TRANSFER AND SURVIVAL OF *LISTERIA MONOCYTOGENES* DURING SLICING, DICING, AND STORAGE OF ONIONS

By

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ABSTRACT

TRANSFER AND SURVIVAL OF *LISTERIA MONOCYTOGENES* DURING SLICING, DICING, AND STORAGE OF ONIONS

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A series of recalls in 2012 involving diced yellow onions contaminated with *Listeria monocytogenes* has heightened public health concerns surrounding the preparation, handling, and use of raw onions. In response, this study aimed to quantify the extent of *L. monocytogenes* transfer during mechanical slicing and dicing of onions, assess sanitizer efficacy during commercial washing practices, and examine *Listeria* growth in diced onions during storage under different in-package atmospheres. Spanish yellow onions (*Allium cepa*) were inoculated with a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H), air-dried, and sliced or diced using a mechanical hand slicer or commercial onion dicer. Transfer from inoculated onions to sequentially processed uninoculated onions was determined by slicing or dicing inoculated followed by uninoculated onions. Samples were collected and analyzed for *Listeria* by direct plating or enrichment. The drum and rolling dicer blades were of greatest concern for the commercial dicer in terms of *Listeria* transfer, containing greater populations ($P < 0.05$) than the paddles and cross-cut blades after dicing uninoculated onions. *Listeria* transfer during onion slicing and dicing was best described using an exponential decay model, with populations on uninoculated product, sliced after an onion inoculated at 10^8 CFU/onion, decreasing to 10^4 CFU/onion after 10 onions, and populations on uninoculated product, diced after 2.2 kg of onion inoculated at 10^4 CFU/g decreasing to ~ 10 CFU/g after 13 kg. All sanitizer treatments effectively reduced cross-contamination from the wash water while an initial packaging atmosphere of 15% CO_2, 5% O_2, 80% N_2, reduced ($P < 0.05$) growth of *Listeria* during storage.

To my fiancée Emily and my Parents

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KEY TO SYMBOLS AND ABBREVIATIONS

°C	Celsius
cb	confidence band
CFU	colony forming unit
cm	centimeter(s)
d	day(s)
g	gram(s)
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
kg	kilogram(s)
lb	pound(s)
mL	milliliter(s)
min	minute(s)
MOX	Modified oxford agar
NRMSE	Normalized Root Mean Squared Error
PBS	phosphate buffered solution
PLA	Polylactic acid
ppm	parts per million
rpm	revolutions per minute
RTE	Ready-to-eat
s	second(s)
TSAYE	Trypticase soy agar containing 0.6% yeast extract

TSBYE	Trypticase soy broth containing 0.6% yeast extract
TSBYE-FE	Trypticase soy broth containing 0.6% yeast extract (w/v), 0.1% esculin (w/v), and 0.05% ferric ammonium citrate (w/v)
UV	Ultraviolet

Chapter 1

Introduction

1.1. Foodborne Illness in the United States

Foodborne illness continues to pose a major public health concern in the United States, with the Center for Disease Control estimating that 48 million cases of illnesses are acquired each year (Scallan et al., 2011) at a cost of ~ $77 billion (Scharff, 2012). Among the 31 recognized bacterial causes of foodborne illnesses, *Listeria monocytogenes* is the third leading cause of deaths, 255 annually, with an economic burden of $2 billion due to the high cost associated with its treatment (Scharff, 2012). Despite increasing food safety efforts by the U.S. government, illnesses causing decreased economic productivity and quality of life continue to occur.

1.2 Minimally Processed Vegetable Industry

As a part of a healthy diet, vegetables are produced in increasingly large quantities in the United States. The onion industry has grown dramatically, with U.S. annual consumption increasing from 12.2 to 20 lbs over the last 30 years and an estimated 20% of onions being processed by the fresh-cut industry (The National Onion Association, 2011). Guidelines for the processing of many different vegetables including dry bulb onions have been established to assist processers in safe practices. However, numerous recalls of raw or minimally processed fruits and vegetables due to contamination by bacterial pathogens have prompted a revision of current regulations related to the safety of fresh produce. Most notably, the Food Safety Modernization Act has placed an increased responsibility for safe food on producers, in addition to increasing the authority of government bodies to hold producers accountable.

1.3 Justification

Onion bulbs are a low acid, high carbohydrate vegetable that may support the growth of various bacteria, yeasts, and molds (Kowalska & Smolinska, 2014). Because of their nutritive value and strong flavor, onions are commonly used in a wide range of cuisines. Widespread use of onions as a food ingredient has led to an increase in production with onions now ranking third among vegetables in the U.S.

The safety of fresh produce is a topic of increasing concern as recalls and outbreaks continue to negatively affect consumer confidence. In addition to leafy greens, cantaloupe, and sprouts, multiple large recalls over the past 7 years involving a total ~22,000 kg of diced onions have caused increased concern over the safety of fresh-cut onions (Food Safety News, 2012; CDPH, 2007). *Listeria monocytogenes* is a common microbial contaminant of food processing facilities that is difficult to remove once colonization has occurred (Tompkin, 2002). Several key characteristics making *Listeria* a concern are its growth at refrigeration temperatures, tolerance of harsh environmental conditions, and its strong attachment to many types of food contact surfaces.

Much research has documented the ability of multiple pathogens to cross-contaminate large quantities of product during slicing, dicing, shredding, and grinding of different foods, including deli meat (Vorst et al., 2006; Lin et al., 2006; Sheen, 2011; Pérez-Rodríguez et al., 2007), lettuce (Buchholz et. al.,2012; Nou, 2010; Beuchat & Brackett, 1990), tomatoes (Chen et al., 2014), celery (Kaminski et al., 2014), and beef (Gilbert, 1969; Flores & Tamplin, 2002). Increased understanding of bacterial transfer is now aiding in the development of scientifically based risk assessments that will guide the decision-making process.

1.4 Objectives

This study, which was designed to fill some critical knowledge gaps surrounding the safety of fresh-cut onions, was split into three main objectives, with the first being to further the understanding of bacterial transfer during onion slicing. This objective aimed to quantify the transfer of *L monocytogenes* during simulated slicing of onions. This was accomplished by slicing dip-inoculated onions, with follow-up sampling of the various equipment surfaces. Thereafter, transfer from inoculated to uninoculated onions was evaluated by slicing or dicing dip-inoculated onions, sampling sequentially sliced or diced uninoculated onions, followed by retrieving samples from the various equipment surfaces. This information was used to compare the extent of contamination at various locations on the equipment before and after processing uninoculated onions in addition to evaluating transfer.

The second objective aimed to 1) quantify the transfer of *L. monocytogenes* during simulated commercial dicing of onions, and 2) assess the efficacy of three popular chemical sanitizers against *Listeria* during washing of diced onions. This work was accomplished by dicing dip-inoculated onions followed by 2 min of washing in a flume tank with samples of water and diced onions collected every 20 s. The onions then passed over a dewatering shaker table, with samples taken for *Listeria* analysis. After processing, surface samples were collected from the flume tank and shaker table to evaluate *Listeria* transfer to the surfaces in the presence of sanitizers.

The third objective, to evaluate the effect of sanitizer and in-package atmosphere on the growth of *Listeria* in diced yellow onions, was performed to assess the potential for growth of *Listeria* during storage. Yellow onions were diced, dip-inoculated, air-dried, washed for 2 min in one of four sanitizer treatments, centrifugally dried, and then packaged under one of three

atmospheres. These results will be useful to processors interested in increasing end product safety during refrigerated storage.

Chapter 2

Literature Review

2.1. *Listeria monocytogenes*

Listeria monocytogenes is a bacterial foodborne pathogen commonly found on raw foods and food contact surfaces in food processing facilities. The ability of *Listeria* to survive in unforgiving environments with attachment to surfaces of various compositions, in addition to a high lethality rate, led to the enactment of a ‘‘zero tolerance’’ policy for *L. monocytogenes* in ready-to-eat (RTE) foods in the United States. The presence of *Listeria* in fresh produce has become increasingly evident in recent years resulting in large, multi-state outbreaks and recalls.

2.1.1. Characteristics of *Listeria*

The genus *Listeria* consists of six species of gram positive, non-spore forming, short rod-shaped bacteria. *Listeria monocytogenes* has been studied extensively as it is a human foodborne pathogen (Wurtzel et al., 2012). *Listeria monocytogenes* is also a psychrotroph. Hence, the ability *L. monocytogenes* to grow at refrigeration temperatures combined with its high tolerance to salt, acid, and low-moisture environments have caused many concerns among food processors.

2.1.2. Listeriosis

While it is estimated that 5-10% of humans are carriers of *Listeria*, the disease caused by *Listeria* infection, termed listeriosis, is a severe illness generally limited to high risk groups (Farber, 1991). Immunocompromised adults, pregnant women, and neonates are at greatest risk of illness. Listeriosis is uncommon with approximately 1600 illnesses resulting in approximately 250 fatalities reported each year in the United States (Scallan et al., 2011). Costs associated to the treatment and long term effects of listeriosis are estimated at approximately $2 billion annually (Scharff, 2012).

2.2. Food safety guidelines

Government enactment of food safety guidelines has continually evolved since the passing of the Federal Food, Drug, and Cosmetic Act in 1938. Since 1938, several key laws have been passed, with the goal of improving the safety of the food supply, including the Food Additive Amendment in 1958, Pathogen Reduction/Hazard Analysis and Critical Control Point (HACCP) Systems in 1996, and most recently the Food Safety Modernization Act. With each amendment or new ruling, more emphasis has been placed on monitoring and government intervention (FDA, 2011). General guidelines have been developed which commonly cover process control similar to Good Manufacturing Practices (GMPs) regarding the inclusion of sanitizers where appropriate and segregation of raw and ready-to-eat product.

2.2.1. "Zero-tolerance"

Listeria has led to the recall of many products since 1981 when it was first recognized as a foodborne pathogen in an outbreak involving contaminated cabbage that was made into coleslaw (Schlech, 1983). The low infective dose and high mortality rate associated with listeriosis led to the current "Zero-tolerance" policy in the United States which requires any ready-to-eat product found to contain *Listeria* in a 25 g sample to be considered adulterated and necessitates its removal from the market.

2.2.2 Food Safety Modernization Act

The Food Safety Modernization Act is now placing responsibility for verifying appropriate preventative steps performed by food processors on the processor instead of the government. These updated laws are intended to move the safety of the U.S. food supply to the forefront and reduce the economic burden of foodborne illness.

2.3. *Listeria* presence in onions

The incidence of *Listeria* in fresh produce is generally considered to be very low with multiple studies failing to identify *Listeria* on onions in retail markets (Kaneko et al., 1999). Various salads containing onions have been contaminated by *Listeria* with no direct link to the onions (Sizmur & Walker, 1988). Onions can become contaminated through contact with soil and food processing surfaces. The possible presence of *Listeria* in food processing facilities is well documented and must not be overlooked (Beuchat & Brackett, 1990; Aguado, 2004), as evidenced by two recent recalls of diced onions due to contamination by *Listeria* supporting the possibility of contamination during processing (Food Safety News, 2012; CDPH, 2007).

2.4. *Listeria* recalls

The presence of *Listeria* in food processing environments is a cause of significant product recalls. The cost to the food industry is estimated at $1.2 – 2.4 billion dollars annually (Ivanek et al., 2004).

In 2007, one recall of yellow diced onions from a major producer resulted in ~20,000 kg being removed from the market due to contamination by *L. monocytogenes* (CDPH, 2007). A second recall issued in 2012 involved more than 907 kg of diced onions from another major producer. Due to the widespread use of onions as an ingredient in many ready-to-eat foods, this second recall led to eleven additional recalls across the United States and Canada, all without incident (FDA, 2012) where the contaminated diced onions were sold and used as an ingredient in a wide range of other products including sandwiches, salads, and stews among other products. During follow-up investigations, the implicated strain of *L. monocytogenes* was recovered from multiple sites within the processing facility, including a chute under an inspection table, a shroud

on a peeling machine, and the loading hopper of a peeling machine (CDPH, 2012). However, onions have not yet been linked to outbreaks involving *Listeria*.

2.5. Characteristics of onions

The onion (*Allium cepa*) is a vegetable that stores large quantities of carbohydrates in a bulb also rich in flavonols (Rodríguez Galdón et al., 2008). Onions have low acidity with a pH of 5.5 (Anon, 1962; Bridges & Mattice, 1939).

The suitability of onions as a growth substrate for microorganisms is well known. The low acidity of onions in addition to the high concentration of soluble solids provides a suitable growth environment for bacteria, yeasts, and molds that commonly infect onion bulbs causing spoilage (Kowalska & Smolinska, 2014) with *Listeria* reportedly able to grow in onion slices when stored 14 d at 10°C (Farber et al., 1998).

2.5.1. Antimicrobial activity of onions

Onions are an important source of flavonols (e.g., quercetin) which possess some antimicrobial activity and act as antioxidants (Rodríguez Galdón et al., 2008; Santas et al., 2010; Hertog et al., 1992). Onion extract was shown to possess antimicrobial activity using the disk diffusion assay (Ramos et al., 2006) with minimum inhibitory concentrations for various bacteria ranging from 100 - >1,000 ppm in presence of a surfactant (Kim et al., 2004); however, at concentrations and conditions encountered in common food matrices, microbial inhibition is limited.

2.5.3. Shelf life of onion

Once sliced, onions have a shelf life of approximately 14 d at 4°C before becoming unacceptable to the average consumer (Gills Onions, 2010). During this time quality decreases as color moves from white to yellow and the odor changes from fresh to sour as the titratable acid increases (Miguel & Durigan, 2007). Degradation is caused in part by the normal respiration of

onion cells and use of onion tissue as a growth substrate by spoilage microorganisms. Temperature control throughout the cold-chain is crucial to maintain the shelf life of onions by reducing the respiration rate and growth of microorganisms (Nunes et al., 2009).

2.6. Minimal processing of fresh produce

Produce that is sliced, diced, shredded, or similarly prepared for consumption without further preparation is known as minimally processed, or fresh-cut. The introduction of fresh-cut produce has greatly expanded the marketability of fresh produce with products appealing to a wide range of consumers. The fresh-cut market has grown consistently, increasing from 1% in 1987 to 15% in 1997 of total produce sales in the U.S. (Dimitri et al., 2003). Demand for fresh-cut products has continued with an 8% increase in fresh-cut vegetable sales and 20% of annual onion production being consumed in fresh-cut processes (The National Onion Association, 2010; Fresh-Cut, 2008).

2.6.1. Fresh-Cut onions

Commercial onion processing begins with "topping and tailing" where the root and sprout ends of the onion bulb are simultaneously removed by blades either before or after the outer skin is peeled (Rani & Srivastava, 2012). Once peeled, onions are commonly washed in chlorine (50-200ppm), and conveyed to a slicer or dicer (Park, 1994). In contrast to diced onions which are washed again, sliced onions are packaged directly without washing.

2.7. *Listeria* transfer

Listeria exhibits strong attachment to various surfaces commonly found in food processing and foodservice operations, most importantly stainless steel, plastic, wood, and glass (Beresford et al., 2001; Stepanović et al., 2004; Wang et al., 2009). Routine cleaning methods have been found to be less than sufficient in removing bacteria, resulting in colonization and

resident *Listeria* populations being identified in processing facilities (Tompkin, 2002; Ferreira et al., 2014). *Listeria* colonization of food-processing environments has led to the same strain being found multiple times over a period of years (Holah et al., 2002).

The presence of *Listeria* in processing environments raises concern regarding transfer between products and surfaces. The potential for such transfer has been well documented in many studies across the food processing industry.

2.8. Sanitary equipment

It is fundamental that equipment used in processing of foods be easily disassembled and sanitized. The need to properly engineer and design readily cleanable and sanitizable equipment has become increasingly apparent with substantial progress now being made (Bilgili, 2006). The ability for processing equipment to be effectively sanitized is crucial for the entire food industry with extreme importance given to manufacturers of ready-to-eat products such as fresh produce which will not undergo additional processing to inactivate pathogens. Manufacturers of equipment used to slice and dice fresh produce have made few changes to the design of equipment since the introduction of automated, commercial scale, equipment.

2.9. Sanitizer use

The use of sanitizers during processing of fresh produce has been a topic of research topic for many years (Gil et al., 2009). Many sanitizers exist with chlorine, peroxyacetic acid, and quaternary ammonia being widely used. More recent sanitizers include electrolyzed water and chlorine dioxide. All sanitizers have various benefits and limitations which are important to evaluate prior to their use in a specific application.

2.9.1. Chlorine-based sanitizers

Chlorine-based sanitizers have been used for many years to reduce microbial populations in various applications including drinking water, food, and surfaces. Chlorine has a high oxidative potential making it very effective at inactivating microorganisms; however, it also reacts strongly with organic matter greatly reducing efficacy (Shen, 2013). Chlorine-based sanitizers are most effective at pH values of 6.5 or below where HOCl is more abundant (White, 1986). Because sodium chlorite has a pH of ~8.5, chlorine-based sanitizers are often pH adjusted to pH 6.5 using weak acids, commonly citric acid.

2.9.2. Peroxyacetic acid-based sanitizers

Peroxyacetic acid sanitizers are being used in applications which involve high organic loads. Research has shown peroxyacetic acid to have increased efficacy in inactivating microorganisms over other commonly used sanitizers (Wang & Ryser, 2014; Al-Nabulsi et al., 2014). The USDA has set a maximum limit of 80 ppm for peroxyacetic acid.

2.9.3. Electrolyzed water

Electrolyzed water is produced when an electrical current is passed through water containing NaCl. Use of electrolyzed water as a sanitizer has been explored for various commodities including beef (Jadeja & Hung, 2014), seafood (Wang et al., 2014), and vegetables (Rahman et al., 2012). Electrolyzed water has gained attention due to its relatively low cost and ease of production.

2.9.4 Chlorine dioxide

Chlorine dioxide is a water soluble gas which is known to inactivate microbial populations commonly found on fruits and vegetables. Studies have shown efficacy on product surfaces and in wash water during processing of grapes (Celikkol & Turkben, 2012) and bay red

chard leaves (Tomás-Callejas et al., 2012). Use of chlorine dioxide has been limited due to its low solubility and the hazards associated with on-site generators.

2.10. Modified atmosphere packaging

The rapid degradation of fresh-cut produce has prompted much research to determine improved packaging methods. An important improvement in packaging design is the inclusion of a modified atmosphere which can greatly increase the shelf life (Blanchard et al., 1996). The research required to assess optimum atmospheres for storage can be costly; hence, it is often limited to products of high economic value such as fruits and vegetables of high economic importance. It has also been shown that pathogens may have increased survival in products that have been sliced, diced, or damaged in some way (Gleeson & O'Beirne, 2005), leading to a need for microbial control.

2.10.1. Presence of super atmospheric oxygen in-package atmosphere

Survival of microorganisms in the presence of oxygen is reliant upon the presence of catalase which prevents oxygen from reacting with cell membrane components. The absence of catalase in many microorganisms suggests that high concentrations of oxygen can potentially be used as a means to limit microbial growth in foods. Jacxsens et. al.(2001) showed *Listeria* to have a prolonged lag phase when grown in an atmosphere containing 95% oxygen. Use of super atmospheric oxygen has been shown to increase shelf-life as a result of reduced yeast and bacterial growth in various products (Jacxsens et al., 2001; Lee et al., 2011).

2.10.2 Presence of carbon dioxide in atmosphere

Survival of microorganisms in the presence of high levels of CO_2 is reliant upon the use of fermentation pathways or alternatives to oxygen as a final electron receptor in the electron

transport chain. The use of packaging atmospheres containing high levels of CO_2 has been shown to reduce microbial growth (Amanatidou et al., 1999).

Chapter 3

Transfer of *Listeria* monocytogenes During Mechanical Slicing of Onions

3.1. MATERIALS AND METHODS

3.1.1. Experimental design

Three avirulent (M3, J22F, J29H) and five virulent strains (R2-499, N3-008, N3-031, J1-110, J1-177) of *L. monocytogenes* were first assessed for attachment, growth characteristics, and survival on onions during storage. Thereafter, transfer of the three avirulent strains from one inoculated to multiple uninoculated onions during mechanical slicing was quantified by direct plating, with these findings then subjected to mathematical modeling to predict the extent of transfer.

3.1.2. Bacterial strains

Three avirulent *L. monocytogenes* strains (M3 1/2a3, J22F 4b, and J29H 4b) obtained from Dr. Sophia Kathariou (North Carolina State University, Raleigh, NC) were used in all slicing experiments. In addition, five virulent strains (R2-499 1/2a deli turkey 2000, N3-008 4b coleslaw outbreak 1981, N3-031 1/2a hot dog 1988, J1-110 4b Mexican style cheese 1985, J1-177 1/2b clinical isolate 1997) previously obtained from Dr. Catherine Donnelly (University of Vermont, Burlington, VT) were used to compare attachment, growth, and survival of the avirulent strains. All cultures were stored at -80°C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. 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°C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35°C transfers in TSBYE. When used as cocktails, the three avirulent strains were combined in equal volumes and appropriately diluted to obtain populations of ~ 8.9, 6.4, or 5.3 log CFU/ml for onion

inoculation, with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI).

3.1.3. Onions

Over 4 months, multiple lots of Spanish yellow onions (*Allium cepa*), weighing 275 ± 50 g, were purchased from a local supplier (Stan Setas Produce Company LLC, Lansing, MI) with each lot stored at 4°C for no more than 7 d before use. The root and sprout ends of each onion were removed using a sterile kitchen knife. After hand peeling the outer skin, the onions were tempered to room temperature (23°C ± 2°C) and weighed prior to slicing.

3.1.4. Attachment, growth, and survival

Attachment was assessed using the microtiter plate assay (Stepanovich et al., 2000). Triplicate wells of a 96 well untreated polystyrene microtiter tissue culture plate (Flat Bottom BD Falcon, Franklin Lakes, NJ) were separately filled with the virulent and avirulent strains serially diluted in sterile TSBYE to ~10^3 CFU/ml with three wells containing TSBYE alone serving as negative controls. Following 48 h of incubation at 20°C, the wells were emptied, rinsed 3 times with sterile 0.1% Phosphate Buffered Solution to remove unattached cells, and air dried. Methanol (Fisher Chemicals, Fair Lawn, NJ) was then added to fix the attached cells followed by 200 µl of 2% Crystal Violet (Remel, Lenexa, KS) for staining. After emptying, thoroughly rinsing and treating the wells with 200 µl of glacial acetic acid 33% (v/v) (Sigma Chemical Company, St. Louis, MO) to solubilize the crystal violet, cellular adherence was quantified by measuring the optical density at 550 nm with a microplate reader (Bio-Tek Instruments Inc. Model SIAFR, Winooski, VT).

Growth was assessed by inoculating 200 ml of TSBYE with ~10^4 CFU in triplicate (3 flasks/ culture). After 0, 3, 6, 9, 12, and 24 h of incubation at 35°C, 1 ml aliquots were

appropriately diluted and plated on TSAYE containing esculin 0.1% (w/v) and ferric ammonium citrate 0.05% (w/v) (TSAYE-EF) with the plates counted after 24 h of incubation at 35°C. Generation times were determined using values obtained during logarithmic growth according to Eq. (1):

$$G = \frac{t}{3.3 \log {}^{b}/_{B}} \qquad (1)$$

where t = time in minutes, b = *L. monocytogenes* population at 3 h, and B = *L. monocytogenes* population at 9 h.

Survival was assessed by dip-inoculating whole peeled onions with either the 3-strain avirulent or 5-strain virulent *L. monocytogenes* cocktail, ~ 6.4 log CFU/ml, for 2 min to achieve ~ 10^6 CFU/onion, followed by air-drying in a biosafety cabinet for 90 min. Initially, and after 4, 24, 48, 120, and 168 h of storage at 4°C, three onions were placed in individual Whirl-pak bags containing 100 ml of PBS, hand rubbed for 90 s, appropriately diluted, and plated on MOX to quantify *Listeria*.

3.1.5. Onion slicer

A manual onion slicer (NEMCO model 56750-2, Hicksville, IN) yielding nine 0.5 cm thick slices was used for all experiments. In order to identify the product contact areas on the slicer for subsequent sampling, Glo Germ (Glo-Germ Company, Moab, UT) was used as reported previously (Vorst 2006, Buchholz 2012). One onion was fully submerged 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). Using this procedure, two 100 cm^2 product contact areas of the slicer – the pusher plates and blades- were identified for subsequent sampling (Figure 3.1).

3.1.6. Sampling protocol

Initially, the distribution of *L. monocytogenes* on the different onion slices was determined by dip inoculating one onion to achieve ~ 8.9 log CFU/onion, slicing the inoculated onion, adding each of the 8 to 9 slices to an individual Whirl-pak bag, and enumerating each on MOX. Following the inoculated onion, one uninoculated onion was then sliced, sampled, and enumerated similarly. Based on the statistical similarity, slice 1, 4, and the last slice of at least half the full thickness were composited for testing in the following slicing experiments.

3.1.7. *Listeria* transfer during slicing

Whole peeled onions were immersed in the 3-strain avirulent cocktail, ~8.9, 6.4, or 5.3 log CFU/ml, for 2 min and then air-dried in a biosafety cabinet for 90 min, giving populations of ~8.6, 6.8, or 5.9 log CFU/onion. Inoculation levels were confirmed by placing two inoculated onions into separate Whirlpak bags containing 100 ml of University of Vermont Medium (UVM, Neogen), hand rubbing for 60 s, and examining for *Listeria*.

Transfer of *L. monocytogenes* to the slicer was assessed by slicing one inoculated onion, after which 100 cm^2 areas of the pusher plates and blades were disassembled and then sampled using the 1-ply composite tissue method (Vorst et al., 2004). Transfer of *L. monocytogenes* was assessed by slicing one inoculated onion immediately followed by 20 uninoculated onions. The sprout, middle, and root end slices from each onion were composited and examined for numbers of *Listeria*. After slicing 20 uninoculated onions, the pusher plates and blades were disassembled with the 100 cm^2 areas again sampled using the 1-ply composite tissue method.

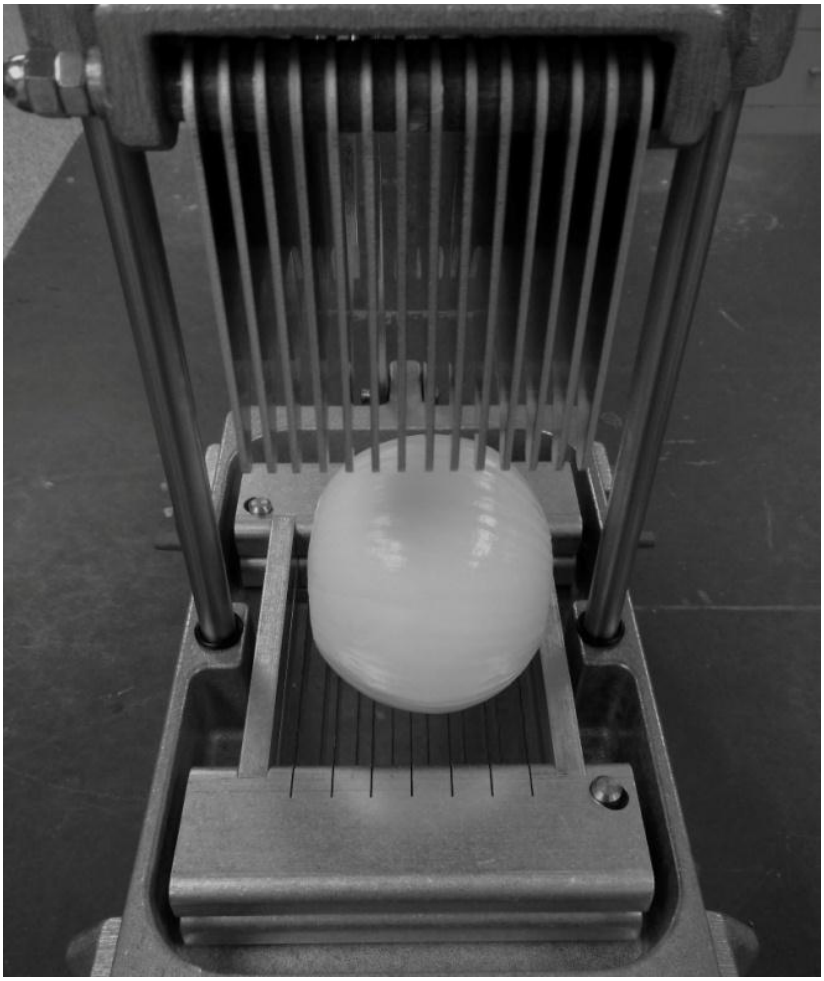


Figure 1: Manual Onion Slicer: A) Pusher plates, B) Blades

All slicing experiments were conducted in triplicate. After each experiment, the slicer was disassembled, washed with a brush to physically remove debris, and then sanitized with 200 ppm Quorum V (Ecolab, Saint Paul, MN), rinsed in tap water, and sprayed with 70% ethanol followed by drying in a biosafety cabinet under UV light for 10 min before reassembly.

3.1.8. Microbial analysis

All samples were added to Whirl-pak bags containing 50 ml of UVM, homogenized by stomaching (Stomacher 400 Circulator, Seward USA, Davie, FL) for 1 min at 300 rpm, appropriately diluted in PBS, and plated, with or without prior filtration (0.45 µm filter) using a vacuum pump (Model E46046, EMD Millipore Corporation, Billerica, MA), on MOX with the UVM-diluted samples incubated at 35^{o}C. All plates were examined for typical *Listeria* colonies after 48 h of incubation at 35°C. After slicing and enumeration in the first of three experiments, multiple colonies were selected and confirmed as *Listeria* using the Neogen Reveal® 2.0 *Listeria* test kit (Neogen). When the MOX plates were negative for *Listeria*, the UVM enrichments were streaked to plates of MOX, incubated at 35°C for 48 h, and then examined for the presence or absence of *Listeria*.

3.1.9. Statistical analysis

All experiments were performed in triplicate. *Listeria* populations were reported as log CFU/onion ± SE or log CFU/100 cm^2 ± SE for the inoculated and uninoculated onions, or surface samples, respectively. The Listeria population/onion was determined as in Eq. (2):

$$S = P \times w \qquad (2)$$

where S is the theoretical sample population (log CFU/onion), P is the known population (log CFU/g), and w is the weight in grams of the whole onion. Samples positive by enrichment were assumed to contain 1 CFU. Surface samples negative by enrichment were assumed to contain 0.5

CFU. Onion samples negative by enrichment were not included in the *Listeria* transfer analysis. The Tukey-Kramer HSD test was performed using JMP 10 (SAS Institute Inc., Cary, NC). Statistical significance was set at $P < 0.05$.

3.1.10 Evaluation of transfer model.

An exponential decay model from a previous study (Sheen and Hwang, 2010) was used to describe the *L. monocytogenes* transfer pattern during onion slicing. The model used to fit the data is shown in Eq. (3):

$$Y = A \cdot e^{X/B} \qquad (3)$$

Where Y (dependent variable) is the log CFU/onion transferred and X (independent variable) is the number of the specific uninoculated onion that was sliced. A and B are transfer model parameters. The above equation was fitted using the *nlinfit* algorithm of MATLAB (R2012a, MathWorks, Natick, MA). The estimated parameters, normalized root mean squared errors (NRMSE) of the model, shown in Eq. (4), and asymptotic 95% confidence intervals of the parameters were then estimated.

$$NRMSE = \frac{RMSE}{(Ymax - Ymin)} \qquad (4)$$

Where NRMSE is the normalized root mean squared error, RMSE is the root mean squared error, Ymax is the maximum value taken by Y, and Ymin is the minimum value taken by Y.

3.2. RESULTS

3.2.1. *Listeria* attachment, growth, and survival

In the microtiter plate assay, avirulent strain M3 exhibited significantly greater attachment (average OD of 0.62) ($P < 0.05$) compared to the remaining seven strains (average OD of 0.11 – 0.13). All strains showed similar doubling times, excluding M3 which had a slower doubling time, with generation times of 50.9 ± 1.7, 33.7 ± 1.0, 32.6 ± 0.6, 36.2 ± 0.8, 33.5 ± 0.3,

39.1 ± 1.3, 40.9 ± 3.0, and 36.8 ± 0.7 min for M3, J22F, J29H, R2-499, N3-031, N3-008, J1-110, and J1-177, respectively. The survival of *L. monocytogenes* on whole peeled onions was similar for both the avirulent and the virulent cocktails, with average populations of 5.2 ± 0.3 and 5.6 ± 0.2 log CFU/onion after 7 d of storage, respectively (Table 3.1). Avirulent *Listeria* populations decreased significantly ($P > 0.05$) after 7 d storage at 4°C.

3.2.2. *Listeria* distribution on onion surface

After slicing one inoculated onion, two uninoculated onions were sliced with *L. monocytogenes* recovered from individual slices and the populations of each onion compiled (Figure 3.2). Estimated *Listeria* populations per onion were not significantly different when the first, fourth and last slices were composited, as compared to sampling each individual slice.

3.2.3. *Listeria* transfer to the slicer

After slicing one inoculated onion, *L. monocytogenes* was recovered from the pusher plates and the blades (Figure 3.3). The pusher plates yielded average *Listeria* populations of 4.8 ± 0.6, 2.6 ± 0.3, and 0.8 ± 0.3 log CFU/100 cm^2 after slicing a single onion inoculated to contain ~ 8.6, 6.8, or 5.9 log CFU/onion, respectively. Numbers of *Listeria* recovered from the blades averaged 5.6 ± 0.5, 3.1 ± 0.5, and 2.9 ± 0.1 log CFU/100 cm^2 at inoculation levels of ~ 8.6, 6.8, or 5.9 log CFU/onion, respectively. When the slicer components were sampled after slicing 20 uninoculated onions, the pusher plates retained 2.9 ± 0.3, 1.3 ± 0.5, and -0.1 ± 0.4 log CFU/100 cm^2 and the blades retained 3.0 ± 0.1, 1.5 ± 0.2 , and 0.4 ± 0.3 log CFU/100 cm^2 at inoculation levels of ~ 8.6, 6.8, or 5.9 log CFU/onion, respectively.

Table 1: Populations (log CFU/onion) of *Listeria* on whole peeled onions during 7 d of storage at 4°C.Populations within each strain were analyzed for changes during storage; values with different letters are significantly ($P < 0.05$) different.

Listeria strain	Day 0	Day 1	Day 2	Day 5	Day 7
Avirulent	6.5 ± 0.1^{a}	6.3 ± 0.1^{a}	5.9 ± 0.1^{a}	5.6 ± 0.2^{a}	5.2 ± 0.3^{b}
Virulent	6.1 ± 0.1^{a}	6.1 ± 0.2^{a}	5.7 ± 0.3^{a}	6.1 ± 0.1^{a}	5.6 ± 0.2^{a}

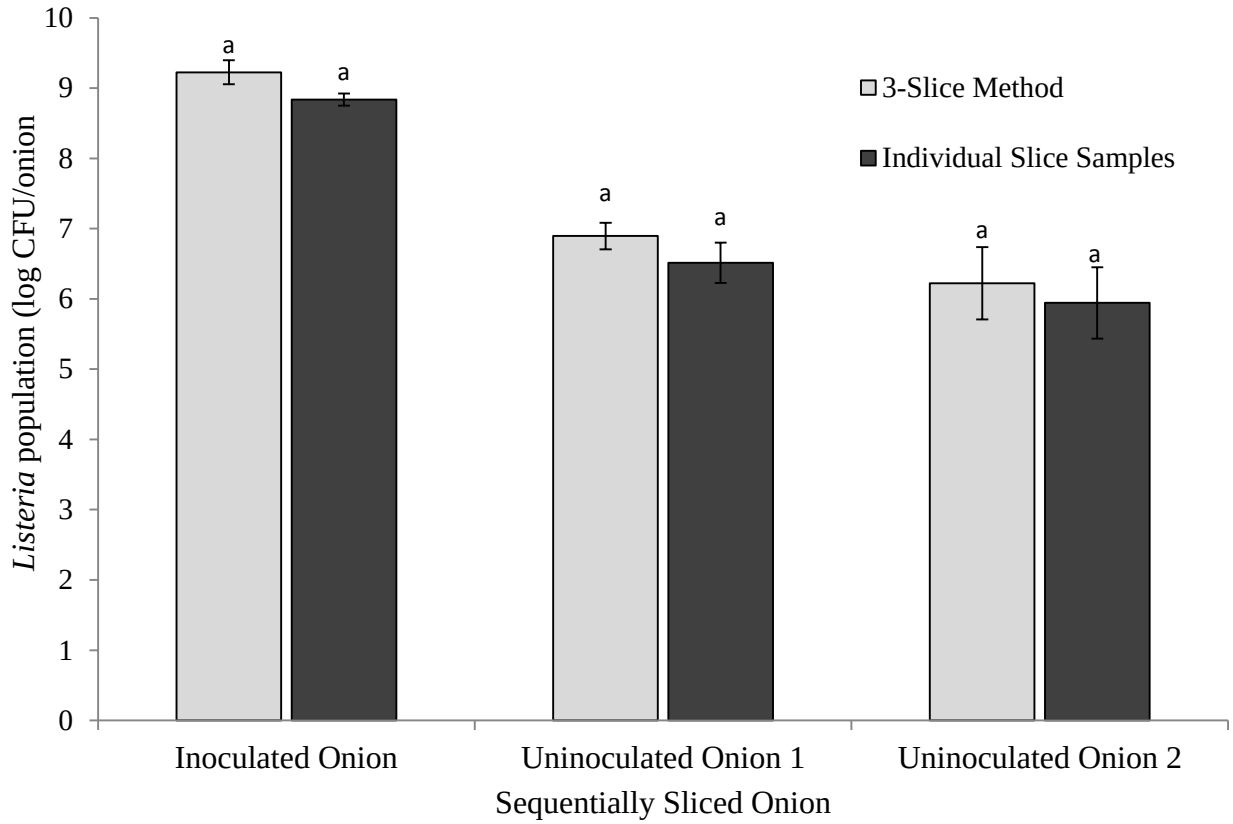


Figure 2: Comparison of the resulting total *Listeria* population transferred after calculating the total *Listeria* population per onion based on the first, fourth, and last onion slice as the average for the total onion. Statistical significance ($P < 0.05$) denoted by different letter for bars at same sequentially sliced onion.

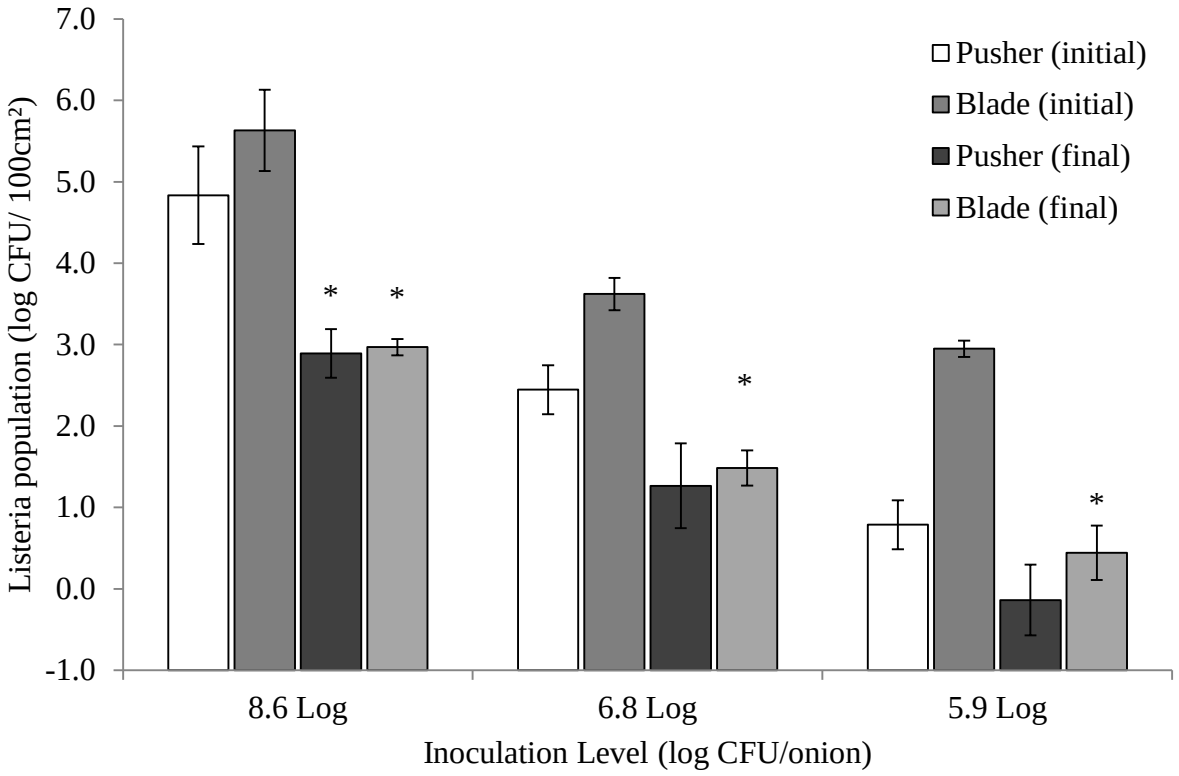


Figure 3: Transfer of *Listeria* to the slicer components after slicing one inoculated onion. Initial and final populations on individual components were compared within inoculation level. Bars with asterisks are significantly ($P < 0.05$) different.

3.2.4. *Listeria* transfer to uninoculated onions from the slicer

After slicing one inoculated followed by 20 uninoculated onions, all but one sample at the lowest inoculation level yielded detectable levels of *Listeria* in 1 or more replicates. At the highest inoculation level of ~8.6 log CFU/onion, onions 1, 10 and 20 yielded average *Listeria* populations of 6.7 ± 0.2, 3.8 ± 0.1, and 2.7 ±0.4 log CFU/onion, respectively (Figure 3.4). *Listeria* was sporadically detected by enrichment in one or more replicates for all onions when the inoculated onion contained ~6.8 log CFU/onion. Similarly, *Listeria* was sporadically recovered after enrichment in one or more replicates from all but the 17th onion, negative by enrichment in all three replicates, when the inoculated onion contained ~5.9 log CFU/onion.

3.2.5. Transfer model and parameter estimation

The parameter estimations after fitting the equation for each transfer scenario are shown in Table 3.2. The model was a better fit for the higher inoculation levels as shown by the lower NRMSE values (Table 3.2). The observed values and predicted lines for transfer were then plotted, along with the confidence band (CB) for the prediction line (Figure 3.4). Although the prediction lines generally reflected the trends in *Listeria* transfer, the CBs were relatively wide, with the CB width between 0.6 – 1.4, 0.5 – 1.1, and 0.7 – 1.5 log CFU/onion at inoculation levels of ~ 8.6, 6.5, and 5.6 log CFU/onion, respectively. The predictions at the lower bacteria populations were far less reliable at the two lower onion inoculation levels.

A)

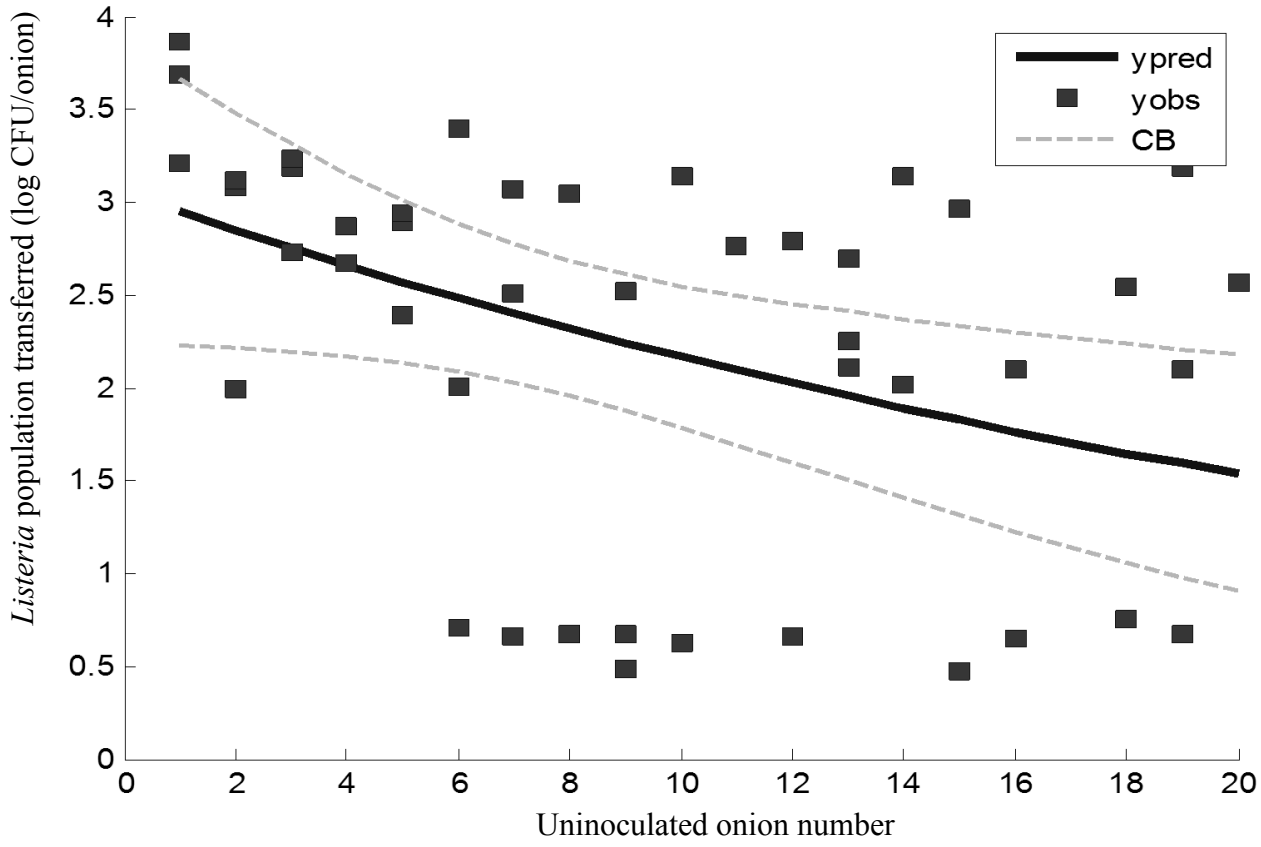
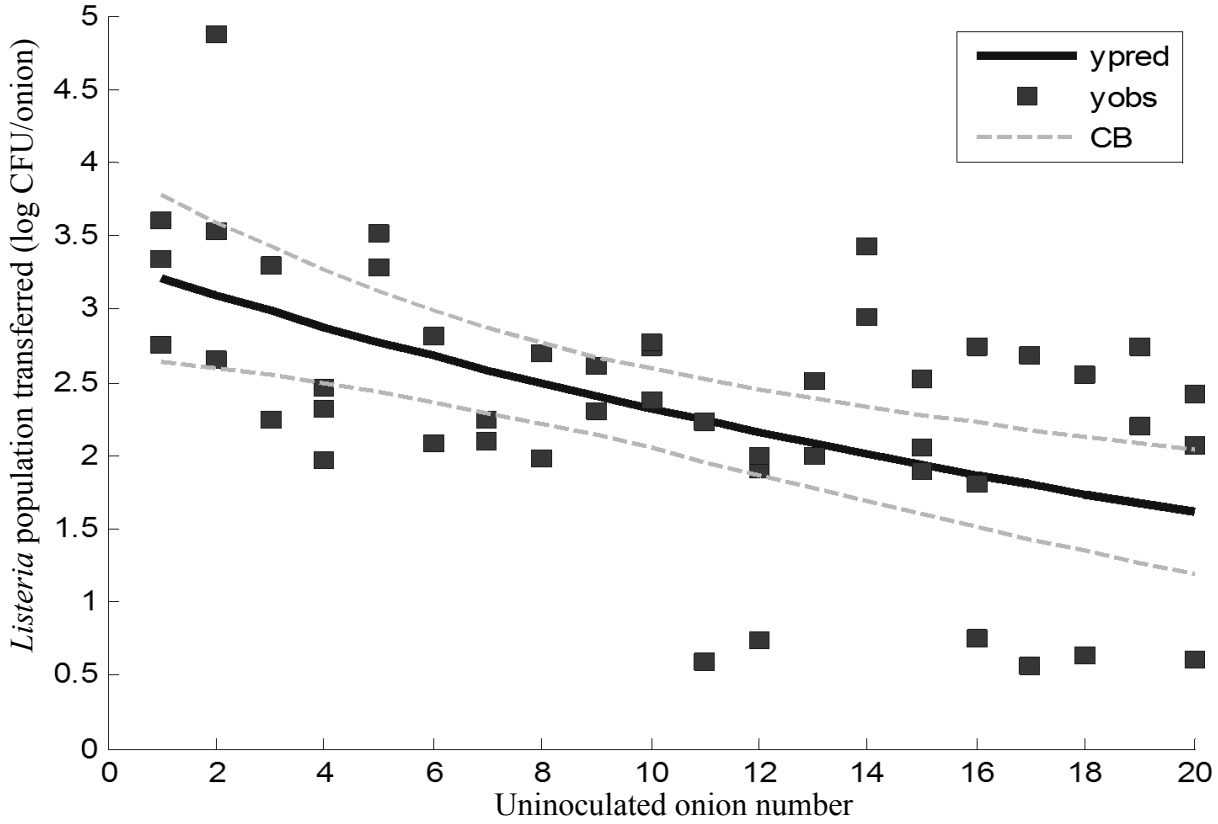


Figure 4: Predicted *L. monocytogenes* transfer from one inoculated onion (A: 5.9 log CUF/onion; B: 6.6 log CFU/onion; C: 8.6 log CFU/onion) to 20 uninoculated onions. ypred is the line of prediction; yobs is the observed line; CB is the confidence band for the line of prediction.

B)


ypred
yobs
CB
Listeria population transferred (log CFU/onion)
Uninoculated onion number
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
2
4
6
8
10
12
14
16
18
20

C)

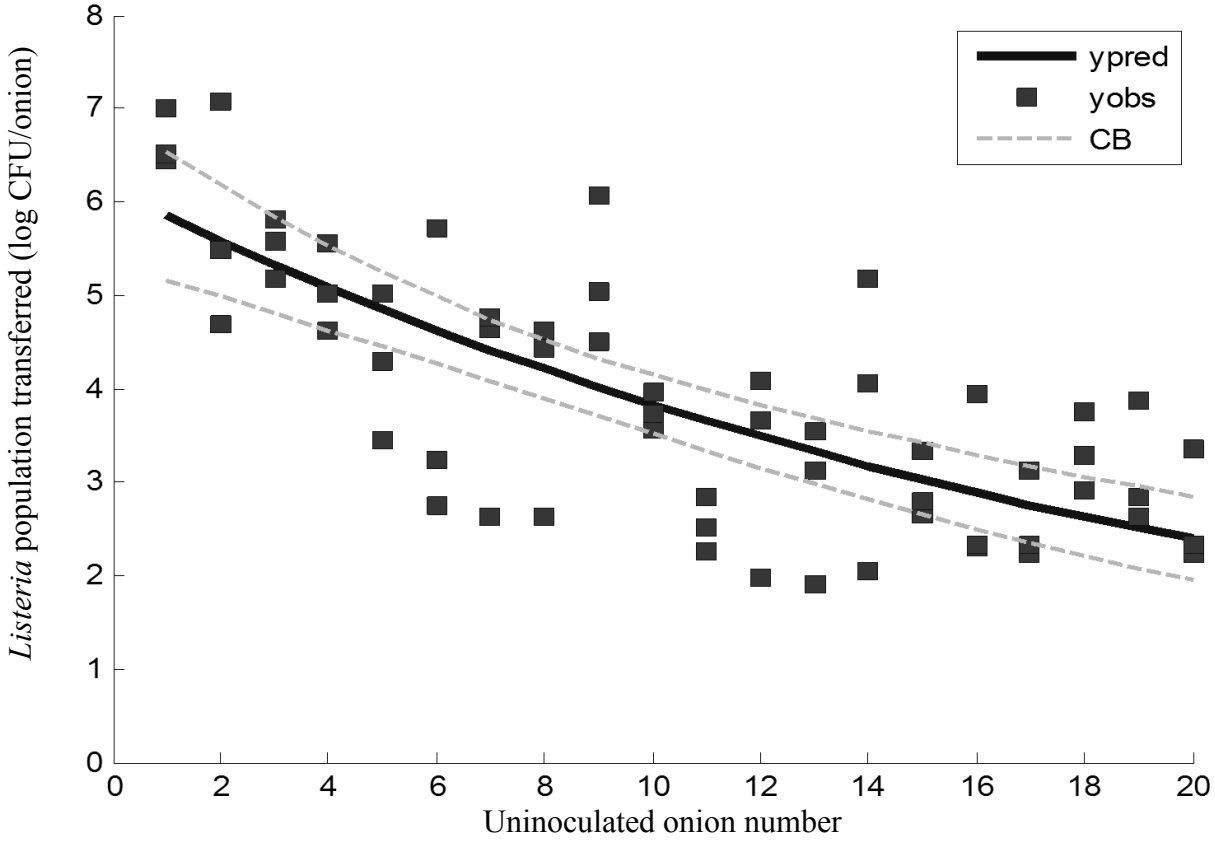

ypred
yobs
CB
Listeria population transferred (log CFU/onion)
Uninoculated onion number
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14
16
18
20

Table 2: Model parameters A and B for *L. monocytogenes* transfer from one inoculated onion to 20 uninoculated onions during slicing.

Inoculated onion level (log CFU/onion)	A (95% CI)[a]	B (95% CI)	NRMSE[b] (log CFU/onion)
5.5	3.05 (2.40, 3.70)	-29.24 (-49.73, -8.76)	0.26
6.4	3.33 (2.82, 3.83)	-27.72 (-39.66, -15.78)	0.19
8.6	6.13 (5.51, 6.76)	-21.28 (-26.32, -16.25)	0.18

[a]Coefficient value (95% confidence interval).
[b]Normalized root mean squared error for the transfer model.

3.3. DISCUSSION

This research clearly shows the potential for cross-contamination from inoculated to uninoculated onions during sequential slicing. After slicing twenty onions, detectable levels of *L. monocytogenes* remained on both the pusher plates and the blades, allowing for further transfer. These observations are consistent with previous cross-contamination studies involving deli meats, lettuce, and celery that showed that foodborne pathogens can readily move between the product and mechanical slicers or shredders (Vorst et al., 2006; Buchholz et al., 2012; Kaminski et al., 2014).

In this study, *Listeria* populations were significantly lower on the blade surfaces while the pusher plates retained a greater proportion after slicing 20 onions. This may be explained by the shear force experienced by the blade surfaces which readily removed loosely attached cells. The limited reduction of *Listeria* cells on the pusher plate surfaces suggests that fewer cells were transferred due to static contact. This transfer would likely continue if additional onions had been sliced.

Once sliced, onions have a shelf life of approximately 14 d at 4°C, which may be sufficient time for *Listeria* to reach potentially hazardous levels (Farber et al., 1998). When an onion is sliced, several sulfur compounds, including *S*-alk(en)yl cysteine sulfoxide metabolites, are released and converted to other types of antimicrobial sulfur compounds (Lancaster & Collin, 1981; Ramos et al., 2006). While onion juice is known to possess antimicrobial activity (Ramos et al., 2006; Kim et al., 2004; Ye, Dai, & Hu, 2013), *L. monocytogenes* was reportedly able to grow on sliced onions when stored at 10°C (Farber et al., 1998), and in the current study, persisted on the surface of whole peeled onions stored at 4°C. Inconsistent temperature control

throughout the cold-chain is also raising concern over microbial growth in fresh-cut produce (Nunes et al., 2009; Zeng et al., 2014).

Appropriate use of GAPs, Good Manufacturing Practices (GMPs), and a Sanitation program as set forth by the FDA are important to minimize contamination prior to processing, reduce cross-contamination during processing, and remove any contaminants after processing. Transfer to and from equipment surfaces, resulting in cross-contamination of product is clearly possible during mechanical slicing of onions. Onions can either be manually pushed by a plate as in this study or fed into a chute, which acts as stationary pusher plate while the blades move perpendicular to the chute. Regardless of the type of slicer, thorough cleaning and sanitizing of both the blades and the pusher plates/chute after use are equally important as evidenced by the continued presence of *Listeria* on component surfaces after slicing 20 uninoculated onions in this study. Due to difficulties associated with cleaning closely spaced (0.64 cm separation) parallel plates, it is important to follow manufacturer cleaning protocols that recommend full disassembly prior to cleaning and sanitizing. Neal (2013) reported that the use of a visual demonstration in combination with written cleaning and sanitizing instructions increased the overall cleanliness of delicatessen slicers, reinforcing the importance of proper staff training by restaurants.

The exponential decay model applied in this study was able to describe the transfer behavior of *L. monocytogenes* during slicing of onions, which is consistent with previous reports (Aarnisalo et al., 2007; Sheen & Hwang, 2010; Sheen, 2011). However, the model was less accurate at the low inoculation level, due to variability in the transfer data as seen by the relatively high NRMSE value (Table 3.2).

When the prediction lines were compared for the different inoculation levels, a much steeper decay pattern was observed for the highest inoculation level (8.6 log CFU/onion) compared to the other two inoculation levels due in part to the nearness of initial inoculation to the limit of detection for the lower inoculations. Variation in observed results may be attributed to a combination of variation in onion diameter and density.

In conclusion, this is the first study to quantitatively assess *L. monocytogenes* transfer during slicing of onions and show through the potential for cross-contamination from one product to sequentially sliced product. Since *L. monocytogenes* has been shown to survive in refrigerated onions and grow during unintentional temperature abuse, vegetable slicers should be properly cleaned and sanitized during prolonged use to minimize the risk of cross-contamination, with maintenance of the cold chain also needed to minimize the potential for *Listeria* growth in the finished product.

Chapter 4

Transfer of *Listeria* monocytogenes during Mechanical Dicing of Onions and Subsequent Reductions during Flume-Washing with Sanitizers

4.1. MATERIALS AND METHODS

4.1.1. Bacterial strains

Three avirulent *L. monocytogenes* strains (M3 1/2a3, J22F 4b, and J29H 4b), obtained from Dr. Sophia Kathariou (North Carolina State University, Raleigh, NC), were used for all dicing experiments. All cultures were stored at -80°C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. These strains were streaked for isolation to plates of trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, Becton, Dickinson and Company) and incubated 24 h at 35°C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35°C transfers in TSBYE. A cocktail was prepared by combining equal volumes of the three avirulent strains, followed by appropriate dilution to obtain populations of ~7.0 or 5.5 log CFU/ml for onion inoculation, with these levels confirmed by surface plating on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI).

4.1.2. Onions

Spanish yellow onions (*Allium cepa*) were purchased from a local supplier (Stan Setas Produce Company, Lansing, MI) and stored at 4°C for no more than 7 d before use. The root and sprout portions of each whole onion were removed using a sterile knife. After hand peeling the outer skin, the onions were tempered to room temperature (23°C ± 2°C) and weighed prior to dicing.

4.1.3. Onion dicing

A large-scale commercial dicer (Model H-A, Urschell, Valparaiso, IN) located in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory (Michigan State University, East Lansing, MI) was used for all experiments (Figure 4.1). Eight 100 cm^2

product contact areas of the dicer – the side and bottom of the loading drum, front and back of the rotating paddles, slicing blade, cross-cut blade, dicing blade, and the discharge chute were chosen for sampling.

4.1.4. *Listeria* transfer during dicing

Whole peeled onions were immersed in the diluted 3-strain avirulent cocktail containing ~7.0 or 5.5 log CFU/mL for 2 min and then air-dried in a biosafety cabinet for 90 min, giving initial populations of ~5.9, or 4.2 log CFU/50 g. Transfer of *L. monocytogenes* to the commercial dicer was assessed by dicing one 2.2 kg batch of inoculated onions. Eight 100 cm^2 areas of the dicer were sampled after disassembly using the 1-ply composite tissue method (Vorst et al., 2004). One 50 g diced onion sample was collected and assessed for numbers of *Listeria* to confirm the initial inoculation level.

Transfer of *L. monocytogenes* was also assessed by dicing one 2.2 kg batch of inoculated onions immediately followed by ten 2.2 kg batches of uninoculated onions. Each of the 11 batches of diced onions was separately collected, mixed by hand, with one 50 g sample chosen at random for quantification of *Listeria*. After dicing the 10 batches of uninoculated onions, the dicer was disassembled and the same eight 100 cm^2 areas again sampled using the 1-ply composite tissue method. All dicing experiments were conducted in triplicate.

4.1.5. Flume washing with various sanitizers

The processing equipment for washing the diced onions was also located in the Department of Food Science and Human Nutrition Fruit and Vegetable Processing Laboratory. This processing line included a water recirculation tank (~1,000-liter capacity), 3.6 m-long flume tank (~130-liter capacity; Heinzen Manufacturing Inc., Gilroy, CA) and a dewatering shaker

A)

B)

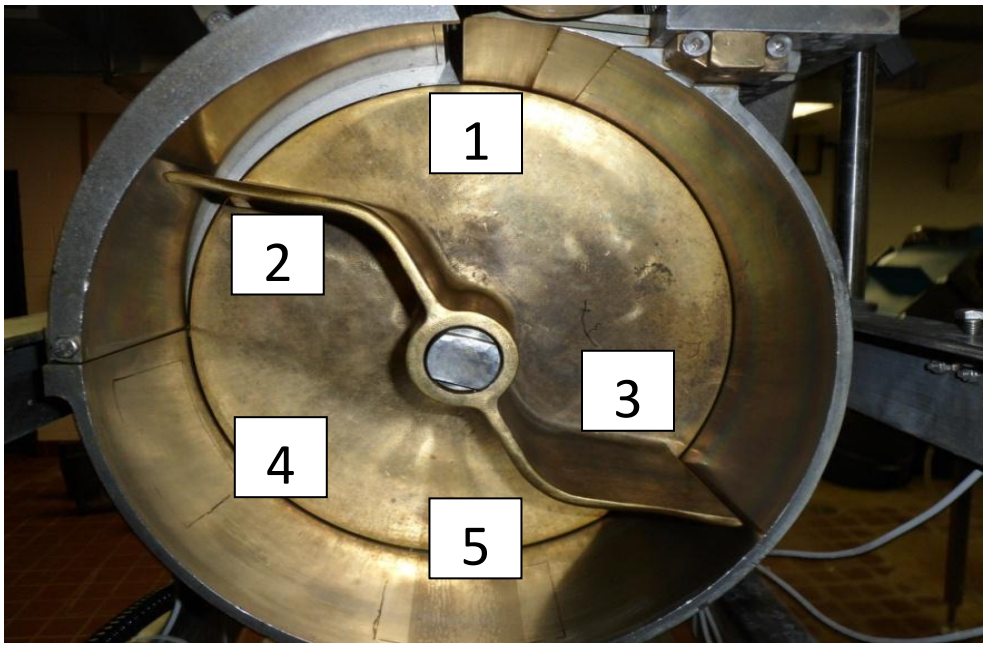
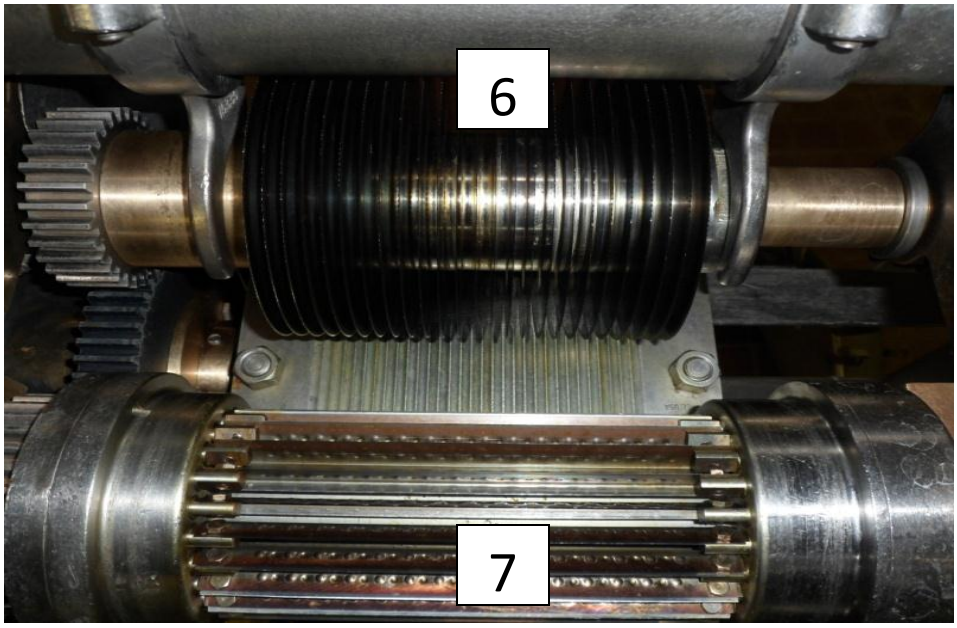


Figure 5: A) Commercial dicer and sampling locations: B) slicer blade (1), paddles (2, 3), and drum (4, 5), C) rolling dicer blades (6) and cross-cut blades (7), and D) chute (8).

C)



D)

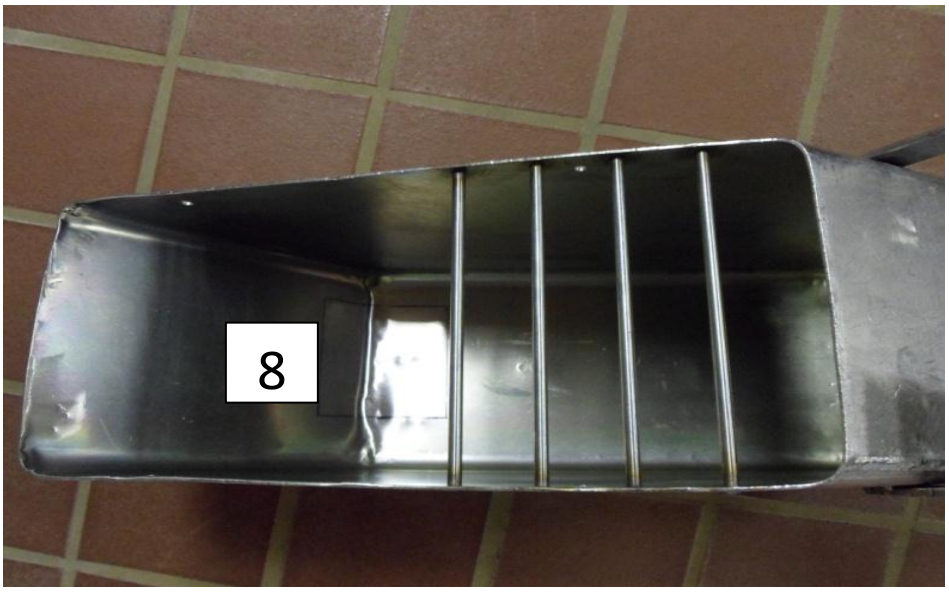


table operated by a 1 horsepower washdown duty motor (Baldor Electric Co., Ft. Smith, AR) at 1,760 rpm. The water recirculation tank containing 700 liters of tap water (~15°C) with or without a sanitizer was connected by a hard plastic discharge hose (4.5 m × 0.1 m) to the flume tank by a centrifugal pump (model XB754FHA, Sterling Electric, Inc., Irvine, CA). A custom-made stainless steel screen was attached to the end of flume tank to retain the diced onions for 2 minutes of washing (Figure 4.2).

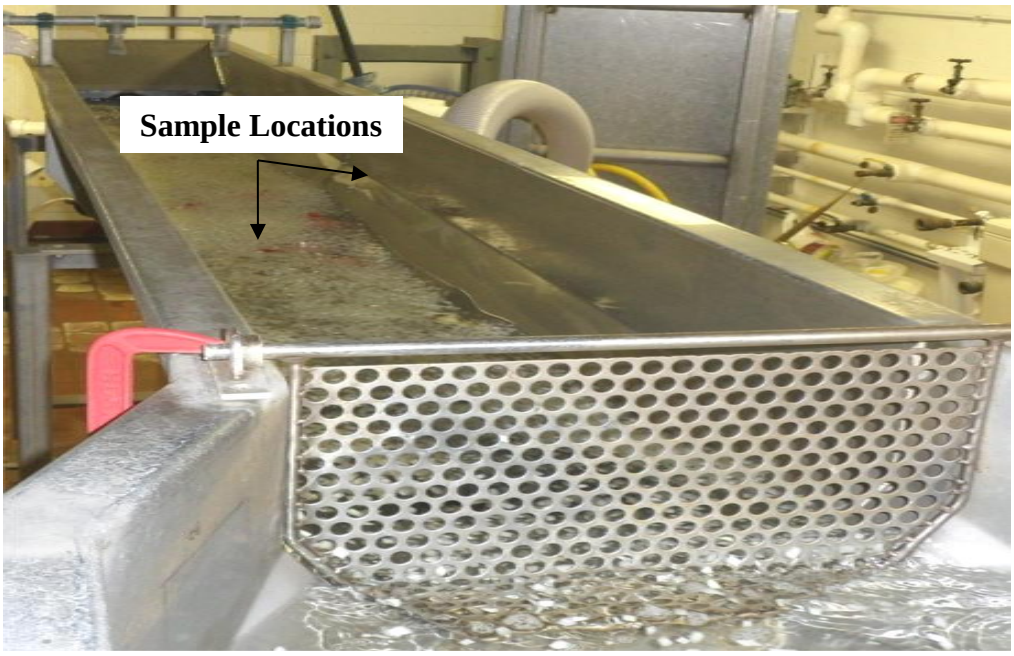
4.1.6. Sanitizers

Three different sanitizer treatments were used in this study. A commercial chlorine-based sanitizer (XY-12, Ecolab, St. Paul, MN) was diluted in tap water to contain 80 ppm free chlorine and adjusted to pH of 6.5 with 10% (w/v) citric acid. Electrolyzed water containing ~55 ppm free chlorine was produced on site using a commercial generator (PathoSans®, Spraying Systems Co., Westfield, IN). A peroxyacetic acid-based sanitizer (Tsunami-100, Ecolab, St. Paul, MN) was diluted in tap water to contain to 80 ppm peroxyacetic acid (PAA). Chlorine test kit 321 (Ecolab) was used to measure chlorine levels, and peroxyacetic acid test kit 311 (Ecolab) was used to measure PAA concentrations.

4.1.7. Evaluation of sanitizer efficacy

Whole peeled onions (9.1 kg) were immersed in the diluted 3-strain avirulent cocktail containing ~7.0 log CFU/mL for 2 min and then air-dried in a biosafety cabinet for 90 min, giving a population of ~4.0 log CFU/g. After dicing the 9.1 kg of inoculated onions with the same commercial dicer, seven 50 g samples were weighed into mesh produce bags (pore size < 0.5 cm), which were then placed in the flume tank containing 90 L of sanitizer-free water, 80 ppm free chlorine, 80 ppm PAA, or 55 ppm free chlorine as electrolyzed water, and vigorously agitated by hand for 2 min.

A)



B)

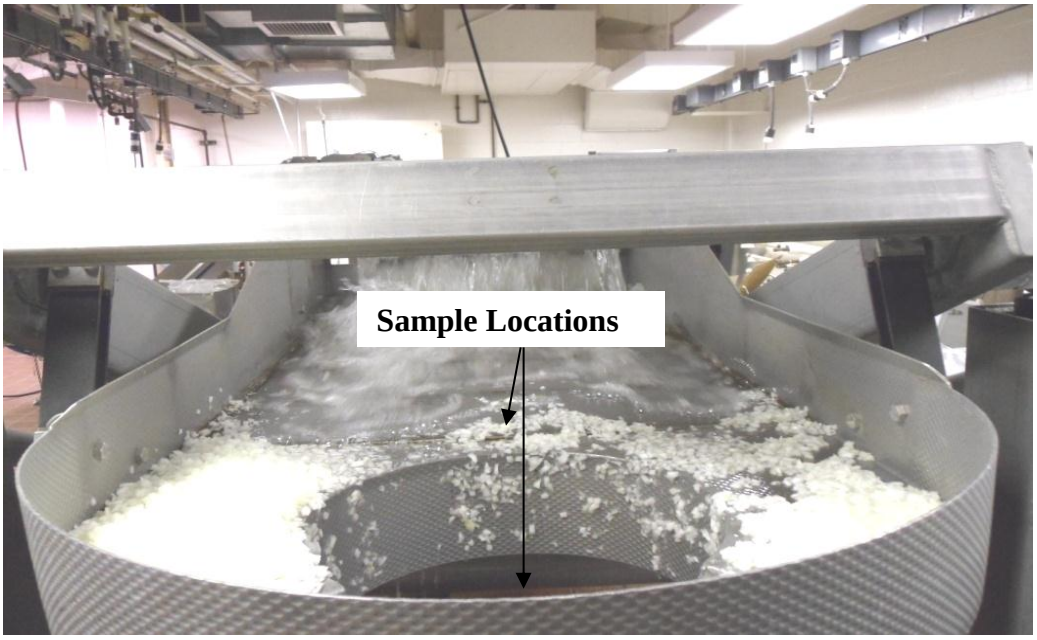


Figure 6: Processing equipment: A) 3.6-m flume tank, B) Dewatering shaker table

During sanitizer exposure, 50 ml water and 50 g onion samples were taken every 20 s. After 2 min of washing, the flume pump was activated, and the screen was lifted to flush the diced onions across the shaker table with a final 50 ml water and 50 g onion sample collected after 20 s. Two surface samples (100 cm^2) were taken each of the flume tank and the shaker table as shown in Figure 4.2.

4.1.8. Decontamination of equipment

After each transfer experiment, the dicer was disassembled, rinsed with a water hose to physically remove debris, sprayed with a quaternary ammonium-based sanitizer (Quorum V, Ecolab) for 5 min, and then rinsed thoroughly again with tap water. The processing line, flume tank, shaker table, bulk tank, and connecting hose were drained and similarly sanitized.

4.1.9. Microbial analysis

All samples from the *Listeria* transfer experiments were added to Whirl-pak® bags containing University of Vermont Medium (UVM, Neogen), homogenized by stomaching (Stomacher 400 Circulator, Seward USA, Davie, FL) for 1 min at 300 rpm, appropriately diluted in PBS, and plated, with or without prior membrane filtration (0.45 µm filter) using a vacuum pump (Model E46046, EMD Millipore Corporation, Billerica, MA), on MOX with the UVM-diluted samples enriched at 35°C. All plates were examined for typical *Listeria* colonies after 48 h of incubation at 35°C. When samples were negative for *Listeria* by direct plating, the UVM enrichments were streaked to plates of MOX, incubated at 35°C for 48 h, and then examined for the presence or absence of *Listeria*. All samples from the sanitizer efficacy studies were added to Whirl-pak® bags containing neutralizer buffer (Difco, BD, Franklin Lakes, NJ), homogenized by stomaching for 1 min at 300 rpm, appropriately diluted in PBS, and plated, with or without prior membrane filtration (0.45 µm filter), on MOX to quantify *Listeria*.

4.1.10. Statistical analysis

All experiments were performed in triplicate. *Listeria* populations were reported in log CFU/50 g ± SE or log CFU/100 cm^2 ± SE for inoculated/uninoculated batches of onions and surface samples, respectively. Samples positive by enrichment were assumed to contain 1 CFU. Samples negative by enrichment were assumed to contain 0.5 CFU. Onion samples negative by enrichment were not included in the *Listeria* transfer analysis. To compare surface populations, the Tukey-Kramer HSD test, $P < 0.05$, was performed using JMP 10 (SAS Institute Inc., Cary, NC).

4.1.11. Evaluation of transfer model. An exponential decay model from a previous study (Sheen and Hwang, 2010) was used to describe the *L. monocytogenes* transfer pattern during dicing of onions. The model used to fit the data is shown in Eq. (3):

$$Y = A \cdot e^{X/B} \qquad (3)$$

where Y (dependent variable) is the log CFU/onion transferred and X (independent variable) is the number of the specific uninoculated batch of onions that was diced. A and B are the transfer model parameters. The above equation was fitted using the *nlinfit* algorithm of MATLAB (R2012a, MathWorks, Natick, MA). The estimated parameters, normalized root mean squared errors (NRMSE) of the model, shown in Eq. (4), and asymptotic 95% confidence intervals of the parameters were then estimated as follows:

$$NRMSE = \frac{RMSE}{(Ymax - Ymin)} \qquad (4)$$

where NRMSE is the normalized root mean squared error, RMSE is the root mean squared error, Ymax is the maximum value taken by Y, and Ymin is the minimum value taken by Y.

4.2. RESULTS

4.2.1. Transfer from inoculated onion to dicer

After dicing one batch of inoculated onions, *L. monocytogenes* was recovered from all of the dicer surfaces sampled with similar trends observed at both inoculation levels (Figure 4.3). The drum and slicer blade yielded average *Listeria* populations of 4.0 ± 0.2 and 4.2 ± 0.5, and 2.7 ± 0.4 and 3.6 ± 0.3 log CFU/100 cm^2 after dicing a single batch of onions inoculated to contain ~ 5.9 or 4.2 log CFU/50 g, respectively. *Listeria* was found to have contaminated the remaining surfaces at varying levels, with the paddles and cross-cut blades yielding lower levels than the other components.

At the lower inoculation level, there was no significant ($P < 0.05$) difference in *Listeria* population retained on the dicer components after dicing 10 batches of uninoculated onions (Figure 4.4). However, at the higher inoculation level, the drum bottom and circular dicing blades retained significantly ($P < 0.05$) higher populations of *L. monocytogenes* than the other components (Figure 4.5).

4.2.2. Transfer from inoculated onions to subsequently diced uninoculated onions

After dicing one batch of inoculated onions followed by 10 batches of uninoculated onions, *Listeria* was detected in at least 1 of 3 replicates by enrichment at the low inoculation level. At the higher inoculation level of ~ 5.9 log CFU/50 g, *Listeria* populations in batches 1, 5 and 10 averaged 4.6 ± 0.1, 3.0 ± 0.1, and 2.3 ± 0.2 log CFU/50 g, respectively (Figure 4.6). The exponential model reasonably fit the lower and higher inoculation level transfer data with NRMSEs of 0.17 and 0.20, respectively (Table 4.1).

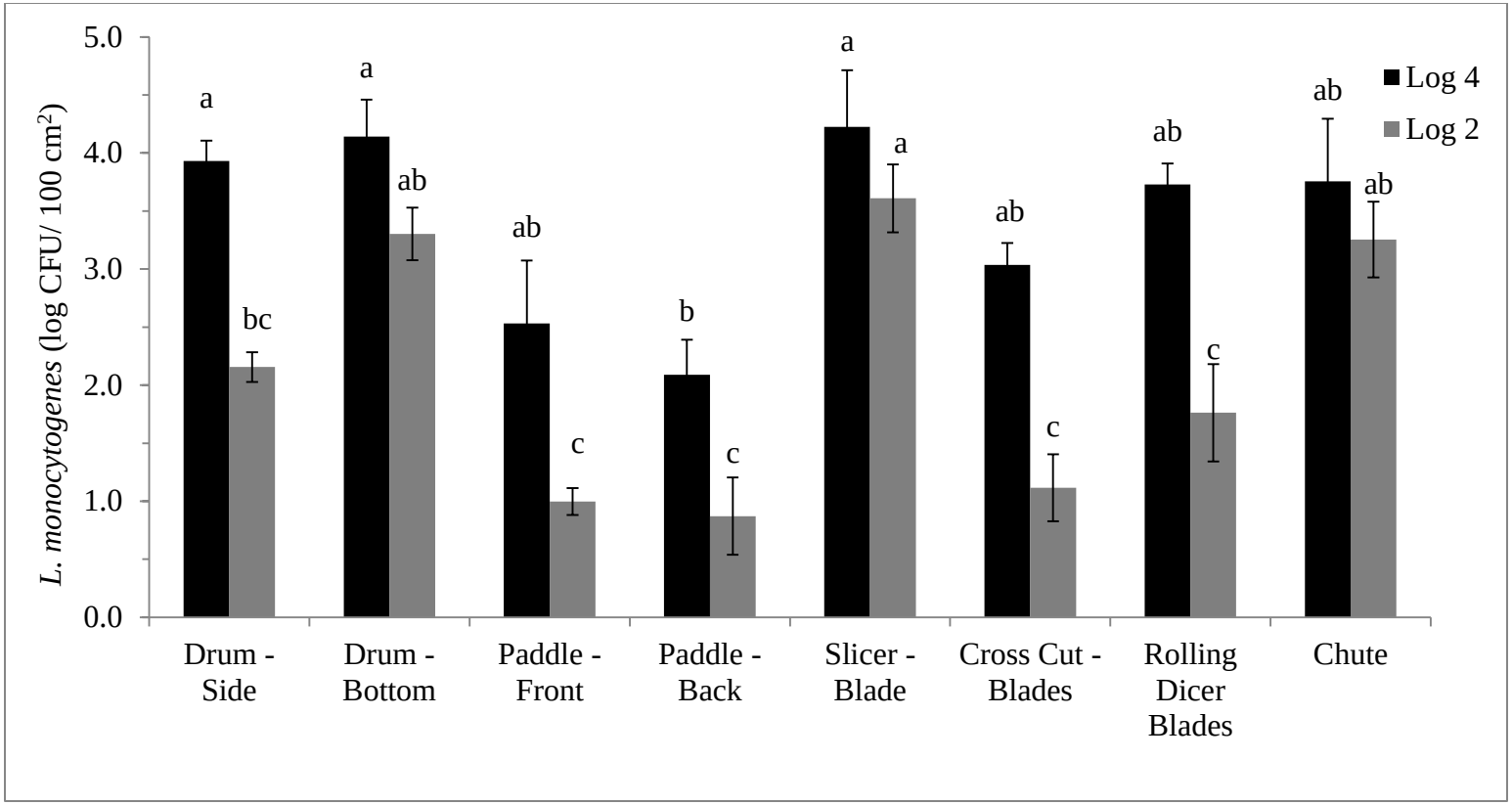


Figure 7: *L. monocytogenes* transfer from inoculated onions (5.9 or 4.2 log CFU/50 g) to different dicer components. Values were compared between components at the same inoculation level.

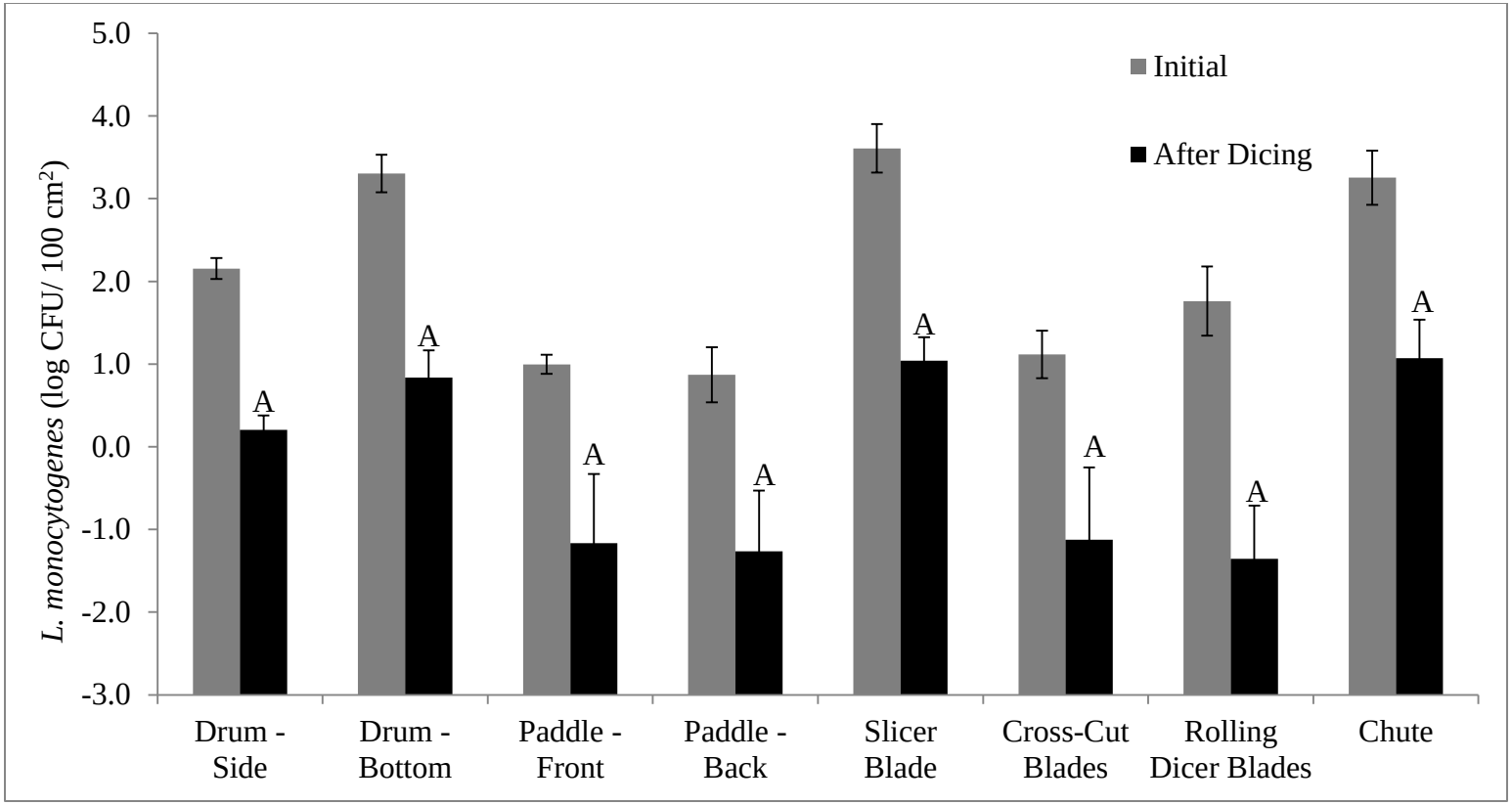


Figure 8: *Listeria* populations remaining on different dicer surfaces after dicing one inoculated (4.2 log CFU/50 g) followed by 10 uninoculated batches of onions. Populations on components were compared after dicing.

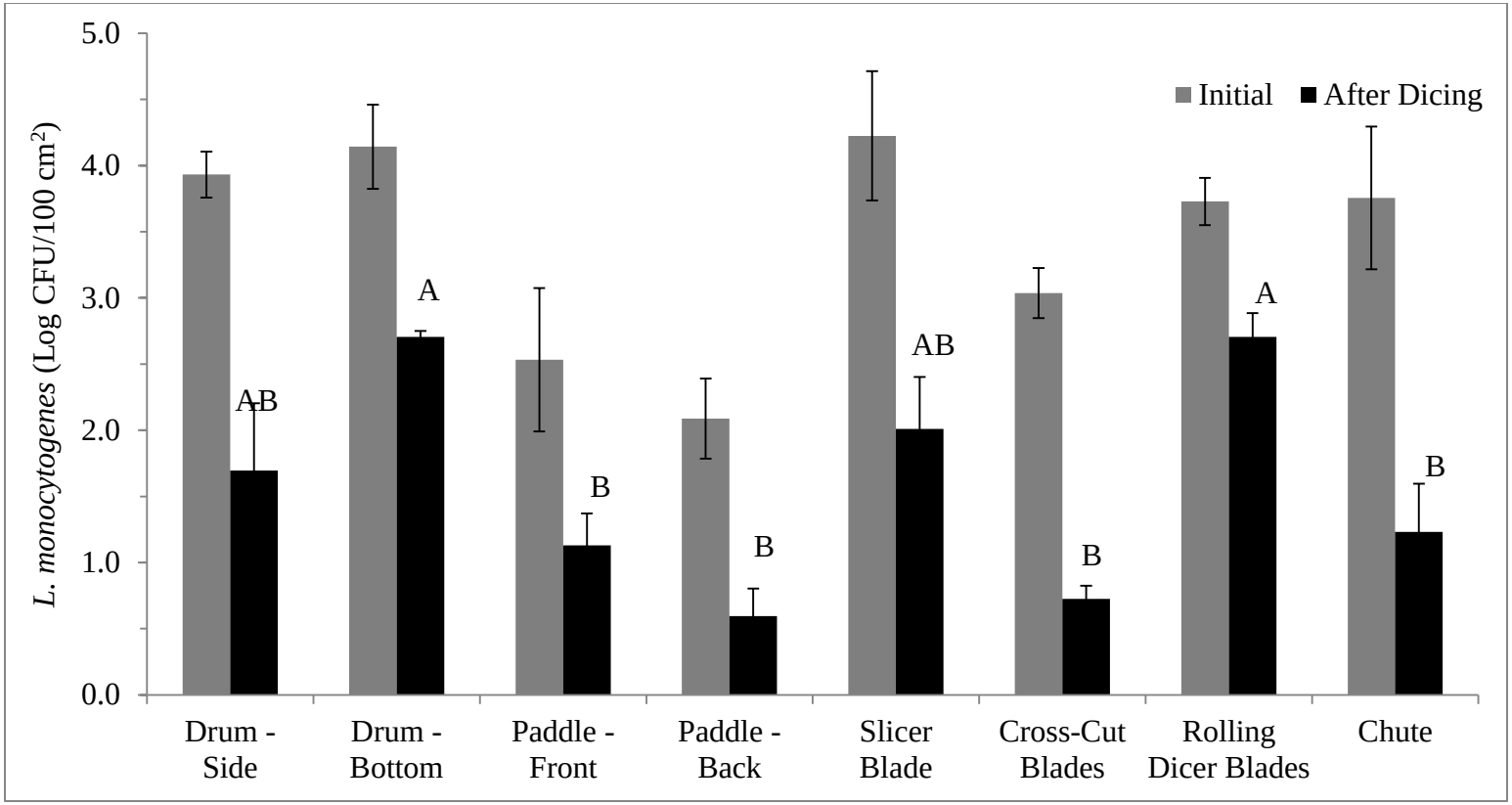


Figure 9: *Listeria* populations remaining on different dicer surfaces after dicing one inoculated (5.9 log CFU/50 g) followed by 10 uninoculated batches of onions. Populations on components were compared after dicing.

(A)

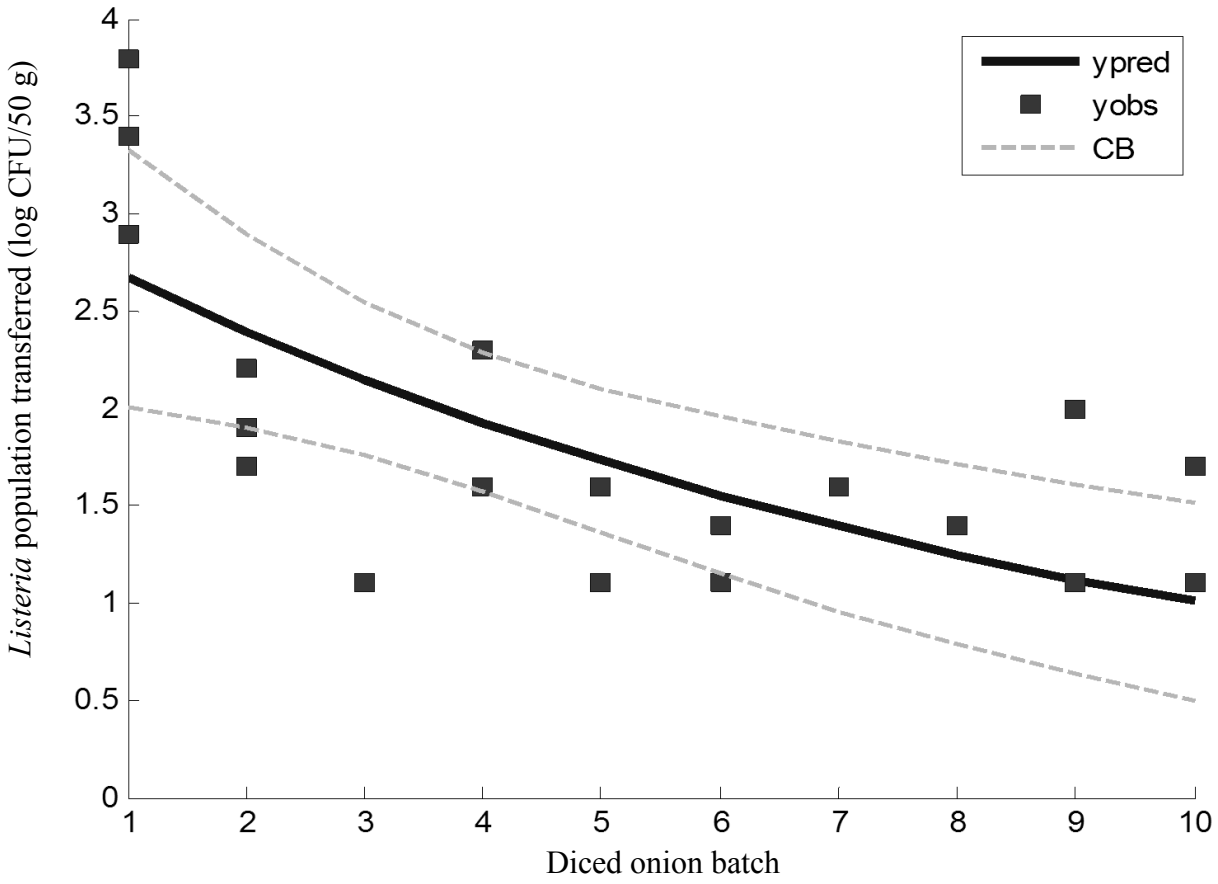


Figure 10: Predicted *L. monocytogenes* transfer from one batch of inoculated onions batch (A: 4.2 log CFU/50 g; B: 5.9 log CFU/50 g) to 10 batches of uninoculated onions. ypred is the prediction line; yobs is the observed line; CB is the confidence band for the prediction line.

(B)

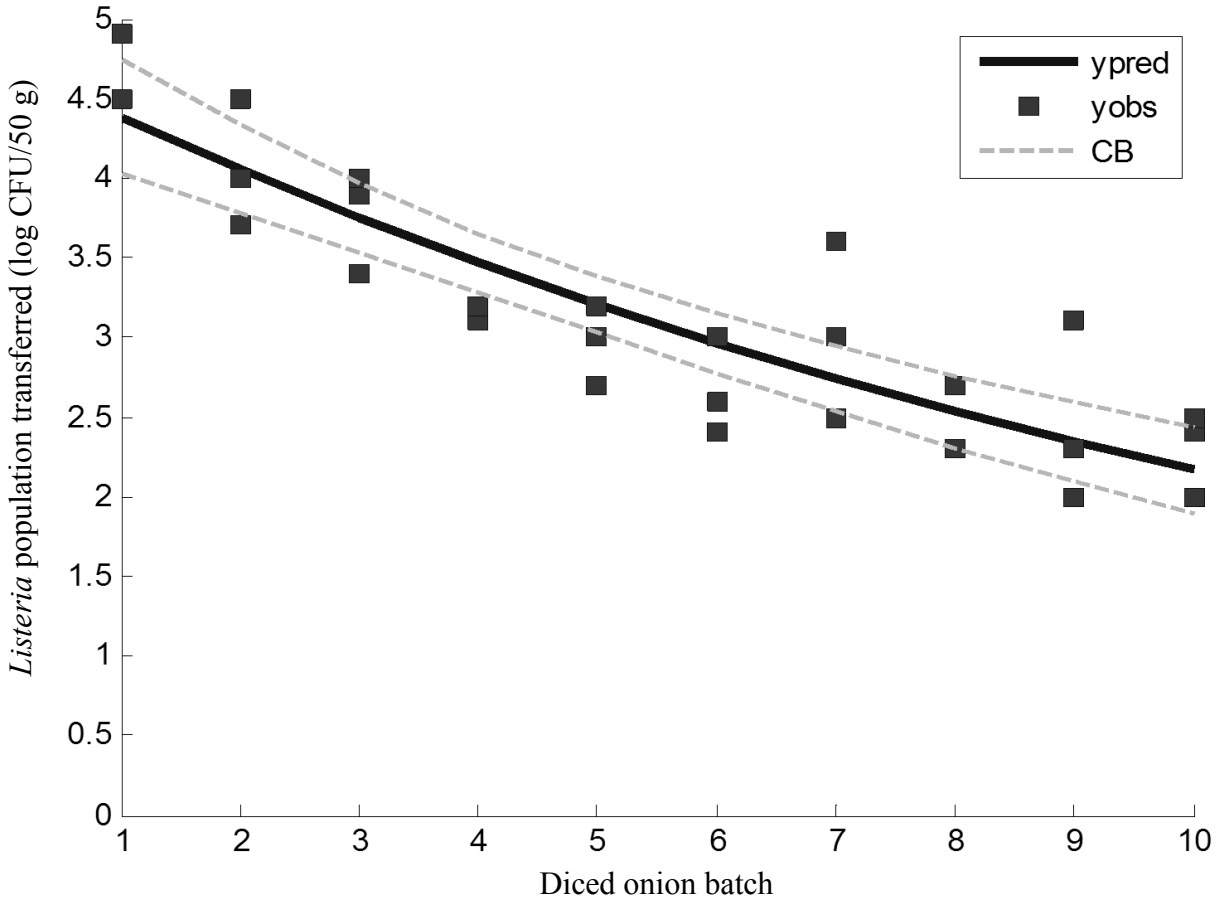

ypred
yobs
CB
Listeria population transferred (log CFU/50 g)
Diced onion batch
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
1
2
3
4
5
6
7
8
9
10

Table 3: Model parameters A and B for *L. monocytogenes* transfer from one inoculated onion to 10 uninoculated batches of onions during dicing.

Inoculation level (log CFU/50 g)	A (95% CI)[a]	B (95% CI)	NRMSE[b] (log CFU/50 g)
4.2	2.98 (2.27 3.68)	-9.23 (-13.96 -4.5)	0.17
5.9	4.74 (4.37 5.11)	-12.76 (-15.29 -10.23)	0.20

[a] Coefficient value (95% confidence interval).
[b] Normalized root mean squared error for the transfer model.

4.2.3. Sanitizer Evaluation

Diced onions yielded *Listeria* populations of 3.6, 2.6, 3.0, and 3.7 log CFU/g after 2 min of washing in tap water, 80 ppm free chlorine, 80 ppm PAA, and 55 ppm free chlorine followed by shaker table dewatering. The chlorine-based sanitizer was significantly ($P < 0.05$) better than tap water, yielding a ~ 1.4 log CFU/g reduction (Figure 4.7).

Listeria populations in sanitizer-free water increased to ~1.2 log CFU/ml during washing (Figure 4.8). During the first minute of washing with a sanitizer, *Listeria* was quantifiable in three water samples at or near the limit of detection, which was attributed to the accumulation of onion particulates on the filter membrane during sample processing. Surface samples yielded significantly ($P < 0.05$) higher *Listeria* populations when sanitizers were not used (Table 4.2). .

4.3. DISCUSSION

The presence of *Listeria* in processing environments has led to numerous investigations on contamination of food contact surfaces and finished (Tompkin, 2002; Buchholz et al., 2012; Beresford et al., 2001; Bierschwale et al., 2013). The *Listeria* transfer results during onion dicing are consistent with other studies showing the ability of foodborne pathogens to cross-contaminate large quantities of product via dicing, slicing, and shredding equipment (Kaminski et al., 2014; Lin et al., 2006; Buchholz et al., 2012). Areas most prone to *Listeria* contamination after onion dicing included the rolling dicing blades, loading hopper, and slicer blade (Fig 4.3).

Proper cleaning protocols for both food and non-food contact surfaces in food processing is important to minimize the risk of product contamination by *Listeria*. Lundén et. al., (2002) showed that a mechanical dicing machine was the vehicle for a resident *Listeria* population that moved between three processing plants, with the dicer blades identified as one source of contamination. The recent recall prompted actions by the responsible party that included a

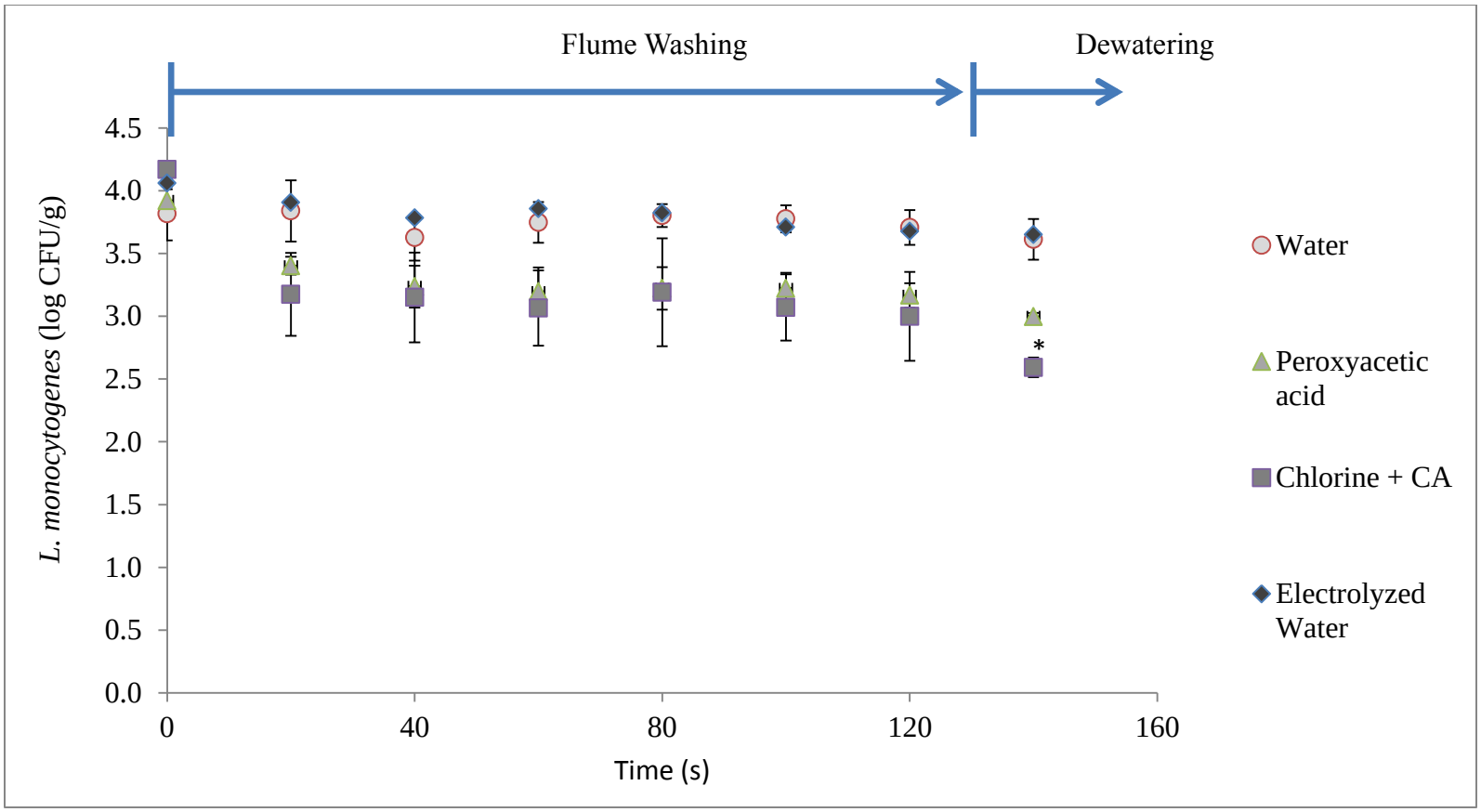


Figure 11: *Listeria* populations on diced onions during washing and shaker table dewatering.
* Denotes significant difference between treatment and water control.

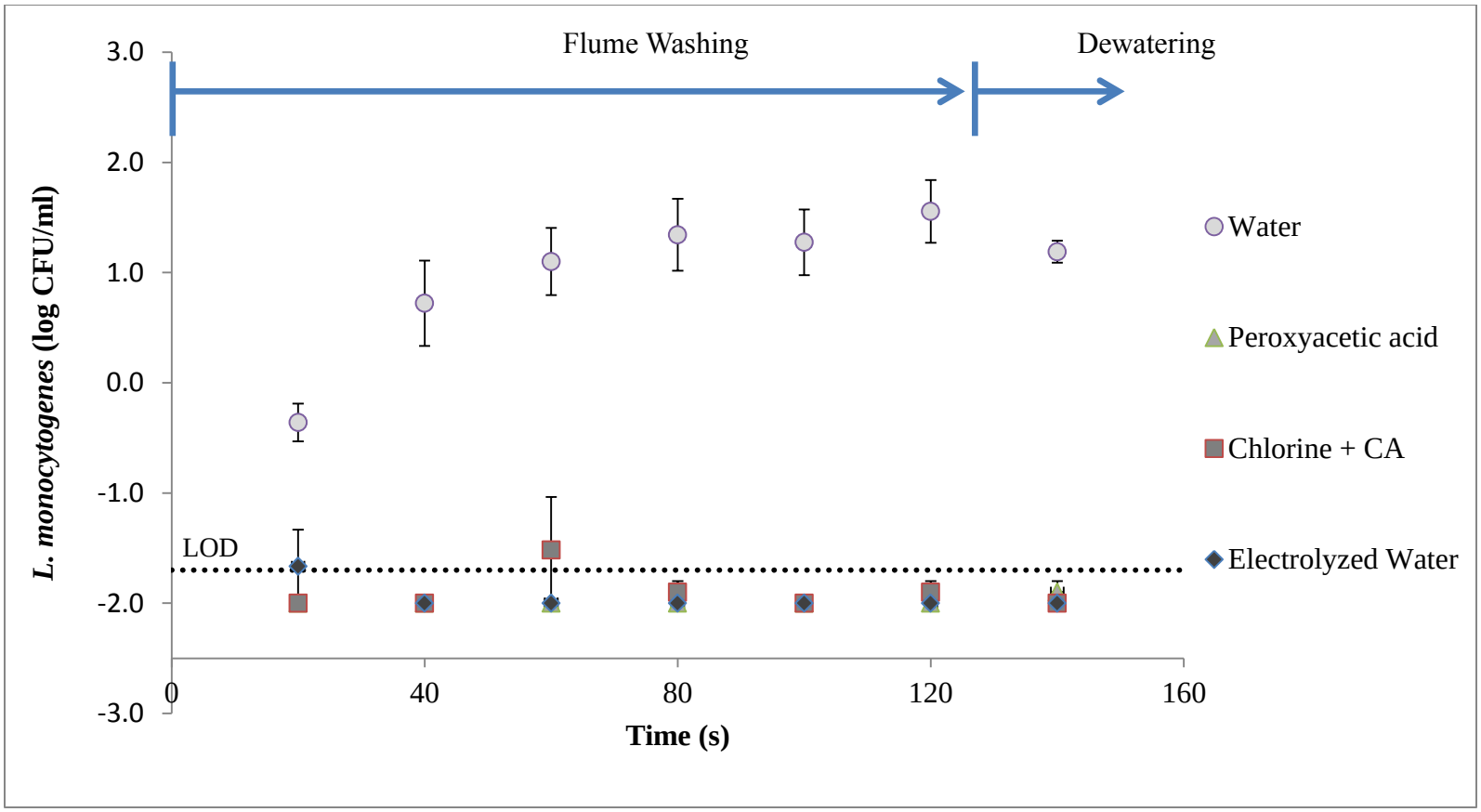


Figure 12: *Listeria* populations in water during washing and shaker table dewatering of diced onions.

Table 4: *Listeria* populations (log CFU/100 cm^2) on the flume tank and shaker table after onion dicing and dewatering.

Equipment Surface	Water	Electrolyzed Water	Chlorine + Citric Acid	Peroxyacetic acid
Flume Tank	1.6 ± 0.3^a *(6/6)	-0.3 ± 0.00^b (0/6)	-0.3 ± 0.0^b (0/6)	-0.1 ± 0.2^b (1/6)
Shaker Table	2.1 ± 0.3^a (6/6)	0.5 ± 0.3^b (4/6)	0.6 ± 0.2^b (5/6)	-0.3 ± 0.1^b (1/6)

*Fraction represents number of quantifiable samples.
Populations on equipment surface were compared across sanitizers.

cleaning protocol in an effort to eliminate *L. monocytogenes* from the facility. However, after actions were taken, the same strain was again identified on facility surfaces, demonstrating the difficulty in effectively removing colonized *Listeria* (Cruse, 2013).

Sanitizers are clearly needed in flume water to minimize cross-contamination during washing (Gil et. al., 2009). This study supports previous work showing that chlorine-based sanitizers are effective when the organic load is low (Davidson et al., 2013; Fatemi & Frank, 1999; Gonzalez et. al., 2004; Tomás-Callejas et al., 2012). The presence of *Listeria* on diced onions after the sanitizer treatment also raises concerns regarding potential migration into the product through cut surfaces. Previous work performed showed that one strain used in the avirulent cocktail exhibited significantly ($P < 0.05$) greater attachment as compared to the three virulent strains tested, suggesting greater migration potential. This study did not evaluate the location of *Listeria* on diced onion after dicing. A possible limitation to evaluation of the sanitizer efficacy during flume tank washing is the presence of *Listeria* below the outer surface of the diced onion. A limitation in the sanitizer study was the lower free chlorine concentration in the electrolyzed water treatment. While effective in minimizing the microbial load in the wash water, a higher concentration may have improved *Listeria* reductions on the diced onion samples.

To our knowledge, this is the first study to show that *Listeria* can transfer and survive during simulated commercial production of diced onions. During washing, the chlorine-based sanitizer used in this study was more effective at inactivating *Listeria* than peroxyacetic acid or electrolyzed water. However, organic matter commonly present in commercial onion wash water would be expected to decrease the efficacy of chlorine. Due to the limited efficacy of sanitizers

to reduce bacterial populations on fresh produce, the use of GAPs is critical to minimizing contamination.

Chapter 5

Effect of Various Sanitizer Treatments and In-Package Atmospheres on *Listeria monocytogenes* Growth in Diced Yellow Onions during Storage

5.1. MATERIALS AND METHODS

5.1.1. Bacterial strains

Three avirulent *L. monocytogenes* strains (M3 1/2a3, J22F 4b, and J29H 4b) obtained from Dr. Sophia Kathariou (North Carolina State University, Raleigh, NC) were used for all dicing experiments. All cultures were stored at -80°C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. All strains were streaked for isolation to plates of trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, Becton, Dickinson and Company) and incubated 24 h at 35°C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35°C transfers in TSBYE. A cocktail was then prepared by combining equal volumes of the three avirulent strains and appropriately diluting to obtain populations of ~5.9 log CFU/ml for onion inoculation with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI).

5.1.2. Onions

Spanish yellow onions (*Allium cepa*) were purchased from a local supplier (Stan Setas Produce, Lansing, MI) and stored at 4°C for no more than 3 d before use. The root and sprout ends of each onion were removed using a sterile knife. After hand peeling the outer skin, the onions were returned to refrigerated storage (4°C ± 2°C) for < 4 h.

5.1.3. Processing and packaging equipment

Processing equipment consisted of an industrial onion dicer (Model H-A, Urschell, Valparaiso, IN), plastic bins (100 L capacity), and a centrifugal dryer (model SD-50-LT, Heinzen Manufacturing, Inc.). A glove box chamber (Labconco 50004 fiberglass glove box,

Kansas City, MO) and an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA) were used to package the onions.

5.1.4. Sanitizers

Sanitizers used for washing diced onions included chlorine (XY-12, Ecolab, St. Paul, MN) diluted to 80 ppm free chlorine in tap water adjusted to pH of 6.5 with 10% (w/v) citric acid, liquid chlorine dioxide (ClO_2,CDG Solution 3000, CDG Environmental, Bethlehem, PA) diluted to 2 ppm in tap water, or peroxyacetic acid (PAA, Tsunami-100, Ecolab) diluted to 80 ppm in tap water with sanitizer-free tap water serving as the control. Chlorine test kit 321 (Ecolab) was used to measure chlorine levels, peroxyacetic acid test kit 311 (Ecolab) was used to measure PAA concentrations, and residual chlorine dioxide test strip CHL-D10 (Precision Laboratories, Cottonwood, AZ) was used to measure ClO_2 concentrations.

5.1.5. Onion Inoculation

After dicing whole peeled onions into a mesh bag, the onions were dip-inoculated with the 3-strain avirulent *L. monocytogenes* cocktail, diluted to contain ~5.9 log CFU/mL, (M3, J22F and J29H) to contain 5.9 log CFU/g for 2 min, and air-dried for 8 min. The onions were then washed for 2 min in 60 L of 4°C water containing either no sanitizer, 80 ppm free chlorine adjusted to pH 6.5 with citric acid, 80 ppm PAA, or 2 ppm ClO_2. After washing, the diced onions were centrifugally dried and then returned to a 4°C cooler for < 4 h prior to packaging.

5.1.6. Gas compositions

Three different in-package atmospheres were evaluated: O_2 (99% O_2, Airgas, Lansing, MI), $CO_2/O_2/N_2$ (15% CO_2, 5% O_2, 80% N_2, Airgas), or air, all using 11 x 11 cm polylactic acid (PLA) film (EVLON EV-HS1, BI-AX International Inc., Wingham, ON, Canada) bags, were double sealed using an impulse sealer (Ceratek, Sencorp Systems Inc., Hyannis, MA). Previously

calculated O_2, CO_2, and water vapor permeability values for the PLA film were $30.34\pm9.07 \times 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}, $5.67\pm1.17 \times 10^{-18}$ kg m m^{-2} s^{-1} Pa^{-1}, and $21.86\pm3.22 \times 10^{-15}$ kg m m^{-2} s^{-1} Pa^{-1}, respectively (Gonzalez-Buesa et al., 2014).

5.1.7. Decontamination of equipment

After each experiment, the dicer was disassembled, rinsed with water to physically remove debris, sprayed with a quaternary ammonia-based sanitizer (Quorum V, Ecolab,) with 5 min of contact, and then rinsed thoroughly with tap water. The washing bins and centrifugal dryer were similarly sanitized. The glove box and impulse sealer were sprayed with 70% (v/v) ethanol and wiped with a paper towel.

5.1.8. Microbial analysis

All samples were added to Whirl-pak bags containing Phosphate Buffer Solution (PBS), homogenized by stomaching (Stomacher 400 Circulator, Seward USA, Davie, FL) for 1 min at 300 rpm, appropriately diluted in PBS, and plated on MOX, with all plates examined for typical *Listeria* colonies after 48 h of incubation at 35^{o}C.

5.1.9. Statistical analysis

All experiments were performed in triplicate. *Listeria* populations in the onions were reported in log CFU/g ± SE. One-way ANOVA and two-way ANOVA with interaction (α = 0.05) was performed using JMP 10 (SAS Institute Inc., Cary, NC), to evaluate the effect of time, sanitizer, in-package atmosphere, and combinations of these variables on the growth of *Listeria* during storage.

5.2. RESULTS

5.2.1. Sanitizer efficacy

Washing inoculated diced onions in water, 80 ppm chlorine, 2 ppm ClO_2, or 80 ppm PAA for 2 min resulted in *Listeria* reductions of 0.9 ± 0.1, 1.0 ± 0.1, 0.9 ± 0.1 and 1.9 ± 0.1 log CFU/g, respectively. Comparison between sanitizer treatments showed greater reduction ($P < 0.05$) using PAA (Figure 5.1).

5.2.2. Effect of atmosphere/sanitizer combination on growth of *Listeria* in diced onions

Figure 5.2 shows the growth of *Listeria* under different treatment combinations during 14 d storage at 7°C. The three factors investigated, sanitizer, atmosphere, and time, had an effect on Listeria populations (Table 5.1). Two-way analysis showed that both PAA and chlorine reduced ($P < 0.05$) growth of *Listeria* in diced onions when compared to the other sanitizers. Comparison of atmospheres resulted in both CO_2 and O_2 reducing ($P < 0.05$) growth of *Listeria* when compared to air, with CO_2 significantly ($P < 0.05$) more effective than O_2. Interactions between time and sanitizer (P = 0.0334) and time and in-package atmosphere (P = 0.0004) affected the growth of *Listeria* (Table 5.1). Several 2-way interactions were found using one-way ANOVA. Some of these interactions are described below. The combination of ClO_2 sanitizer and CO_2 resulted in significantly ($P < 0.05$) less growth than ClO_2 sanitizer and the other atmospheres between 0 and 7 d (Table 5.2). The combination of chlorine sanitizer and atmosphere, other than air, resulted in significantly ($P < 0.05$) less growth than chlorine sanitizer and air at day 14 (Table 5.2). The combination of air atmosphere and chlorine sanitizer resulted in significantly ($P < 0.05$) less growth than air atmosphere and ClO_2 sanitizer between 3 and 10 d (Table 5.2).

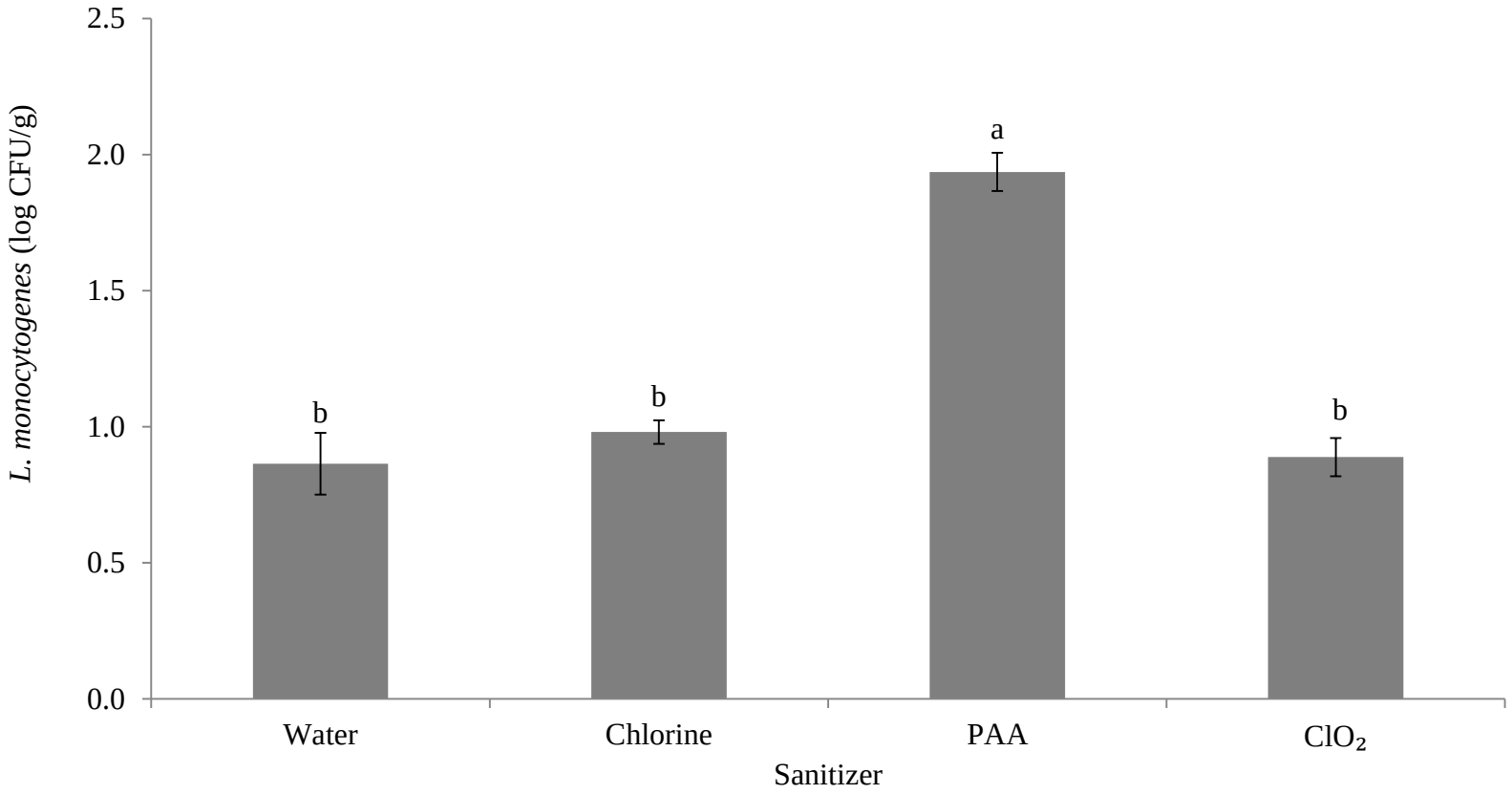


Figure 13: Reduction of *L. monocytogenes* in diced onions after 2 min of washing in water, 80 ppm chlorine, 2 ppm ClO_2, or 80 ppm PAA. Different letters signify significantly ($P < 0.05$) different values.

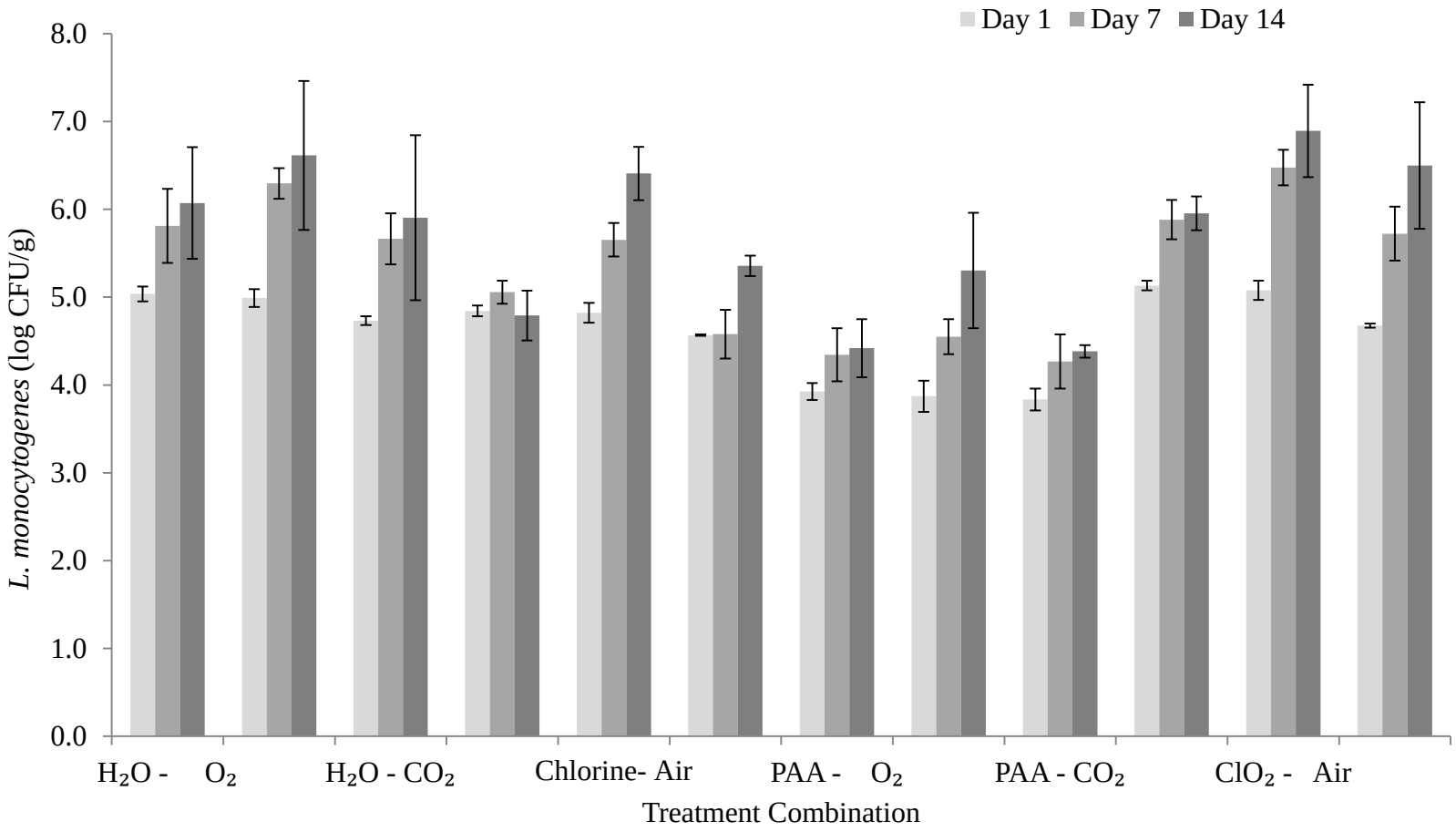


Figure 14: Change in population of *L. monocytogenes* in sanitizer-treated diced onions packaged under different atmospheres during 14 d of storage at 7°C.

Table 5: P values resulting from ANOVA (*P < 0.05 indicates effect of factor (single or combined)).

	Parameter
Factor	**Listeria**
Time	<.0001*
Sanitizer	<.0001*
Atmosphere	<.0001*
Day*Sanitizer	0.0334*
Day*Atmosphere	0.0004*
Sanitizer*Atmosphere	0.9366
Day*Sanitizer*Atmosphere	0.4457

Table 6: Effect of atmosphere/sanitizer combinations on change in *Listeria* populations in diced onions during 14 d storage at 7°C.

		Treatment											
	Storage Time (Days)	O_2 H_2O	Air H_2O	CO_2 H_2O	O_2 Chlorine	Air Chlorine	CO_2 Chlorine	O_2 PAA	Air PAA	CO_2 PAA	O_2 ClO_2	Air ClO_2	CO_2 ClO_2
Change in *Listeria* population (log CFU/g)	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	1	-0.1 ± 0.1* aAB1	-0.1 ± 0.2 abA1	-0.4 ± 0.1 bA1	-0.2 ± 0.1 aB1	-0.2 ± 0.2 aA1	-0.4 ± 0.0 aA1	-0.1 ± 0.2 aAB1	-0.1 ± 0.3 aA1	-0.2 ± 0.2 aA1	0.2 ± 0.1 aA1	-0.2 ± 0.2 aA1	-0.2 ± 0.0 bA1
	3	0.2 ± 0.2 aA1	0.4 ± 0.1 aAB1	-0.2 ± 0.1 bAB1	0.1 ± 0.2 aA1	0.0 ± 0.1 aB1	-0.5 ± 0.1 bB1	0.2 ± 0.1 aA1	0.1 ± 0.2 aAB1	-0.1 ± 0.1 aA1	0.5 ± 0.2 aA1	0.6 ± 0.3 aA12	-0.1 ± 0.1 bAB1
	5	0.8 ± 0.8 aA1	1.1 ± 0.6 aAB1	0.1 ± 0.3 aA1	0.3 ± 0.4 aA1	0.4 ± 0.1 abB1	-0.3 ± 0.2 bA1	0.1 ± 0.5 aA1	0.6 ± 0.5 aAB1	0.0 ± 0.3 aA1	1.0 ± 0.3 aA1	1.5 ± 0.2 aA123	0.0 ± 0.4 bA1
	7	0.7 ± 0.7 aA1	1.2 ± 0.3 aAB1	0.6 ± 0.5 aA1	0.1 ± 0.2 abA1	0.7 ± 0.3 aB1	-0.4 ± 0.5 bA1	0.3 ± 0.5 aA1	0.6 ± 0.3 aB1	0.3 ± 0.5 aA1	1.0 ± 0.4 aA1	1.6 ± 0.3 aA123	0.8 ± 0.5 aA1
	10	0.9 ± 1.1 aA1	1.2 ± 0.7 aAB1	0.6 ± 1.2 aA1	0.0 ± 0.5 aA1	0.5 ± 0.4 aB1	-0.4 ± 0.8 aA1	0.2 ± 0.4 bA1	1.0 ± 0.3 aAB1	-0.4 ± 0.5 bA1	0.9 ± 0.4 aA1	2.1 ± 0.6 aA23	0.8 ± 1.1 aA1
	14	1.1 ± 1.1 aA1	1.6 ± 1.5 aA1	0.9 ± 1.6 aA1	-0.1 ± 0.5 bA1	1.5 ± 0.5 aA2	0.5 ± 0.2 bA1	0.4 ± 0.6 aA1	1.3 ± 1.1 aA1	0.4 ± 0.1 aA1	1.0 ± 0.3 aA1	1.9 ± 0.9 aA3	1.5 ± 1.2 aA1

Table 6 (cont'd)

* means sharing the same lowercase letter in the same row for each sanitizer show no significant ($P < 0.05$) difference caused by gas composition; means sharing the same uppercase letter in the same row for each gas composition show no significant ($P < 0.05$) difference caused by sanitizer; means sharing the same number in the same column show no significant ($P < 0.05$) difference caused by time.

5.3. DISCUSSION

Individual use of sanitizers or modified atmospheres has been assessed for many different types of fresh produce under a wide range of storage conditions (Gonzalez et al., 2004; Gonzalez-Buesa et al., 2014; Harris & Harvey, 1973). While the outcome from using a particular sanitizer or in-package atmosphere may now be anticipated, the combination of multiple microbial hurdles cannot be assumed to yield the most desirable end product due to possible negative or positive interactions. Assessing the effect of combined treatments, including different sanitizers and packaging atmospheres, is an integral step in assuring both the safety and quality of fresh-cut produce (Castaigne et al., 1996; Couey & Wells, 1970).

Sanitizer use during commercial washing of fresh fruits and vegetables remains a critical step in limiting cross-contamination (Buchholz et al., 2012a; Buchholz et al., 2012b; Davidson et al., 2013; López-Gálvez et al., 2010). Similar to other studies, sanitizer efficacy has shown to be variable for different types of produce (Gil,et al., 2009), (Gonzalez et al., 2004). In this study, PAA provided significantly ($P < 0.05$) greater reduction before storage in *Listeria* populations on diced onions.

Different in-package modified atmospheres are commonly used to extend the microbial shelf life of fresh produce with high CO_2 concentrations used for strawberries and broccoli (Couey & Wells, 1970; Steen et al., 2003). The reduction in growth observed when using modified atmospheres is specific to the target organism being investigated with some molds and pathogens more affected by high CO_2 (Couey & Wells, 1970), (Harris & Harvey, 1973), (Bennik et al., 1995). *Listeria* has been shown to have an increased lag phase under high O_2 atmospheres which may allow for microbial competition to further inhibit growth over time (Jacxsens et al., 2001). This study showed that an in-package atmosphere with high CO_2 concentrations resulted

in the least *Listeria* growth over 14 d of storage at 7 °C, and a high O_2 atmosphere also proved effective at reducing *Listeria* growth as compared to packaging in air.

In the current study, as determined by two-way ANOVA with interaction, no synergistic interaction was observed between the sanitizer treatment and in-package atmosphere during storage; however, further investigation of different atmosphere and sanitizer combinations is still needed to determine the most suitable treatment for extending product quality and shelf life.

The current study encountered limitations in assessing the sanitizer efficacy during washing due to a short drying time post inoculation. This is a result of adhering to common practice of completing the packaging process shortly after dicing and the goal of assessing the effect of modified in-package atmospheres. Increasing the post-inoculation drying time prior to sanitizer treatment may result in lower reductions of *Listeria* population.

Due to the varying tolerance of fresh produce to environmental changes, the suitability of any sanitizer or modified atmosphere must be tailored to the specific product in question and then tested in the laboratory before full scale commercial use. This work shows the clear benefit of peroxyacetic acid or a chlorine-based sanitizer adjusted to pH 6.5 in combination with an initial in-package atmosphere air over the industry standard of ClO_2 and air in minimizing *Listeria* growth in diced onions during refrigerated storage. Additionally, the use of a chlorine sanitizer in combination with an initial in-package atmosphere of 99% O_2 or 15% CO_2 was shown to be the most effective at minimizing *Listeria* growth in diced onions during refrigerated storage.

Chapter 6

Conclusions and Future Recommendations

The risk associated with contamination of yellow onions by *Listeria* is relatively low, with few recalls and no outbreaks having been identified. This research was conducted with the intention of filling several important knowledge gaps related to the extent of *Listeria* transfer during slicing and dicing, the ability of various sanitizers to minimize cross-contamination during commercial washing of diced onions and the impact of different in-package atmospheres on subsequent survival of *Listeria* during refrigerated storage. The results of this work have provided some useful insight into all of these areas.

The current research showed the ability of *Listeria* to both survive on the surface of whole peeled onions at 4°C and grow in diced onions at 7°C during storage. This research, in addition to other work, supports the suitability of sliced and diced onions as a growth menstrum for *Listeria* during extended storage.

The transfer of *Listeria* during slicing, dicing, and washing of onions was evaluated, with potential for extensive transfer shown in all processes. Residual *Listeria* populations on the hand slicer and commercial dicer surfaces reinforce the importance of all food contact surfaces in the transfer of bacteria to and from product. It is suggested that cutting surfaces contribute to rapid transfer of high bacteria populations while static contact experienced by pushing plates likely serves as a point of widespread, low level transfer. This, in addition to limited reductions achieved during flume washing with sanitizers, shows the importance of effective sanitation methods.

Modified atmosphere packaging is an important technology which, when economically advantageous, should be explored for its potential to prolong the shelf-life of many products.

This research shows potential benefits in changing current commercial practices, with the use of chlorine as a sanitizer and an atmosphere of either O_2 or CO_2 as an in-package atmosphere proving to be most effective at reducing growth of *Listeria* in diced onions during storage. This study also supports the work of Jacxsens et al., (2001), who showed that the use of O_2 can limit the growth of *Listeria*. This information will be useful for assessing the appropriateness of establishing a packaging system.

The assessment of common commercial practices, such as shipping whole peeled onions to facilities, slicing, dicing, washing, and packaging onions, will assist in improving practices to reduce the risk of future recalls and outbreaks associated with onions. The data produced in this series of studies will be of use in further economic cost/benefit analyses to the onion industry.

Future work is necessary to better understand the role of sanitizers during slicing and dicing, with these findings being applied to all products in the fresh-cut industry. Future work should include:

- Determining the effect of various sanitizers, and application methods, to limit the transfer of bacteria to product and component surfaces.
- Assessing the effect of various sanitizers on industrial equipment over time to rule out accelerated deterioration of metal components.

APPENDICES

APPENDIX A

Slicing of onions using hand slicer

Table 7: Raw data for transfer of *Listeria* from one inoculated onion (5.9 log CFU/onion) to sequentially sliced onions.

	Rep 1	Rep 2	Rep 3
Inoculated Onion	5.6	6.0	6.0
Uninoculated Onion 1	3.2	3.9	3.7
Uninoculated Onion 2	2.0	3.1	3.1
Uninoculated Onion 3	3.2	2.7	3.2
Uninoculated Onion 4	Negative	2.7	2.9
Uninoculated Onion 5	2.9	2.9	2.4
Uninoculated Onion 6	2.0	3.4	0.7
Uninoculated Onion 7	2.5	3.1	0.7
Uninoculated Onion 8	Negative	3.0	0.7
Uninoculated Onion 9	0.5	0.7	2.5
Uninoculated Onion 10	Negative	3.1	0.6
Uninoculated Onion 11	Negative	2.8	Negative
Uninoculated Onion 12	Negative	2.8	0.7
Uninoculated Onion 13	2.3	2.7	2.1
Uninoculated Onion 14	Negative	3.1	2.0
Uninoculated Onion 15	Negative	3.0	0.5
Uninoculated Onion 16	2.1	0.7	Negative
Uninoculated Onion 17	Negative	Negative	Negative
Uninoculated Onion 18	Negative	2.5	0.8
Uninoculated Onion 19	2.1	3.2	0.7
Uninoculated Onion 20	Negative	2.6	Negative

Table 8: Raw data for transfer of *Listeria* from one inoculated onion (6.8 log CFU/onion) to sequentially sliced onions.

	Rep 1	Rep 2	Rep 3
Inoculated Onion	6.8	7.0	6.5
Uninoculated Onion 1	3.6	3.4	2.8
Uninoculated Onion 2	3.5	2.7	4.9
Uninoculated Onion 3	3.3	2.3	3.3
Uninoculated Onion 4	2.3	2.0	2.5
Uninoculated Onion 5	Negative	3.5	3.3
Uninoculated Onion 6	2.8	2.1	Negative
Uninoculated Onion 7	Negative	2.2	2.1
Uninoculated Onion 8	Negative	2.7	2.0
Uninoculated Onion 9	Negative	2.6	2.3
Uninoculated Onion 10	2.8	2.4	2.8
Uninoculated Onion 11	Negative	2.2	0.6
Uninoculated Onion 12	2.0	1.9	0.7
Uninoculated Onion 13	2.0	2.5	2.0
Uninoculated Onion 14	Negative	3.0	3.4
Uninoculated Onion 15	2.1	1.9	2.5
Uninoculated Onion 16	0.8	1.8	2.8
Uninoculated Onion 17	Negative	2.7	0.6
Uninoculated Onion 18	0.6	0.6	2.6
Uninoculated Onion 19	Negative	2.7	2.2
Uninoculated Onion 20	2.4	2.1	0.6

Table 9: Raw data for transfer of *Listeria* from one inoculated onion (8.6 log CFU/onion) to sequentially sliced onions.

	Rep 1	Rep 2	Rep 3
Inoculated Onion	8.8	8.6	8.5
Uninoculated Onion 1	6.5	7.0	6.5
Uninoculated Onion 2	7.1	4.7	5.5
Uninoculated Onion 3	5.6	5.2	5.9
Uninoculated Onion 4	5.6	4.7	5.1
Uninoculated Onion 5	4.3	3.5	5.1
Uninoculated Onion 6	5.8	3.3	2.8
Uninoculated Onion 7	4.8	2.7	4.7
Uninoculated Onion 8	4.7	4.5	2.7
Uninoculated Onion 9	6.1	4.5	5.1
Uninoculated Onion 10	3.8	3.6	4.0
Uninoculated Onion 11	2.3	2.9	2.6
Uninoculated Onion 12	3.7	4.1	2.0
Uninoculated Onion 13	3.2	3.6	1.9
Uninoculated Onion 14	5.2	4.1	2.1
Uninoculated Onion 15	3.4	2.7	2.8
Uninoculated Onion 16	4.0	2.4	2.3
Uninoculated Onion 17	2.3	2.4	3.2
Uninoculated Onion 18	3.8	3.3	3.0
Uninoculated Onion 19	2.7	2.9	3.9
Uninoculated Onion 20	2.3	3.4	2.4

APPENDIX B

Dicing of onions using commercial dicer

Table 10: Raw data for transfer of *Listeria* from one batch inoculated onion (4.2 log CFU/50 g) to sequentially diced onions.

	Rep 1	Rep 2	Rep 3
Inoculated Onion Batch	4.2	4.2	4.2
Uninoculated Onion Batch 1	2.9	3.8	3.4
Uninoculated Onion Batch 2	1.7	1.9	2.2
Uninoculated Onion Batch 3	Negative	Negative	1.1
Uninoculated Onion Batch 4	Negative	1.6	2.3
Uninoculated Onion Batch 5	1.1	Negative	1.6
Uninoculated Onion Batch 6	Negative	1.4	1.1
Uninoculated Onion Batch 7	Negative	Negative	1.6
Uninoculated Onion Batch 8	Negative	Negative	1.4
Uninoculated Onion Batch 9	1.1	1.1	2.0
Uninoculated Onion Batch 10	Negative	1.1	1.7

Table 11: Raw data for transfer of *Listeria* from one batch inoculated onion (5.9 log CFU/50 g) to sequentially diced onions.

	Rep 1	Rep 2	Rep 3
Inoculated Onion Batch	5.9	6.0	5.8
Uninoculated Onion Batch 1	4.5	4.9	4.5
Uninoculated Onion Batch 2	3.7	4.5	4.0
Uninoculated Onion Batch 3	4.0	3.9	3.4
Uninoculated Onion Batch 4	3.2	3.2	3.1
Uninoculated Onion Batch 5	3.0	3.2	2.7
Uninoculated Onion Batch 6	2.6	3.0	2.4
Uninoculated Onion Batch 7	3.6	3.0	2.5
Uninoculated Onion Batch 8	2.7	2.7	2.3
Uninoculated Onion Batch 9	2.3	3.1	2.0
Uninoculated Onion Batch 10	2.4	2.5	2.0

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