IMPACT OF SELECTED UNIT OPERATIONS ON THE SPREAD OF *ESCHERICHIA COLI* O157:H7 DURING PILOT-SCALE PRODUCTION OF FRESH-CUT LETTUCE

By

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ABSTRACT

IMPACT OF SELECTED UNIT OPERATIONS ON THE SPREAD OF *ESCHERICHIA COLI* O157:H7 DURING PILOT-SCALE PRODUCTION OF FRESH-CUT LETTUCE

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In response to continued foodborne outbreaks and recalls, two separate studies were conducted to assess the importance of various unit operations in spreading *E. coli* O157:H7 during production of fresh-cut leafy greens. Initially, *E. coli* O157:H7 transfer from a contaminated shredder and conveyor belt to previously uncontaminated iceberg lettuce, wash water, and the equipment was quantified during pilot-plant production of fresh-cut lettuce using 9.1 kg of dip-inoculated radicchio (10^6 CFU/g, colored surrogate for iceberg lettuce) to contaminate a pilot-plant-scale shredder or conveyor. After processing 90.7 kg of previously uncontaminated lettuce, average *E. coli* O157:H7 populations on the shredder and conveyor belt decreased from 3.7 to 0.9 and from 4.1 to 2.5 log CFU/100 cm^2, respectively. *E. coli* O157:H7 levels in twenty 4.5 kg batches of lettuce collected after exiting the contaminated shredder and conveyor also decreased significantly ($P < 0.05$) from 2.7 to 0.9 and from 1.8 to 0.9 log CFU/g during processing. Thereafter, *E. coli* O157:H7 transfer from a single inoculated lettuce leaf (6.9 log CFU/g) to wash water was assessed at different water velocities in a custom-made pipe system. Shedding of *E. coli* O157:H7 from lettuce was significantly greater at a laminar flow rate of 0.01 m/s (5.1 log CFU) ($P < 0.05$) compared to turbulent flow at 0.07 m/s (3.8 log CFU) and lower laminar flow at 0.005 m/s (3.2 log CFU) with numbers of *E. coli* O157:H7 peaking in the water after 10, 4, 4, and 3 s at flow rates of 0.005, 0.01, 0.04, and 0.540 m/s, respectively. All of these findings will be useful in the development of bacterial transfer models for current and future risk assessments.

To my mother Guizhi Fu, my father Xiao Ren, my husband Bo Song, and my daughter Meeya Song

谨以此献给我亲爱的父母，我的爱人和我可爱的女儿

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Lin Ren

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KEY TO SYMBOLS AND ABBREVIATIONS

ANOVA Analysis of Variance

CDC Centers for Disease Control and Prevention

CFU Colony forming unit(s)

CIP Clean in place

CSPI Center for Science in the Public Interest

d days(s)

EHEC Enterohemorrhagic *Escherichia coli*

FDA Food and Drug Administration

g grams(s)

GAPs Good Agricultural Practices

GFP Green fluorescent protein

h hour(s)

HUS Hemolytic uremic syndrome

L liter(s)

LOD limit of detection

min minute(s)

ml milliliter(s)

PBS Phosphate Buffered Saline

ppm Parts per million

PVC Polyvinyl chloride

Re Reynold's number

s second(s)

TSA-YE Trypticase Soy Agar with 0.6 % Yeast Extract

TSB-YE Trypticase Soy Broth with 0.6 % Yeast Extract

US United States of America

USDA United States Department of Agriculture

µl microliter(s)

INTRODUCTION

Changes in eating habits are now leading to increased human consumption of fresh vegetables. Although considered healthy and nutritious, leafy greens can also be a source of bacterial foodborne pathogens. In 2009, leafy greens were ranked as the riskiest food by the Food and Drug Administration, responsible for 363 outbreaks and 13,568 reported cases of illness (Klein et al., 2009). In 2011, another risk ranking study concluded that enterohemorrhagic *Escherichia coli* (EHEC) in leafy greens ranked first among the 53 pathogen-produce commodity pairs considered, based on scenario and sensitivity analyses (Anderson et al., 2011). Between 2000 and 2007, lettuce contaminated with *E. coli* O157:H7 was responsible for 10 outbreaks and 242 illnesses (Anonymous, 2011). According to the 2012 annual report from CDC, among the 29 *E. coli* outbreaks, which caused 500 illnesses and 98 hospitalizations, 3 were associated with contaminated lettuce consumption (CDC, 2012).

Cross-contamination can occur at any point during the farm-factory-fork continuum. At the farm, lettuce can become contaminated with *E. coli* O157:H7 from domestic and wild animals shedding the organism, as well as from contaminated irrigation water and soil (Solomon et al., 2003; Oliveira et al., 2012).

Workers can further spread pathogens to large quantities of produce during harvesting and subsequent handling. Although Good Agricultural Practices (GAPs) have been constantly emphasized by FDA (FDA, 2006), pathogen contamination can still occur with improper storage or transportation leading to pathogen growth and further spread in packing facilities.

The number of *E. coli* O157 leafy green-related outbreaks and recalls has risen sharply during the past 10 years. In 2006, one nationwide *E. coli* O157:H7 outbreak was linked to bagged baby spinach which resulted in 205 illnesses, 103 hospitalizations, and 3 deaths. In the same year, *E. coli* O157:H7-contaminated lettuce was responsible for two additional outbreaks,

which sickened 105 people (CSPI, 2008). In 2010, a multistate outbreak of *E. coli* O145 infections was linked to shredded lettuce from a single processing facility (CDC, 2011). In a *Salmonella* outbreak reported in Australia, shredded lettuce was contaminated by cutting wheel of a shredder in a processing facility (Stafford et al., 2002).

Buchholz et al. (2012a) previously assessed the number of *E. coli* O157:H7 cells transferred from equipment surfaces to lettuce during processing in a small-scale production line with sanitizer-free water. In this work, 90.8 kg of previously uninoculated lettuce was contaminated with *E. coli* O157:H7 from the processing equipment, which was initially contaminated by processing inoculated lettuce containing *E. coli* O157:H7 at 10^6 and 10^4 log CFU/g. Even at the lowest initial contamination level of 10^2 log CFU/g, 0.1 and 0.3% of the original *E. coli* O157:H7 inoculum spread to previously uninoculated iceberg lettuce after processing (Buchholz et al., 2012a). Improper post-harvest handling increases the potential for cross-contamination during washing (Wachtel and Charkowski, 2002). Commercial processing, especially washing, further facilitates the spread of foodborne pathogens to large volumes of leafy greens (CSPI, 2008). Given the above outbreaks, a better understanding of pathogen transfer during commercial flume washing is warranted. Several laboratory-scale studies assessing the efficacy of various sanitizers have shown 1-3 log reductions of foodborne pathogens on lettuce (Beuchat et al., 2004; Gil et al., 2009; Parish et al., 2003). In our previous work, *E. coli* O157:H7 transfer from inoculated iceberg lettuce to wash water was quantified during simulated commercial processing, with a 1 log reduction observed (Buchholz et al., 2012b). However, commercial washing is a complicated process, with the extent to which bacterial removal are impacted by a range of biological and physical factors. Lelièvre et al. (2002) found that the removal of *Bacillus cereus* spores from a clean-in-place line was strongly

dependent on the hydrodynamic conditions during washing. In another study, removal of *E. coli* O157:H7 from pre-inoculated apples and cantaloupe during washing increased with the water flow velocity and agitation rate (Wang et al., 2007). However, the three flow velocities that were assessed all fell in the laminar flow range, which is much lower than often seen in commercial practice.

Consequently, the objectives of this research were to:

1. Quantify the spread of *E. coli* O157:H7 from a product contaminated shredder or conveyor to subsequent product, other equipment surfaces, and the water during simulated commercial production of fresh-cut iceberg lettuce.
2. Assess the impact of laminar, transition, and turbulent flow on *E. coli* O157:H7 transfer from lettuce to water during washing.

CHAPTER 1:

Review of Pertinent Literature

1.1 Fresh-cut production and consumption

Lettuce is one of the most popular leafy green vegetables. Due to many perceived health benefits such as reduction of chronic diseases, hypertension, ischemic stroke, and cancer (Hung et al., 2004; López-Gálvez et al., 2010), consumption of leafy greens continues to increase. According to Cook (2012), per capita consumption of head lettuce in United States was 12.5 pounds in 2013 , with romaine and leaf lettuce being 7.7 and 3.6 pounds, respectively (USDA/ERS, 2014) (Figure 1.1). As shown in Figure 1.2, bagged fresh-cut salads comprised the largest portion of produce sales in 2013 - approximate $3 billion in selected US retailers. In 2010, 200 million pounds of head lettuce and 300 pounds of lettuce were imported, while the total exported reached 535 million pounds (USDA/ERS, 2011). Due to the significant economic and nutritional value, lettuce is grown year- round in California and Arizona as well as in Colorado, New Mexico, Washington, New Jersey, New York, Ohio, and Florida. Approximately 98 percent of commercial domestic output of lettuce is from California and Arizona.

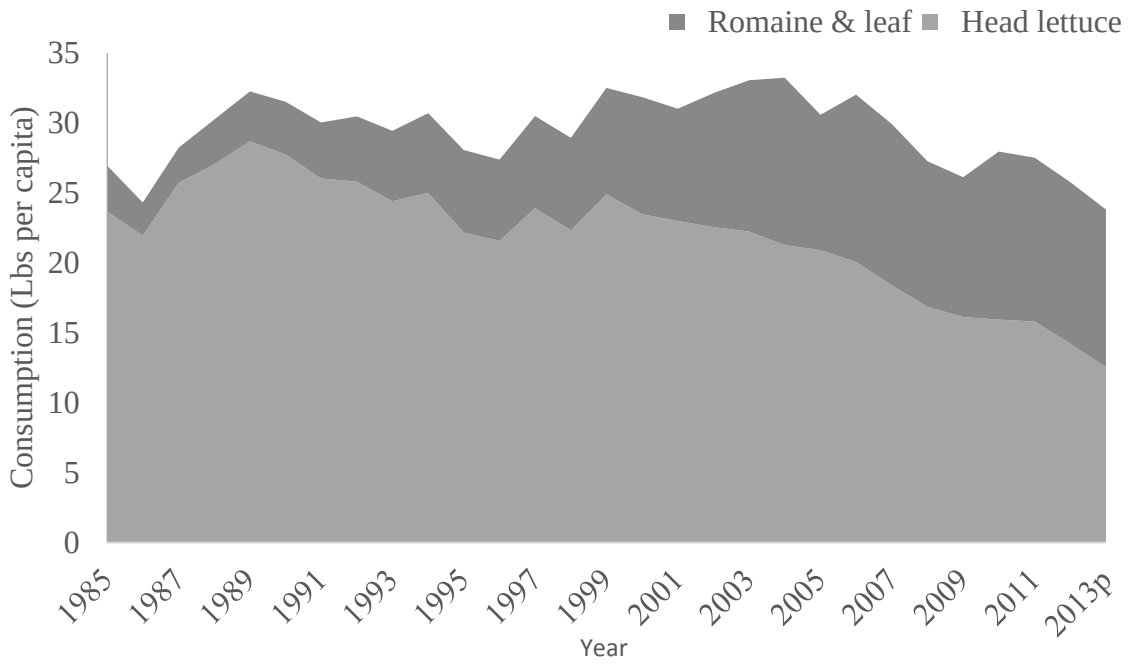

Figure 1.1: *Consumption of lettuce by type in United States from 1985 to 2013 (USDA/ERS, 2014).*

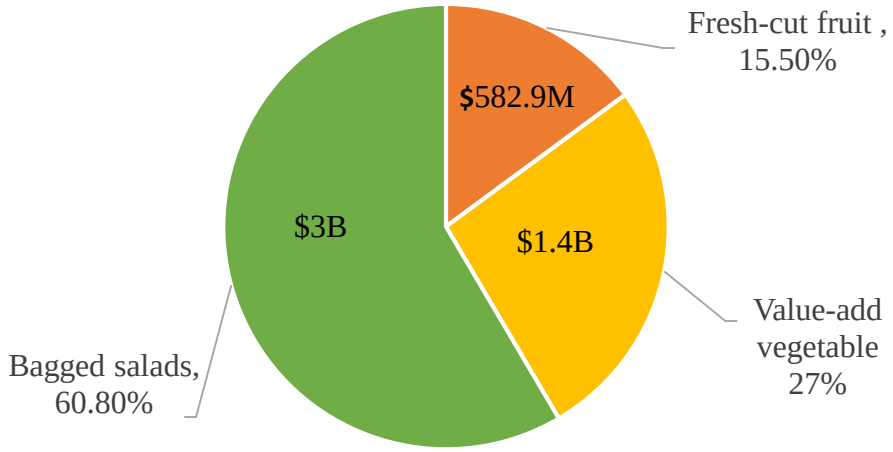

Figure 1.2: *Estimated value-added produce sales in select US retailers estimated by Cook from various sources (Cook, 2012).*

1.2 Fresh-cut safety issues and related outbreaks

Fresh vegetables are inherently prone to bacterial contamination from infected wild and domestic animals, soil, contaminated irrigation water, soil amendments, sewage, air and poor worker hygiene (Nguyen-the & Carlin 1994, Nou & Luo 2010). Processing of fresh-cut fruits and vegetables introduce wounds and cuts to the tissues, which leads to an increased respiration rate (Watada et al., 1996). Vegetables are prone to bacterial attack and invasion which can lead to various types of spoilage depending on the product pH (Heard, 2002). Damaged cells at the cut surfaces of vegetables increase the sugar supply and provide microbial pathogens an opportunity to adapt and grow.

As Americans change their eating habit towards more healthy food, the incidence of foodborne outbreaks related to fresh produce has continued to increase. Among the 25 major monitored microbial pathogens, *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* are the bacterial foodborne pathogens of most concern in fresh produce (FDA, 2008). *Escherichia coli* O157:H7 and *Salmonella* enterica have been most frequently linked to illnesses involving fresh produce (Sivapalasingam et al., 2004), and have received considerable attention following two leafy green outbreaks in 2006 and 2007.

Leafy greens are leafy vegetables that include Romaine lettuce, green leaf lettuce, red leafy lettuce, baby leafy lettuce, escarole, endive, spring mix, spinach, cabbage, kale, and chard (Matthews, 2014). From 1998 to 2008, the number of annual cases of foodborne illnesses was estimated at 9.6 million, among which leafy vegetable-related illnesses attributed to 22% of the cases in United States (Painter et al., 2013). Overall, 57,000 of 9.6 million individuals were hospitalized, with 14% of these hospitalizations caused by leafy vegetables. Leafy greens accounted for 6% of the 1451 fatalities annually (Painter et al., 2013). The Food and Drug

Administration ranked leafy greens as the riskiest food in 2009, responsible for 363 outbreaks, and 13,568 illnesses (Klein et al., 2009), with enterohemorrhagic *E. coli* having the greatest impact on human health. *E. coli* outbreaks since 1999 associated with leafy green consumption are listed in Table 1.1 (CDC, 2014).

Table 1.1: Leafy greens associated *E. coli* outbreaks from 1999 to 2013. Information based on Foodborne Outbreak Online Database (CDC). The October 2013 outbreak are based on CDC (CDC, 2013).

Date	Location	Pathogen	Illnesses Reported	Produce
Feb. 99	NE	*E. coli* O157:H7	65	Lettuce based salad
Jul. 99	OH	*E. coli* O157:H7	18	Coleslaw
Aug. 99	Multistate	*E. coli* O157:H7	14	Romaine lettuce
Oct. 99	OH	*E. coli* O157:H7	47	Salad
Oct. 99	Multistate	*E. coli* O157:H7	45	Romaine lettuce
Nov. 01	TX	*E. coli* O157:H7	20	Lettuce-based salads
Jul. 02	WA	*E. coli* O157:H7	55	Caesar salad
Nov. 02	Multistate	*E. coli* O157:H7	16	Iceberg lettuce
Sep. 03	CA	*E. coli* O157:H7	51	Lettuce-based salads
Oct. 03	CA	*E. coli* O157:H7	16	Spinach
Nov. 04	NJ	*E. coli* O157:H7	6	Lettuce
Sep. 05	Multistate	*E. coli* O157:H7	34	Lettuce, prepackaged
Oct. 05	Multistate	*E. coli* O157:H7	12	Lettuce, prepackaged
Aug. 06	Multistate	*E. coli* O157:H7	238 (5 deaths)	Spinach
Nov. 06	Multistate	*E. coli* O157:H7	77	Lettuce
Nov. 06	Multistate	*E. coli* O157:H7	80	Lettuce
Jun. 07	AL	*E. coli* O157:H7	26	Lettuce-based salad
May 08	WA	*E. coli* O157:H7	10	Spinach
Aug. 08	Multistate	*E. coli* O157:H7	13	Spinach
Apr. 09	Multistate	*E. coli* O157:H7	16	Lettuce
Sep. 09	Multistate	*E. coli* O157:H7	22	Lettuce
Apr. 10	Multistate	*E. coli* O145	31	Romaine lettuce

Table 1.1 (cont'd)

Date	Location	Pathogen	Illnesses Reported	Produce
Oct. 11	MN	*E. coli* O157:H7	*2*	Romaine lettuce
Oct. 11	Multistate	*E. coli* O157:H7	26	Lettuce
Oct. 11	Multistate	*E. coli* O157:H7	60	Romaine lettuce
Apr. 12	CA	*E. coli* O157:H7	12	Lettuce
May. 12	GA	*E. coli* O157:H7	2	Iceberg lettuce
Jun. 12	Multistate	*E. coli* O157:H7	52	Romaine lettuce
Sep. 12	PA	*E. coli* O157:H7	9	Romaine lettuce
Oct. 12	Multistate	*E. coli* O157:H7	33	Prepackaged leafy greens
Oct. 12	Multistate	*E. coli* O145	16	Lettuce
Nov. 12	WA	*E. coli* O157:H7	4	Spinach
Nov. 12	MA	*E. coli* O157:H7	8	Leaf lettuce
Oct. 13	Multistate	*E. coli* O157:H7	33	Salad

E. coli O157:H7 outbreaks have continued to occur with the majority of the produce-related outbreaks associated with lettuce and spinach. In August 2006, a multistate *E. coli* O157:H7 outbreak which caused 238 illnesses and 5 deaths was linked to consumption of bagged spinach. FDA traced the spinach back to a company in California's Salinas Valley. Four spinach fields were possible origins of contamination with the same outbreak strain isolated from cattle and a wild boar in nearby fields.

The *E. coli* O145 outbreak in April 2010 was related to the consumption of shredded Romaine lettuce from a single processing facility (CDC, 2011). Therefore, the outbreaks indicated that the microbial hazards present during pre-harvest and harvest cannot be completely eliminated by post-harvest handling or processing.

1.3 *Escherichia coli* O157:H7

Escherichia coli is a Gram- negative, facultatively anaerobic rod-shaped bacteria which is commonly found in the intestinal tract of humans and animals. Most strains are not harmful, however, *Escherichia coli* serotype O157:H7 (expressing the O-antigen 157 and the H-antigen 7) belongs to the group of pathogenic enterohemorrhagic *E. coli* (EHEC). EHEC are virulent due to their ability to produce shiga- like toxins, adherence factors and enterohemolysin. *E. coli* O157:H7 produces fimbriae that aid in attachment and colonization of intestinal mucosal cells (Ashkenazi et al., 1992). Clinical symptoms of *E. coli* O157:H7 infections vary from non-bloody diarrhea to hemorrhagic colitis and hemolytic uremic syndrome (HUS) which is the leading cause of acute renal failure in children (Proulx et al., 2001).

E. coli O157:H7 was first recognized as a foodborne pathogen in 1982 when under cooked hamburgers were linked to a large outbreak. Cattle are known as a main reservoir for *E. coli* O157:H7, and can shed the pathogen in feces at level as high as 10^6 CFU/g (Chase-Topping et al., 2007). However, some other species such as sheep were also reported to shed *E. coli* O157:H7 at similar levels (Ogden et al., 2005). *E. coli* O157:H7 can survive for long periods of time at a pH as low as 3.4 (Benjamin & Datta. 1995) in apple cider (Zhao et al., 1993) and mayonnaise (Raghubeer et al., 1995). Furthermore, the low infectious dose of 10 - 100 cells (FDA 2012) is problematic when fresh produce is consumed raw without further processing.

1.4 Lettuce contamination during pre-harvest and post-harvest handling

1.4.1 Pre-harvest

Before harvest, foodborne pathogens can harbor and persist in contaminated irrigation water (Erickson et al., 2010; Islam et al., 2005; Solomon et al., 2002), sewage, soil (Ma et al., 2014), dust, insects (Hancock et al., 1998; Rahn et al., 1997), animals (Doane et al., 2007), animal feces (Kudva et al., 1998; Wang et al., 1996), and improperly treated soil amendments

(Islam et al., 2005; Jensen et al., 2013; Johannessen et al., 2005). Field workers can further contaminate the lettuce during harvesting. Pathogens can attach to the leaf surfaces and/or internalize into the lettuce tissue or be up taken by the roots from soil or irrigation water into internal tissues. Pathogens tend to attach to the stomata, lenticels or sites of biological or physical damage on the lettuce surface. Survival, contamination and internalization are affected by many factors such as humidity, temperature, health and maturity of the plant (Kroupitski et al., 2009; Pu et al., 2009), and motility of the pathogen (Kroupitski et al., 2009).

In most cases, foodborne pathogens are difficult to distinguish from microorganisms on produce. The microbial populations on lettuce may not be sufficient to cause decay but may be enough to cause human infection due to the low infectious dose of pathogens. Therefore, perfection in appearance is not a guarantee for microbial safety. Increased outbreaks of *E. coli* O157:H7 in recent years have prompted significant efforts towards the development and implementation of improved Good Agricultural Practices (GAPs) to minimize pathogen contamination in the field.

1.4.2 At-harvest

Lettuce is harvested as soon as the plants reach acceptable size and firmness (Salunkge and Kadam, 1998). In most cases, lettuce is field packed and usually cut with a long-handled sharp knife. Heads can be wrapped or bagged in water vapor permeable plastic film (Salunkge and Kadam, 1998) by the cutter or packer. Cross-contamination might occur due to these harvesting activities. Taormina reported that a coring knife contaminated by soil containing 2.72 and 1.67 log CFU/g *E. coli* O157:H7 further contaminated all 10 subsequent lettuce heads by cutting and coring. Prolonged time for post-inoculation of knife did not decrease the likelihood of lettuce being contaminated (Taormina et al., 2009). More cuts and damage introduced to

lettuce during harvest facilitates the survival, internalization and growth of pathogens, and make the control and disinfection of the product during post-harvest handling even difficult.

1.4.3 Post-harvest

Soon after harvest, lettuce is usually transported from the field to a cooling warehouse to be pre-cooled to -1 °C at 98%-100% relative humidity (Salunkge and Kadam, 1998). This pre-cooling step is the most commonly used treatment to improve quality and prolong the shelf life of lettuce. However, Li et al. (2008) reported that vacuum cooling had a significant impact on *E. coli* O157:H7 cell infiltration into lettuce tissue. With vacuuming, a significantly larger portion of cells were internalized in the lettuce tissue than that for the control group. Internalized bacterial cells are unlikely to be removed during washing at fresh-cut processing facilities (Aruscavage et al., 2006).

After pre-cooling, lettuce is transported in refrigerated trucks to maintain quality on the way to the grocery store. After being sent to stores, lettuce is generally not stored for long periods at 2°C. However, precise temperature control during transportation between the manufacture and retailer are challenging due to exchange with extreme outside temperatures during produce loading and unloading. Koseki & Isobe (2005) reported that the temperature for lettuce in the supply chain fluctuated between 3° and 15°C. In a study conducted by McKellar et al. (2012), increased temperatures were also observed during transport although the temperature remained below the minimal growth temperature for *E. coli* O157:H7. However, this study was conducted in winter with greater temperature increases to be expected during the warmer months. The impact of temperature on *E. coli* O157:H7 growth during storage has been assessed in many studies (Chua et al., 2008; Luo et al., 2010; McEvoy et al., 2009; Oliveira et al., 2010). If *E. coli* O157:H7 is able to survive during post-harvest handling and processing, it is likely to

recover and grow during refrigerated storage (Delaquis et al., 2002; Diaz & Hotchkiss, 1996; Oliveira et al., 2010).

1.5 Contamination during post-harvest processing

Lettuce harvested for processing is placed in large bulk bins for transportation to the pre-cooling or processing facility. Lettuce can be cored either in the field or at local processing facility. At the processing facility where the temperature is generally between 0-5°C (Kitinoja and Gorny, 1999), lettuce which is processed for salads is cut by shredder, washed in cold, chlorine-containing water, centrifuged to remove the extra moisture and then packaged. The equipment used in processing facilities varies, but mostly includes a shredder, conveyor, flume tank, and dewatering centrifugal dryer. Any human foodborne pathogens contaminating lettuce in the field or at harvest could be transferred to a large quantity of product during subsequent processing. Outbreaks traced back to packaging facilities have been reported, therefore, Good Manufacturing Practices (GMPs) play an important role in preventing the risk of foodborne pathogens contamination during processing and packing.

1.5.1 Shredding

At the shredding step, lettuce passes through the shredder/cutter and is cut into certain sizes according to commercial needs. Shredder surface/ blades can be contaminated by field-contaminated lettuce or even animal feces/contaminated soil particles carried by the lettuce. In May of 2001, 41 cases of *Salmonella* Bovismorbificans infection were reported. The infections were associated with consumption of shredded lettuce. An investigation was conducted and suggested that the contamination was from cutting equipment in a processing plant (Stafford et al., 2002). If the shredding equipment is contaminated and efficient cleaning and sanitation cannot be implemented immediately, it is highly possible that pathogens persist on surfaces and

contamination may be spread to other product during continued production. Moore et al. (2003) conducted a study to evaluate the ability of *Salmonella* Typhimurium and *Campylobacter jejuni* to transfer from stainless steel surfaces to both wet and dry romaine lettuce through direct contact. For *S.* Typhimurium, 66% of the population transferred from dry stainless steel to dry lettuce after 60 minutes of direct contact. In contrast, 23% to 31% of the *Salmonella* population transferred from dry stainless steel to wet lettuce after 120 minutes of direct contact. Transfer of *C. jejuni* to dry and wet lettuce was similar, ranging from 16% to 38% and from 15% to 27% after 80 minutes of contact, respectively. These results suggest that effective cleaning and sanitizing protocols should be developed since bacteria can attach to equipment surfaces and produce biofilms.

Although the temperature in processing facilities is low, it is still possible for bacteria to form biofilms. Research by Rossi et al. (2013) showed that at 4°C, out of the 32 strains isolated from milk and vegetables, 20 strains (62.5%) were able to form biofilms on stainless steel in 24 hours, and 28 and 27 strains formed biofilms after 48 and 72 hours, respectively. At 20°C, more strains were found to form biofilms than at 4°C.

1.5.2 Conveying

After being shredded, lettuce is step-conveyed and then flume washed. Conveyor can be present at many different points of the processing line and play a major role in transportation. Conveyors used in the leafy green industry are mainly smooth belted with one seam. Various materials can be used for conveyor belts such high-density polyethylene, polypropylene, and acetyl (Buchholz et al., 2010). In one study by Allen et al. (2005) in Florida, the survival of *Salmonella* on a packing line was evaluated. Five types of surface materials were assessed, including stainless steel, polyvinyl chloride (PVC), sponge rollers, conveyor belts and an

unfinished oak surface. During simulated spring conditions with a temperature of 30°C and a relative humidity of 80%, *Salmonella* were able to survive on these conveyors for 3 days while in simulated fall/ winter conditions with temperature of 20°C and humidity of 60%, *Salmonella* were able to survive on the same materials for 21 days.

Although conveyor belt surfaces are visually smooth, crevices and holes can be observed by scanning electron microscopy (Mafu et al., 1990). These crevices and holes serve as attachment sites for bacteria. Therefore, cleaning and sanitizing conveyors remains challenging. Rossi et al. (2013) reported, if the sponges used to clean stainless steel and polyethylene surfaces were contaminated with fecal coliforms at 5 log CFU, then transfer varied from 3.3 to 5.5 log CFU/cm^2 for stainless steel and from 3.5 to 5.6 log CFU/cm^2 for polyethylene. More than 1 log CFU/cm^2 survived after 24 hours on both surfaces.

1.5.3 Flume washing

In commercial processing, shredded lettuce is washed in recirculating cold water with sanitizer for 30 seconds to 2 minutes to remove soil and debris (Herdt and Feng, 2009). Commercial sanitizers used in leafy green industry include chlorine-based sanitizers, peroxyacetic acid and mixed peracid (Herdt and Feng, 2009), with chlorine used most commonly. The purposes of the washing are to reduce the temperature of the produce, provide an appealing appearance, prolong shelf life and reduce the microbial load, which are also critical for microbial safety (Herdt and Feng, 2009). However, the efficacy of the most commonly used chlorine-based sanitizer in commercial washing is questionable since antimicrobial activity is affected by organic load. In leafy green processing, the organic load of wash water consists of debris, cellular fluids released from cut leaves, soil particles, insects, and microbes (Herdt and Feng, 2009). It accumulates and increases during washing (Nou and Luo, 2010). The decreased

efficacy of sanitizers make the wash water an ideal vehicle of transferring microorganisms to a much larger volume of produce and increases the risk of cross-contamination.

1.5.4 Dewatering and drying

After flume washing, excess water must be removed before packing to maintain product quality and optimal shelf life. Leave that are wet with microorganisms or pathogens increase the possibility of microbial proliferation and encourage spoilage or even microbial hazards. Various types of dewatering and drying equipment can be used such as shaker/ spinner, forced-air drying tunnel, blower, or centrifugal dryer (Buchholz et al., 2010; Turatti, 2011; Krasaekoopt and Bhandari, 2011). Intensity is the main factor that needs to be adjusted for various products. Shredded iceberg and romaine lettuce is normally spin blow dried followed by centrifugal drying or centrifugally dried alone. Other products that cannot withstand intensive drying such as centrifugal drying can be treated with other drying processes such as spin drying or drying in drying tunnels. If wash water is contaminated, it is highly possible that lettuce leaves with contaminated water further spread the contamination to drying equipment. In the work of Buchholz et al. (2012b), leafy greens previously inoculated with *E. coli* O157:H7 that were washed in sanitizer-free water were able to transfer ~90% of the original inoculum to wash water and contaminate the whole processing line. Centrifugal drying, followed by conveying and shredding, picked up more *E. coli* O157:H7 from product than the flume tank and shaker table.

1.6 Industry washing

In the fresh produce industry, washing is a critical step to eliminate potential chemical, physical and microbial hazards. Since fresh-cut leafy greens are consumed raw, washing is even more crucial to assure the quality as well the safety of the product.

1.6.1 Industry washing system

Leafy greens are crisp and subject to bruising and damage, therefore, other washing aids such as surface brushing can be used for apples and tomatoes but not leafy greens. Therefore, a well-designed washing system and an effective washing process are needed.

Multiple washes is more effective than a single wash if the produce is washed with fresh water (Gil et al., 2013). Normally, an optimal washing system consists of three tanks (Gil et al., 2013), with each tank having its own specific function in washing. The first of these tanks, is used as a pre-washing procedure and aims to eliminate soil particles or other debris attached to produce and conveys the product to later washing steps. The significance of the first step washing is maximum removal of organic matter that might hinder sanitizer efficacy and ensure washing effectiveness. In most cases, the water flow rate in this tank is comparatively low. In some cases, the washing system includes some pipes at the bottom that blow high volumes of air to ensure that the produce is floating on the water surface (Simons and Sanguansri, 1997). It also generates a strong jaccuzi effect that causes produce to move and tumble, creating the mechanical action needed to loosen and remove dirt and debris (FSAI, 2001). This dirt and debris floating on water surface or sinking to the bottom of the tank, is either removed by an incorporate system (Turatti, 2011) or cleaned by release through a periodic draining system. The organic load and microbial counts accumulate rapidly in this tank, therefore, proper water management is necessary. Proper sanitizing agents and treatment conditions should be applied and frequently monitored to ensure water quality.

The second tank is where sanitation/decontamination takes place. Wash water added with sanitizing agent further reduces the microbial load and prevents cross-contamination (Herdt and Feng, 2009; Luo et al., 2011). The type of tank varies, it could be a flume tank with/without

overhead jets or a submersion tank with/or without spray disinfection application (FDA, 2008; FDA, 2008). Generally, the flow rate in the flume tank is much higher than in the dump tank. Water in the flume tank is recirculated, organic matter accumulates in the water which consumes sanitizer and decreases the ability of the sanitizer to minimize cross-contamination (Zhang et al., 2009), thereby the sanitizer concentration needs to be monitored and adjusted frequently. In some cases, the flume tank is equipped with overhead jets. In addition to shear forces generated by liquid flow and turbulence, wash water ejected by these jets generates a strong force which acts on the produce surface (Dezuniga et al., 1991) to prolong the contact time with the sanitizer. A submersion tank filled with sanitizer can be used as another method for decontamination. Residence time of produce and concentration of sanitizer in wash water are key factors for microbial reduction (FSAI, 2001). Sometimes, a submersion tank is used as a prewash step, after which a sanitizer spray is applied to the produce surface for decontamination.

The third tank is not always present in all cases, however, the main purpose of the last washing step is to eliminate sanitizer residues on the produce, give the produce a final wash and reduce the temperature of the produce. A tank used here should be filled with sanitizer-free water with the quality of water maintained in good condition. In some cases, a sanitizer-free spray is used instead (Herdt and Feng, 2009).

1.6.2 Problems in flume washing

Generally, in postharvest processing of fresh produce, washing is the only intervention activity. It is a key procedure to reduce potential microbiological hazards. Any foodborne pathogen that contacted and contaminated produce in the earlier stage of harvesting is supposed to be eliminated.

To increase washing efficacy, several sanitizing agents are used in the fresh-cut processing industry to reduce potential microbial hazards, including chlorine-based sanitizers, peroxyacetic acid and mixed peracid (peroxyacetic acid, organic acids, hydrogen peroxide, acetic acid) (Herdt and Feng, 2009), among which, chlorine is the most widely used (López-Gálvez et al. 2009) due to its ease of use and relatively low cost. Numerous researchers (López-Gálvez et al., 2009; Parish et al., 2003; Pirovani et al., 2004, Singh et al., 2002; Gil et al., 2013) have assessed the reduction of pathogens on lettuce using sanitizers. However, the organic load builds up quickly in dump and immersion tanks during washing. In addition, chlorine will react with organic and inorganic matter in the wash water, with the efficacy of chlorine deceasing rapidly as the organic load increases (Gil et al., 2013). Davidson et al. (2013) evaluated the efficacy of some other sanitizers. After washing a 5.4 kg batch of pre-inoculated lettuce containing *E. coli* O157:H7 at 6 log CFU/g with water alone and water with peroxyacetic acid, the processing water yielded 3.47 and 3.01 log CFU/ml *E. coli* O157:H7, respectively, after 90 s of washing. Mixed peracid and peroxyacetic acid yielded similar results in terms of *E. coli* O157:H7 reduction. Hence, use of a sanitizer does not ensure a microbiologically safe final product. In some European countries, including Germany, the Netherlands, Denmark, Switzerland and Belgium, use of antimicrobial agents such as chlorine in during washing of fresh-cut produce washing is forbidden (Artés & Allende 2005; Nguyen-the and Carlin, 1994).

Cleaning of the processing/washing equipment is another potential problem. Most fresh-cut processing plants are using clean-in-place (CIP) cleaning. CIP means that the interior surfaces of pipes, vessels, process equipment, filters and associated fittings are cleaned without disassembly. If a sanitizer is not functioning during washing due to the increased organic load and solid content in water, fresh produce carrying foodborne pathogens has chance to

contaminate wash water, other produce and the processing equipment. Biofilms (Ryu and Beuchat, 2005) can be formed by *E. coli* O157:H7 on stainless steel and protect microorganisms from sanitizers.

In industry, washing system vary from plant to plant. It is hard to define a best washing system due to the different intrinsic properties of fresh produce being washed and different requirements in microbial reduction, product appearance and washing costs. The exact sanitizer type, wash time, flow rate, pH and temperature etc. in washing conditions for optimal washing of produce remain unknown.

1.7 Washing conditions

Washing, in particular, is a sanitizing practice, the efficiency of which is often affected by different factors (Adams et al., 1989). Washing conditions vary depending on the processing plant and produce characteristics. Both the chemical factors, such as the types (Beuchat et al., 2004; Davidson et al., 2013; Keskinen and Annous, 2011; Keskinen et al., 2009; López-Gálvez et al., 2009; Singh et al., 2002) and concentrations (Keskinen and Annous, 2011; Kim et al., 2008; Luo et al., 2011; Barrera et al., 2012) of sanitizer, and physical factors, such as wash water flow rate, water hardness, washing time (Barrera et al., 2012), pH (Akbas & Ölmez 2007; Park et al., 2011; Yang et al. 2003), temperature (Baur et al., 2005; Martin-Diana et al., 2005), water-product ratio (Lu et al. 2007; FDA, 2008) and organic load can influence the effectiveness of washing (FDA, 2008). In addition, surface roughness of the washed object, and adhesion force of different bacteria are also considered as factors that affect washing efficiency.

1.7.1 Flow rates

In fluid dynamics, flow rate refers to the mass flow rates or volumetric flow rate. The volumetric flow rate is the volume of fluid which passes per unit time, whereas mass flow rate is

the mass of a fluid which passes per unit time (Semat and Katz, 1958). Under steady-state conditions, the mass and volumetric flow rates remain constant.

Flowmeters are used to measure flow rates. End-line and in-line flowmeters are the two broad categories, with end-line flowmeter used at the outlet or discharge of the flow and in-line flowmeter placed in line with the pipe, respectively (Cimbala, 2009). End-line flowmeter is also called discharge flowmeter: it calculate the flow rate by measuring the time to fill up a container of known volume. Positive-displacement flowmeter is a commonly used in-line flowmeter which measures the volumetric flow rate by collecting a fixed volume of fluid and then counting the time needed for collection. Various units are used depending on the application and industry, but might include gallons per minute, liters per second or cubic meters per second.

Flow velocity is the distance liquid particles travel per unit of time (Semat and Katz, 1958). It mathematically describes the motion of a fluid.

1.7.2. Flow regime and Reynolds number

Three different flow regimes are recognized - laminar, transitional, and turbulent flow (Singh and Heldman, 2014). Laminar flow occurs when a fluid travels smoothly or in parallel layers. In contrast, turbulent flow occurs when a fluid undergoes irregular fluctuations with lateral mixing. Between laminar and turbulent flow, there is an intermediate level with both laminar and turbulent called transitional flow. A simple dye can be used to characterize the type of flow. At low flow rates, the dye moves in a linear manner in the axial direction, which is characteristic of laminar flow. As the flow rate increases to some intermediate level, the dye begins to blur at a certain distance away from the injection point, which is due to some of the dye moving in a radial direction. At high flow rates of turbulent flow, the dye blurs immediately at the point of injection and spreads in a random manner along both the radial and axial directions.

The Reynolds number (*Re*) is a dimensionless number (Munson et al., 2009), obtained from the ratio of the inertial resistance to viscous resistance for a flowing fluid (as shown in the equation below). It is a critical value for quantitatively describing the characteristics of a fluid flowing either in a pipe or on the surfaces of objects with different shapes. It can be used to specifically identify how a given liquid would behave under selected flow conditions.

$$N_{Re} = \frac{\rho \bar{u} D}{\mu}$$ (Singh and Heldman, 2014)

In this equation, ρ is the liquid density, D is tube diameter, $\bar{u}$ is the average fluid velocity, and μ is the liquid viscosity.

In a pipe, laminar flow has a Reynolds number of 2100 or less. When the Reynolds number is greater than 4000, the type of flow is turbulent. Any Reynolds number between 2100 and 4000 signifies a transitional flow rate (Singh and Heldman, 2014).

1.7.3 Precious studies

1.7.3.1 Wash flow rate and shear force

The prime goal of produce washing is to reduce the microbial load (Ukuku and Fett, 2002). Because bacteria usually attach to the surface of produce by binding or adhesion forces, additional force, particular shear force, is needed to break these bonds and detach the cells. Bacterial binding to the produce surface depends on the surface characteristics of both the bacterium and the material surface. For example, in ligand/receptor mediated attachment, the number of bonds is a function of ligand and receptor density (Hubble et al., 1996). The number of effective bonds between bacteria and the substrate surface determines the amount of additional shear stress needed to detach the organism. There is an optimum flow rate (shear force) for bacterial attachment that reflects the balance between the rate of delivery and the force acting on attached bacteria (An and Friedman, 1998; Liu and Tay, 2002). Generally, high shear

rates on produce surfaces during washing increase detachment of bacteria (Chang et al., 1991). At higher flow rates, a higher shear force is applied to attached bacteria, and the potential to form higher numbers of bonds is of much greater significance (Hubble et al., 1996). When bacteria are exposed to a sanitizer, increased turbulence during washing may aid in breaking up bacterial clumps, thus increasing accessibility of the sanitizer (Wang et al. 2007). However, in some cases, increased shear force tends to enhance the attachment of bacteria and their binding surfaces. Thomas et al. (2004) and Rangel et al. (2013) reported higher shear forces facilitated the binding of *E. coli* O157:H7 from FimH (a lectin-like protein responsible for adhesion properties of type 1 fimbriae).

Flow rate is an important factor that affects the efficiency of washing. Most laboratory studies have focused on laminar flow. Wang et al. (2007) assessed *E. coli* O157:H7 reductions from the surfaces of cantaloupe rind and cut apples under different washing conditions. After 3-min of washing in a flow-through chamber, *E. coli* O157:H7 populations on cantaloupe rind and cut apples decreased 2.5 and 2.3 log CFU/cm^2, respectively, as the flow velocity increased from 0.0 to 0.52, and 0.65 to 0.8 m/min. Populations of *E. coli* O157:H7 decreased as the flow velocity increased in both chambers (Wang et al., 2007). However, contradictory results were reported by (Huang et al., 2013). In their research, a comparison in *L. monocytogenes* reductions was made between dipping and washing with tap water. At an initial inoculation level of 3 log CFU/g, the reduction caused by washing under running tap water (2 L/min) was less than immersing in water. However, the treatment times for dipping (5 min) and washing (1 min) were different; thus, it was difficult to conclude that dipping achieve a better performance than washing. In order to quantitatively investigate the relationship between flow rate and bacterial detachment, a number of studies have been conducted (Huang et al., 2013; Katsikogianni and

Missirlis, 2010; Mbaye et al., 2013; Yoshihara et al., 2014). Almost all of them suggested that bacteria reduction was dependent on flow rate, with higher flow rates resulting in greater reductions.

1.7.3.2 Washing time

Simply prolonging the washing period offers no significant improvement in bacterial removal, except for the initial stage of washing. Adams et al., (1989) observed a rapid drop in numbers of bacteria during the first 5 min of washing. Increasing the wash time for fresh lettuce with hypochlorite did not result in further microbial reductions, even though 60% of the original free available chlorine remained after 30 min of washing. In addition, 20 min of produce washing yielded similar results, with a 10 min extension yielding no further improvement (Adams et al., 1989)

Similar results were observed by Wang et al. (2007) who assessed removal of *E. coli* O157:H7 during washing of fruits. During the first 2 to 3 min, a relatively rapid reduction in *E. co*li O157:H7 was observed. However, extending the wash time an additional 3 min did not significantly increase bacteria removal at the different flow velocities (Wang et al., 2007).

At the beginning of washing, time is an important factor regarding the reduction of bacterial population. (Frank, 2001) proposed that washing lettuce under running tap water permitted a maximum of 1 log CFU/g reduction in approximately 30 s; however, 10 s of washing resulted in only a 0.6 log CFU/g reduction, with no difference in reduction seen after 5 and 15 min.

1.7.3.2 Bacterial binding ability, attachment to surfaces, and the surrounding environment

The ability to attach and detach from surfaces is an intrinsic characteristic of microorganisms. Bacterial attachment is the initial stage of pathogen infections, which allows

bacteria to further grow and survive. Attachment is influenced by the physical/chemical properties of the cell surface, contact surface, and the surrounding environment (Frank, 2001). Capsule and fimbriae are two major functional structures on bacterial cell surface for attachment. The capsule of a bacterial cell often contains acidic residues which that impart a negative charge to the bacteria surface (Sutherland, 1985). This hydrophilic capsule prefers a hydrophilic surface and prevents adhesion between bacteria and hydrophobic surfaces (Ofek and Doyle, 1994). The amino acids in fimbriae contain numerous nonpolar side chains that impart hydrophobicity to the structure (Bitton and Marshall, 1980; Frank, 2001). The FimH adhesin is a two-domain protein located on the tip of type 1 fimbriae. The *E. coli* adhesin FimH has two highly conserved cysteines that form a disulphide bond under its ligand-binding (Choudhury et al., 1999). In the research of (Nilsson et al., 2007), FimH was found to bind to mannose-coated surfaces through catch bonds, which persist longer under higher tensile force. Thus, fluid flow-induced tensile forces can significantly enhance the binding strength of bacteria (Nilsson et al., 2007).

Surface properties of produce can also contribute to microbial adhesion. These properties include surface roughness and hydrophobicity. Surface roughness influences bacterial attachment and removal from a surface (Wang et al., 2009). Han (2000) suggested that *E. coli* O157:H7 preferentially attached to coarse or injured surfaces compared to uninjured surfaces of green peppers. Hydrophobicity relates to the physical properties of the surface of produce, which tends to be non-polar and repel water. However, the relationship between surface hydrophobicity and surface roughness of fresh produce is still unknown, although some comparison studies have been conducted to evaluate the influence of surface roughness. (Fernandes et al., 2014) proposed that surface roughness was not a dominant factor for bacterial adhesion. Both green peppers (average roughness (Ra) =13.0 ± 2.7 nm) and melons (Ra =33.5 ± 7.9 nm) showed bacterial

adhesion (7.3 and 7.0 log CFU/cm^2 for *E. coli* and *S. enterica* Enteritidis, respectively). These findings suggest that adhesion is a multifactorial process (Fernandes et al., 2014).

Bacterial attachment and detachment is also influenced by temperature (Jaglic et al., 2011; Rajkowski and Fan, 2008; Shwartz et al., 2003) and pH (Lewis et al., 1989). Normally, bacterial detachment increases with increasing temperature and pH. This can be explained by the thermal and pH inactivation of bacterial pathogens (Dixon et al., 2012; Spinks et al., 2006).

1.8 Overall goals.

In commercial fresh-cut processing facilities, microbial contaminants including bacterial pathogens can spread to large volumes of product during washing, particularly when chlorine-based sanitizers become less effective in the presence of high organic loads. On one *Salmonella* Bovismorbificans outbreak in May of 2001 (Stafford et al., 2002), contamination spread from a shredder to subsequent product being processed. Therefore, it is important to assess the potential contribution of mechanical shredding and conveying as well as washing at different flow rates in spreading *E. coli* O157:H7 during simulated commercial production of fresh-cut leafy greens. Such work is critical for the development of predictive mathematical models for pathogen transfer during production of fresh-cut produce.

CHAPTER 2:

Spread of *Escherichia coli* O157:H7 from a Contaminated Shredder or Conveyor Belt to Fresh-Cut Iceberg Lettuce during Simulated Commercial Production

2.1 OBJECTIVE

The objectives of this study were to 1assess the transfer of *E. coli* O157:H7 from a product-contaminated shredder and conveyor to iceberg lettuce, wash water, and equipment surfaces during the production of fresh-cut iceberg lettuce

2.2 MATERIALS AND METHODS

2.2.1 Overall experimental design. A small-scale commercial leafy green processing line consisting of a shredder, conveyor, flume tank, shaker table and centrifugal dryer was used to assess *E. coli* O157:H7 transfer during production of fresh-cut iceberg lettuce. Uninoculated lettuce (22.7 kg) was first shredded or shredded and conveyed after which 9.1 kg of radicchio (previously dip-inoculated with *E. coli* O157:H7 at 10^6 CFU/g) was processed to contaminate the shredder or conveyor belt. In order to separately quantify transfer from the shredder and conveyor, the conveyor and shredder were then respectively cleaned and sanitized before processing an additional 90.7 kg of uninoculated iceberg uninoculated lettuce. Within a 5 min processing, 15 radicchio-free lettuce samples were collected immediately after exiting the contaminated shredder or conveyor. These samples, along with additional lettuce, sanitizer-free water and equipment surface samples collected throughout processing were then quantitatively examined for *E. coli* O157:H7.

2.2.2 Leafy greens. Individually wrapped heads of iceberg (*Lactuca sativa* L.) (24 heads per case) and radicchio (*Cichorium intybus*) (2.3 kg per case) lettuce were obtained from a local wholesaler (Stan Setas Produce Co., Lansing, MI), stored in a 4^{o}C walk-in cooler, and used within 2 days of delivery. All heads were hand-cored immediately before use.

2.2.3 Bacterial strains. Four non-toxigenic (stx_1^- and stx_2^-), green fluorescent protein (GFP)-labeled, ampicillin-resistant strains of *E. coli* O157: H7 (6980-2, 6982-2, ATCC 43888, and CV2b7) previously obtained from Dr. Michael Doyle (Center for Food Safety, University of Georgia, Griffin, GA) were stored in tryptic soy broth (Difco, BD, Sparks, MD) containing 0.6% (w/vol) yeast extract (TSBYE; Difco, BD) and 10% (vol/vol) glycerol (Sigma Chemical Co., St. Louis, MO) at -80^{o}C until use. Working cultures were prepared by streaking each stock culture

onto plates of tryptic soy agar (Difco, BD) containing 0.6% (w/vol) yeast extract and 100 ppm of ampicillin (ampicillin sodium salt, Sigma Life Science, St. Louis, MO) (TSAYE plus amp). After 18 to 24 h of incubation at 37°C, a single colony was transferred to 9 ml of TSBYE containing 100 ppm ampicillin (TSBYE plus amp) and incubated at 37°C for 24 h.

2.2.4 Inoculum preparation. From those 9 ml of culture, a 0.2 ml aliquot of each non-toxigenic *E. coli* O157: H7 was added to 200 ml of TSBYE plus amp and incubated at 37°C for 24 h. As determined previously by Buchholz et al. (2012a), the growth rates for these four *E. coli* O157: H7 strains were similar. Therefore, the four strains were combined in equal volumes to obtain an 800 ml cocktail, which was added to 80 L of tap water in a 121 L plastic container (Rubbermmaid, Wooster, OH) to obtain a population of ~7 log CFU/ml.

2.2.5 Radicchio inoculation. After removing the outer leaves, the radicchio heads (9.1 kg) were cored with a sterile knife, placed in a centrifugation basket, immersed into the inoculum (Preparation described in 2.2.4) for 15 min, and then drained at room temperature (22 °C) for 1 h before being spun in a commercial dewatering centrifuge (described in 2.2.6) to eliminate any residual inoculum from the lettuce head. Based on triplicate analysis of 25-g samples collected from random layers, the radicchio contained an *E. coli* O157:H7 population of ~6 log CFU/g before processing.

2.2.6 Processing line. A small-scale commercial leafy green processing line was used consisting of a shredder (model TRS 2500 Urschel TranSlicer, Valparaiso, IN), step conveyor (ThermoDrive, Mol Industries, Grand Rapids, MI), 3.3-m long stainless steel flume tank (Heinzen Manufacturing, Inc., Gilroy, CA) with two overhead spray jets, stainless steel shaker table operated by a 1 HP Baldor washdown duty motor (Baldor Electric Co., Fort Smith, AR), a ~1000 L capacity stainless steel water recirculation tank, and a 22.7 kg capacity centrifugal Spin

Dryer (Model SD50-LT, Heinzen Manufacturing, Inc.)

2.2.7 Lettuce processing. Initially**,** 22.7 kg of iceberg lettuce were shredded or shredded and conveyed to prime the processing line, followed by 9.1 kg of radicchio containing *E. coli* O157:H7 at 10^6 CFU/g to contaminate the shredder or shredder and conveyor, respectively. In the contaminated conveyor study, the shredder were sanitized, rinsed thoroughly and air dried to assess transfer exclusively from the conveyor. Thereafter, iceberg lettuce (90.7 kg) was shredded, conveyed to the flume tank, washed for 90 s in sanitizer-free water, and dewatered using the shaker table. After collecting 20 5.5-kg samples of dewatered lettuce and removing the shreds of radicchio by visual inspection, every four baskets of lettuce were combined and spun in the centrifugal dryer for 80 s.

2.2.8 Sample collection. Three iceberg lettuce samples were collected before processing as the control. Fifteen radicchio-free iceberg lettuce samples were also collected immediately after contacting the pre-contaminated shredder/conveyer during processing, with 20 and 4 radicchio-free iceberg lettuce samples collected after shaker table dewatering and centrifugal drying, respectively. Three 50-ml water samples were collected from the water return spout above the recirculation tank at the beginning, middle, and end of processing. During centrifugal drying, five water samples (50 ml each) were collected from the centrifuge drain. The equipment surface sampling locations (100 cm^2 each) as previously described by Buchholz et al (2012) were shown in Figure 2.1, 2.2, and 2.3. The 1-ply composite tissue method was used for surface sampling as described by Vorst et al. (2004). In the contaminated shredder study, 7 equipment surface samples (Figure 2.1) were collected before and after processing the 90.7 kg of iceberg lettuce, with 8, 11, 9, and 8 surface samples respectively collected from the conveyor belt (Figure 2.2), flume tank (Figure 2.3), shaker table (Figure 2.3), and centrifugal dryer (Figure 2.3)

immediately after processing.

2.2.9 Microbiological analyses. All 25-g lettuce samples were added to 100ml sterile PBS, homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min and then quantitatively assayed for *E. coli* O157:H7 by plating appropriate dilutions on TSAYE with ampicillin. As described previously, the lettuce and equipment surface samples were either directly plated on TSAYE with amp or concentrated by membrane filtration, with a limit of detection (LOD) of 0.2 CFU/g and 2 CFU/100cm^2, respectively. Water samples were concentrated by membrane filtration, with a LOD of 0.01 CFU/ml. All plates were incubated at 37^{o}C for 24 h, and observed for green fluorescing colonies of *E. coli* O157:H7 under ultraviolet light (365 nm, Black-Ray, Ultra-violet Product Inc. San Gabriel, CA).

2.2.10 Statistical analysis. *E. coli* O157:H7 counts from triplicate experiments were converted to log CFU/ml, log CFU/g or log CFU/100cm^2, averaged and then subjected to an analysis of variance (ANOVA) using JMP 11.0 (SAS Institute Inc., Cary, NC). Values equaling half the limit of detection were used for samples yielded no *E. coli* O157:H7 counts. The Tukey-Kramer HSD test was used with P values of ≤ 0.05 considered significantly different.

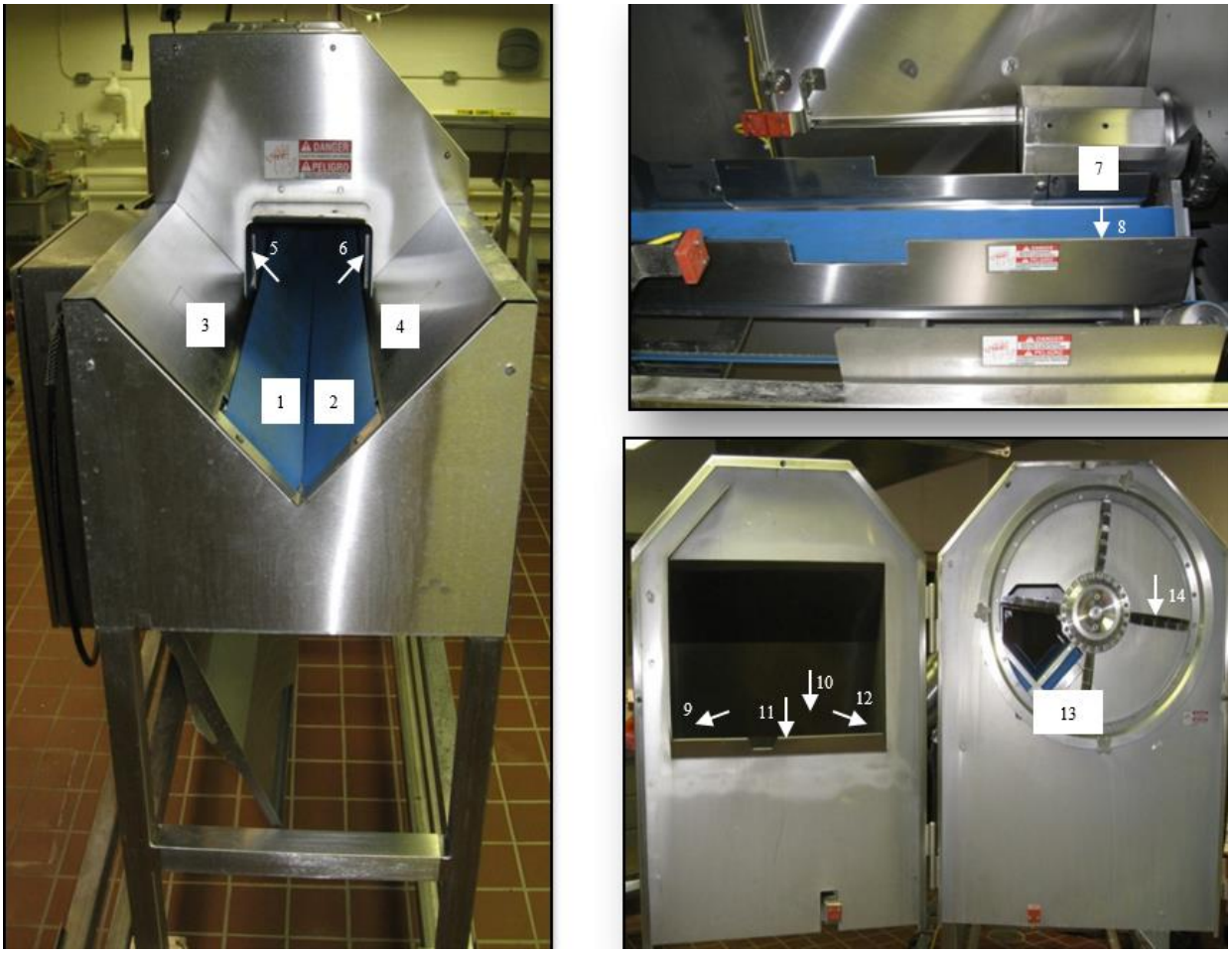

Figure 2.1: *Sampling locations on the shredder. Locations with number of 1,3,5,7,9,11, and 13 were sampled before 90.7 kg of iceberg lettuce was processed. Locations with number 2, 4, 6, 8, 10, 12, and 14 were sampled after 90.7 kg of iceberg lettuce was processed*

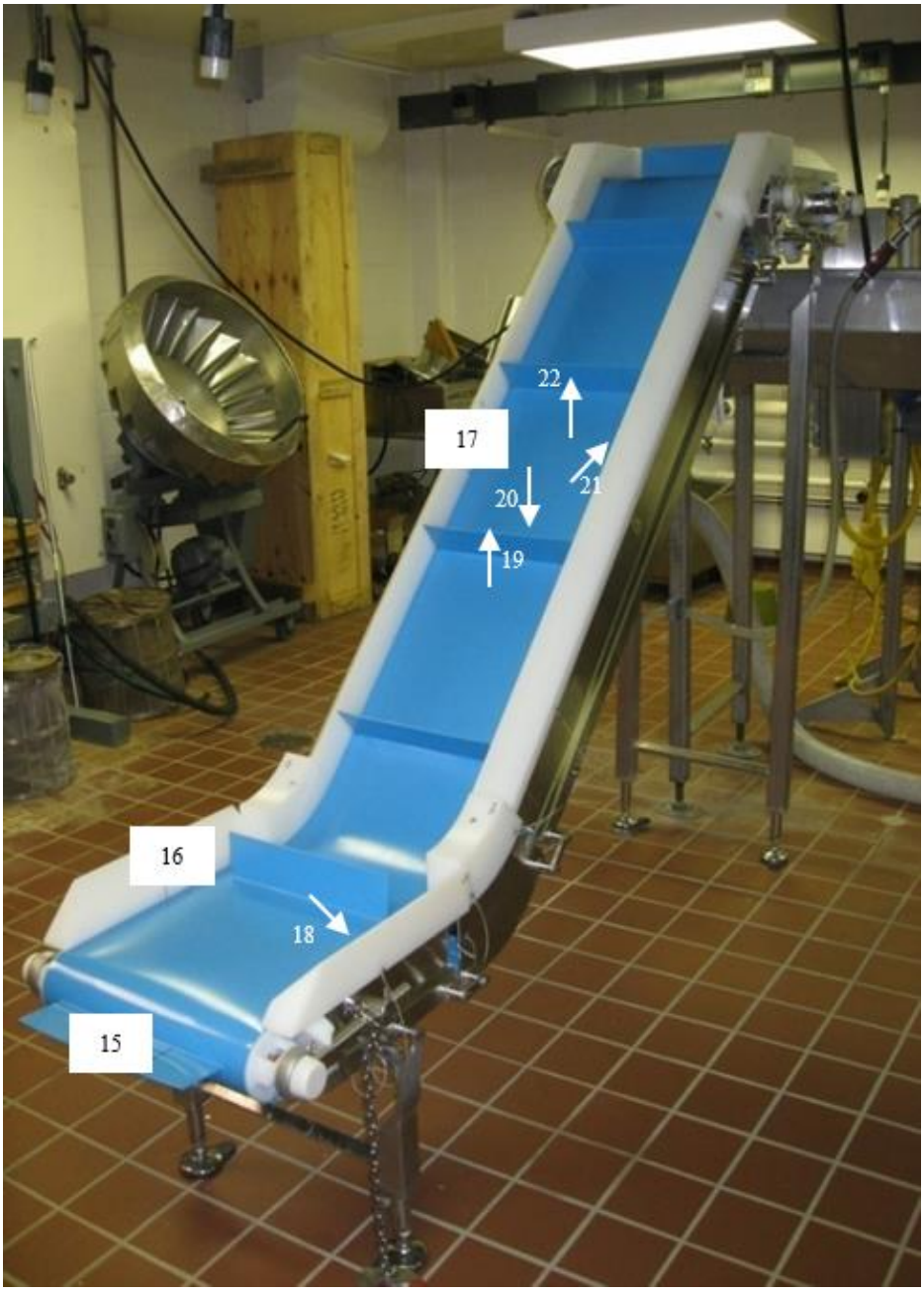

Figure 2.2: *Sampling locations on the conveyor. All sampling locations were sampled after 90.7 of iceberg lettuce was processed when the shredder was contaminated. While the conveyor was contaminated, locations with number of 15, 17, 19, and 21 were sampled before 90.7 kg of iceberg lettuce was processed. Locations with number 16, 18, 20 and 22 were sampled after 90.7 kg of iceberg lettuce was processed.*

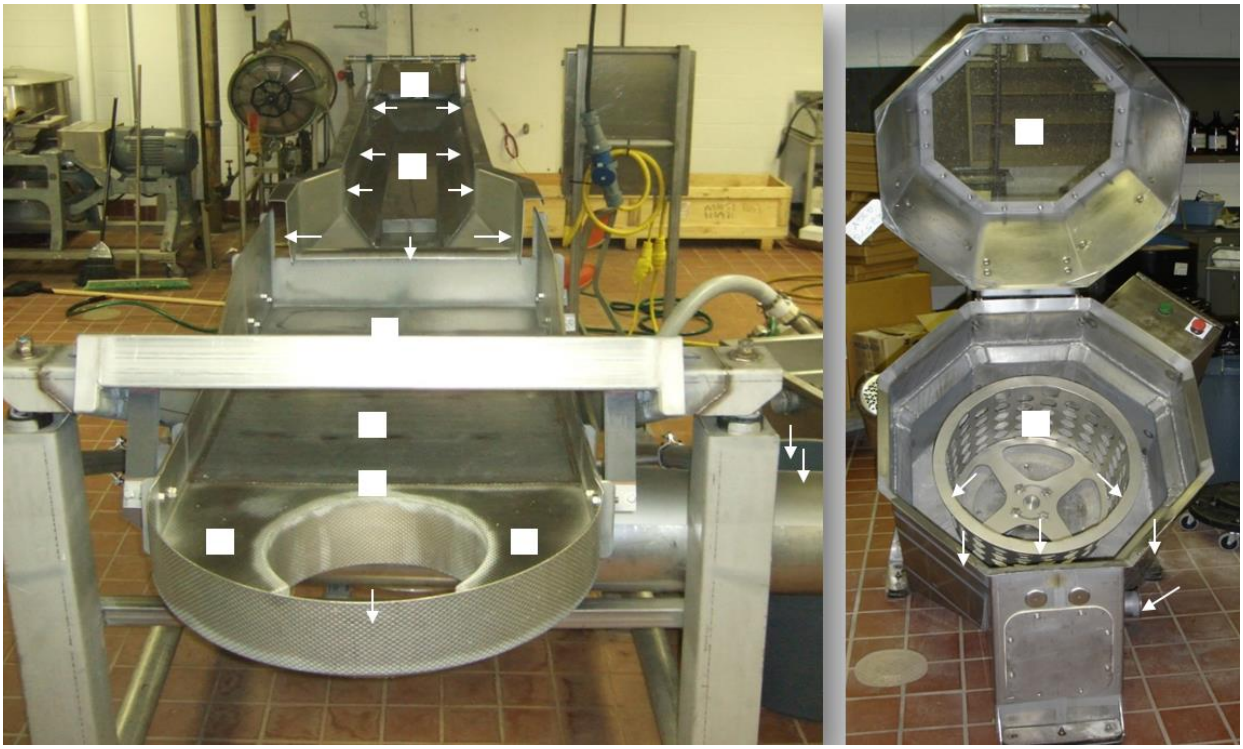

Figure 2.3: *Sampling locations on the flume tank, shaker table and centrifugal dryer.*

2.3 RESULTS

2.3.1. Radicchio. Before processing, radicchio was inoculated to contain *E. coli* O157:H7 at 6.1 ± 0.1 log CFU/g. After processing 90.7 kg of iceberg lettuce, pieces of radicchio pieces were retrieved from all surfaces of the shredder, conveyor, and shaker table. The weight of radicchio recovered from each small basket of iceberg lettuce after shaker table dewatering decreased ($P < 0.05$) from 13.9 to 0.2 g and from 9.7 to 0.1 g when the shredder and conveyor were contaminated, respectively (Figures 2.4, 2.5, 2.6 and 2.7). *E. coli* O157:H7 populations on the radicchio decreased from 6 to 5.4 log CFU/g after processing 90.7 kg of iceberg lettuce. The amounts of radicchio retrieved from the shredder, conveyor, flume tank, shaker table and dewatering centrifuge are shown in Table 2.1.

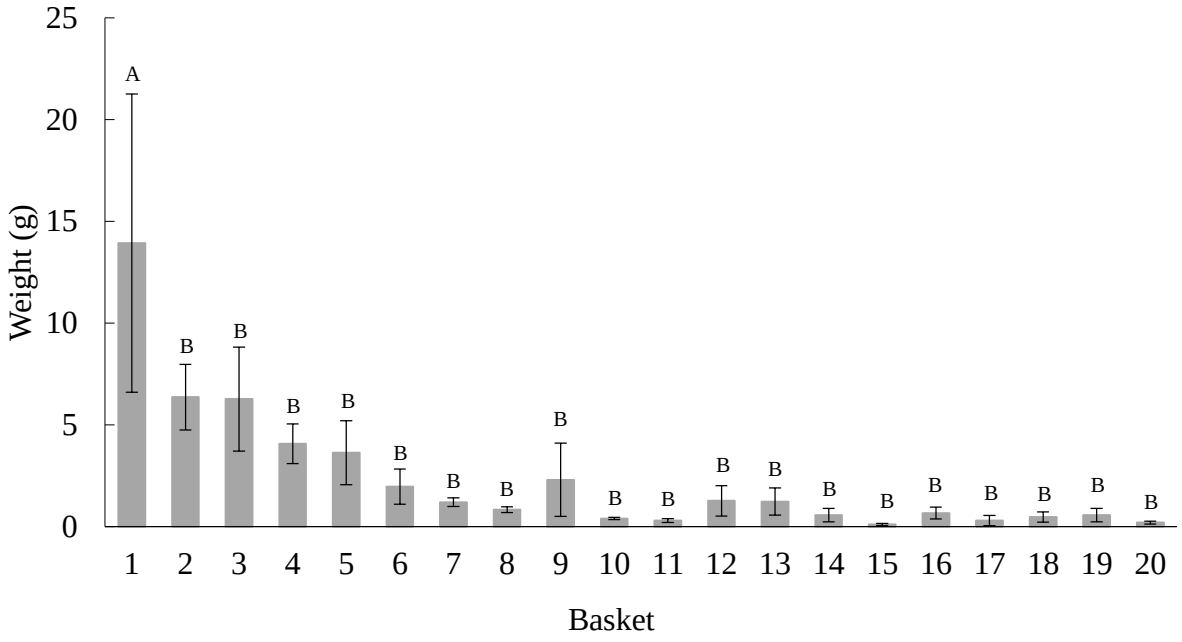

Figure 2.4: *Mean (±SE) weight of radicchio retrieved after shaker table dewatering while the shredder is contaminated (n=3). Mean weights from baskets without common letters are significantly different (*P *< 0.05).*

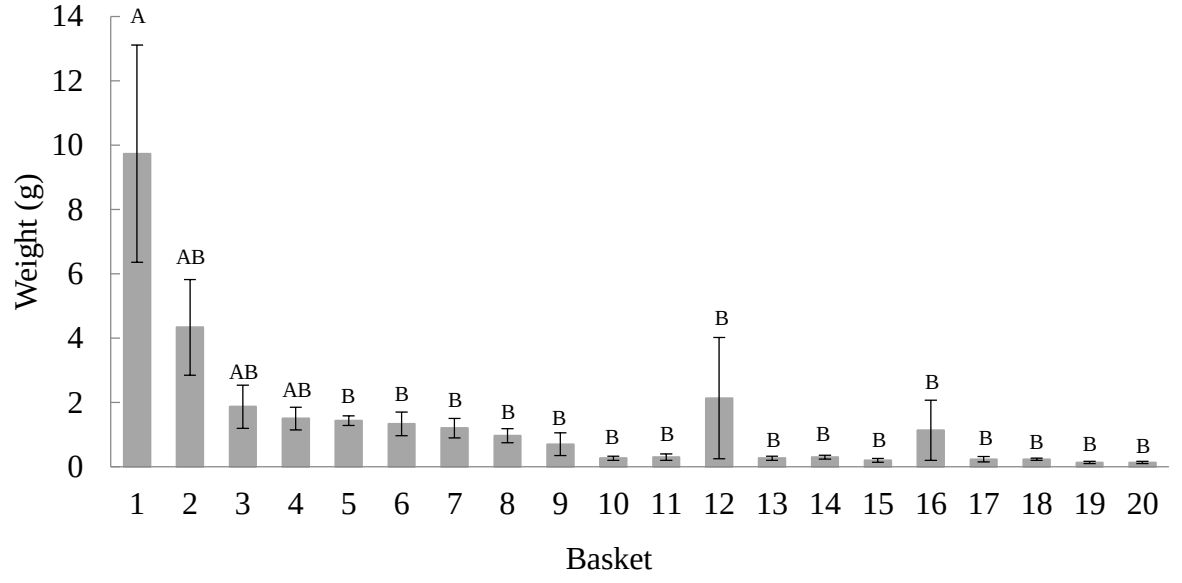

Figure 2.5: *Mean (±SE) weight of radicchio retrieved after shaker table dewatering while the conveyor is contaminated (n=3). Mean weights from baskets without common letters are significantly different (*P *< 0.05).*

Table 2.1: *Mean (±SD) weight (g) of radicchio and mean (±SD)* E. coli *O157:H7 populations on equipment surfaces after processing (log CFU/cm2) (n=3). NA stands for not applicable.*

	Contaminated shredder			Contaminated conveyor		
	Radicchio	*E. coli* populations on equipment (log CFU/cm^2)		Radicchio	*E. coli* populations on equipment (log CFU/cm^2)	
	Weight(g)	Mean(±SD)	SE	Weight(g)	Mean(±SD)	SE
Shredder	15.6± 7.4	0.9± 1.8	0.7	NA	NA	NA
Conveyor	3.7± 6.0	1.4± 0.7	0.1	6.5± 0.9	2.5± 0.0	0.0
Flume tank	5.7± 9.1	2.1± 0.7	0.4	2.0± 1.7	1.1± 0.3	0.2
Shaker table	1.2± 0.8	1.6± 0.2	0.1	3.8± 3.6	1.4± 0.2	0.1
Centrifuge	NA	1.1± 1.0	0.6	NA	0.1± 0.4	0.2

2.3.2. Iceberg lettuce. After contacting the contaminated shredder or conveyor, all 15 radicchio-free samples of iceberg lettuce contained *E. coli* O157:H7, with populations of 2.7 to 0.9 log CFU/g for the shredder and 1.8 to 0.9 log CFU/g for the conveyor. When the shredder was contaminated, *E. coli* O157:H7 populations decreased significantly with time ($P < 0.05$). In contrast, iceberg lettuce collected after contacting the contaminated conveyor belt was only randomly contaminated, with no significant differences seen between samples ($P > 0.05$) (Figure 2.6). When the shredder was contaminated, *E. coli* O157:H7 populations on iceberg lettuce decreased ~ 0.6 log CFU/g after flume washing and shaker table dewatering. Significant differences ($P < 0.05$) in *E. coli* O157:H7 populations were only seen for the first ten washed iceberg lettuce samples. No significant differences in *E. coli* O157:H7 populations were seen when the conveyor was contaminated ($P > 0.05$) (Figure 2.7). Statistically similar *E. coli* O157:H7 populations ($P > 0.05$) were recovered from iceberg lettuce after centrifugal drying, while the shredder or conveyor contaminated.

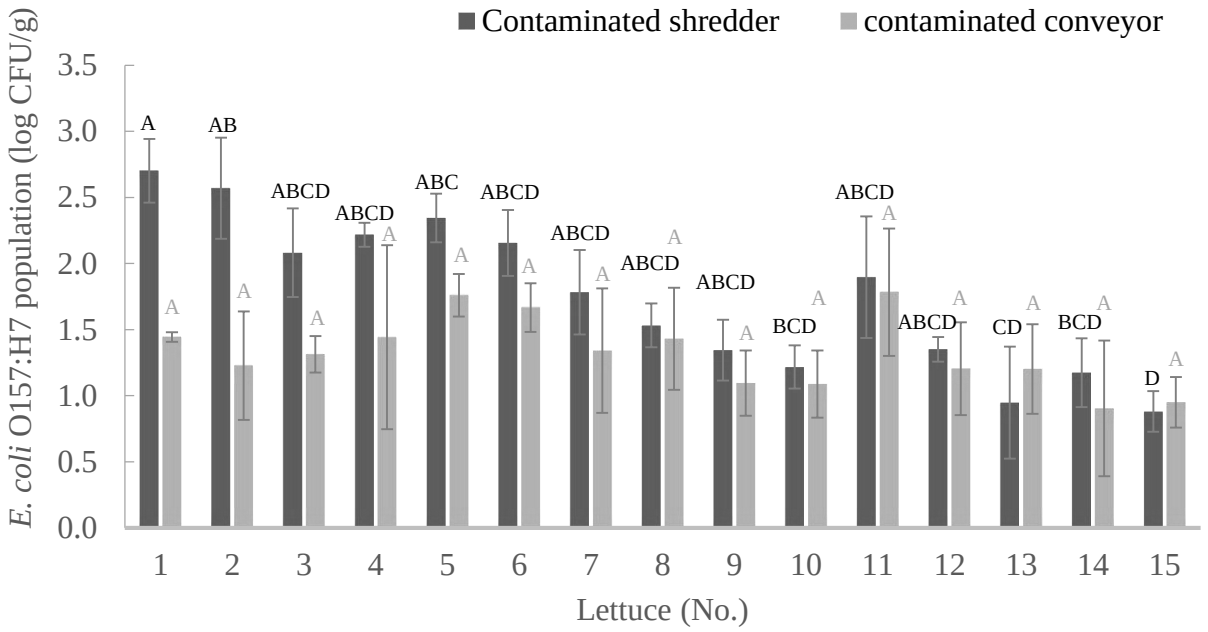

Figure 2.6: *Mean (±SE)* E. coli *O157:H7 populations on iceberg lettuce after contacting the contaminated shredder and conveyor (n=3). Means without common letters for the shredder/ conveyor data sets are significantly different (*P < *0.05).*

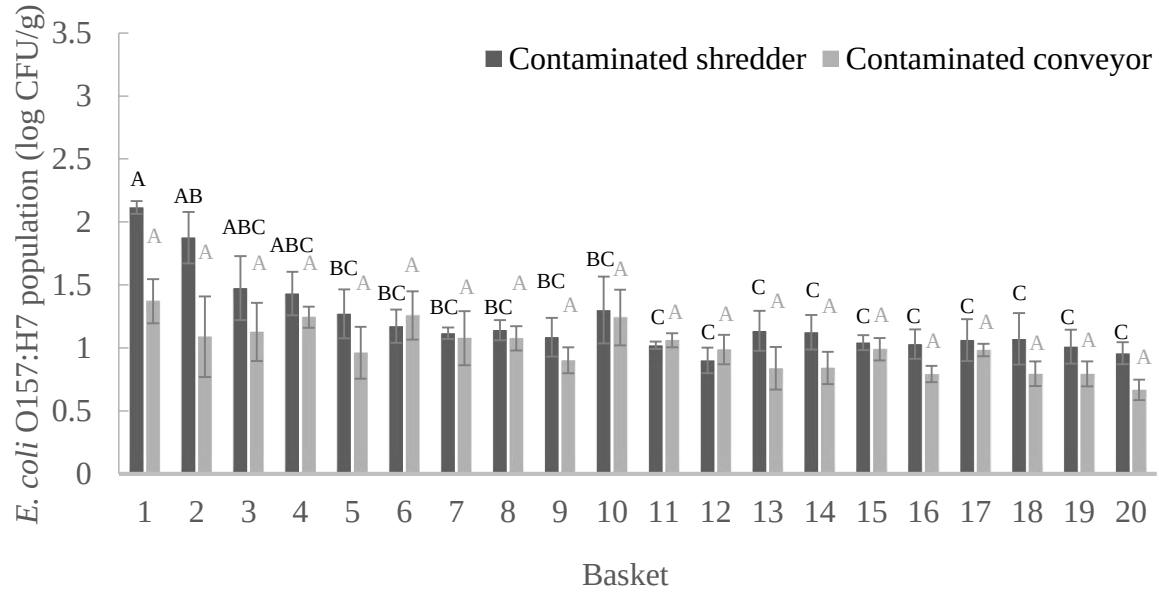

Figure 2.7: *Mean (±SE)* E. coli *O157:H7 populations on iceberg lettuce collected from each collection basket after shaker table dewatering (n=3), while the shredder / conveyor was contaminated. Means without common letters for the shredder/ conveyor data sets are significantly different (*P < *0.05).*

2.3.3. Contaminated shredder/ conveyor. When the shredder surface was contaminated to contain 3.7 log *E. coli* O157:H7 CFU/cm^2 by processing inoculated radicchio, 0.9 log CFU/cm^3 remained on the shredder after processing 90.7 kg of iceberg lettuce ($P < 0.05$), with 99.8% transferred from the conveyor to the lettuce, wash water and product contact surfaces of the processing line. Similarly, when the conveyor initially contained 4.1 log CFU/cm^2, 2.5 log CFU/cm^2 remained after conveying 90.7 kg iceberg lettuce ($P < 0.05$) (Figure 2.8), with only 97.5% subsequently transferred during processing. After processing, all equipment surfaces examined beyond the initially contaminated shredder and conveyor yielded *E. coli* O157:H7 (Table 2.1).

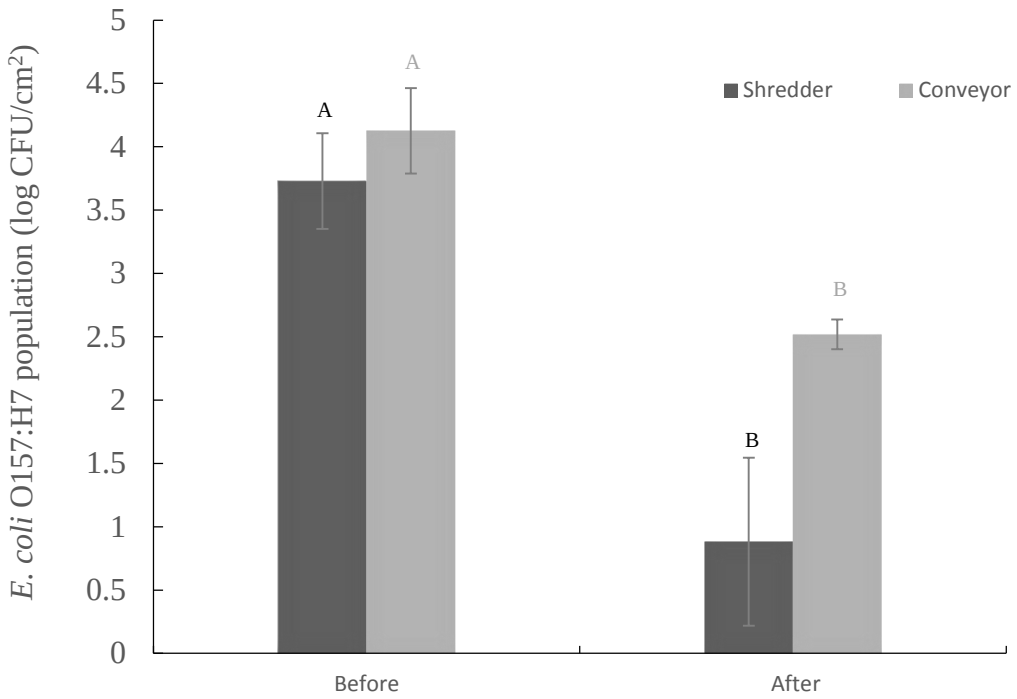

Figure 2.8: *Mean (±SE)* E. coli *O157:H7 populations on the shredder and conveyor (after processing 9.1 kg of inoculated radicchio) before and after processing 90.7 kg of iceberg lettuce. Means without common letters are significantly different (*$P < 0.05$*).*

2.3.4. Water. *E. coli* O157:H7-free wash water was immediately contaminated from the lettuce during flume washing. No significant differences were observed for the water samples collected from the recirculation spout at the beginning, mid-point, and end of processing ($P > 0.05$) when either the shredder or conveyor were initially contaminated, with *E. coli* O157:H7 populations of 1.4 and 0.6 log CFU/ml, respectively. Similar results were observed for the water samples collected from the centrifuge drain for both contamination scenarios ($P > 0.05$) (Figure 2.9).

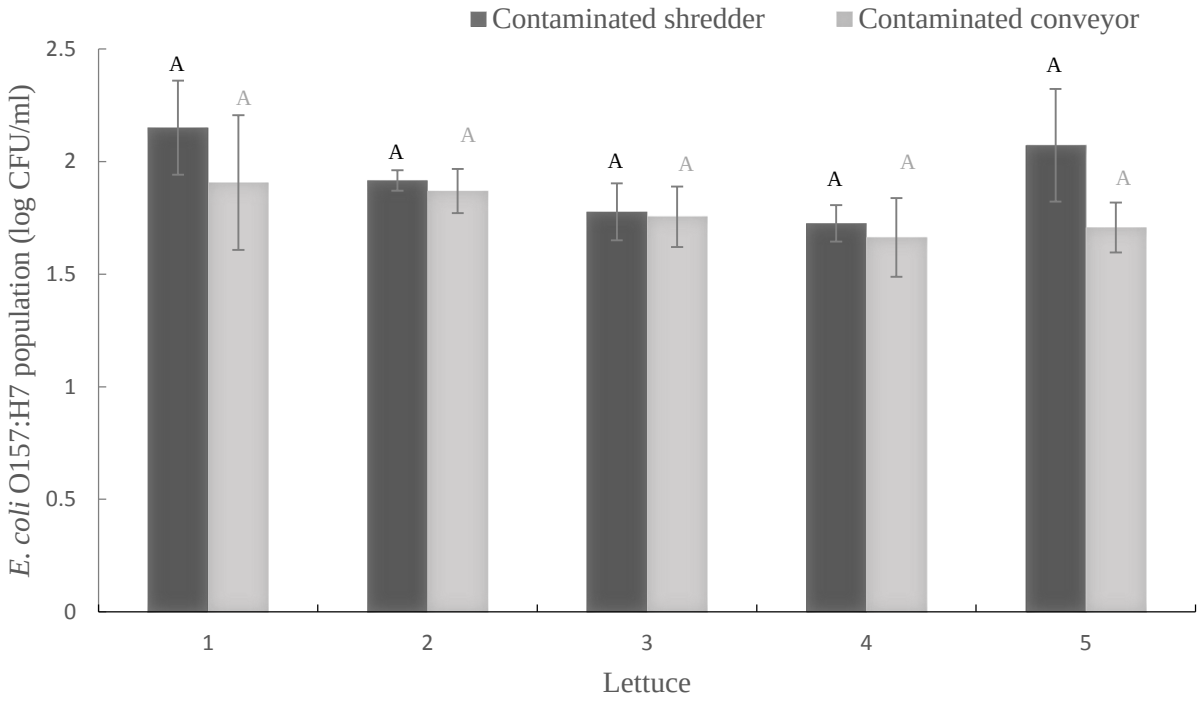

Figure 2.9: *Mean (±SE)* E. coli *O157:H7 populations in centrifugation water when the shredder and conveyor were initially contaminated. Mean E. coli O157:H7 populations with same letter are not significantly different (*P *> 0.05).*

2.4 DISCUSSION

An unusually high *E. coli* O157:H7 inoculum level (10^6 log CFU/g) was used to contaminate both the shredder and conveyor in order to generate quantifiable results for subsequent mathematical modeling. However, in field-grown lettuce, exposure to feces from "super-shedder" cattle and sheep (Chase-Topping et al., 2007; Ogden et al. 2005) or highly contaminated irrigation water remains plausible.

Radicchio was used as a colored surrogate for iceberg lettuce as in previous work (Buchholz et al., 2014), which allowed the physical separation of inoculated and previously uncontaminated product. Over 50% of the radicchio processed was recovered from the first three collection baskets for washed iceberg lettuce. The higher populations of *E. coli* O157:H7 observed in radicchio-free lettuce from these same three baskets also supports pathogen transfer via direct radicchio/lettuce contact during removal of the radicchio prior to analysis. Thereafter, the decrease in *E. coli* O157:H7 populations on radicchio-free iceberg lettuce in subsequent baskets paralleled the decrease in the radicchio/iceberg lettuce ratio. Taormina et al. (2009) also observed a decrease in *E. coli* O157:H7 populations during hand-coring of lettuce.

When the initial contamination level was ~4 log CFU/cm^2 on shredder and conveyor, *E. coli* O157:H7 populations decreased to ~1 log CFU/cm^2 on the shredder as compared to ~2.5 log CFU/cm^2 on the conveyor after processing 90.7 kg of previously uncontaminated lettuce. Over 99.8% of the initial *E. coli* O157:H7 population was transferred from the contaminated shredder to previously uncontaminated lettuce, wash water, and equipment surfaces. Using the same processing line, Buchholz et al. (2012) also reported higher *E. coli* O157:H7 populations on the conveyor belt compared to the shredder, flume tank, shaker table, and dewatering centrifuge. This observation is supported by several studies showing greater bacterial adherence to

polyurethane as compared to stainless steel (Sinde & Carballo 2000; López-Gálvez et al., 2009). When the conveyor was contaminated by conveying previously inoculated radicchio, the conveyor belt was contaminated at certain areas, where the radicchio had contacted. *E. coli* O157:H7 recovery from sequentially collected lettuce samples was inconsistent, with no discernable pattern, suggesting that the conveyor belt was also non-uniformly contaminated.

During flume washing, *E. coli* O157:H7 population on the lettuce decreased significantly, with the numbers of *E. coli* O157:H7 becoming homogenized mid-way through processing. No significant difference was found after the tenth lettuce sample when either the shredder or conveyor was initially contaminated, indicating that flume washing soon led to uniform distribution of *E. coli* O157:H7 in the water. Furthermore, similar *E. coli* O157:H7 populations were recovered from the centrifugation water for each batch of product.

Although chemical sanitizers are routinely used in commercial flume washing systems to minimize contamination from the water (López-Gálvez et al., 2009), the efficacy of chlorine-based sanitizer efficacy decreases significantly at higher organic load (Buchholz et al., 2012b). In addition, some of the lettuce - contaminated or otherwise - will tend to linger in the processing line during continued operation and gradually spread to subsequent product, as shown in this study using radicchio.

Given the ability of mechanical conveyors to continually shed bacteria and contaminated product over time, their use in commercial operations should be minimized with lettuce directly shredded into the flume washing system to minimize the chance of cross-contamination. With a great ability of the shredder to spread contamination and implication of a lettuce shredder in the previously outbreak of salmonellosis in Australia (Stafford et al., 2002), introduction of a continuous sanitizer spray within the shredder may be useful in minimizing product build-up and

decreasing the subsequent spread of microbial contaminants. However, appropriate use of chemical sanitizers during flume washing along with effective pre- and post-operative cleaning and sanitation programs remain critically important for maintaining the safety of fresh-cut produce.

CHAPTER 3:

Effect of Water Velocity on *Escherichia coli* O157:H7 Transfer from Inoculated Lettuce to Wash Water in a Custom-made Pipe System

3.1 OBJECTIVE

The objective of this study was to assess the impact of laminar, transition, and turbulent flow on *E. coli* O157:H7 transfer from lettuce to water during washing.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design. The spread of *E. coli* O157:H7 from inoculated pieces of iceberg lettuce to wash water under different flow rates was assessed by horizontally inserting 5 x 5 cm pieces of iceberg lettuce dip-inoculated to contain *E. coli* O157:H7 at 6.7± 0.4 log CFU/g into a continuously flowing stream of water pumped through a 10-cm diameter custom-made pipe at 0.005, 0.01, 0.04, and 0.07 m/s. Water and lettuce samples were collected at 1-, 5-, or 10-s intervals for 2 or 5 min and quantitatively essayed for *E. coli* O157:H7 by plating appropriate dilutions with/without membrane filtration on TSAYE with ampicillin. The ability of the custom-made pipe and sampling method to recover bacterial populations from the water was measured and calibrated by adding 1 ml of a 4-strain non-toxigenic *E. coli* O157:H7 cocktail (~7.6 log CFU/ml) using the respective flow rates and the same recovery method. Data from triplicate experiments were assessed for significance using the Tukey-Kramer HSD test.

3.2.2 Leafy greens. Individually wrapped heads of iceberg lettuce (*Lactuca sativa L.*) were purchased from a local grocery store (Meijer, Okemos, MI), stored in a 4^{o}C walk-in cooler, and used within 2 days of purchase.

3.2.3 Bacterial strains. Identical to 2.2.3.

3.2.4 Inoculum preparation. A 25 µl aliquot of each non-toxigenic *E. coli* O157: H7 strain was added to 25 ml of TSBYE plus amp and incubated at 37^{o}C for 24 h. Since the growth rates for the four *E. coli* O157: H7 strains were previously shown to be similar (Buchholz et al., 2012b), these strains were combined in equal volumes to obtain a 100 ml *E. coli* O157:H7 cocktail, and then added to 200 ml of tap water (~15^{o}C) to make 300 ml of inoculum containing ~8.1 log CFU/ml.

3.2.5 Lettuce inoculation. Iceberg lettuce heads were hand-cored, with the leaves

removed and cut into 5 x 5 cm pieces with a sterilized knife. After immersing in the inoculum for 15 min (preparation described in 3.2.4) followed by 15 min of drying in a bio-safety hood at room temperature, the leaf pieces contained *E. coli* O157:H7 at 6.7± 0.4 log CFU/g based on direct plating (n=3).

3.2.6 Custom-made pipe system. A custom-made pipe system consisted of a 10-cm diameter PVC pipe (Figure 3.1), with an inlet and an outlet on the ends, an inoculum/lettuce insertion port on the top, and a water collecting spout on the bottom. An eye valve preceding the inlet was used to obtain different water flow rates. A centrifugal pump (B633, Magnetek, Menomonee Falls, WI) was connected by a flexible hose to pump tap water (~15^{o}C) through the pipe from a ~1000 L capacity stainless steel tank.

3.2.7 Flow rates. Four different flow listed below (Table 3.1) were achieved by adjusting the valve and then verified by the time required to fill a 3.78 L container, with these times being 96, 50, 12, and 7 s, respectively.

Table 3.1: *Flow properties.*

Flow velocity (m/s)	Volumetric flow rate (L/s)	Flow regime	Reynolds number *(Re)*
0.005	0.039	Laminar	506
0.01	0.076	Laminar	1012
0.04	0.315	Transitional	3846
0.07	0.549	Turbulent	7085

3.2.8 Processing. To calibrate the pipe system and sampling method, 1 ml of the *E. coli* O157:H7 cocktail containing ~7.6 ± 0.3 CFU /ml was rapidly pipetted at point ‘a’ into water flowing at 0.005, 0.01, 0.04 or 0.07 m/s (Figure 3.1). To evaluate the impact of flow rate on the transfer of *E. coli* O157:H7 from lettuce, a 5 x 5 cm piece of iceberg lettuce dip-inoculated to contain *E. coli* O157:H7 at 6.7± 0.4 log CFU/g were secured to a thin-wired clamp (4.2 x 4.2 cm) and inserted at point ‘a’ (shown in Figure 3.1) into water flowing at the same rates. Based on

results from preliminary experiments, wash times of 5, 2, 2 and 2 minutes were used for flow rates of 0.005, 0.01, 0.04 m/s and 0.07 m/s, respectively. Differences in *E. coli* O157:H7 transfer were accordingly related to differences in the flow rate and flow regime.

Figure 3.1: *Custom-made pipe system. Point 'a' is the inoculation port in the water study and the lettuce insertion port in the lettuce study. Point 'b' is the water sample spout. Valve 'c' is an eye valve for controlling water flow rates.*

3.2.9 Sample collection. Water samples (500 ml each) were analyzed for *E. coli* O157:H7 by membrane filtration, which yielded a lower limit of detection (LOD) of 0.002 CFU/ml. Water samples (50 or 500 ml) were collected in Whirl-Pak™ bags (Nasco, Fort Atkinson, WI) from the sampling spout at 1-, 5-, or 10-s intervals for 2 min (flow rates of 0.01, 0.04, and 0.07 m/s) or 5 min (flow rates of 0.005 m/s) with collection starting either 5 s before inserting inoculum or lettuce (flow rates of 0.07 m/s) or at time 0 (flow rates of 0.005, 0.01, and 0.04 m/s). After each experiment, the inoculated lettuce piece was removed and added to a Whirl-Pak™ bag (Nasco, Fort Atkinson, WI) containing 25 ml of Phosphate Buffered Saline (PBS) for further analysis.

3.2.10 Microbiological analyses. All lettuce samples were homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min and then quantitatively essayed for *E. coli* O157:H7 by plating appropriate dilutions on TSAYE plus ampicillin. Water samples were concentrated by membrane filtration on TSAYE with amp plates, with limits of detection (LOD) of 0.02 and 0.002 CFU/ml for the 50 and 500 ml samples, respectively. All plates were incubated at 37ºC for 24 h and then observed for green fluorescing colonies under an ultraviolet light (365 nm, Black-Ray, Ultra-violet Product Inc. San Gabriel, CA), which were counted as *E. coli* O157:H7.

3.2.11 Percentage of *E. coli* O157:H7 cells recovered. The percentage of *E. coli* O157:H7 cells recovered and the adjusted *E. coli* O157:H7 populations during wash of inoculated lettuce at the four different flow rates were determined as follows:

$$\%\ E.coli\ \text{O157:H7 population recovered} = \frac{E.coli\ \text{O157:H7 population recovered to water}}{E.coli\ \text{O157:H7 population released to water}} \times 100\%$$

$$\text{Adjusted } E.coli\ \text{O157:H7 population recovered} = \frac{E.coli\ \text{O157:H7 population recovered in water}}{\%\ E.coli\ \text{O157:H7 population recovered}}$$

3.2.12 Statistical analysis. Identical to 2.2.10.

3.3 RESULTS

3.3.1 Percentage of *E. coli* O157:H7 cells recovered. When a 1 ml aliquot of *E. coli* O157:H7 (7.6 ± 0.3 CFU/ml) was pipetted into water flowing at 0.005 m/s, water samples collected during 5 to 180 s yielded the organism. In contrast, at the higher flow rates of 0.01 and 0.04 m/s, *E. coli* O157:H7 was only recovered from samples collected during 5 to 30 s and 3 to 15 s, respectively. At the highest flow rate of 0.07 m/s, *E. coli* O157:H7 was only observed during 2 to 10 s. *E. coli* O157:H7 populations peaked in the water after 20, 10, 5, and 3 s at 3.2, 3.5, 3.3, and 4.1 log CFU/ml for flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively (Figure 3.2). *E. coli* O157:H7 persisted in the water for 175, 25, 12, and 8 s at flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively. Cumulative *E. coli* O157:H7 populations after processing were 6.7, 6.0, 6.3, and 6.3 log CFU for flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively. Percent recovery decreased from 98 to 92, 88 to 84% as the water flow rate increased.

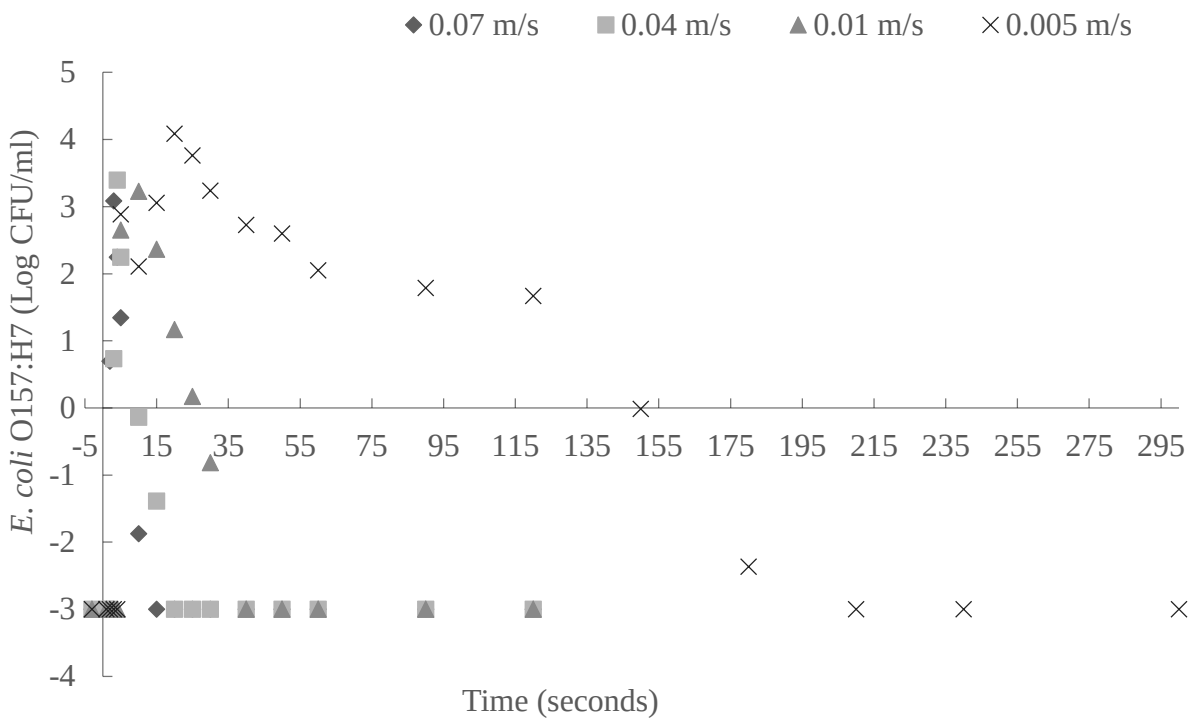

Figure 3.2: *Mean* E. coli *O157:H7 populations in water samples collected during washing of lettuce at four different flow rates. Half the limit of detection was used to calculate the mean log value when a 500 ml water sample yielded no colonies by membrane filtration.*

3.3.2 Wash of inoculated lettuce.

3.3.2.1 Lettuce. After washing iceberg lettuce pieces (initially containing *E. coli* O157:H7 populations of 6.7± 0.4 log CFU/g) at flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, 6.1, 6.2, 6.3, and 6.1 log CFU were shed into the water, respectively (Figure 3.3), none of which were significantly different. Shedding of *E. coli* O157:H7 into the water ceased after 2 min at flow rates of 0.01, 0.04, and 0.07 m/s, and after 5 min at a flow rate of 0.005 m/s.

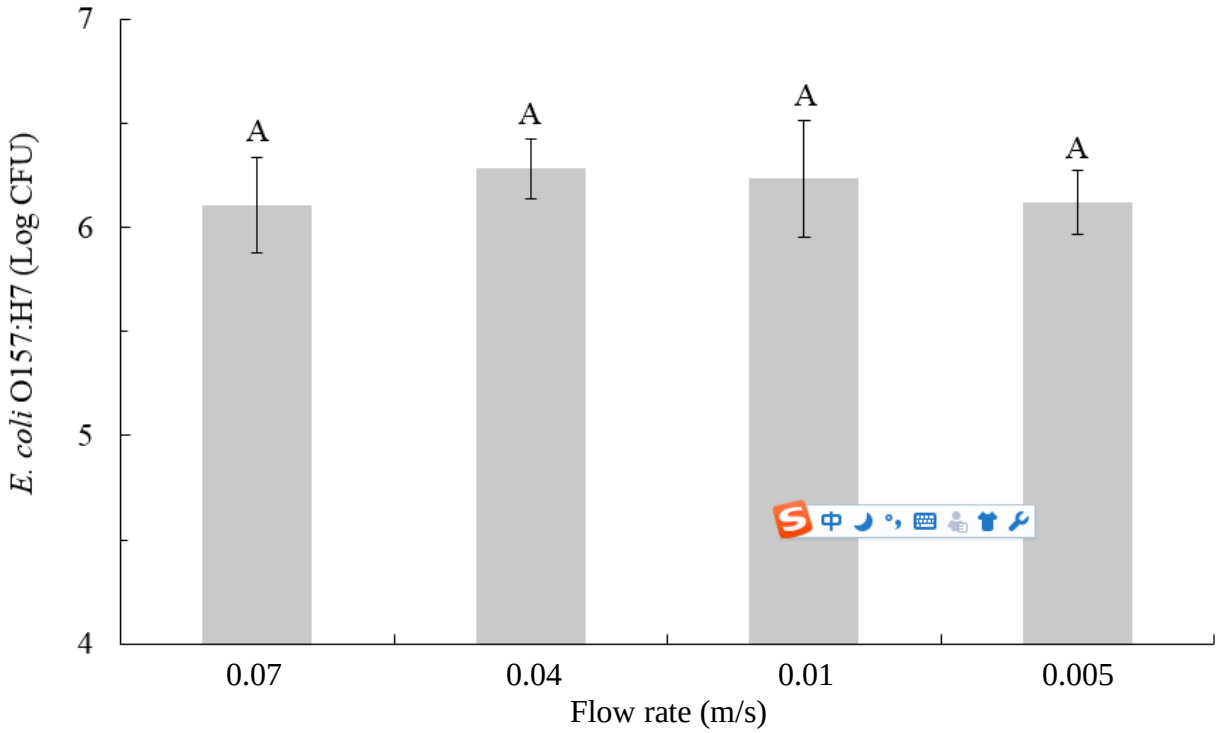

Figure 3.3: *Mean (±SD)* E. coli *O157:H7 populations shed from inoculated lettuce into the water based on the populations before and after washing. Different water flow rates with same letter are not significantly different (*P > *0.05).*

3.3.2.2 Water samples. At the lowest flow rate of 0.005 m/s, *E. coli* O157:H7 was found in water samples collected after 3 to 180 s. In contrast, at the next two higher flow rates of 0.01 and 0.04 m/s, water samples collected from 5 to 60 s and from 3 to 15 s yielded *E. coli* O157:H7, respectively (see Figure 3.4). At the highest flow rate of 0.07 m/s, *E. coli* O157:H7 was only observed in water samples collected after 3 to 5 s. *E. coli* O157:H7 persistence in the water decreased from 177 to 57, 12, and 2 s at the flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively. *E. coli* O157:H7 populations peaked in water samples at 10, 4, 4, and 3s at 0.7, 2.3, 2.1, and 0.7 log CFU/ml for flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively. The total populations of *E. coli* O157:H7 recovered from water samples collected after processing were 3.2 ± 0.2, 5.1 ± 0.2, 4.6 ± 0.8, and 3.8 ± 0.0 log CFU for flow rates of 0.005, 0.01, 0.04, and 0.07 m/s, respectively. *E. coli* O157:H7 populations in the water were significantly higher ($P < 0.05$) at a flow rate of 0.01 m/s (5.1 log CFU) compared to 0.07 m/s (3.8 log CFU) and 0.005 m/s (3.2 log CFU) (Figure 3.5). The adjusted *E. coli* O157:H7 populations shed from lettuce during washing (using the equations in 3.2.11) were 3.3 ± 0.2, 5.5 ± 0.2, 5.3 ± 0.8, and 4.1 ± 0.0 log CFU, respectively, which were significantly affected by flow rates ($P < 0.05$). For laminar and transition flow, the higher the flow rate, the more transfer to the water from lettuce. At the higher flow rates (0.540 and 0.04 m/s), significantly less transfer ($P < 0.05$) was seen after 10 s, whereas at the lower flow rates (0.01 and 0.005 m/s), significantly less transfer ($P < 0.05$) was seen after 4 s.

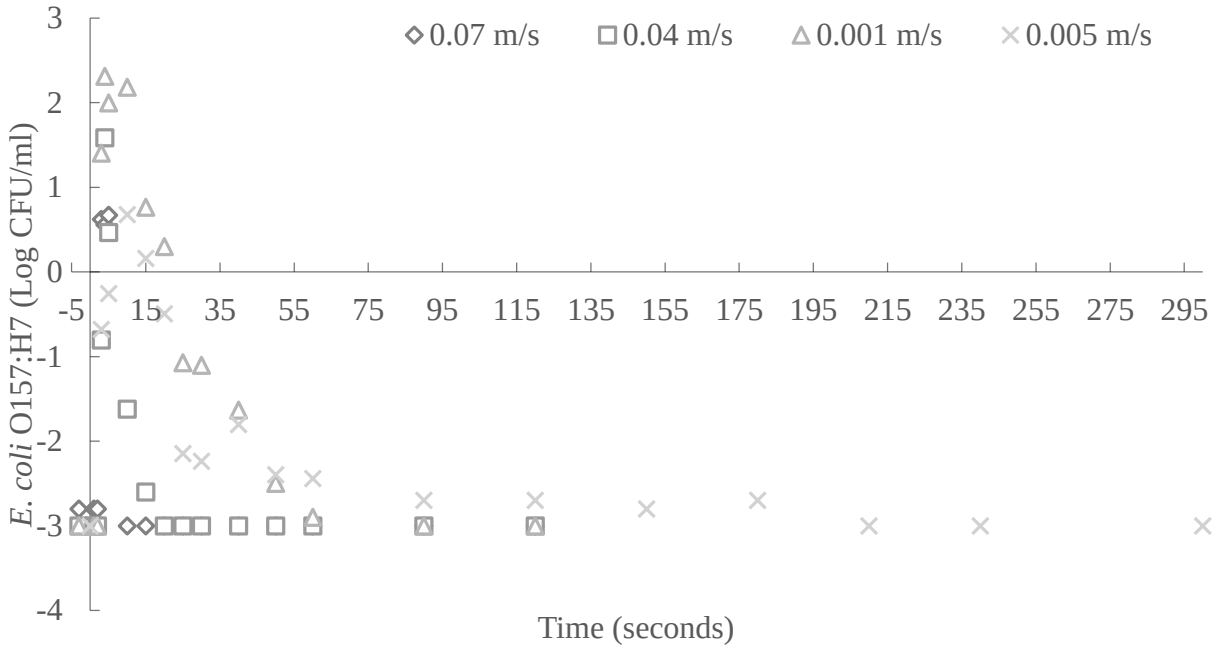

Figure 3.4: *Mean log* E. coli *O157:H7 populations in water samples collected during washing of lettuce at four different flow rates. Half the limit of detection was used to calculate the mean log value when a 500 ml water sample yielded no colonies by membrane filtration.*

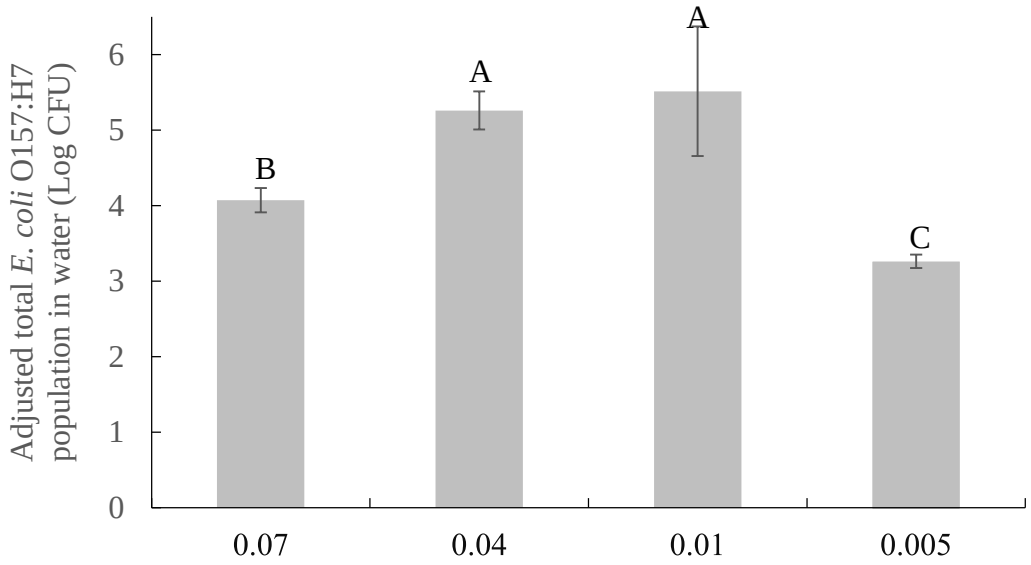

Figure 3.5: *Mean (±SD) adjusted log* E. coli *O157:H7 populations shed from inoculated lettuce pieces to wash water. Means of the different water flow rates with different letter are significantly different (*P *< 0.05).*

3.4 DISCUSSION

The flow rates achieved using the present pipe system more closely resembled those seen for commercial dump tanks and water flow conveyors than commercial leafy green flume washing systems (~3.6 m/s). However, various conditions of laminar, transitional, and turbulent flows were obtained with their impacts on bacterial transfer investigated for the first time during washing.

Dip-inoculation was used to ensure uniform distribution of *E. coli* O157:H7 on the lettuce pieces (Lang et al., 2004). Although *E. coli* O157:H7 could potentially internalize into lettuce through the cut edges (Erickson, 2012) during inoculation, such internalized cells would not affect the transfer results since homogenization by stomaching recovers very few internalized cells (Davidson et al., 2013).

When 1 ml of inoculated water was pipetted to the continuously flowing stream of water in the pipe, *E. coli* O157:H7 persistence in the water increased as the flow rate decreased. This observation is in accordance with the high velocity of rapidly moving water (Semat and Katz, 1958), which validates the custom-made pipe system and sampling method. Similarly, during the washing of inoculated lettuce, a rapid initial increase in numbers of *E. coli* O157:H7 was seen at all flow rates. However, this initial increase was of short duration with *E. coli* O157:H7 populations decreasing in the water after 10 s. The time used to reached thresholds for initial detection increased with the decrease of flow rates, which can be explained by classic fluid mechanics since higher flow rates generate higher shear forces (Semat and Katz, 1958). Eventually, the *E. coli* O157:H7 populations reached a steady state, below limit of detection of 0.002 CFU/ml (half the LOD of -3 log CFU/ml was used to calculate the mean log), regardless of the flow rate, which is consistent with other reports (Adams et al., 1989; Doménech et al.,

2013; Ukuku and Fett 2002). The declining numbers of *E. coli* O157:H7 shed into the water are also related to the flow rate, with a higher flow rate more rapidly decreasing the residual population of the *E. coli* O157:H7 on the lettuce surface as also observed by Wang et al. (2007). Consequently, washing at a higher low rate leads to more efficient removal of surface bacteria.

Based on the populations before and after washing, total *E. coli* O157:H7 populations shed from the inoculated lettuce into water increased with flow rate within the laminar and transitional flow regimes. The population decreased to the lowest level at a flow rate of 0.07 m/s in the turbulent regime. However, these data were too scattered to confirm this finding. Alternatively, the total numbers of *E. coli* O157:H7 shed in the wash water can be calculated. In the lettuce study, total *E. coli* O157:H7 reductions were obtained by adjusting the populations recovered from the water samples with the recovery rates for the four different flow rates in the water study (as explained in 3.2.11). Again, bi-phasic removal was observed during washing. Because of physical limitations in the experimental design, the possibility of multi-phase (more than one phase) *E. coli* O157:H7 reductions at different flow rate cannot be ruled out.

During laminar flow (0.005 and 0.01 m/s), bacterial transfer increased with the increase of flow rate, since shear force increases with velocity. The wall shear force at the lettuce surface is likely partly responsible for shedding of *E. coli* O157:H7 into the water. In support of these observations, Wang et al. (2007) also reported more effective bacterial transfer from cantaloupe rinds to water at higher shear forces. However, neither transitional nor turbulent flow was assessed in their study. From our observations, bacterial transfer to the water was lowest at the highest flow rate of 0.07 m/s (turbulent flow); therefore, bacterial detachment is not always increased with the increase of flow rate/shear force. Normally, a higher flow rate leads to an increase in shear force which can affect bacterial adhesion to a surface (Rusconi et al., 2014).

This shear stress-enhanced adhesion of *E. coli* O157:H7 to surfaces is not simply due to fluid transport or an increased number of bonds formed, but is consistent with the formation of catch bonds that strengthen as the force increases (Forero et al., 2004; Nilsson et al., 2006; Thomas et al., 2004; Thomas et al., 2006).

Typically, at a low shear force, bacterial cells will pass over a surface unhindered. However, higher shear forces tend to enhance surface binding (Rangel et al., 2013; Thomas et al., 2004) with FimH (a lectin-like protein responsible for adhesion properties of type 1 fimbriae) expressed by *E. coli* O157:H7 (Thomas et al., 2004). FimH has two functional states corresponding to weak and strong bonds. At a low shear force, most FimH proteins that interact with the substrate are in the unstressed equilibrium state. These weak bonds can be easily broken, allowing the bacterial cell to detach from the surface. Under such conditions, the rate of bacterial removal is fairly high. As the shear force increases, more bacteria tend to roll forward instead of detach. In the "rolling" state, a faster flow rate could rotate and carry bacteria forward faster, with the weak bonds still leading to rapid rolling (Chang et al., 2000). As the shear force increases further, mechanical force fosters another change in FimH, in which the interdomain linker chain is disassociated from the lectin domain to form a strong long-lasting bond. At this point, most bacterial cells would have been immobilized on the surface leading to decreased removal (Thomas et al., 2004). Since lettuce is a non-anti-FimH material, the surface interactions between lettuce and FimH need to be considered when assessing *E. coli* transfer. Due to physical limitations regarding the power of the pump and the feasibility of water sampling, higher flow rates could not be achieved. However, given a sufficiently strong shear force to break the bonds between FimH and the lettuce surface; increased removal of *E. coli* O157:H7 might be possible in the regime of turbulent flow.

In conclusion, this study assessed the relationship between bacterial removal rates and water velocity. Overall, flow rate significantly impacted the rate of bacterial transfer from lettuce to water. Transfer of *E. coli* O157:H7 from lettuce to wash water increased with flow rate within the laminar flow regime due to the increased shear force; however, when the flow rate entered the turbulent flow regime, the greater shear force may have facilitated adhesion of *E. coli* O157:H7 with the lettuce, decreasing transfer to the water.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Fresh produce is now responsible for more than half of all cases of foodborne illness with fresh-cut leafy greens posing a major public health concern as evidence by recent recalls and outbreaks. Although sanitizers are commonly used during production of fresh-cut produce, their antimicrobial efficacy is difficult to maintain due to the rapid built up of organic material in the wash water. Therefore, the presence of sanitizers cannot guarantee effective washing.

The first objective of this research was to quantify the spread of *E. coli* O157:H7 from a product-contaminated shredder or conveyor to subsequent product, other equipment surfaces, and the water during simulated commercial production of fresh-cut iceberg lettuce. The results indicated that while the shredder was contaminated by previously inoculated radicchio, 99.8% of the *E. coli* O157:H7 was spread to the wash water and other equipment surfaces. After processing 90.7 kg of previously uninoculated iceberg lettuce, 2.5 log CFU/cm^2 remained on the conveyor while the conveyor belt was initially contaminated to contain ~4 log CFU/cm^2 *E. coli* O157:H7. Considering the ability of the shredder to spread *E. coli* O157:H7 during processing, a continuous sanitizer spray should be introduced within the shredder to minimize the spread of microbial contaminants. The use of mechanical conveyors after shredding should be eliminated or minimized due to their ability to transfer bacteria over a long period of time. Therefore shredding lettuce directly into the flume washing system is strongly recommended.

Being the only post-harvest microbial reduction step for, washing remains critical for enhancing end-product safety. The second objective of this research assessed the impact of laminar, transition, and turbulent flow on *E. coli* O157:H7 transfer from lettuce to water during washing. Persistence of *E. coli* O157:H7 in the wash water increased with the decrease in flow rate. The time needed for *E. coli* O157:H7 to reach the detection threshold decreased with increasing flow rate. Within the laminar regime, removal of *E. coli* O157:H7 from inoculated lettuce increased with the increase in flow rate, however, at the highest flow rate achieved by the

custom-made pipe system, *E. coli* O157:H7 removal was lowest compared to the lower flow rates. In order to better understand the impact of flow rate on *E. coli* O157:H7 transfer during washing of lettuce, at least two additional flow rates within the turbulent regime should be included in the future research. This additional work would reveal if an increase in flow rate leads to less removal of *E. coli* O157:H7 from inoculated lettuce during washing. Additionally, the product-to-water ratio should be considered since this is another critical factor affecting washing efficacy. A much greater product-to-water ratio should be obtained to mimic commercial washing conditions.

APPENDIX

Table AI. 1: E. coli *O157:H7 populations (log CFU/g) on previously uninoculated lettuce collected (over time along processing) after contacting contaminated shredder.*

Lettuce sample (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	2.5	2.4	3.2	2.7	0.4
2	1.8	3.0	2.9	2.6	0.7
3	1.8	2.8	1.7	2.1	0.6
4	2.0	2.4	2.2	2.2	0.2
5	2.3	2.7	2.1	2.3	0.3
6	1.7	2.3	2.5	2.2	0.4
7	1.8	2.3	1.2	1.8	0.6
8	1.6	1.2	1.7	1.5	0.3
9	1.7	1.4	0.9	1.3	0.4
10	1.4	0.9	1.3	1.2	0.3
11	2.8	1.2	1.7	1.9	0.8
12	1.4	1.2	1.5	1.4	0.2
13	1.7	0.2	1.0	0.9	0.7
14	1.7	0.9	0.9	1.2	0.5
15	0.8	0.6	1.2	0.9	0.3

Table AI. 2: E. coli *O157:H7 populations (log CFU/g) on previously uninoculated lettuce collected (over time along processing) after contacting contaminated conveyor.*

Lettuce sample (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	1.4	1.4	1.5	1.4	0.1
2	0.6	2.0	1.1	1.2	0.7
3	1.6	1.3	1.1	1.3	0.2
4	0.6	2.8	0.9	1.4	1.2
5	1.5	2.1	1.7	1.8	0.3
6	1.9	1.8	1.3	1.7	0.3
7	1.2	2.2	0.6	1.3	0.8
8	1.2	2.2	0.9	1.4	0.7
9	0.6	1.3	1.4	1.1	0.4
10	0.6	1.2	1.5	1.1	0.4
11	2.7	1.4	1.2	1.8	0.8
12	0.9	1.9	0.8	1.2	0.6
13	0.6	1.8	1.2	1.2	0.6
14	1.2	1.6	-0.1	0.9	0.9
15	1.3	0.9	0.6	0.9	0.3

Table AI. 3: E. coli *O157:H7 populations (log CFU/g) on previously uninoculated lettuce collected from each of the 20 collection baskets after washing and shaker table dewatering while the shredder was contaminated.*

Basket (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	2.1	2.0	2.2	2.1	0.1
2	1.7	2.3	1.6	1.9	0.4
3	1.2	2.0	1.2	1.5	0.4
4	1.4	1.7	1.2	1.4	0.3
5	1.5	1.5	0.9	1.3	0.3
6	1.0	1.4	1.1	1.2	0.2
7	1.0	1.1	1.2	1.1	0.1
8	1.0	1.2	1.2	1.1	0.1
9	1.0	1.4	0.9	1.1	0.3
10	0.9	1.2	1.8	1.3	0.5
11	1.0	1.0	1.1	1.0	0.1
12	1.1	0.9	0.7	0.9	0.2
13	1.0	1.4	0.9	1.1	0.3
14	1.0	0.9	1.4	1.1	0.2
15	0.9	1.1	1.1	1.0	0.1
16	1.2	1.1	0.8	1.0	0.2
17	1.4	1.0	0.8	1.1	0.3
18	1.4	1.2	0.7	1.1	0.4
19	1.3	0.9	0.8	1.0	0.2
20	0.9	1.1	0.9	1.0	0.2

Table AI. 4: E. coli *O157:H7 populations (log CFU/g) on previously uninoculated lettuce collected from each of the 20 collection baskets after washing and shaker table dewatering while the conveyor was contaminated.*

Basket (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	1.0	1.4	1.7	1.4	0.3
2	0.4	1.4	1.4	1.1	0.6
3	0.7	1.5	1.2	1.1	0.4
4	1.3	1.1	1.4	1.2	0.1
5	1.3	0.9	0.6	1.0	0.4
6	0.9	1.6	1.2	1.3	0.3
7	0.7	1.5	1.0	1.1	0.4
8	0.9	1.2	1.2	1.1	0.2
9	0.8	1.1	0.8	0.9	0.2
10	1.6	0.9	1.2	1.2	0.4
11	1.1	1.1	1.0	1.1	0.1
12	0.8	1.0	1.2	1.0	0.2
13	0.5	0.9	1.1	0.8	0.3
14	0.6	0.9	1.0	0.8	0.2
15	0.9	0.9	1.2	1.0	0.2
16	0.7	0.9	0.8	0.8	0.1
17	1.1	0.9	0.9	1.0	0.1
18	0.6	0.9	0.9	0.8	0.2
19	0.6	1.0	0.8	0.8	0.2
20	0.7	0.7	0.5	0.7	0.1

Table AI. 5: E. coli *O157:H7 (log CFU/g) populations on previously uninoculated lettuce collected (over time along processing) for each centrifugal drying batch while the shredder was contaminated.*

Lettuce sample (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	1.4	2.4	1.3	1.7	0.6
2	0.7	1.2	0.9	1.0	0.2
3	0.7	1.3	1.4	1.1	0.4
4	0.7	1.0	0.6	0.8	0.2
5	0.8	1.8	1.3	1.3	0.5

Table AI. 6: E. coli *O157:H7 (log CFU/g) populations on previously uninoculated lettuce collected (over time along processing) for each centrifugal drying batch while the conveyor was contaminated.*

Lettuce sample (No.)	*E. coli* O157:H7 populations (log CFU/g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	0.9	1.4	1.2	1.2	0.3
2	1.2	0.2	1.0	0.8	0.5
3	0.5	1.3	0.9	0.9	0.4
4	1.0	2.0	1.6	1.5	0.5
5	0.9	0.8	0.7	0.8	0.1

Table AI. 7: E. coli *O157:H7 populations (log CFU/cm2) on equipment surfaces while the shredder was contaminated. * representing half of the LOD was used to calculate mean log value for the surface samples yielded no E. coli O157:H7 colonies on the plates.*

Surface sample (No.)/ Surface of equipment	*E. coli* O157:H7 populations (log CFU/cm^2)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	4.7	3.7	3.6	4.0	0.6
2	0.0*	0.0*	0.0*	0.0	0.0
3	1.3	4.4	2.4	2.7	1.6
4	0.0*	0.0*	0.0*	0.0	0.0
5	2.4	4.0	3.5	3.3	0.8
6	0.0*	0.0*	0.3	0.1	0.2
7	3.6	1.3	4.0	3.0	1.5
8	0.0*	0.0*	1.6	0.5	0.9
9	3.0	4.2	2.8	3.3	0.7
10	3.3	0.3	0.0*	1.2	1.8
11	2.3	5.0	4.8	4.1	1.5
12	4.1	5.0	4.9	4.6	0.5
13	5.8	5.5	5.8	5.7	0.2
14	0.0*	0.3	0.0*	0.1	0.2
15	0.0*	0.3	1.7	0.7	0.9
16	0.0*	1.2	1.8	1.0	0.9
17	0.3	0.3	3.2	1.3	1.6
18	0.3	2.5	1.9	1.6	1.1
19	2.4	2.8	1.8	2.3	0.5
20	2.1	2.2	0.8	1.7	0.8
21	0.0*	2.9	1.3	1.4	1.4
22	0.0*	3.3	1.8	1.7	1.6
Flume tank	3.0	1.9	1.5	2.1	0.7
Shaker table	1.8	1.5	1.5	1.6	0.2
centrifuge	1.4	1.9	0.1	1.1	0.9

Table AI. 8: E. coli *O157:H7 populations (log CFU/cm2) on equipment surfaces while the conveyor was contaminated. * representing half of the LOD was used to calculate mean log value for the surface samples yielded no E. coli O157:H7 colonies on the plates. NA stand for not applicable*

Surface sample (No.)/ Surface of equipment	*E. coli* O157:H7 populations (log CFU/cm^2)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1-14	NA	NA	NA	NA	NA
15	4.9	4.0	4.0	4.3	0.5
16	2.9	2.8	2.6	2.8	0.2
17	3.9	5.0	5.5	4.8	0.8
18	2.4	2.8	2.7	2.7	0.2
19	4.9	4.1	3.7	4.2	0.6
20	2.8	1.9	2.3	2.3	0.5
21	2.6	3.7	3.2	3.2	0.6
22	2.1	2.5	2.3	2.3	0.2
Flume tank	1.0	1.4	0.9	1.1	0.3
Shaker table	1.5	1.2	1.4	1.4	0.2
centrifuge	1.4	0.8	0.8	1.0	0.3

Table AI. 9: E. coli *O157:H7 (log CFU/ml) populations in water samples for each centrifugal drying batch while the shredder was contaminated.*

Water sample (No.)	*E. coli* O157:H7 populations (log CFU/ml)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	1.9	2.6	2.0	2.2	0.4
2	1.8	2.0	1.9	1.9	0.1
3	1.6	2.0	1.8	1.8	0.2
4	1.6	1.9	1.7	1.7	0.1
5	1.6	2.5	2.1	2.1	0.4

Table AI. 10: E. coli O157:H7 (log CFU/ml) populations in water samples for each centrifugal drying batch while the conveyor was contaminated.

Water sample (No.)	*E. coli* O157:H7 populations (log CFU/ml)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	1.4	1.9	2.4	1.9	0.5
2	1.7	1.9	2.0	1.9	0.2
3	1.6	1.7	2.0	1.8	0.2
4	1.3	1.9	1.8	1.7	0.3
5	1.6	1.6	1.9	1.7	0.2

Table AI. 11: *Weight of radicchio (g) retrieved from each of the 20 collection baskets after washing and shaker table dewatering while the shredder was contaminated.*

Basket (No.)	Weight of radicchio (g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	4.2	28.3	9.3	13.9	12.7
2	5.8	9.4	3.9	6.4	2.8
3	2.4	11.1	5.3	6.3	4.4
4	2.2	5.5	4.5	4.1	1.7
5	0.5	5.3	5.1	3.6	2.7
6	0.3	3.2	2.4	2.0	1.5
7	0.9	1.6	1.1	1.2	0.4
8	1.1	0.6	0.8	0.8	0.3
9	0.7	0.3	5.9	2.3	3.1
10	0.3	0.5	0.4	0.4	0.1
11	0.5	0.2	0.2	0.3	0.2
12	0.0	1.2	2.6	1.3	1.3
13	0.1	2.4	1.2	1.2	1.2
14	0.1	1.2	0.4	0.6	0.6
15	0.1	0.2	0.0	0.1	0.1
16	0.2	0.6	1.2	0.7	0.5
17	0.0	0.8	0.1	0.3	0.4
18	0.0	0.9	0.5	0.5	0.4
19	1.1	0.4	0.2	0.6	0.5
20	0.1	0.3	0.2	0.2	0.1

Table AI. 12: *Weight of radicchio (g) retrieved from each of the 20 collection baskets after washing and shaker table dewatering while the conveyor was contaminated.*

Basket (No.)	Weight of radicchio (g)				
	Replicate 1	Replicate 2	Replicate 3	Mean	STDEV
1	13.5	3.0	12.7	9.7	5.8
2	2.6	3.1	7.3	4.3	2.6
3	1.1	1.3	3.2	1.9	1.2
4	2.2	1.2	1.1	1.5	0.6
5	1.2	1.7	1.4	1.4	0.3
6	1.8	0.6	1.6	1.3	0.6
7	1.6	1.4	0.6	1.2	0.5
8	0.7	1.4	0.8	1.0	0.4
9	1.4	0.4	0.3	0.7	0.6
10	0.4	0.2	0.2	0.3	0.1
11	0.5	0.2	0.2	0.3	0.2
12	5.9	0.3	0.2	2.1	3.3
13	0.4	0.2	0.2	0.3	0.1
14	0.4	0.3	0.2	0.3	0.1
15	0.3	0.1	0.2	0.2	0.1
16	0.3	0.1	3.0	1.1	1.6
17	0.2	0.1	0.4	0.2	0.2
18	0.3	0.2	0.2	0.2	0.1
19	0.1	0.1	0.2	0.1	0.1
20	0.1	0.2	0.1	0.1	0.1

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