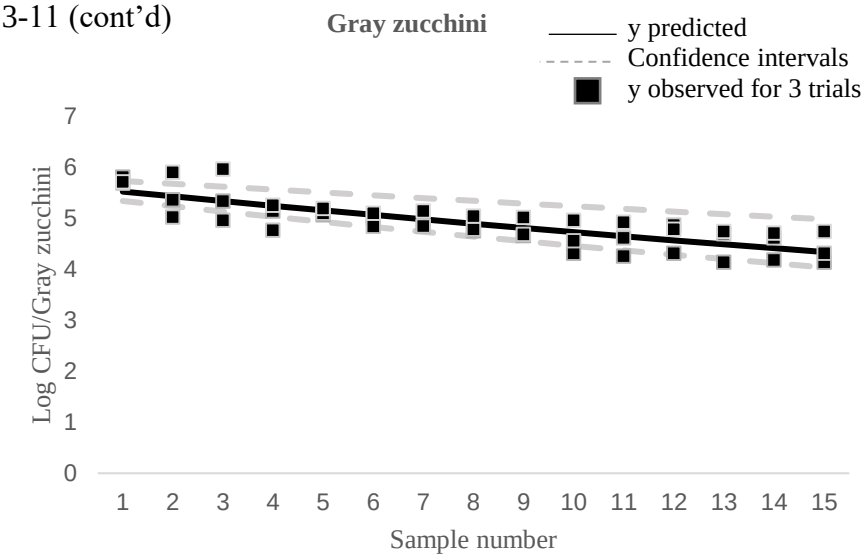


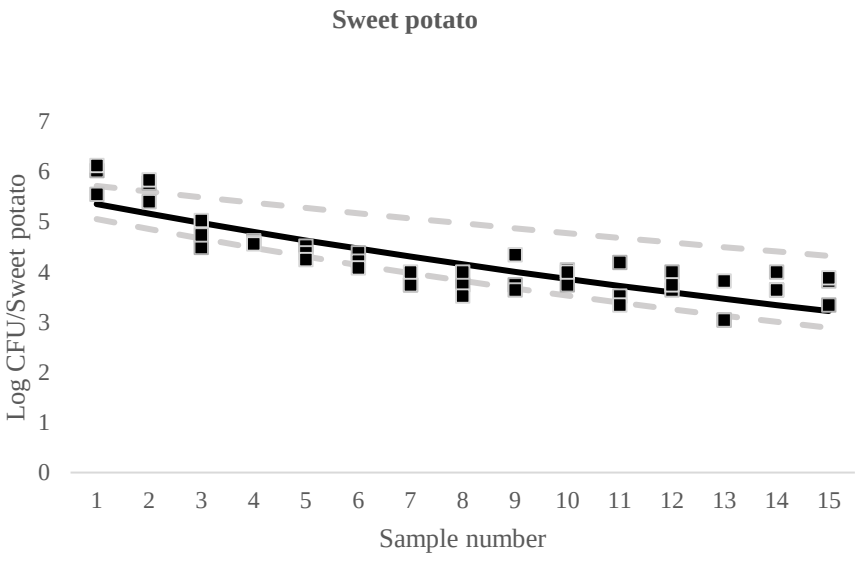


Table 3-3: Multiple comparison summary for the decay rate parameter (B)

	t value	df	*P*-value	adjusted *P*-value
Gray zucchini vs Tomatoes	13.4	88	5.3E-23	1.9E-21*
Tomatoes vs Cucumber	11.3	88	7.5E-19	2.7E-17*
Radish vs Cucumber	8.0	88	3.8E-12	1.3E-10*
Gray zucchini vs Radish	7.9	88	5.5E-12	2.0E-10*
Radish vs Zucchini	7.1	88	2.3E-10	8.5E-09*
Tomatoes vs Potatoes	7.0	88	3.4E-10	1.2E-08*
Radish vs Potatoes	6.8	88	9.0E-10	3.2E-08*
Sweet potato vs Cucumber	6.1	88	2.7E-08	9.8E-07*
Radish vs Pear	6.0	88	4.2E-08	1.5E-06*
Sweet potato vs Radish	5.7	88	1.2E-07	4.6E-06*
Tomatoes vs Zucchini	5.5	88	2.7E-07	9.8E-06*
Cantaloupe vs Cucumber	5.2	88	1.1E-06	4.2E-05*
Radish vs Onion	5.1	88	1.6E-06	5.7E-05*
Gray zucchini vs Cantaloupe	5.0	88	1.9E-06	7.0E-05*
Radish vs Apple	4.9	88	3.5E-06	0.0001*
Radish vs Tomatoes	4.9	88	4.2E-06	0.0001*
Gray zucchini vs Potatoes	4.4	88	2.3E-05	0.0008*
Potatoes vs Cucumber	4.2	88	5.4E-05	0.001*
Cantaloupe vs Radish	4.2	88	6.3E-05	0.002*

Table 3-3 (cont'd)

Cantaloupe vs Zucchini	3.9	88	0.0001	0.005*
Cucumber vs Onion	3.4	88	0.0007	0.02*
Sweet potato vs Zucchini	3.4	88	0.0009	0.03*
Cantaloupe vs Potatoes	3.3	88	0.001	0.04*
Tomatoes vs Pear	3.1	88	0.002	> 0.05
Sweet potato vs Tomatoes	2.7	88	0.006	> 0.05
Sweet potato vs Potatoes	2.7	88	0.006	> 0.05
Zucchini vs Onion	2.5	88	0.01	> 0.05
Cantaloupe vs Pear	2.4	88	0.01	> 0.05
Tomatoes vs Apple	1.9	88	> 0.05	> 0.05
Pear vs Cucumber	1.8	88	> 0.05	> 0.05
Potatoes vs Zucchini	1.8	88	> 0.05	> 0.05
Cantaloupe vs Apple	1.8	88	> 0.05	> 0.05
Gray zucchini vs Pear	1.6	88	> 0.05	> 0.05
Sweet potato vs Cantaloupe	1.6	88	> 0.05	> 0.05
Tomatoes vs Onion	1.5	88	> 0.05	> 0.05
Potatoes vs Onion	1.5	88	> 0.05	> 0.05
Sweet potato vs Pear	1.4	88	> 0.05	> 0.05
Cantaloupe vs Onion	1.2	88	> 0.05	> 0.05
Pear vs Zucchini	1.2	88	> 0.05	> 0.05
Pear vs Onion	1.1	88	> 0.05	> 0.05
Apple vs Cucumber	1.1	88	> 0.05	> 0.05
Gray zucchini vs Apple	0.9	88	> 0.05	> 0.05

Table 3-3 (cont'd)

Sweet potato vs Apple	0.9	88	> 0.05	> 0.05
Gray zucchini vs Cucumber	0.8	88	> 0.05	> 0.05
Apple vs Zucchini	0.8	88	> 0.05	> 0.05
Apple vs Onion	0.8	88	> 0.05	> 0.05
Cucumber vs Zucchini	0.3	88	> 0.05	> 0.05
Cantaloupe vs Tomatoes	0.1	88	> 0.05	> 0.05
Pear vs Potatoes	0	88	> 0.05	> 0.05
Sweet potato vs Onion	0	88	> 0.05	> 0.05
Apple vs Potato	0	88	> 0.05	> 0.05
Apple vs Pear	0	88	> 0.05	> 0.05
Gray zucchini vs Zucchini	0	88	> 0.05	> 0.05
Gray zucchini vs Onion	0	88	> 0.05	> 0.05

Table 3-4: Physicochemical characteristics of produce

Produce	Water content (%)	Cutting force (N)	pH	soluble solids content (SSC) º Brix	Surface hydrophobicity º	Surface roughness (*Ra*)
Apples	82.8±1.1 cd	17.3±1.0 b	4.5±0.2 c	15.0±0.3 a	93.4±1.4 b	75±6.4 b
Cantaloupe	89.5±1.0 b	3.2±0.0 e	6.5±0.1 a	10.0±0.3 c	<5.0 e	167.1±13.7 ab
Cucumbers	95.6±1.0 a	17.3±1.0 b	5.8±0.1 ab	4.0±0.3 f	93.0±2.6 b	367.6±155.4 a
Gray zucchini	95.2±0.1 a	11.7±0.1 cd	6.1±0.05 a	5.8±0.7 e	63.0±11.5 c	236.3±41.2 ab
Onions	85.4±0.6 bc	13.1±0.2 bc	5.8±0.2 b	8.0±0.3 d	98.1±2.0 ab	100.2±24.0 b
Pears	85.1±0.2 cd	15.2±0.6 bc	4.5±0.1 c	12.5±0.2 b	112.1±7.4 a	122.1±9.1 ab
Potatoes	85.4±0.2 de	13.1±1.1 bc	6.2±0.0 ab	5.0±0.0 ef	51.3±1.4 cd	145.1± 36.1 ab
Radishes	94.8±0.1 a	16.2±0.9 b	6.1±0.1 ab	4.0±0.0 f	37.1±0.9 d	161.4±30.6 ab
Sweet potatoes	80.3±1.9 e	33.5±3.8 a	5.8±0.1 ab	10.1±0.2 c	<5.0 e	112.2±14.1 ab
Tomatoes	95.3±0.7 a	7.5±0.8 de	4.7±0.0 c	5.0±0.3 ef	103.0±1.6 ab	108.7±7.3 ab
Zucchini	95.4±0.6 a	14.5±0.7 bc	6.2±0.3 ab	4.0±0.3 f	93.1±6.9 b	153.6±14.2 ab
Carrots	88.1±0.5	28.8±0.3	6.3±0.2	9 ± 0.5	103 ± 9.5	113.7±2.9

Table 3-5: Regression analysis of variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	13	0.01686732	0.001297	4.4158
Error	19	0.00558274	0.000294	**Prob > F**
C. Total	32	0.02245006		0.0018*

Table 3-6: Effect tests of the regression analysis

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Product	10	10	0.01474480	5.0182	0.0013*
Cutting force	1	1	0.00037030	1.2603	0.2756
pH	1	1	0.00065365	2.2246	0.1522
Surface hydrophobicity	1	1	0.00052127	1.7741	0.1986

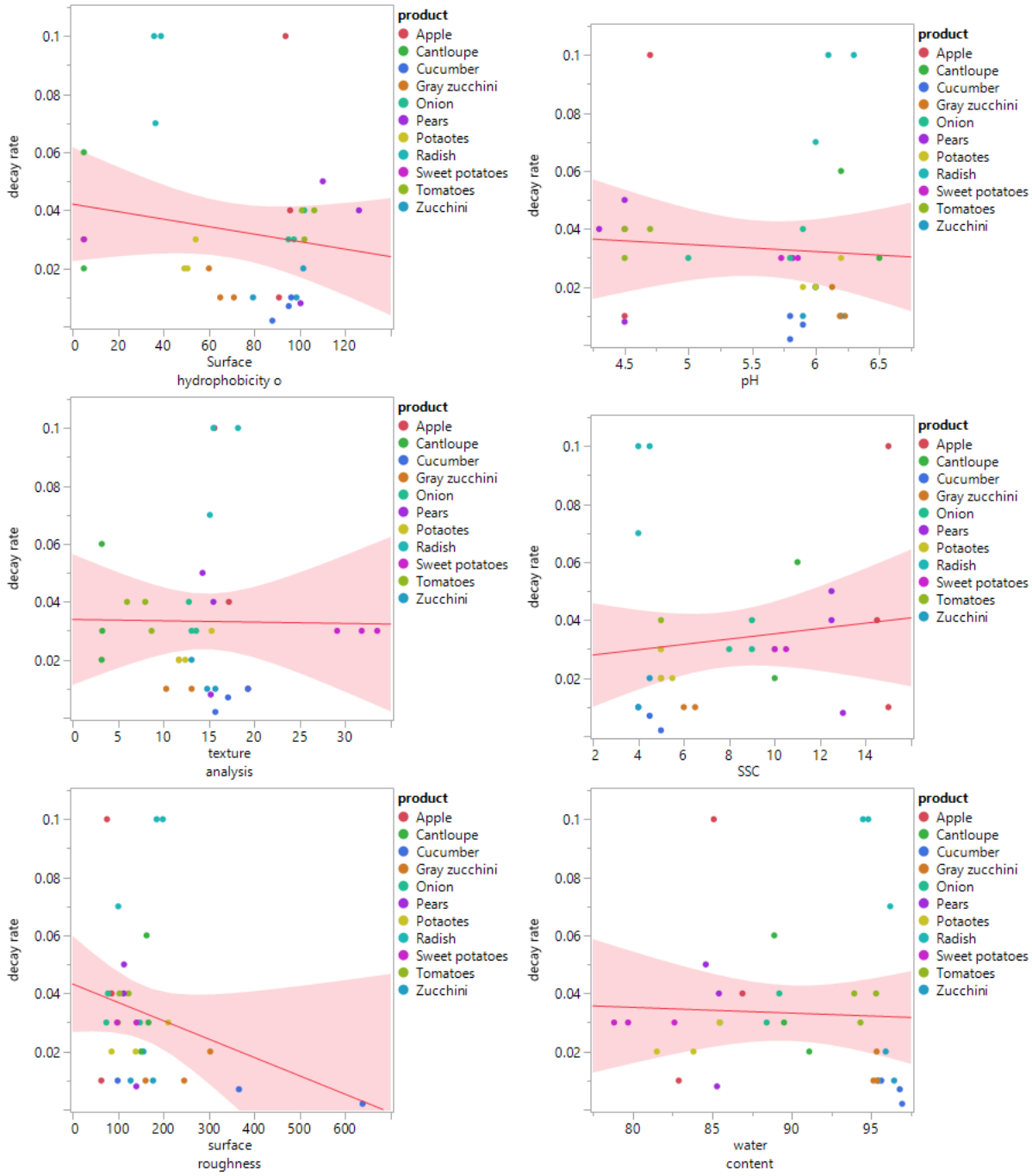


Figure 3-12: Individual component of the multiple regression model

3.4 DISCUSSION:

The findings of this research clearly show that cross-contamination from inoculated to uninoculated produce occurs at different rates during sequential slicing. After slicing one inoculated followed by 15 uninoculated samples, detectable levels of *L. monocytogenes* remained on both the pusher plates and the blades, allowing for further transfer in all products. However, *L. monocytogenes* populations remaining on the slicer varied significantly across different products. In the present study, percent sequential transfer was the highest for cantaloupe compared to other products sliced. These finding are consistent with those of Miranda and Schaffner (2016) who showed that more bacteria transferred to watermelon (~ 0.2 to 97%) than to any other food examined regardless of the contact time. Given the significantly higher water content of watermelon (0.99 ± 0.01) compared to the other products tested, these findings support the impact of water content on bacterial transfer. The recovery percentage (mass balance) of *Listeria* after slicing 15 uncontaminated products varied greatly. Mass balance calculation or percentage recovery is a highly variable process due to the nature of bacterial transfer and it has been observed by other research groups. For instance, Buchholz (2012) reported recovery of *E. coli* transferred during processing of baby spinach as high as 147.2±50.4 % compared to 52.6±43.0 % recovered from romaine lettuce. These variances are attributed to the errors inherent to microbial collection from surfaces and enumeration techniques.

Bacterial transfer during slicing of foods is a complex process. In this study, eleven products were evaluated individually for the transfer of *Listeria* during mechanical slicing. Additionally, the physicochemical characteristics of these products were also measured. An exponential decay model used to describe the transfer of *Listeria* during slicing resulted in

significantly different decay rates for different products. These observations are in partial agreement with those of Vorst *et al*. (2006) who evaluated the transfer of *L. monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. These products, which vary in fat and moisture content, yielded different *Listeria* populations on the slicer blade and other components after repeated slicing. Moreover, these observations are consistent with previous cross-contamination studies in which foodborne pathogens were shown to readily transfer to or from slicers or mechanical shredders to deli meats (Vorst *et al*. 2006), lettuce (Buchholz et al. 2012a, 2012b), and celery (Kaminski *et al*. 2014) during simulated commercial processing (Herman *et al.* 2015).

The impact of the physicochemical characteristics of products on decay rates was examined in this research. The multiple linear regression preformed showed that decay rate of products during slicing is highly depended on the product itself with a statistical significate ($P \leq 0.05$). However, the model showed that pH, cutting force, and surface hydrophobicity had the most pronounced effect on the decay rate compared to surface roughness, soluble solid content, and water content. The impact of texture/cutting force on decay rate have been studied before. Wang and Ryser (2016) reported significantly lower transfer decay rates for *Salmonella* when slicing Rebelski and Bigdena as compared to Torero tomatoes which had significantly ($P \leq 0.05$) tougher texture and lower water content compared to the other two varieties. In this study, the impact of pear texture/ cutting force was also investigated. Given their ability to ripen over time, pears were used assess the effect of product firmness on bacterial transfer. Except for cutting force, all of the remaining (water content, pH, soluble solids content and surface hydrophobicity) remained similar. The slicer used for this study also provided a constant force to minimize

variability. Cutting force seemed to have no significant effect on transfer as the reduction of bacteria on the slicer and the exponential decay rate were statistically similar ($P > 0.05$). Although these finding contradict those of Wang and Ryser (2016) where cutting force did influence bacterial transfer during slicing of tomatoes, the different tomato varieties that were used varied in water content as well, suggesting that the combination of cutting force and water content can potentially affect bacterial transfer.

Few studies have measured and reported the impact physicochemical characteristics of products on bacterial transfer. For instance, Wang *et al*. (2009) showed a positive linear correlation between average surface roughness (***Ra***) and the adhesion rate of *E. coli* O157:H7 for Golden Delicious apples (1.43 ± 0.13 μm), navel oranges (10.94 ± .07 μm), avocadoes (9.58 ± 0.27 μm) and cantaloupe (14.18 ± 0.25 μm), while surface hydrophobicity for these same products was 77.27 ± 4.57, 78.23 ± 8.37, 56.33 ± 5.16 and 47.20 ± 18.52 θ, respectively. In a meta-analysis of bacterial transfer data, Mazon (2017) found that five studies included surface roughness for salami (8.04 μm), ham (5.17 μm), tomatoes (2.88 μm), onions (0.3 μm) and lettuce (20 μm) with these same studies showing that product roughness affected bacterial transfer. Based on the meta-analysis by Mazon (2017) that included the impact of pH on bacterial attachment to food products, both the intercept and rate parameter decreased with increasing pH.

All of the studied mentioned before examined the transfer as a function of one variable (water content, pH, cutting force ...etc.). to our knowledge, this is the first study to analyze bacterial transfer as a function of multiple physicochemical characteristics. Although the model

accurately predicted the decay rate for the different product, the predictions were heavily dependent on the type of product analyzed.

In summary, based on product type, some fresh products are more prone to cross-contamination than others during slicing**.** Creating a generalized model to predict decay rates of product based on their inherent characteristic is a challenging task due to the complexity, dynamics, and variables involved in bacterial transfer during slicing. However, these findings should lay the foundation for future research and narrow the focus of variables affecting bacteria transfer during slicing to improve our understanding of this phenomena.

CHAPTER 4:

Conclusions and Recommendations for Future Work

4.1 CONCLUSIONS OF THIS DISSERTATION

This dissertation includes three research chapters pertaining to bacterial transfer during slicing of fresh produce and the effect of physicochemical properties of produce on bacterial transfer. The findings from this research illustrate the overall interactions between physicochemical characteristics of fresh produce and transfer of *Listeria* during slicing, and more importantly provide a new approach for modeling *Listeria* transfer data, which should in turn lead to the development of more effective strategies to minimize cross-contamination.

The first research chapter -"Chapter 2: Microbial Cross-Contamination of Cucumber and Zucchini during Slicing as Impacted by Mechanical Slicer Type, Slicing Speed and Water content", demonstrated that slicing direction (vertical vs horizontal) impacted *Listeria* transfer. After slicing one inoculated sample followed by fifteen uninoculated samples, *Listeria* populations on different parts of the stationary slicer decreased significantly ($P \leq 0.05$). However, different parts of the rotating slicer were able to retain a greater proportion of the initial *Listeria* population transferred to the same parts during continued use. Moreover, using floral foam to evaluate the effect of water content on bacterial transfer, a statistically similar decay rate was observed for all moisture content examined. This chapter concluded that both the type of slicer and type of product sliced affected the numbers of *Listeria* transferred.

Chapter 3 focused on the effect of physicochemical properties of fresh produce and *Listeria* transfer during mechanical slicing. Using three pear firmness categories - firm (10-15 N), medium (6 - 9 N) and soft (< 6 N) to study the impact of firmness on *Listeria* and *Salmonella* transfer during slicing, similar transfer ($P > 0.05$) decay rates were observed for firm, medium, and soft pears, indicating that bacterial transfer is a multifactorial process. When a range of fresh produce (onions, radishes, tomatoes, potatoes, carrots, zucchini, cantaloupe, apples, sweet

potatoes, grey zucchini and cucumbers) were assessed for *Listeria* transfer, the different products yielded different transfer decay rates. Further investigation of the physicochemical properties of fresh produce indicated that decay rates are significantly ($P \leq 0.05$) dependent on product tested. In summary, based on product characteristics, some types of fresh produce are more prone to cross-contamination than others during slicing**.** These findings should improve our understanding of bacterial transfer, help define the order in which different products are sliced and aide in the development of improved predictive models for risk assessment.

4.2 RECOMMENDATIONS FOR FUTURE WORK

As shown in this research, bacterial transfer during slicing is a very complex process that involves a biological agent “bacteria” on biological surfaces “produce”, therefore, more replications are needed to minimize variation in the data collected which will result in better predictions. While the results from this study can provide valuable information, in the future, increasing the number of products sliced when collecting experimental data could help improve model predictions.

Most bacterial transfer studies have used one product and one microorganism, which makes it difficult to draw general conclusions on what factors affect bacterial transfer during slicing. It would be extremely beneficial for future research to focus on the effect of extreme differences of physiochemical characteristic on bacterial transfer. This could be achieved by genetically engineer a product to have for instance, low and high water content.

One of the main observations from this work is that bacterial transfer during slicing is a multifactorial process. Identifying and evaluating new factors related to produce, bacteria and the physical process of slicing is vital. For instance, the impact of new produce characteristics such

as cell size and cell wall polysaccharides, which contribute to the amount of liquid released from products during slicing, will advance our understanding of the bacterial transfer phenomena.

APPENDICES

APPENDIX A:

Microbial Cross-Contamination of Cucumber and Zucchini during Slicing as Impacted by Mechanical Slicer Type, Slicing Speed and Water Content

Table A-1: Mean *L. monocytogenes* distribution on produce slices from inoculated and uninoculated cucumber and zucchini after slicing with a rotating slicer.

	***Listeria* population (log CFU/cm^2)**			
Produce slices order	Inoculated cucumber	Un-inoculated cucumber	Inoculated zucchini	Un-inoculated zucchini
1st	4.7	3.8	5.3	3.0
2nd	4.4	3.8	4.2	2.7
15th	4.3	3.7	4.0	2.4
16th	4.4	3.8	4.1	2.6
29th	4.2	3.7	4.2	2.6
Last slice	4.7	3.4	5.0	2.4

Table A-2: *Listeria* distribution (mean ± SE) on different components of the rotating slicer before and after slicing 15 uninoculated zucchini and cucumber.

	***Listeria* population (log CFU/component)**			
Slicer component	Before slicing cucumber	After slicing cucumber	Before slicing zucchini	After slicing zucchini
Slicing plate	4.0	3.3	4.2	3.4
Bottom	3.3	2.2	3.4	4.5
Pusher	3.9	3.0	4.5	3.3

Table A-3: *Listeria* distribution (mean ± SE) on different components of the stationary slicer before and after slicing 15 uninoculated zucchini and cucumber.

	***Listeria* population (log CFU/component)**			
Slicer component	Before slicing cucumber	After slicing cucumber	Before slicing zucchini	After slicing zucchini
Blade	4.0	3.1	4.4	3.1
Pusher	5.0	4.0	5.1	4.4

Table A-4: *Listeria* populations (mean ± SE) on different locations of a zucchini and cucumber slice.

	***Listeria* population (log CFU/cm^2)**			
Slicer type	Skin cucumber	Flesh cucumber	Skin zucchini	Flesh zucchini
Rotating	5.55	2.56	6.17	3.25
Stationary	5.53	2.27	6.19	2.52

Table A-5: *Listeria* transfer from an inoculated stationary slicer (~ 7 log CFU/ product) to 15 inoculated zucchini and cucumber.

	***Listeria* population (log CFU/product)**					
	Cucumber			Zucchini		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.9	5.1	5.6	4.3	5.4	6.4
2	4.6	5.6	4.5	4.9	5.6	4.8
3	4.6	4.9	4.6	4.5	4.0	5.0
4	4.9	4.6	5.0	3.3	4.3	5.0
5	4.9	4.8	4.6	4.6	4.6	5.2
6	4.7	4.8	4.8	4.8	3.8	5.5
7	4.5	4.9	5.1	3.9	4.2	5.0
8	4.2	4.6	5.2	4.3	3.8	4.6
9	4.9	5.0	4.8	4.3	3.6	4.5
10	4.7	4.5	4.9	4.5	4.5	5.1
11	4.6	4.7	5.0	3.3	2.9	4.4
12	3.9	4.4	4.9	3.5	4.0	4.3
13	4.4	4.3	5.1	3.3	3.6	5.1
14	4.0	4.3	4.7	4.2	3.2	5.0
15	4.1	4.5	4.7	4.3	3.8	4.9

Table A-6: *Listeria* transfer from an inoculated rotating slicer (~ 7 log CFU/ product) to 15 inoculated zucchini and cucumber.

	***Listeria* population (log CFU/product)**					
	Cucumber			Zucchini		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3

Table A-6 (cont'd)

1	6.0	6.1	5.4	5.6	5.7	5.7
2	6.0	5.2	4.6	5.4	5.6	6.0
3	4.7	4.8	5.0	4.8	5.4	5.7
4	3.7	4.7	4.7	6.1	5.7	5.4
5	3.6	4.4	4.5	5.3	5.0	5.7
6	3.6	3.9	4.1	5.2	4.5	5.6
7	3.3	4.6	4.0	6.0	5.1	5.1
8	3.4	4.1	3.6	5.7	5.7	4.6
9	3.6	3.4	4.9	6.1	5.8	4.7
10	3.3	3.9	4.6	4.9	5.4	4.7
11	3.5	4.6	4.4	4.8	5.4	5.0
12	3.3	4.5	5.0	4.8	4.8	5.1
13	4.0	4.4	3.5	4.6	4.8	5.0
14	2.8	4.4	4.7	3.9	5.7	4.8
15	2.8	3.5	3.1	5.0	4.9	5.0

Table A-7: *L. monocytogenes* transfer from inoculated to uninoculated zucchini during slicing at high speed and low speed.

	***Listeria* population (log CFU/zucchini)**					
	High speed			Low speed		
Uninoculated zucchini	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	4.3	5.7	6.4	5.4	5.1	5.8
2	4.9	5.7	4.8	5.1	5.7	5.8
3	4.5	5.6	5.0	4.8	4.8	6.3
4	3.3	5.7	5.0	4.6	5.6	6.1
5	4.6	5.3	5.2	4.3	4.3	6.3
6	4.8	5.4	5.5	4.8	5.0	5.8
7	3.9	5.5	5.0	3.9	5.6	5.1
8	4.3	5.4	4.6	5.2	4.7	6.0
9	4.3	5.5	4.5	4.4	5.0	5.6
10	4.5	5.1	5.1	5.6	5.1	5.6
11	3.3	4.9	4.4	4.3	5.6	5.4
12	3.5	5.3	4.3	4.9	4.7	4.7
13	3.3	5.2	5.1	4.4	5.1	4.6
14	4.2	5.1	5.0	4.4	5.1	4.3
15	4.3	5.3	4.9	3.9	5.0	4.4

Table A-8: *L. monocytogenes* transfer from inoculated to uninoculated cucumber during slicing at high speed and low speed.

	***Listeria* population (log CFU/cucumber)**					
	High speed			Low speed		
Uninoculated cucumber	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	6.0	5.2	5.7	5.5	4.9	5.5
2	4.7	5.6	4.6	5.8	5.2	6.1
3	4.7	5.0	4.7	4.8	5.2	5.8
4	5.0	4.6	5.1	4.4	4.7	5.2
5	5.0	4.8	4.6	4.8	4.9	5.4
6	4.8	4.9	4.9	5.4	5.1	6.3
7	4.6	5.0	5.2	5.0	5.0	6.0
8	4.3	4.7	5.3	5.0	4.9	5.6
9	5.0	5.1	4.9	5.1	5.2	5.4
10	4.8	4.6	5	5.2	4.2	4.2
11	4.7	4.8	5.1	5.1	4.9	4.7
12	4	4.5	5.0	4.8	5.0	4.8
13	4.5	4.4	5.2	5.0	5.0	4.2
14	4.1	4.4	4.8	4.2	5.2	4.5
15	4.2	4.6	4.7	4.7	5.0	4.7

Table A-9: *Listeria* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated pieces of floral foam at water saturation levels of 97.6, 96.7, and 95.1%.

	***Listeria* population (log CFU/component)**					
Slicer component	Before slicing moisture levels of 97.6%	After slicing moisture levels of 97.6%	Before slicing moisture levels of 96.7%	After slicing moisture levels of 96.7%	Before slicing saturation levels of 95.1%	After slicing moisture levels of 95.1%
Blade	4.0	3.23	4.0	3.13	4.0	3.4
Pusher	5.08	3.45	5.0	3.7	5.0	4.1

Table A-10: Sequential transfer during slicing of floral foam at water saturation levels of 97.6, 96.7, and 95.1%

	***Listeria* population (log CFU/floral foam)**								
	Percent moisture 97.6%			Percent moisture 96.7%			Percent moisture 95.1%		
Uninoculated floral foam	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	4.9	5.0	2.7	5.6	<LOD	5.5	4.7	4.1	4.9
2	3.9	4.5	<LOD	4.1	<LOD	4.2	3.9	<LOD	2.7

Table A-10 (cont'd)

3	2.7	3.8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3.2
4	2.7	3.4	2.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	2.7	3.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	2.7	<LOD	2.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	2.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8	<<LOD	<LOD	<LOD	<LOD	<LOD	2.7	<LOD	<LOD	<LOD
9	2.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
13	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
14	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

APPENDIX B:

Quantify *Listeria* transfer during slicing of different fresh cut produces as impacted by produce firmness and other physiological characteristics. :

Table B-1: *Salmonella* distribution (mean ± SE) on different components of a stationary slicer before and after slicing 15 uninoculated firm, medium and soft pear. Columns with asterisks are significantly different ($P \leq 0.05$) from the corresponding component.

	***Salmonella* population (log CFU/component)**					
Slicer component	Before slicing firm pear	After slicing firm pear	Before slicing medium pear	After slicing medium pear	Before slicing medium pear	After slicing medium pear
Blade	3.50	2.83	3.36	2.59	3.89	2.84
Pusher	3.79	2.74	4.00	3.60	4.03	3.10

Table B-2: *Salmonella* sequential transfer during slicing of pears.

	***Salmonella* population (log CFU/pear)**								
	firm pear			medium pear			soft pear		
Uninoculated pear	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	4.9	4.5	4.7	5.7	5.5	5.0	6.2	5.9	5.2
2	4.6	4.6	4.4	4.8	4.4	4.3	4.5	4.7	4.7
3	4.3	4.1	3.9	4.8	4.5	4.3	4.8	4.6	4.0
4	4.3	2.7	4.5	4.7	4.4	3.0	4.6	4.3	2.7
5	2.7	4.1	2.7	2.7	4.0	4.1	2.7	4.4	2.7
6	2.7	3.3	2.7	4.0	4.1	4.4	4.8	2.7	4.4
7	4.1	4.0	3.5	4.1	3.6	3.4	4.3	4.3	4.2
8	2.7	2.7	4.2	4.7	3.1	4.3	4.1	4.4	4.1
9	4.1	2.7	2.7	2.7	3.7	4.2	3.4	4.1	3.8
10	2.7	3.4	2.7	3.0	2.7	2.7	3.7	2.7	3.9
11	2.7	2.7	3.0	2.7	3.0	2.7	4.2	2.7	2.7
12	2.7	2.7	2.7	2.7	2.7	2.7	2.7	4.2	4.4
13	2.7	2.7	2.7	4.2	2.7	3.9	3.5	2.7	2.7
14	2.7	3.1	2.7	3.9	3.9	2.7	4.3	2.7	4.2
15	2.7	2.7	2.7	2.7	2.7	3.3	3.6	3.9	2.7

Table B-3: *Listeria* distribution (mean±SE) on different components of a stationary slicer before and after slicing 15 uninoculated firm, medium and soft pear. Columns with asterisks are significantly different ($P \leq 0.05$) from the corresponding component.

	***Listeria* population (log CFU/component)**					
Slicer component	Before slicing firm pear	After slicing firm pear	Before slicing medium pear	After slicing medium pear	Before slicing medium pear	After slicing medium pear
Blade	3.47	2.00	3.50	2.44	3.66	2.27
Pusher	4.07	2.61	4.02	3.11	4.17	2.43

Table B-4: *Listeria* sequential transfer during slicing of pears.

	***Listeria* population (log CFU/pear)**								
	firm pear			medium pear			soft pear		
Uninoculated pear	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.8	4.5	4.3	5.9	5.7	5.7	5.5	5.7	5.7
2	4.1	4.4	4.2	4.6	3.9	4.0	3.9	4.6	4.8
3	3.6	3.7	4.1	4.7	4.1	3.8	4.0	5.1	4.3
4	<LOD	3.3	4.0	4.5	4.1	4.1	3.6	3.7	4.1
5	<LOD	<LOD	3.9	4.3	3.4	3.5	3.3	4.3	3.6
6	<LOD	3.3	4.1	4.1	<LOD	3.7	<LOD	<LOD	4.5
7	2.7	3.8	3.8	3.6	3.8	3.6	<LOD	2.7	3.3
8	2.7	3.1	3.9	3.6	2.7	3.4	<LOD	<LOD	3.6
9	2.7	3.4	3.9	3.1	<LOD	3.4	<LOD	3.9	4.0
10	<LOD	2.7	4.0	3.0	2.7	2.7	<LOD	<LOD	3.3
11	<LOD	3.0	3.6	3.4	0	4.4	<LOD	3.1	3.1
12	2.7	<LOD	3.8	2.7	2.7	3.1	<LOD	<LOD	3.5
13	<LOD	<LOD	3.7	3.9	2.7	3.1	2.7	3.5	3.3
14	2.7	<LOD	3.7	3.7	<LOD	3.4	<LOD	3.4	3.7
15	2.7	<LOD	4.0	3.1	<LOD	2.7	<LOD	3.4	3.9

Table B-5: *Listeria* distribution (mean±SE) on different component of a stationary slicer before and after slicing 15 uninoculated produce.

Produce	***Listeria* population (log CFU/component)**			
	Blade		Pusher	
	Before slicing	After slicing	Before slicing	After slicing
cucumber	4.36	3.02	5.29	3.16
zucchini	4.81	2.82	5.62	3.10
onion	4.40	3.28	4.72	3.32
tomatoes	3.98	3.42	4.33	3.37
potatoes	4.17	3.76	5.07	4.07
radish	4.40	3.48	4.31	2.41
cantaloupe	4.00	1.90	3.77	3.30
apple	3.16	2.36	3.71	1.80
pear	3.47	2.00	4.07	2.61
Gray zucchini	4.39	3.25	4.60	3.39
Sweet potato	4.74	3.67	5.11	3.83

Table B-6: Sequential transfer of *Listeria* during slicing of fresh cut produce.

	***Listeria* population (log CFU/product)**								
	Cucumber			Zucchini			onion		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.9	5.1	5.6	4.3	5.4	6.4	5.5	4.7	4.7
2	4.6	5.6	4.5	4.9	5.6	4.8	4.9	4.2	4.2
3	4.6	4.9	4.6	4.5	4.0	5.0	4.7	4.0	3.4
4	4.9	4.6	5.0	3.3	4.3	5.0	4.4	3.3	3.5
5	4.9	4.8	4.6	4.6	4.6	5.2	4.6	3.3	3.0
6	4.7	4.8	4.8	4.8	3.8	5.5	4.7	2.9	2.9
7	4.5	4.9	5.1	3.9	4.2	5.0	5.0	3.3	2.6
8	4.2	4.6	5.2	4.3	3.8	4.6	4.5	4.1	3.0
9	4.9	5.0	4.8	4.3	3.6	4.5	4.1	2.6	2.6
10	4.7	4.5	4.9	4.5	4.5	5.1	3.7	4.1	2.6
11	4.6	4.7	5.0	3.3	2.9	4.4	4.3	2.6	2.6
12	3.9	4.4	4.9	3.5	4.0	4.3	3.0	2.6	2.6
13	4.4	4.3	5.1	3.3	3.6	5.1	3.6	2.6	2.6
14	4.0	4.3	4.7	4.2	3.2	5.0	2.9	2.9	2.6
15	4.1	4.5	4.7	4.3	3.8	4.9	3.9	2.9	2.6
	Tomatoes			Potatoes			Sweet potatoes		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.6	5.7	5.8	6.1	6.2	6.6	6.0	5.5	6.1
2	5.1	4.9	4.7	5.3	5.9	5.7	5.7	5.3	5.8
3	4.9	4.5	4.7	4.8	5.0	5.3	4.4	4.7	5.0
4	4.8	4.1	4.5	5.1	5.0	5.2	4.6	4.5	4.5
5	4.4	4.4	4.6	4.7	4.7	4.8	4.5	4.3	4.2
6	4.6	4.0	4.1	4.6	4.9	4.9	4.3	4.2	4.0
7	3.6	3.8	4.3	4.7	4.7	5.0	3.7	3.7	3.9
8	3.8	3.8	4.2	4.4	4.3	4.6	3.7	3.9	3.5
9	3.9	3.5	3.8	4.2	4.5	4.9	3.7	3.6	4.3
10	3.4	3.7	4.1	4.2	4.6	4.8	3.7	4.0	3.9
11	3.0	3.0	3.9	4.0	4.0	4.6	4.1	3.5	3.3
12	3.6	3.2	3.9	4.3	3.8	4.3	3.9	3.6	3.7
13	3.7	2.7	3.6	4.1	4.0	4.5	3.0	3.0	3.8
14	3.0	2.7	3.4	3.7	4.3	4.3	3.6	3.9	3.6
15	2.7	<LOD	3.3	3.5	4.4	4.4	3.3	3.8	3.8
	Grey zucchini			Radish			Cantaloupe		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	5.7	5.6	5.7	3.2	2.1	3.8	2.8	2.2	2.7
2	5.3	5.0	5.8	2.0	1.9	3.1	2.9	2.8	2.8
3	5.3	4.9	5.9	1.7	1.9	2.5	3.1	2.3	2.7

Table B-6 (cont'd)

4	5.1	4.7	5.2	1.7	1.9	2.5	2.9	2.1	2.2
5	5.0	5.0	5.1	1.6	2.2	2.4	2.9	2.7	2.2
6	4.9	4.8	5.0	1.5	1.6	2.4	2.8	2.4	1.7
7	4.8	4.8	5.1	1.5	1.3	1.9	2.5	2.1	1.6
8	4.7	4.7	5.0	1.3	1.3	1.5	2.4	2.4	1.6
9	4.6	4.6	5.0	1.2	1.2	1.5	2.4	2.0	1.4
10	4.3	4.5	4.9	1.0	0.3	1.3	2.3	2.0	1.8
11	4.2	4.6	4.9	1.2	1	1.2	2.1	1.9	1.3
12	4.3	4.8	4.7	0.9	1.3	1.0	2.2	1.6	2.2
13	4.1	4.6	4.7	0.7	0.3	0.8	1.9	1.8	1.4
14	4.1	4.6	4.7	0.7	1.7	0.5	1.8	1.7	0.6
15	4.1	4.3	4.7	0.3	0.9	1.0	1.9	1.7	0.9

	Apples			Pears			Carrots		
Uninoculated product	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
1	3.8	4.5	4.6	5.8	4.5	4.3	0	0	0
2	2.6	4.4	3.4	4.1	4.4	4.2	0	0	0
3	<LOD	4.5	3.7	3.6	3.7	4.1	0	0	0
4	<LOD	3.8	3.1	<LOD	3.3	4.0	0	0	0
5	<LOD	3.4	<LOD	<LOD	<LOD	3.9	0	0	0
6	<LOD	3.3	<LOD	<LOD	3.3	4.1	0	0	0
7	<LOD	4.0	<LOD	2.7	3.8	3.8	0	0	0
8	<LOD	<LOD	<LOD	2.7	3.1	3.9	0	0	0
9	<LOD	<LOD	<LOD	2.7	3.4	3.9	0	0	0
10	<LOD	<LOD	<LOD	<LOD	2.7	4.0	0	0	0
11	<LOD	<LOD	<LOD	<LOD	3.0	3.6	0	0	0
12	3.2	<LOD	<LOD	2.7	<LOD	3.8	0	0	0
13	2.6	<LOD	<LOD	<LOD	<LOD	3.7	0	0	0
14	<LOD	<LOD	<LOD	2.7	<LOD	3.7	0	0	0
15	<LOD	<LOD	<LOD	2.7	<LOD	4.0	0	0	0

APPENDIX C:

Survival and Growth of Foodborne Pathogens In Fresh Juice

C-1 Introduction:

The trend toward healthier lifestyles has resulted in increased juice production and consumption at juice bars and in the home. Juices and their preparation areas are at risk for pathogen contamination, leading to health risks. A recent multistate *Salmonella* outbreak was traced to a packaged salad product containing spinach, kale, chard, and carrots (1). Another outbreak of foodborne Hepatitis A was investigated with epidemiologic evidence indicating that frozen strawberries used in smoothies were the source (4). This study addressed how physical properties of common juices affect the ability of foodborne pathogens to grow and survive in different environments. Juices analyzed included orange, apple, pear, cucumber, spinach, carrot, and kale.

C-2 MATERIALS AND METHODS:

C-2.1 Overall experimental design

Juices extracted from retail oranges, apples, pears, cucumbers, spinach, carrots, kale, celery, and kiwi using a Hamilton Beach 67608 Juicer were inoculated with inoculated with 3-strain virulent cocktails of *Listeria monocytogenes, Salmonella,* and *E. coli* O157:H7 to contain ~3 log CFU/ml. Thereafter, juice samples were divided into 50 ml aliquots and stored at 4, 10, and 22°C. Juices were analyzed for *L. monocytogenes, Salmonella,* and *E. coli* O157:H7 daily over a period of 5 days.

C-2.2 Physicochemical characteristics measurements of produce

For all juices, the pH and the soluble solids content (SCC) were measured. The pH was measured by using a HANNA® HI 221 pH meter with a penetration electrode. After the pH reading, the soluble solids content (SSC) was determined by using a handheld optical refractometer Fisher Scientific® refractometer at 20 ºC.

C-2.3 Bacterial strains

Three avirulent *L. monocytogenes* strains - M3 serotype 1/2a (Hly^-, parent strain Mackaness), J22F serotype 4b (Hly^+, purB mariner-based mutant of H7550- Cd^S, parent strain NCTC 10527), and J29H serotype 4b (Hly^-, parent strain NCTC 10527) (obtained from Dr. Sophia Kathariou, North Carolina State University, Raleigh, NC) were used for inoculating the different products. All strains were stored at -80°C in trypticase soy broth containing 0.6% yeast extract (TSBYE) and 10% glycerol, and subjected to two successive transfers (24 h at 37°C) in TSBYE before inoculation. The three avirulent strains were combined in equal volumes and appropriately diluted to obtain populations of ~ 3.0 log CFU/ml for produce inoculation, with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen

Corp., Lansing, MI). Produce juice was inoculated in the *L. monocytogenes* cocktail and storiedat . similarly, a 3-strain *Salmonella* cocktail including *Salmonella* Montevideo MDD22 (tomato outbreak, human isolate), *Salmonella* Poona MDD237 (cantaloupe outbreak, human isolate), and *Salmonella* Newport MDD314 (tomato outbreak, environmental isolate) (Dr. Lawrence Goodridge, Colorado State University, Fort Collins, CO) were used for inoculation. *E. coli* O157:H7.

C-2.4 Microbiological analysis

All juice samples were homogenized by stomaching in 50 ml of phosphate buffered saline (PBS) in a Whirl-pak bag® for 1 min and then quantitatively analyzed for *Listeria* by surface-plating appropriate dilutions on Modified Oxford Agar (MOX). All colonies resembling *Listeria* were counted after 48 h of incubation at 35 °C. *Salmonella* was enumerated by surface-plating appropriate dilutions on trypticase soy agar (BD) containing 0.6% yeast extract (BD), 0.05% ferric ammonium citrate (Sigma) and 0.03% sodium thiosulfate (Fisher Science Education, Hanover, IL) (TSAYE-FS). Plates were incubated at 37°C for 24 h, after which all black colonies were counted as *Salmonella*. *E. coli* O157:H7 was enumerated with Sorbitol MacConkey Agar (SMAC) or SMAC containing cefixine and tellurite.

C-2.5 Statistical analysis

All growth experiments were performed in triplicate. *Listeria*, *E coli*, and *Salmonella* populations were converted to CFU per ml unit and subjected to analysis of variance using JMP 12.0 (SAS Institute Inc., Cary, NC). For all tests, a P value of < 0.05 was considered significant.

13.1.1 C-3 Results:

After 5 days of storage at 10°C, higher pathogen populations were observed in spinach (5.00 - 5.75 log CFU/ml) compared to apple (3.29 - 4.45 log CFU/ml), orange (3.54 - 4.35 log

CFU/ml), pear (3.58 - 4.70 log CFU/ml), and kiwi juice (3.60 – 4.14 log CFU/ml) which reflects the higher pH (Table 1). After 5 days of storage at 10°C, cucumber juice experienced the greatest growth in *Salmonella* population (2.41 log CFU/ml growth) compared to all other juices (0.66 – 1.83 log CFU/ml growth). Growth of *Salmonella* in cucumber, spinach, and carrot juices (1.8 – 2.41 CFU/ml growth) was greater when compared to growth of *Salmonella* in fruit juices including apple, orange, pear, and kiwi (0.66-1.43 CFU/ml growth). The greatest growth of *E. Coli* in juices stored for 5 days at 10°C was observed in carrot juice (2.9 CFU/ml growth) compared to the other juices (0.59 – 2.62 CFU/ml growth). The lowest growth of *E. Coli* was observed in kiwi juice (0.59 CFU/ml growth). *Listeria* growth after 5 days of storage at 10°C was highest for spinach (2.65 CFU/ml growth) compared to the other juices (Figure1). The lowest amount of *Listeria* growth was observed in apple juice (0.38 CFU/ml). Carrot juice did not support growth of *Listeria* which is consistent with previous studies involving whole and cut carrots (2).

Overall, pathogen growth after 5 days of storage at 4°C was lower compared to growth after 5 days of storage at 10°C. At 4°C, the highest growth of *Listeria, E. Coli,* and *Salmonella* was observed in spinach juice (1.16 – 2.36 CFU/ml growth). After 5 days of storage at 4°C, the lowest average pathogen growth was observed in celery juice (0.44 CFU/ml) compared to all other juices (0.68 – 1.87 CFU/ml).

Overall, *Salmonella* grew better than *Listeria* and *E. coli* O157:H7 in the juices studied. All juices were spoiled after 2 days of storage at 22°C. Fresh juices should be refrigerated to inhibit the growth of foodborne pathogens.

Table C-1: Physicochemical Measurements of produce

	Orange	**Apple**	**Pear**	**Cucumber**	**Kale**	**Spinach**	**Carrot**	**Celery**	**Kiwi**
pH	4.07	3.79	4.12	6.09	6.73	6.19	5.81	6.12	3.41
Sugar Content (°Brix)	11.3	12.0	12.7	2.70	3.80	8.00	6.30	3.70	11.8

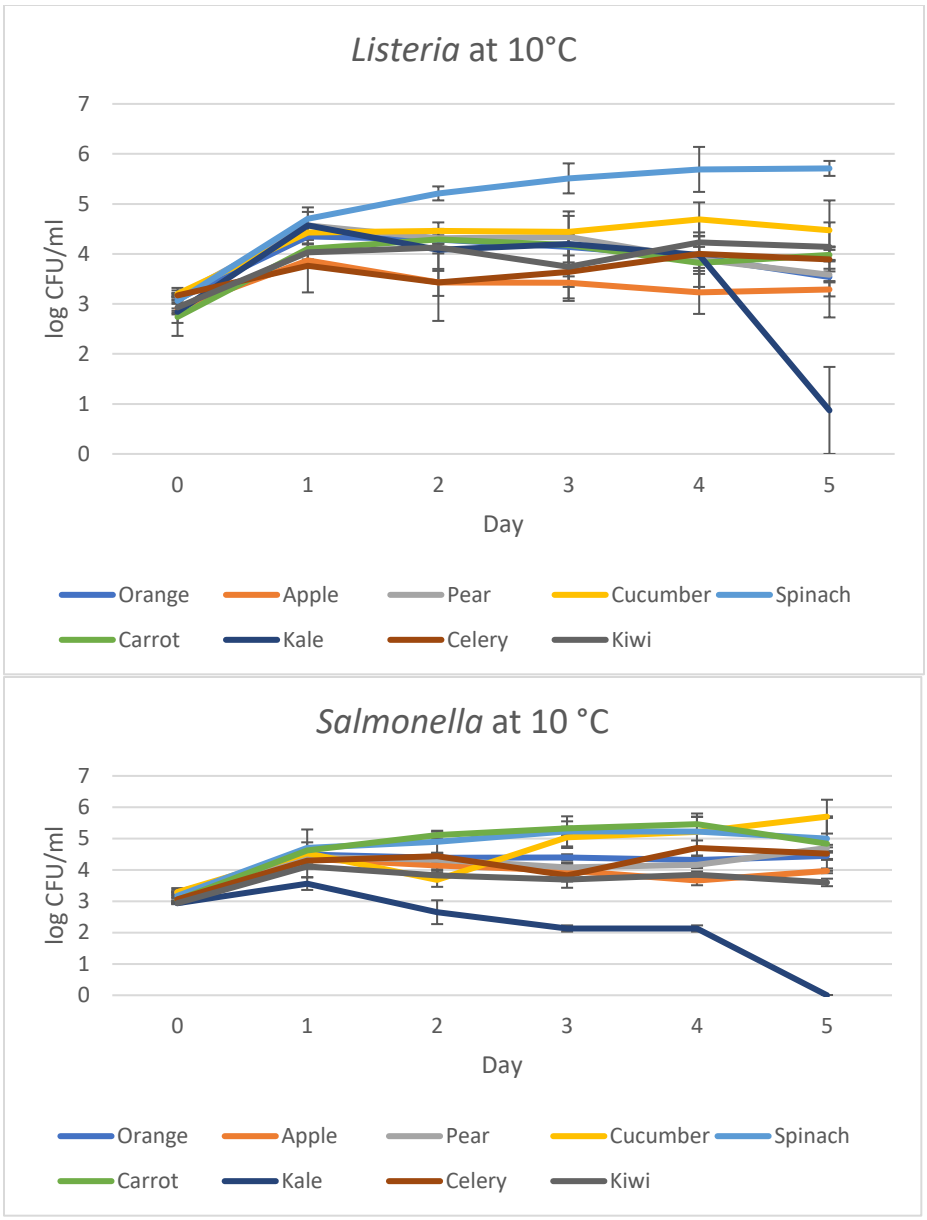


Figure C-1: Pathogen Growth at 4°C and 10°C Over a 5 Day Period. Juices with an asterisk are significantly different ($P < 0.05$).

Figure C-1 (cont'd)

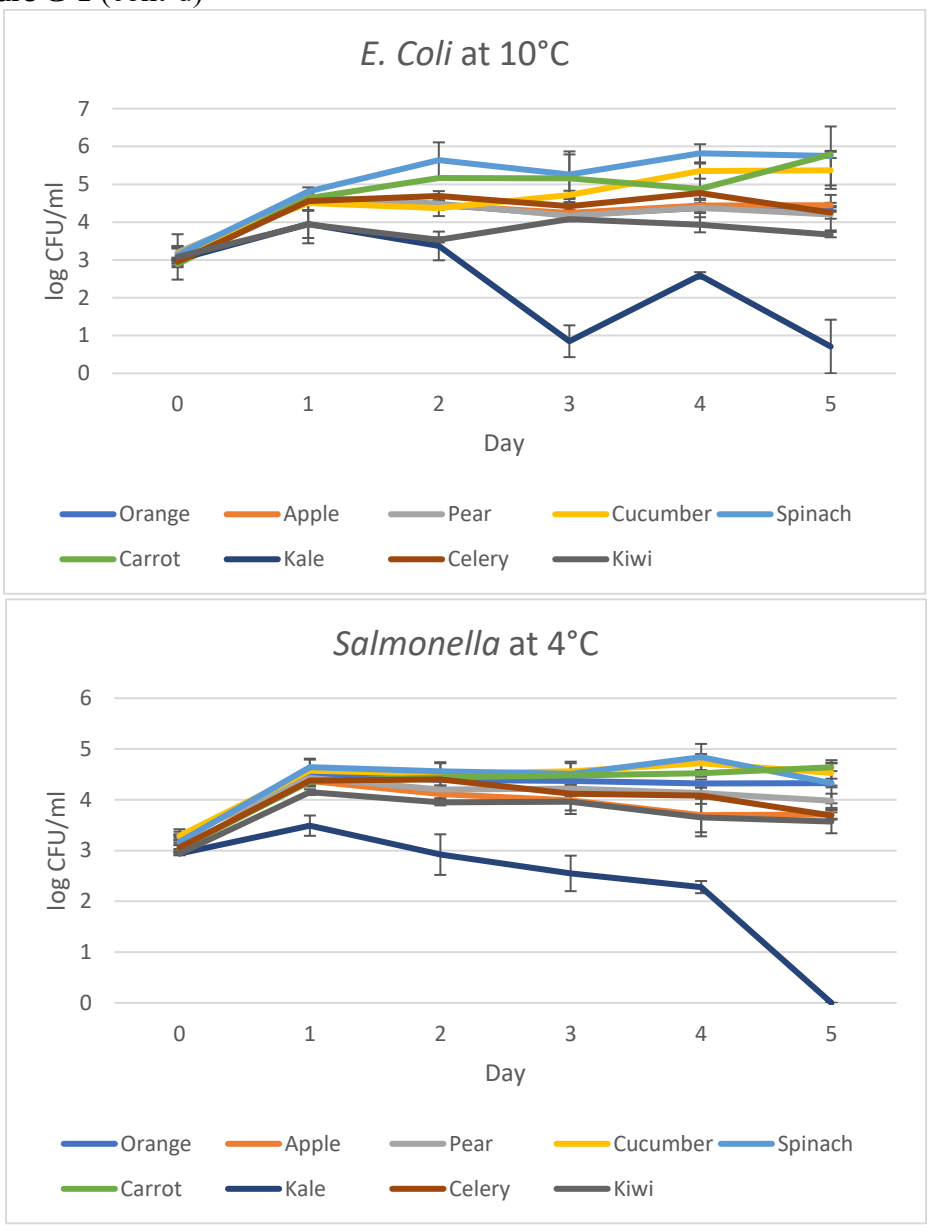
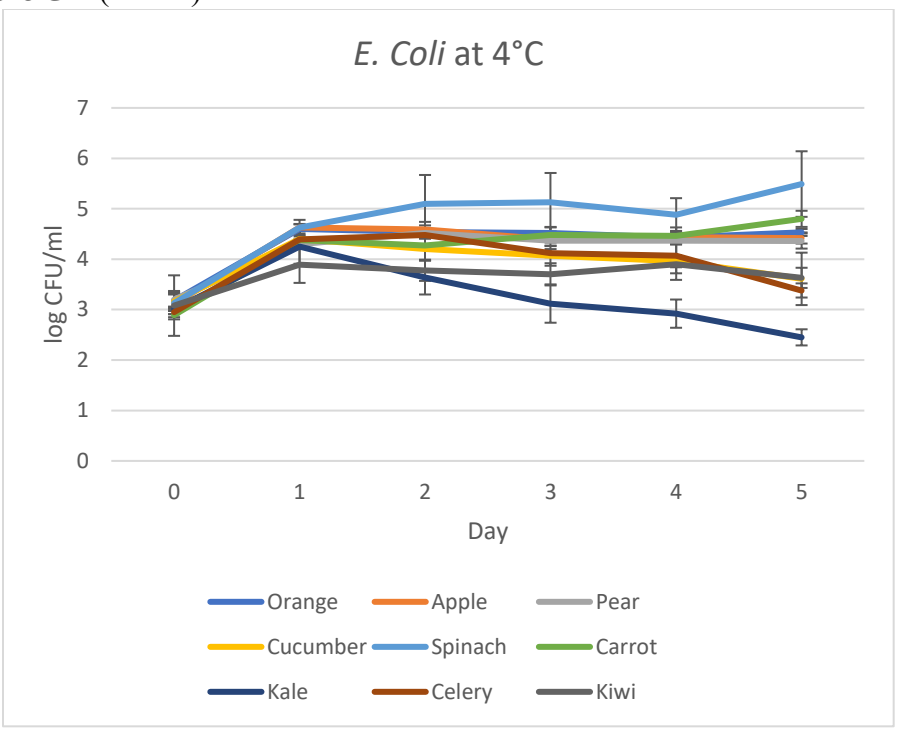
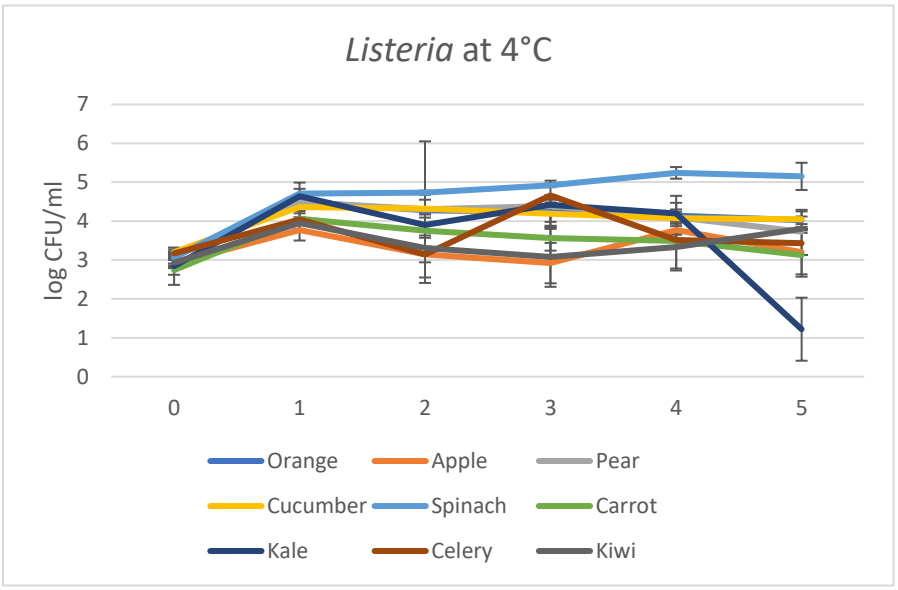


Figure C-1 (cont'd)





C-4 Conclusion:

- Kale supported significantly ($P < 0.05$) less growth than all other juices, suggesting the presence of a naturally occurring antimicrobial agent as has been reported for other cruciferous vegetables (*3*).
- After 5 days of storage at 10°C, higher pathogen populations were observed in spinach (5.00 - 5.75 log CFU/ml) compared to apple (3.29 - 4.45 log CFU/ml), orange (3.54 - 4.35 log CFU/ml), pear (3.58 - 4.70 log CFU/ml), and kiwi juice (3.60 – 4.14 log CFU/ml) which reflects the higher pH (Table 1).
- Carrot juice did not support growth of *Listeria* which is consistent with previous studies involving whole and cut carrots (*2*).
- Overall, *Salmonella* grew better than *Listeria* and *E. coli* O157:H7 in the juices studied.
- All juices were spoiled after 2 days of storage at 22°C.
- Fresh juices should be refrigerated to inhibit the growth of foodborne pathogens.

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