IMPACT OF ORGANIC LOAD ON SANITIZER EFFICACY AGAINST *ESCHERICHIA COLI* O157:H7 DURING PILOT-PLANT PRODUCTION OF FRESH-CUT LETTUCE

By

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ABSTRACT

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Addition of chemical sanitizers during commercial flume washing of leafy greens remains the sole microbial mitigation strategy. However, continued *Escherichia coli* O157:H7 outbreaks have raised concerns regarding recirculation of wash water, with the accumulation of organic load during processing leading to decreased efficacy of chlorine-based sanitizers. Reliable methods to quantify the impact of organic load on sanitizing efficacy do not yet exist.

Initially, the efficacy of six different wash treatments (water alone, 50 ppm peroxyacetic acid, 50 ppm mixed peracid, or 50 ppm available chlorine either alone or acidified to pH 6.5 with citric acid (CA) or T-128) was assessed using 5.4 kg of iceberg lettuce inoculated to contain 10^6 CFU/g of a 4-strain non-toxigenic, GFP-labeled, ampicillin-resistant cocktail of *E. coli* O157:H7 in a pilot-scale leafy green processing line consisting of a commercial shredder, conveyor, flume tank, shaker table, and centrifugal dryer. Without an organic load in the water, none of the sanitizers were more effective ($P \leq 0.05$) than water alone at reducing *E. coli* O157:H7 populations on lettuce, with reductions ranging from 0.8 to 1.4 log CFU/g. However, chlorine, chlorine + CA, and chlorine + T-128 were generally more effective ($P \leq 0.05$) than the other treatments against *E. coli* O157:H7 in the flume water, with reductions of 3.8, 5.5, and 5.4 log CFU/ml after 90 s of processing, respectively.

Thereafter, a novel and cost-effective carboy system was developed to assess the efficacy of the same five sanitizing agents against *E. coli* O157:H7 in wash water containing an organic

load of 0 to 10% (w/v) blended lettuce. After iceberg lettuce previously inoculated to contain *E. coli* O157:H7 at 10^6 CFU/g was washed for 90 s, *E. coli* O157:H7 persistence was subsequently correlated to various physicochemical parameters of the wash water. Organic load negatively impacted the efficacy of chlorine, chlorine + CA, and chlorine + T-128 ($P \leq 0.05$), with typical *E. coli* O157:H7 reductions of < 1 log CFU/ml after 10 min of exposure. However, the efficacy of peroxyacetic acid and mixed peracid was unaffected by organic load ($P > 0.05$), with average *E. coli* O157:H7 reductions of ~4.8 and ~5.5 log CFU/ml, respectively, after 10 min of exposure.

Finally, efficacy of the same five sanitizer treatments was assessed against *E. coli* O157:H7 on iceberg lettuce, in wash water, and on surfaces of a pilot-scale processing line using flume water containing an organic load of 0 to 10% (w/v) blended lettuce. Organic load negatively impacted the efficacy of all three chlorine treatments ($P \leq 0.05$), with typical *E. coli* O157:H7 reductions of > 5 log CFU/ml by the end of processing with no organic load in the wash water and 0.9 – 3.7 log CFU/ml with a 10% organic load. Organic load rarely impacted ($P > 0.05$) the efficacy of either peroxyacetic acid or mixed peracid, with typical reductions of > 5 log CFU/ml in wash water throughout processing for all organic loads. Sanitizer efficacy against *E. coli* O157:H7on lettuce was seldom impacted by organic load. In both the carboy system and the pilot-scale processing line, reduced sanitizer efficacy generally correlated to increases ($P \leq 0.05$) in total solids, chemical oxygen demand and turbidity, and decreases ($P \leq 0.05$) in maximum filterable volume, indicating that these tests may be effective alternatives to the industry standard of oxygen/reduction potential. These findings demonstrate that monitoring of both sanitizer concentration and wash water quality is critical to minimizing the likelihood of amplifying a previously isolated contamination event.

To my parents, Brad and Janet Davidson

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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)
COD	Chemical oxygen demand
CPS	Center for Produce Safety
CSPI	Center for Science in the Public Interest
d	days(s)
EHEC	Enterohemorrhagic *E. coli*
FDA	Food and Drug Administration
FSMA	Food Safety Modernization Act
g	grams(s)
GFP	Green fluorescent protein
GRAS	Generally recognized as safe
h	hour(s)
HACCP	Hazard Analysis and Critical Control Point
HUS	Hemolytic uremic syndrome
min	minute(s)
MFV	Maximum filterable volume
ml	milliliter(s)
ORP	Oxidation/reduction potential
PBS	Phosphate Buffered Saline

PFGE	Pulsed-field Gel Electrophoresis
PMACS	Portable Multi-use Automated Concentration System
ppm	Parts per million
RMSE	Root-Mean-Square Error
s	second(s)
SAS	Statistical Analysis Systems
Stx	Shiga toxin
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)
µm	micron(s)

INTRODUCTION

The Centers for Disease Control and Prevention (CDC) ranked leafy vegetables as the leading food commodity for foodborne illnesses in the United States (22%) and the second most frequent cause of hospitalizations (14%) between 1998 and 2008 *(89)*. In 2009, leafy greens were ranked as the riskiest food regulated by the Food and Drug Administration (FDA), accounting for 363 outbreaks and 13,568 reported cases of illness *(36)*. Between 1995 and 2006, leafy green-associated outbreaks increased by 39% while consumption increased by only 9% *(63)*. Leafy greens were responsible for 363 separate outbreaks involving 13,568 individual cases of illness through 2009 *(36)*, with most of the *E. coli* outbreaks attributed to lettuce.

The widely-publicized nationwide outbreak of *E. coli* O157:H7 that was traced to baby spinach in 2006 resulted in 205 confirmed infections, 103 hospitalizations, and three deaths *(28, 38, 45)*. Two additional *E. coli* O157:H7 outbreaks in November and December of 2006 were linked to shredded iceberg lettuce that was served at two different Mexican chain restaurants *(123, 124)*. These two outbreaks resulted in 150 illnesses *(35, 123, 124)*.

Bacterial pathogens can contaminate fresh produce at any point during the farm-to-fork continuum *(83)*. Major on-farm areas of concern now recognized by the FDA include agricultural water, biological soil amendments (e.g., manure), domesticated and wild animals, field worker health and hygiene, and the cleanliness of harvesting equipment, tools and buildings *(127)*. Processing of leafy greens involves a series of steps that can either decrease or promote the growth of pathogenic microorganisms. These steps include harvesting, cold storage, trimming, shredding, washing and rinsing, draining, packaging, cold storage and distribution

(118). The sole microbial mitigation strategy during production of fresh-cut leafy greens is the use of sanitizers during flume washing.

Commercial processing facilities recirculate flume water in order to reduce operational costs *(80)*. Sanitizing agents are routinely added to recirculating flume water as a means to prevent the water from becoming a source of microbial contamination, thereby preventing the spread of pathogens in large, centralized processing facilities. However, the efficacy of various sanitizing agents has been questioned as product recalls and outbreaks have continued to occur. Numerous bench-top studies have shown that produce sanitizers reduce pathogen populations only 1 to 3 logs on lettuce *(17, 47, 55, 91, 100)*, with water alone decreasing *E. coli* O157:H7 levels about 1 log on lettuce during pilot-scale processing *(22)*.

Chlorine-based sanitizers are most commonly used by commercial processors to minimize cross-contamination during processing, due to their relatively low cost compared to other sanitizers, and minimal negative impact on end-product quality *(31, 62, 81, 87, 91)*. However, chlorine use has raised concerns regarding potentially hazardous by-products, worker safety, environmental damage, and, most importantly, decreased efficacy in the presence of an increasing organic load in recirculating flume water, which has heightened interest in other alternatives, such as peroxyacetic acid-based sanitizers *(100, 113)*.

An organic load, consisting of plant tissues and cellular fluids released during cutting, in addition to soil, insects, and microbes *(62)*, will accumulate in recirculating flume water as produce is washed, decreasing the ability of sanitizers to minimize cross-contamination from the water during processing *(57, 110, 139)*. While organic load impacts the efficacy of sanitizing agents, most notably chlorine, the means to quantify sanitizer efficacy against pathogens such as *E. coli* O157:H7 have not yet been determined. The Center for Produce Safety currently ranks the

identification of methods to validate the efficacy of flume water used to wash fruits and vegetables as one of their top priorities *(33)*.

It is hypothesized that 1) organic load decreases the efficacy of sanitizers used during simulated commercial processing of leafy greens and 2) *E. coli* O157:H7 persistence can be correlated to various physicochemical parameters of the wash water.

In the absence of any major improvements in the methods of growing, harvesting, processing, transporting, and displaying leafy greens, outbreaks of illness associated with the consumption of fresh-cut leafy greens have continued to occur in the United States. Given the need for a safe end-product, the overall objective of this research was to develop methods that can be employed by processors to determine the efficacy of various sanitizers during commercial lettuce processing in order to minimize the transfer of pathogens during washing.

The research reported in this dissertation had four primary objectives: 1) assess the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 during simulated commercial processing of iceberg lettuce in a pilot-scale leafy green processing line; 2) determine the persistence of *E. coli* O157:H7 in wash water containing various sanitizers and organic loads in a novel and cost-effective model bench-top carboy system; 3) determine the impact of organic load on sodium hypochlorite and peroxyacetic acid efficacy, against *E. coli* O157:H7 during simulated commercial processing; and 4) assess the relationship between various physicochemical parameters and organic load of the wash water on sanitizer efficacy against *E. coli* O157:H7 in both the carboy model and pilot-scale leafy green processing line.

CHAPTER 1:

Review of Pertinent Literature

1.1 The leafy greens industry

Consumption of vegetables has increased dramatically in recent years as Americans have moved to towards healthier eating habits *(27)*. Vegetables are rich sources of fiber, vitamins, minerals and essential nutrients, especially carbohydrates *(27)*. Processing methods such as canning, drying and freezing lead to significant nutrient losses; however, these treatments are necessary to improve product shelf life and quality, enhance palatability, and inactivate nutritional inhibitors *(27)*. Additional advancements in modified atmosphere storage and minimal processing technologies have led to the commercial production of a wide for a variety of fresh, convenient, and ready-to-eat products including leafy greens *(2)*.

Commercially processed leafy greens are "value added" products and represent a multi-billion dollar industry. California and Arizona are the main producers of iceberg lettuce and baby spinach, with California's peak production season in May and June, while Arizona's is in December through February, with production occurring nearly year-round *(121)*. Prior to the series of leafy green outbreaks in the fall of 2006, the pre-washed salad market brought in $2.6 billion annually, with the spinach industry worth $286 million *(119)*. Earthbound Farms, under its parent company Natural Selection Farms, had grown from selling spinach at a roadside stand in 1986 into a $360 million industry *(119)*.

The leafy green industry as a whole was hit particularly hard after the 2006 outbreaks, with sales rapidly declining due to the extensive media coverage during the outbreaks and the slow rate of the outbreak investigation. By mid-October of 2006, Natural Selection's sales of conventional salads were down by 70% and down by 10% for products sold under the Earthbound Farms name *(119)*. The company was forced to lay off 164 employees, and it has been estimated that Earthbound Farms and Dole may have to pay up to $110 million to settle

cases with the victims of the outbreaks *(119)*. Spinach losses alone totaled $205.8 million following the outbreaks in 2006 *(8)*.

1.2 Leafy green associated recalls and outbreaks

It is estimated that approximately 37.2 million illnesses are caused by 31 different pathogens annually in the United States *(103)*. As Americans attempt to improve their eating habits, outbreaks of illness attributed to fresh produce are increasingly being reported. Between 1998 and 2006, produce was responsible for ~40 illnesses per outbreak, while poultry, beef, and seafood were responsible for ~25, 23 and 9 illnesses per outbreak, respectively *(34)*. The Centers for Disease Control and Prevention recently ranked leafy vegetables as the leading cause of foodborne illnesses in the United States (22%) and the second most frequent cause of hospitalizations (14%) between 1998 and 2008 *(89)*. In 2009, leafy greens were ranked as the riskiest food regulated by the Food and Drug Administration (FDA), accounting for 363 outbreaks and 13,568 reported cases of illness *(36)*. Between 1995 and 2006, leafy green-associated outbreaks increased by 38.6% while consumption increased by only 9% *(63)*. During this same period, *E. coli* O157:H7 outbreaks increased notably in 2005 and 2006 compared to 1998 – 2004, as seen in Figure 1.1 *(34)*.

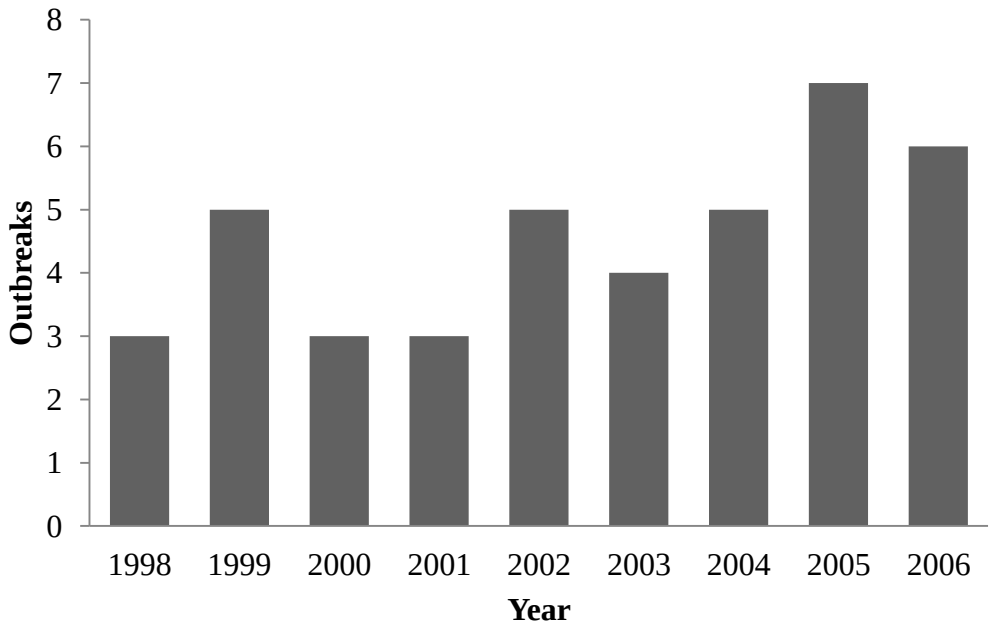


Figure 1.1: *Produce outbreaks attributed to* E. coli *O157:H7 from 1998 – 2006 (34).*

As seen in Figure 1.2, the Center for Science in the Public Interest also ranked the specific commodities for produce-linked outbreaks between 1998 and 2006 *(34)*. Salads were responsible for the majority of outbreaks, followed by potatoes, tomatoes, melons and sprouts. Salads alone, which include various types of leafy greens, were responsible for over one-third of the outbreaks during that time period, with lettuce responsible for an additional 8%.

Bacterial pathogens can contaminate fresh produce at any point during the farm-to-fork continuum *(83)*. Major on-farm areas of concern now recognized by the FDA include agricultural water, biological soil amendments (e.g., manure), domesticated and wild animals, field worker health and hygiene, and the cleanliness of harvesting equipment, tools and buildings *(127)*. However, leafy greens are also prone to contamination during commercial processing, packing *(24)*, distribution, marketing *(138)*, and in-home preparation *(90)*.

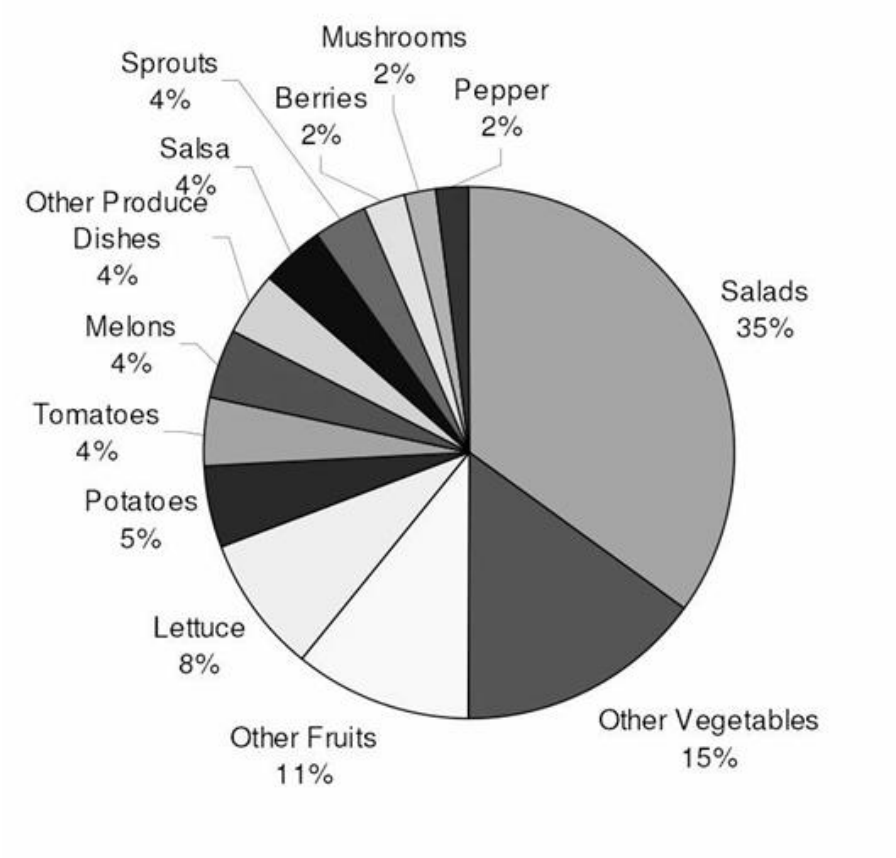


Figure 1.2: *Produce-linked outbreaks between 1998 and 2006, ranked according to commodity (34).*

The term "leafy greens" is broadly used to describe arugula, baby leaf lettuce, butter lettuce, cabbage, chard, endive, escarole, green leaf lettuce, iceberg lettuce, kale, red leaf lettuce, romaine lettuce, spinach and spring mix *(29)*. Leafy greens were responsible for 363 separate outbreaks involving 13,568 individual cases of illness through 2009 *(36)*, with most of the *E. coli* outbreaks attributed to lettuce, much of which is grown in the Salinas Valley in California. A selection of *E. coli* outbreaks since 1995 can be seen in Table 1.1, (adopted from Mandrell *(84)*), which has been updated to include more recent outbreaks.

The nationwide outbreak of *E. coli* O157:H7 that was traced to baby spinach in 2006 resulted in 205 confirmed infections, 103 hospitalizations, and three deaths *(28, 38, 45)*. The outbreak strain was isolated from numerous feral pigs on a ranch approximately one mile from

the spinach field implicated in the outbreak. Additionally, 33.8% of the cattle tested on the same ranch tested positive for the outbreak strain. Evidence of feral pig intrusion, including fecal droppings in the field and adjacent vineyards, signs of rooting, and tracks were present on the same ranch *(67)*. Molecular typing by pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis were used to confirm that the strain isolated from the pigs was in fact the outbreak strain. It is hypothesized that the pigs accessed the spinach field and contaminated the product.

Two additional *E. coli* O157:H7 outbreaks in November and December of 2006 were linked to shredded iceberg lettuce that was served at two different Mexican chain restaurants *(123, 124)*. These two outbreaks resulted in 150 illnesses in the Midwest and northeastern United States *(35, 123, 124)*. One of the *E. coli* O157:H7 outbreak strains matched isolates from samples from a dairy farm near the growing region in central California *(124)*, however the source of the other strain could not be confirmed *(123)*.

Table 1.1: *Select leafy greens associated outbreaks from 1995 through 2012*

Date	Pathogen	Location	Reported Illnesses	Product	Source Region
Jul. 95	*E. coli* O157:H7	MT	74	Lettuce, Romaine	MT, WA
Sept. 95	*E. coli* O157:H7	ME	30	Lettuce, Iceberg	Unknown
Sept. 95	*E. coli* O157:H7	ID	20	Lettuce, Romaine	Unknown
Oct. 95	*E. coli* O157:H7	OH	11	Lettuce	Unknown
May 96	*E. coli* O157:H7	IL, CT	61	Lettuce, Mesclun mix	CA
Jun. 96	*E. coli* O157:H7	NY	7	Lettuce, Mesclun	Unknown
May 98	*E. coli* O157:H7	CA	2	Lettuce, salad	Unknown
Sep. 98	*E. coli* O157:H7	MD	4	Lettuce	Unknown
Feb. 99	*E. coli* O157:H7	NE	65	Lettuce, salad	Unknown
Sep. 99	*E. coli* O157:H7	CA	8	Lettuce, Romaine	CA
Sep. 99	*E. coli* O157:H7	WA	6	Lettuce, Romaine	CA
Oct. 99	*E. coli* O157:H7	OH, IN	47	Lettuce, salad	Unknown
Oct. 99	*E. coli* O157:H7	OR	3	Lettuce, Romaine hearts	CA
Oct. 99	*E. coli* O157:H7	PA	41	Lettuce, Romaine	CA

Table 1.1 (cont'd)

Jul. 02	*E. coli* O157:H7	WA	29	Lettuce, Romaine	CA
Nov. 02	*E. coli* O157:H7	IL, WI, MN, SD, UT	24	Lettuce	CA
Sep. 03	*E. coli* O157:H7	CA	57	Lettuce, Iceberg/Romaine	CA
Sep. 03	*E. coli* O157:H7	ND	5	Lettuce, mixed w/ Romaine	Unknown
Oct. 03	*E. coli* O157:H7	CA	16	Spinach	CA
Nov. 04	*E. coli* O157:H7	NJ	6	Lettuce	CA
Aug./Sep. 06	*E. coli* O157:H7	26 states	>200	Spinach, baby, bagged	CA
Nov. 06	*E. coli* O157:H7	NJ, NY, PA, DE	71	Lettuce, Iceberg	CA
Nov./Dec. 06	*E. coli* O157:H7	MN, IA, WI	81	Lettuce, Iceberg	CA
May 08	*E. coli* O157:H7	WA	10	Lettuce, Romaine	CA
Sep. 08	*E. coli* O157:H7	MI, IL, NY, OR, OH, Ontario	74	Lettuce, Iceberg	CA
Apr. 09	*E. coli* O157:H7	MN	16	Lettuce, prepackaged	Unknown
Sep. 09	*E. coli* O157:H7	NY, WI, UT, NC, CO, SD	29	Lettuce, Iceberg/Romaine	CA
May 10	*E. coli* O145	MI, OH, NY, PA, TN	26	Lettuce, Romaine	AZ
Oct./Nov. 11	*E. coli* O157:H7	10 States	58	Lettuce, Romaine	Unknown

Table 1.1 (cont'd)

Apr. 12	*E. coli* O157:H7	CA, Quebec	28	Lettuce, Romaine	CA
Oct./Nov. 12	*E. coli* O157:H7	CN, MA, NY, PA, VA	33	Spinach and lettuce mix	MA

Information for the September 2008 outbreak based on Foodborne Illness Outbreak Database *(49)*, the April 2009 outbreak is based on Foodborne Illness Outbreak Database *(52)*, the September 2009 outbreak is based on Foodborne Illness Outbreak Database *(50)*, the May 2010 outbreak is based on CDC *(39)*, The October/November 2011 outbreak is based on Foodborne Illness Outbreak Database and the CDC *(40, 53)*, the April 2012 outbreak is based on Foodborne Illness Outbreak Database *(51)*, and the October/November 2012 outbreak is based on CDC *(41)*, all other outbreaks were summarized by Mandrell *(84)*.

1.3 *Escherichia coli* O157:H7

E. coli O157:H7, a facultative anaerobic, Gram-negative, rod-shaped bacterium, is an unusually virulent enterohemorrhagic strain of *E. coli* (EHEC). It is a cause of enteric disease resulting in bloody diarrhea and severe abdominal pain and in some cases, hemolytic uremic syndrome (HUS). Characterized by hemolytic anemia, thrombocytopenia and hemolytic uremic syndrome (acute kidney failure) *(69),* HUS appears mainly in children, as first described by Karmali and others *(70). E. coli* O157:H7 is a common inhabitant of the bovine gastrointestinal tract and has historically been a major source of concern in the ground beef industry *(58).* This organism first became recognized as a foodborne pathogen in 1982 after an outbreak tied it to the consumption of undercooked hamburgers, which ultimately sickened 47 individuals *(94).* Since its discovery, *E. coli* O157:H7 has been most often linked to outbreaks associated with ground beef *(15, 94),* produce *(67, 123, 124),* fruit juices *(37, 44),* unpasteurized milk *(58, 71),* and contaminated drinking water *(114).*

E. coli O157:H7 is the most well-known EHEC strain, but non-O157 strains now cause an estimated 36,000 illnesses, 1,000 hospitalizations and 30 deaths annually in the United States. The six most common serotypes of non-O157 EHEC are O26, O111, O103, O121, O45, and O145 *(20). E. coli* is characterized by a specific combination of the O (somatic), H (flagellar) and sometimes K (capsular) antigens which define the various serotypes *(69).* Strains of pathogenic *E. coli* use multistep schemes of pathogenesis starting with colonization of the intestinal mucosa, evading the hosts immune system, multiplication and damage to the host *(69).* Four classes (virotypes) of *E. coli* in addition to EHEC that cause diarrheal diseases are currently recognized: enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli* and enteroaggregative *E. coli.*

E. coli O157:H7 has an extremely low oral infectious dose of less than 100 cells *(85).* Enterohemorrhagic strains including *E. coli* O157:H7, destroy the normal microvillar structure of the colon by expressing the virulence factor protein, intimin, which allows intimate attachment of the bacteria and inducement of an attaching and effacing lesion. Shiga toxin (Stx) is the major virulence factor of EHEC and also commonly known as verocytotoxin. Stx is a family of structurally similar cytotoxins, with related biological activity, including two main groups, Stx1 and Stx2, which share about 40% amino acid similarity. Stx is composed of five identical B subunits that bind the holotoxin to the glycolipid globotriaosylceramide on the host cell surface and an A subunit, which cleaves ribosomal RNA to disrupt protein synthesis, ultimately killing the epithelial and endothelial cells. Stx causes local damage to the colon resulting in bloody diarrhea, hemorrhagic colitis and intestinal perforation. HUS results when Stx, produced in the colon, travels through the bloodstream to the renal endothelial cells in the kidney, causing inflammation by obstructing the microvasculature through both direct toxicity and inducing local cytokine and chemokine production *(30, 69, 92, 116).*

1.4 Pre-harvest contamination and control

The increasing frequency of reported foodborne outbreaks associated with fresh fruits and vegetables is of major concern in the United States. These outbreaks are the driving force for changes needed by the produce industry in the way products are grown, harvested, and processed. Contamination can occur at any point during the farm-to-fork continuum. Produce can become contaminated on the farm through fecal contamination from domestic and wild animals, water runoff from nearby livestock operations, improperly composted manure, dust/air, insects, contaminated irrigation water, or poor handling practices during the harvesting process *(19).*

The mechanisms that *E. coli* O157:H7 uses to attach to the surfaces of leafy greens are receiving increased interest. One group has recently demonstrated that EHEC strains of *E. coli* O157:H7 and non-O157:H7 both attach to the leaf surfaces of spinach, arugula and lettuce through the EspA filamentous type III secretion system that has also been shown to play an important role in the organisms colonization of bovine and human hosts. This finding indicates that EHEC uses plant leaves solely as a transmission vector rather than acting like a plant pathogen *(105).* Additionally, flagella play a role in attachment because *fliC* gene mutants have been shown to be significantly impaired in their ability to adhere to the surfaces of spinach and lettuce compared to the parental strains *(136).* Adhesion has also been shown to be temperature and time dependent. While intimin adhesion is vital for attachment to the host organism, it is not required for colonization of leafy greens. Based on this, colonization of leafy greens by EHEC strains of *E. coli* O157:H7 is likely a means to survive the processing environment and to allow transmission to the human host *(136).*

Pathogens are most likely to attach to stomata, irregularities on intact surfaces, cut surfaces, or cracks on the external surfaces *(55, 91, 100, 102, 111)* and can be protected from sanitizers by biofilms *(104).* Biofilms are defined as an aggregate of microbes that are embedded in an extracellular matrix, which encapsulates the cells and provides a physical barrier against environmental stresses such as sanitizing agents, temperature changes, desiccation or ultraviolet rays *(7, 99, 117).* Bacterial attachment and biofilm formation on the surfaces of leafy greens greatly hinder the removal of microbial contaminants during washing *(32, 101).* When Lang and others *(75)* assessed the impact of post-inoculation drying times on *E. coli* O157:H7 survival on parsley and iceberg lettuce, equal or greater populations were recovered from the leafy greens after 2 h of drying at 22°C compared to 2 h drying at 22°C followed by 22 h at 4°C. Hence, the

earlier in the farm-fork continuum the product is contaminated, the less effective bacterial mitigation strategies are likely to be.

Following passage of the Food Safety Modernization Act (FSMA) into law in 2011, the FDA released a set of proposed rules in January of 2013 for growing, harvesting, packing, and holding produce for human consumption. According to the FDA, 16.8% of produce-related outbreaks are attributed to fresh-cut fruits and vegetables, with the original contamination likely occurring during growing, harvesting, packing, or holding *(128)*. The major provisions and regulatory actions of the proposed rule would set new standards for the following areas: worker training, health and hygiene; sanitary agricultural water; biological soil amendments; proximity and monitoring procedures for domestic and wild animals; sanitary conditions for equipment, tools, and buildings; and sprout growth, harvesting, and handling *(128)*. The proposed regulation is estimated to prevent 1.75 million cases of foodborne illness annually, with an associated financial benefit of $1.04 billion and an estimated cost of $460 million in total for domestic farms *(128)*. Even when contamination occurs at the growing or harvesting stages, conditions during post-harvest processing can intensify and spread what would normally be just a small, contained contamination event, resulting in a widespread outbreak *(19, 26, 128)*.

1.5 Post-harvest processing

Minimal processing of leafy greens involves a series of steps that can either amplify or promote the growth of pathogenic microorganisms. These steps include: harvesting, cold storage, trimming, shredding, washing and rinsing, draining, packaging, cold storage and distribution *(118)*. After harvesting, microbial contamination can come from many different sources such as the water used for cooling and washing, equipment surfaces and workers. The equipment used in

leafy green processing facilities varies, but most processors use the same general types of equipment- including a shredder, conveyer belt, flume tank, shaker table, and dewatering centrifugal dryer. Microbial contaminants can spread to multiple batches of product during processing (e.g., washing, peeling, shredding, slicing, drying and sorting) which can lead to a potential outbreak of illness.

Human foodborne pathogens, including *E. coli* O157:H7, *Salmonella*, *Listeria* and *Cryptosporidium* that may inadvertently contaminate fresh fruits and vegetables in the field or at harvest can be readily transferred to much larger quantities during subsequent product handing and processing. These same foodborne pathogens can also contaminate the product during processing. In one outbreak of salmonellosis traced to shredded lettuce, Stafford and others *(108)* recovered *Salmonella* Bovismorbificans from the cutting wheel of a mechanical shredder during an environmental audit, with insufficient cleaning and sanitizing of the shredder cited as a key factor in this outbreak. In a study examining the microbial changes of lettuce during processing and storage, a 1 log CFU/g increase was observed after shredding *(3)*, indicating that the shredder may be a critical in-plant vehicle for amplifying contamination of leafy greens during processing *(54)*.

After shredding, leafy greens are washed to remove soil and debris, which decreases the microbial load, improves quality and appearance, and enhances product shelf life and safety *(62)*. Flume washing during leafy green processing is a common but highly variable practice. Commercial washing of leafy greens can vary in terms of the number of wash steps *(62)*, product contact time with the flume water (30 s to 2 min) *(62)*, mechanical agitation of the product in the water, sanitizing agents and concentrations used, rate of sanitizer and water replenishment,

amount and kinds of product washed per shift, and capacity of the flume system. Flume washing is typically the sole microbial mitigation strategy during processing.

1.6 Purpose of sanitizer addition to wash water

Numerous bench-top studies have shown that produce sanitizers reduce pathogen populations only 1 to 3 logs on lettuce *(47, 55, 91, 100)*, with water alone decreasing *E. coli* O157:H7 levels about 1 log on lettuce during pilot-scale processing *(22)*. Table 1.2 describes a selection of sanitizing agents typically used by commercial produce processors. While washing is the only intervention step against microbial populations on product, the primary goal of sanitizing agents is to reduce the microbial populations in flume water to prevent cross-contamination during washing *(137)*. Commercial processing facilities recirculate flume water in order to reduce operational costs *(80)*. Sanitizing agents are routinely added to recirculating flume water as a means to prevent the water from becoming a microbial carrier, thereby preventing the spread of pathogens in large, centralized processing facilities. However, the efficacy of various sanitizing agents has been questioned as product recalls and outbreaks have continued to occur.

Previous work done by our group involved quantification of *E. coli* O157:H7 transfer during pilot-scale leafy green processing using sanitizer-free wash water. A major finding from one of the studies was that ~90% of *E. coli* O157:H7 inoculum transferred from 22.7 kg of iceberg lettuce inoculated at 10^6 log CFU/g to the 890 L of flume water used during processing *(22)*. These results emphasize that effective sanitizing agents in flume water are critical to prevent the wash water from becoming a carrier of microbial contaminants.

Chlorine-based sanitizers are most commonly used by commercial processors to minimize cross-contamination during processing, due to their relatively low cost compared to other sanitizers, and minimal negative impact on end-product quality *(31, 62, 81, 87, 91).* However, chlorine use has raised concerns regarding potentially hazardous by-products, worker safety, environmental damage and, most importantly, decreased efficacy in the presence of an increasing organic load in recirculating flume water, which has heightened interest in other alternatives, such as peroxyacetic acid-based sanitizers *(100, 113).*

Table 1.2: *Commercial produce sanitizers used during flume washing of leafy greens* [a]

Sanitizing Agent	Active Ingredients	Designated Use	Maximum Allowed Concentration (ppm)	Advantages	Disadvantages
Chlorine	Sodium hypochlorite	Sanitizing food contact equipment, potable water treatment, fruit and vegetable washing	$\leq$ 200	Cost effective, effective against all microbial forms	pH dependent, high reactivity with organic solids, corrosive to metals
Peroxyacetic acid	Peroxyacetic acid Hydrogen peroxide	Pathogen reduction in fruit and vegetable processing water	$\leq$ 80	Low reactivity with organic solids, no hazardous breakdown products	Strong oxidant, concentrated solutions may be hazardous, costly
Mixed Peracid	Peroxyacetic acid Organic acids Hydrogen peroxide Acetic acid	Reduction of yeasts, molds and bacteria in water and on fruit and vegetable surfaces			

[a] Adapted from text in Hedt and Feng *(62)*.

1.7 Sodium hypochlorite use in wash water

Sodium hypochlorite (i.e., chlorine) - the most commonly employed sanitizing agent in the fresh produce industry *(31)*, has a long history of use and is relatively inexpensive when compared to other sanitizing agents. The following reactions occur when sodium hypochlorite is combined with water:

$NaOCl + H_2O \leftrightarrow HClO + NaOH$

$HClO \leftrightarrow H^+ + OCl^-$

$HClO + HCl \leftrightarrow H_2O + Cl_2$

Hypochlorous acid (HClO) is the main active ingredient formed and is the form of free available chlorine that has the highest bactericidal activity of all active components. The dissociation of HClO into OCl^- is pH dependent, with the concentration of HClO increasing with decreasing pH *(62, 91)*, predominating at pH 6.5 *(16, 109)*. Solutions containing sodium hypochlorite below a pH of 4 will cause toxic off-gassing and corrosion to processing equipment *(16, 109)*. Due to the potential of harmful disinfection byproducts, several European countries have banned the use of chlorine in fresh-cut operations *(61)*. Hypochlorous acid functions by oxidation, with HClO allowing oxygen to combine with components of the cell wall, resulting in cell death *(62)*. More specifically, Rosen and others *(96)* proposed that hypochlorous acid inactivates membrane proteins involved in DNA replication.

Free chlorine concentrations in clean commercial flume water generally range from 10 to 200 ppm *(55, 91, 122)*. Chlorine is highly reactive with organic compounds, which is a major issue considering that chlorine is the most commonly used sanitizer in the fresh produce industry. Chlorine exists as two forms, free chlorine (i.e., available chlorine) and total chlorine.

Free chlorine refers to the hypochlorite ion (OCl^-) and hypochlorous acid (HClO) concentration in solution *(62)*. Chloromines, such as monochloramine, dichloramine, or trichloramine, form when chlorine is exposed to ammonia or organic nitrogen and are referred to as combined chlorine *(60)*. Additionally, the pH of a solution has an impact on combined chlorine formation, with the reaction of chlorine with ammonia promoted above a pH of 7 *(60, 62)*. Total chlorine is the sum of combined chlorine and free chlorine. In systems with no organic load present in the water, free chlorine is equivalent to total chlorine; however, as organic load increases in the water, free chlorine levels drop *(60)*.

Modification of pH is often achieved through the use of a weak acid, such as citric acid, although newly-developed products, such as T-128 (SmartWash Solutions, Salinas, CA) are now being marketed as effective alternatives. T-128 is a generally recognized as safe (GRAS) acidifying agent comprised of phosphoric acid and propylene glycol, designed to stabilize free chlorine *(76)*. Previous research by Nou and others *(87)* showed that chlorine acidified with T-128 ($P < 0.001$) decreased the rate of free chlorine degradation in the presence of organic load. Additionally, T-128 significantly reduced cross-contamination when lettuce inoculated with *E. coli* O157:H7 was washed with uninoculated lettuce. An additional study by the same group showed that chlorine acidified to pH 5.0 with T-128 significantly reduced *E. coli* O157:H7 cross-contamination during pilot-scale processing of baby spinach when compared to the control, where chlorine was acidified to pH 6.5 with citric acid *(81)*. While exhibiting weak bactericidal activity when used alone, T-128 does not play a significant role in pathogen inactivation *(87)*, which emphasizes the products role as means to enhance the efficacy of chlorine.

Numerous small-scale laboratory studies have assessed sodium hypochlorite efficacy under a variety of conditions. More specifically, the impact of organic load in simulated produce

wash water on sanitizer efficacy has been previously examined *(57, 61, 78, 80, 87, 107, 139)*. Gonzalez and others *(57)* noted than an organic load correlating to a chemical oxygen demand (COD) value of 3,500 mg O_2/L resulted in significant reduction of sodium hypochlorite efficacy against *E. coli* O157:H7 on carrots and in water. Haute and others *(61)* showed that maintaining a free chlorine concentration of 1 ppm could reduce *E. coli* O157:H7 populations below the lower limit of detection in process water containing COD values of 500 and 1,000 mg O_2/L. Additionally, they constructed regression models that incorporated chlorine dose and COD to predict *E. coli* O157:H7 inactivation. Lopez-Galvez and others *(78)* found that wash water containing an organic load with a COD value of 500 – 700 mg O_2/L reduced of *E. coli* populations by 2 logs on lettuce after 1 min of exposure to 40 ppm chlorine, with populations in the wash water falling below the lower limit of detection. Luo *(80)* showed that washing Romaine lettuce in water containing 100 ppm chlorine and a COD value of 1861 mg O_2/L resulted in lactic acid bacteria populations that were 0.8 – 1.6 log CFU/g higher than those washed in clean water (COD value 9.8 mg O_2/L) at the end of storage under modified atmosphere packaging. More recently, Shen and others *(107)* showed that inactivation of *Salmonella*, *E. coli* O157:H7, and non-O157 shiga toxin-producing *E. coli* in chlorinated wash water significantly ($P < 0.0001$) depended on initial free chlorine concentration and contact time. Additionally, they showed that pathogen inactivation was specifically dependent on the residual free chlorine concentration when an organic load (COD values 186 – 460 mg O_2/L) was present in the wash water. According to Zhang and others *(139)*, wash water containing 30 ppm chlorine did not significantly reduce *E. coli* O157:H7 in wash water containing a 10% organic load when

compared to the sanitizer-free control. In addition to the research completed on leafy greens, studies have also assessed chlorine efficacy on carrots *(57, 97)*, apples *(1, 21)*, nuts *(18)* and melons *(6)* with generally similar findings.

Sodium hypochlorite has a long history of use in the fresh produce industry. It has become increasingly evident that commercial produce processors must take steps to monitor, at least, chlorine concentration and pH in order to minimize the dangers associated with toxic off-gassing. Additionally, increasing organic loads are well known to negatively impact the efficacy of chlorine, which is increasing the interest in alternative sanitizers, such as peroxyacetic acid. However, since chlorine is still widely used throughout the fresh-produce industry, improved monitoring methods must be developed for chlorine efficacy to minimize the spread of microbial contaminants during processing.

1.8 Peroxyacetic acid use in wash water

Peroxyacetic acid (i.e., peracetic acid or POAA) is receiving increasing interest as an alternative to sodium hypochlorite, due to its non-hazardous breakdown products and resistance to organic material in wash water. Peroxyacetic acid ($C_2H_4O_3$) is produced when acetic acid (CH_2CO_2H) reacts with hydrogen peroxide (H_2O_2) *(62)* as follows:

$$CH_3CO_2H + H_2O_2 \leftrightarrow CH_3CO_3H + H_2O$$

Peroxyacetic acid functions by oxidation, with the suggested mechanism of action being the oxidation of sulfhydryl bonds in proteins, enzymes and other metabolites, resulting in increased cell wall permeability *(62, 64)*. It has also been proposed that peroxyacetic acid is involved in enzymatic blockage of enzymatic and transport systems within microorganisms *(74)*.

Peroxyacetic acid breaks down into water, oxygen, and acetic acid *(9, 46, 74)*. The FDA has established, under the Code of Federal Regulations *(43)*, a peroxyacetic acid limit of 80 ppm in wash water to be used for fruit and vegetable washing.

While chlorine efficacy has been studied extensively, only a few studies have assessed the efficacy of peroxyacetic acid in simulated processing water *(1, 17, 65, 78, 95, 129)* and even fewer have examined the impact of organic load on peroxyacetic acid efficacy *(57, 64, 97, 139)*. Gonzalez and others showed that the efficacy of 80 ppm peroxyacetic acid in wash water was not significantly impacted by an organic load (COD 3,500 mg/L), resulting in *E. coli* O157:H7 populations below the limit of detection after washing inoculated shredded carrots. Hilgren and others *(64)* showed that the efficacy of peroxyacetic acid against *Bacillus anthracis* spores was rarely impacted by the presence of whole milk, egg yolk or flour paste. Ruiz-Cruz and others *(97)* found that the antimicrobial activity of 40 ppm peroxyacetic acid was not impacted by organic load (COD 3,500 mg/L), resulting in *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* reductions of 2.1, 1.24 and 0.83 log CFU/g, respectively, on carrot shreds after 2 min of washing. Zhang and others *(139)* showed that 20 ppm peroxyacetic acid and 20 ppm peroxyacetic acid mixed with octanoic acid (i.e., mixed peracid) significantly reduced ($P < 0.05$) *E. coli* O157:H7 transfer from inoculated lettuce leaves to uninoculated leaves in wash water containing a 10% organic load comprised of blended lettuce solids.

The addition of octanoic acid, a fatty acid, to peroxyacetic acid has been examined as a means to increase the fungicidal activity of peroxyacetic acid while also increasing the efficacy against coliform bacteria. Hilgren and Salverda *(65)* found that peroxyacetic acid in combination with octanoic acid (80 ppm) was significantly more effective ($P < 0.05$) than peroxyacetic acid alone (80 ppm) at reducing numbers of yeasts and molds in potato wash water and on the

surfaces of potatoes. However, no significant difference ($P > 0.05$) in coliform reductions on celery, cabbage, and potatoes was seen between peroxyacetic acid and peroxyactiec acid in combination with octanoic acid *(65)*.

The major advantages of peroxyacetic acid are its non-hazardous byproducts and increased resistance to organic material in wash water. However, no studies have yet explained the phenomenon of peroxyacetic acid resistance to organic load. Major disadvantages of peroxyacetic acid-based sanitizers include the increased cost and relatively short history of use when compared to sodium hypochlorite. Addition of peroxyacetic acid will increase the organic load in the wash water due to the acetic acid content *(73)*. However, this increase in organic load will not impact peroxyacetic acid efficacy, since acetic acid is native to the sanitizer *(73, 78)*, but the cost of the waste water disposal may be higher. The foremost disadvantage of peroxyacetic acid is the cost, which is four to five times higher as compared to sodium hypochlorite in the United States *(73)*. Kits *(73)* hypothesized than an increased demand of peroxyacetic acid would reduce this cost, making peroxyacetic acid-based sanitizers more economically feasible for processors.

1.9 Organic load accumulation in wash water

An organic load, consisting of plant tissues and cellular fluids released during cutting, in addition to soil, insects, and microbes *(62)*, will accumulate in recirculating flume water as produce is washed, decreasing the ability of sanitizers to minimize cross-contamination from the water during processing *(57, 110, 139)*. Based on a series of personal site visits to various commercial leafy-green processing facilities, numerous factors were observed that will affect the rate of accumulation and maximum organic load in flume water, such as the type and amount of product

being processed, product shred size, the rate of processing, and the means by which the organic load is decreased in the water during processing (e.g., separating screens, foam removal, filters, clarifiers, precipitators).

Numerous small-scale laboratory experiments have previously assessed the impact of organic load on sodium hypochlorite *(57, 78, 80, 87, 107, 139),* chlorine dioxide *(10),* electrolyzed water *(56, 79),* and peroxyacetic acid *(11, 57, 64, 97, 139).* In general, sodium hypochlorite efficacy dramatically decreases, but peroxyacetic acid maintains some activity in the presence of an organic load. Given the various commercial sanitizers available and the variable rates at which organic material accumulates in flume water during processing, commercial leafy green processors are clearly in need of better means to both predict and monitor the efficacy of sanitizers during processing.

1.10 Strategies for monitoring wash water efficacy

A variety of methods have been developed to either directly or indirectly monitor the efficacy of sanitizing agents in wash water, with these ranging from titration kits to digital probes or even fully automated systems that monitor and adjust sanitizing agent concentrations as needed. Titration test kits are often used by smaller operations to rapidly quantify the level of total chlorine or peroxyacetic acid content in wash water or as a validation procedure alongside high-tech automated systems. The principle of the chlorine titration method (i.e., iodometric method) is that chlorine liberates iodine in the acidified test solution containing potassium iodide, with the liberated iodine titrated with a standard solution of sodium thiosulfate to a starch endpoint. Free chlorine concentrations also can be quantified by using the DPD (N, N diethyl-p-phenylenediamine) method, which is based on the oxidation of an amine by chlorine into

Würster dye, which is then read by a colorimeter *(60)*. Electronic probes - either hand-held models or as part of an automated system, are also available. Considering that both free and total chlorine concentrations are variable and do not necessarily correlate to the efficacy of chlorine, methods to monitor the oxidizing capacity of the water have been employed.

As part of the proposed FSMA regulation, the FDA recommends, at minimum, monitoring and adjusting as necessary the concentration of the active component of the sanitizing agent and the pH of wash water, especially if sodium hypochlorite is used *(128)*. The active component of chlorine, HClO, is most abundant around pH 6.5, resulting in a recommended range of 6.5 – 7.5 *(16, 62, 82, 111, 125)*. Some commercial processors that use chlorinated wash water for leafy greens have set their critical limit at a pH of < 7 *(82)*. However, peroxyacetic acid-based sanitizers remain effective over a wider pH range *(62, 129)*, generally making pH adjustments unnecessary.

The temperature at which washing occurs varies widely throughout industry, with some processors opting for ambient water temperatures followed by air-cooling subsequent to washing. The maximum solubility of chlorine occurs at 4°C *(91)*, allowing the retention of antimicrobial activity for longer periods *(62)*. It is often reported that the overall bactericidal activity of water increases with temperature *(140)*; however, so does chlorine volatility *(109)*. In contrast to chlorine, peroxyacetic acid efficacy is maintained over a wider temperature range *(64, 129)*.

Oxidation/reduction potential (ORP) is the potential (voltage) at which oxidation occurs at the anode and reduction occurs at the cathode of an electrochemical cell *(112)*. It is used by processors to monitor the oxidizing capacity in millivolts (mV) of sanitizing flume water as an alternative or in addition to monitoring sanitizer concentration. An advantage of monitoring ORP

is that it is a direct measurement of the ability of the wash water to oxidize microbial contaminants, thought to be independent of the water quality. Commodity-specific guidelines by the Florida Department of Agriculture initially required 150 ppm of free chlorine at a pH of 6.5 – 7.5 for flume water processing of tomatoes *(125)*. However, the option to maintain a minimum ORP of 650 mV was later included due to uncertainty of processors' ability to maintain such a chlorine concentration over time *(125)*. No such regulation has been established for leafy greens; however, some commercial processors have also adopted this same ORP of 650 mV as a critical limit in their HACCP programs. More recently, the relationship between ORP and sanitizer strength was shown to be nonlinear, raising questions regarding the ability of ORP to predict sanitizer efficacy *(120)*.

Increased interest in wash water safety has shifted the focus from direct monitoring of sanitizer strength or efficacy to methods that can quantify and predict sanitizer efficacy based on the organic load and other physicochemical parameters of the wash water. Chemical oxygen demand (COD), which measures the amount of dissolved oxygen (O_2) per L of solution, is an indirect but quantitative means to determine organic load. In this method involves the reaction of organic matter with a strong acid solution in the presence of a known excess of potassium dichromate ($K_2Cr_2O_7$). After 2 h of digestion at 150°C, unreduced $K_2Cr_2O_7$ is quantified by titrating with ferrous ammonium sulfate. The amount of oxidizable matter is calculated in terms of oxygen equivalent *(12)*. While by no means a rapid method, COD has shown to be an effective means of quantifying organic load in wash water, with increasing COD correlating to increased organic matter in both produce processing and waste water *(10, 78, 87)*.

Additional methods to quantify organic load in wash water include direct methods (e.g., total solids and maximum filterable volume) and indirect methods (e.g., turbidity). Measurement of total solids in water is a direct method to quantify the amount of solids in suspension or dissolved in a set volume of liquid. The procedure involves determining the mass of dried solids remainingin a crucible after evaporation of the liquid in an oven *(14)*. Maximum filterable volume (MFV) is a direct method to quantify organic load that was developed by our group. This simple and rapid procedure is based on the volume of liquid pulled through a 0.45 µm filter after 1 min of vacuum filtration at -80 kPa. However, this test is not as sensitive as that for total solids. Measuring the turbidity (i.e., absorbance) of a water sample provides another rapid and indirect means to quantify organic load, which was also developed by our group. This procedure first involves a pre-filtration step to remove suspended solids from the water sample to reduce interference in the spectrophotometer. The absorbance of the filtrate is then measured at 663 nm, a wavelength previously shown to correlate to chlorophyll content *(133)*. However, these results are likely sanitizer-specific, due to the different oxidizing strengths impacting the measured chlorophyll content. While the methods above can be used to directly or indirectly assess sanitizer efficacy and organic load in wash water, knowledge gaps still remain as to what physicochemical parameters best correlate to *E. coli* O157:H7 persistence during simulated commercial processing.

1.11 Current challenges

Flume water is recirculated during leafy green processing to reduce water waste and cost. Previous work by Buchholz and others *(22)* reported that approximately 90% of the *E. coli* O157:H7 populations on dip-inoculated leafy greens transferred to sanitizer-free water during

washing in a pilot-scale processing line. This reinforces the importance of adding an effective sanitizer to the wash water to minimize cross-contamination via the water to subsequently processed product. Oxidation/reduction potential (ORP) is most commonly used commercially to monitor sanitizer efficacy is *(112)*, however, with multi-state outbreaks and recalls still occurring, it is clear that isolated contamination events are being amplified during processing of leafy greens. Organic load, which contains produce tissue, cellular fluids released during cutting, soil, insects, and microbes *(62)*, will continually accumulate in recirculating flume water as produce is washed *(86)*, decreasing the ability of sanitizers to minimize cross-contamination from the water during processing *(57, 110, 139)*. While organic load impacts the efficacy of sanitizing agents, most notably chlorine, the means to quantify sanitizer efficacy against pathogens such as *E. coli* O157:H7 have not yet been determined. The Center for Produce Safety (CPS) currently ranks the identification of methods to validate the efficacy of flume water used to wash fruits and vegetables as one of their top priorities *(33)*. Physicochemical parameters that either directly or indirectly quantify the organic load in flume water may be better predictors of sanitizer efficacy than ORP. Intervention steps (e.g., dumping of flume water or use of technologies to remove organic load) are also needed to ensure that the target physicochemical parameters are being maintained in the flume water, so as to not amplify possible microbial contamination events due to sanitizer failure.

CHAPTER 2:

Efficacy of Commercial Produce Sanitizers against *Escherichia coli* O157:H7 during Processing of Iceberg Lettuce in a Pilot-Scale Leafy Green Processing Line

2.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 on iceberg lettuce, in wash water, and on equipment during simulated commercial production in a pilot-scale processing line. Iceberg lettuce (5.4 kg) was inoculated to contain 10^6 CFU/g of a 4-strain cocktail of non-toxigenic, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 and processed after 1 h of draining at ~22°C. Lettuce was shredded using a Urschel TransSlicer, step-conveyed to a flume tank, washed for 90 s using six different treatments (water alone, 50 ppm peroxyacetic acid, 50 ppm mixed peracid, or 50 ppm available chlorine either alone or acidified to pH 6.5 with citric acid (CA) or T-128), and then dried using a shaker table and centrifugal dryer. Various product (25 g) and water (50 ml) samples collected during processing, and equipment surface samples (100 cm^2) from the flume tank, shaker table and centrifugal dryer, were homogenized in neutralizing buffer and plated on tryptic soy agar containing 0.6% yeast extract and 100 ppm ampicillin with or without prior 0.45 μm membrane filtration to quantify *E. coli* O157:H7 under UV light. During and after iceberg lettuce processing, none of the sanitizers were significantly more effective ($P \leq 0.05$) than water alone at reducing *E. coli* O157:H7 populations on lettuce, with reductions ranging from 0.8 to 1.4 log CFU/g. Regardless of the sanitizer treatment used, the centrifugal dryer surfaces yielded *E. coli* O157:H7 populations of 3.5 to 5 log CFU/100 cm^2. In terms of the flume water, chlorine, chlorine + CA, and chlorine + T-128 were generally more effective ($P \leq 0.05$) than the other treatments, with reductions of 3.8, 5.5, and 5.4 log CFU/ml after 90 s of processing, respectively. This indicates that chlorine-based sanitizers will likely

prevent wash water containing low organic loads from becoming a vehicle for cross-contamination.

2.2 INTRODUCTION

In 2009, leafy greens were ranked as the riskiest food category regulated by the Food and Drug Administration, accounting for 363 outbreaks and 13,568 reported cases of illness *(36).* Between 1995 and 2006, leafy green-associated outbreaks increased by 38.6% while consumption increased by only 9% *(63)*. The nationwide outbreak of *E. coli* O157:H7 that was traced to baby spinach in 2006 resulted in 205 confirmed infections, 103 hospitalizations, and three deaths *(28, 45)*. Following two additional *E. coli* O157:H7 outbreaks in 2006 linked to shredded iceberg lettuce resulting in 150 illnesses *(35),* at least 9 more outbreaks responsible for nearly 300 cases of *E. coli* O157:H7 infection have been documented in the United States through 2012 *(40),* heightening continued safety concerns surrounding fresh-cut leafy greens.

Bacterial pathogens can contaminate leafy greens at any point during the farm-to-fork continuum *(83)*. Major on-farm areas of concern now recognized by the Food and Drug Administration include agricultural water, biological soil amendments (e.g., manure), domesticated and wild animals, field worker health and hygiene, and the cleanliness of harvesting equipment, tools and buildings *(127).* However, leafy greens are also prone to contamination during commercial processing, packing *(24),* distribution, marketing *(138),* and in-home preparation *(90)*. Regarding leafy greens, pathogens are most likely to attach to stomata, irregularities on intact surfaces, cut surfaces, or cracks on the external surfaces *(55, 91, 100, 102, 111)* and can be protected from sanitizers by biofilms *(104).* Since sanitizers in the wash water cannot be relied upon to inactivate attached or internalized pathogens during processing, it is imperative that growers and harvesters follow Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs) to reduce the likelihood of contamination *(48).*

Washing of leafy greens remains important for removing soil and debris, decreasing the microbial load, improving quality and appearance, and enhancing product shelf life and safety *(62)*. Numerous small-scale laboratory studies have shown that produce sanitizers reduce pathogen populations only 1 to 3 log CFU on lettuce *(17, 47, 55, 91, 100)* with water alone decreasing *E. coli* O157:H7 levels about 1 log CFU on lettuce during pilot-scale processing *(22)*. Recirculation of this wash water during processing can further magnify the spread of contaminants at large, centralized processing facilities *(62, 80)*. Hence, the addition of sanitizers to processing water is imperative to minimize cross contamination during commercial production of fresh-cut leafy greens *(5, 81, 100, 126)*.

Chlorine-based sanitizers are preferred for commercial flume washing systems due to their relatively low cost compared to other sanitizers and minimal negative impact on end product quality *(31, 62, 81, 87, 91)*. Since the active component of chlorine, hypochlorous acid (HClO), is most abundant at pH 6.5 – 7.0 *(16)*, the pH of the wash water typically needs to be lowered by adding a weak acid, most commonly citric acid *(62)*. A new, generally recognized as safe (GRAS) acidifying agent comprised of phosphoric acid and propylene glycol, known as T-128 (SmartWash Solutions, Salinas, CA), has been developed to improve the stability of chlorine *(76, 81, 87, 106)*. However, chlorine use has raised concerns regarding potentially hazardous bi-products, worker safety, environmental damage and, most importantly, decreased efficacy in the presence of an increasing organic load in recirculating flume water, which has heightened interest in other alternatives such as peroxyacetic acid-based sanitizers *(100, 113)*.

Numerous small-scale laboratory studies have assessed sanitizer efficacy against pathogens on leafy greens *(4, 17, 66, 72, 78, 82, 88, 115, 139, 140)*. However, these findings are difficult to extrapolate to large-scale commercial production facilities. Previous work completed

by our group was performed without chemical sanitizers to quantify *E. coli* O157:H7 transfer during pilot-plant production of fresh-cut leafy greens *(22, 23)*. Since chemical sanitizers remain the sole intervention strategy to prevent cross-contamination during commercial production of fresh-cut leafy greens, it is imperative that these sanitizers be re-evaluated under conditions that more closely resemble commercial operations. Consequently, the objective of this study was to assess the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 during processing of iceberg lettuce in a pilot-scale leafy green processing line.

2.3 MATERIALS AND METHODS

2.3.1 Experimental design. The efficacy of five different sanitizing treatments was assessed in triplicate against *E. coli* O157:H7 by processing a 5.4 kg batch of iceberg lettuce inoculated at 10^6 CFU/g, with sanitizer-free water serving as the control. All lettuce was processed by shredding, conveying, fluming, shaker table dewatering, and/or centrifugal drying, during and/or after which various product, water, and equipment surface samples were collected and quantitatively examined for *E. coli* O157:H7.

2.3.2 Leafy greens. Individually wrapped heads of iceberg lettuce (*Lactuca sativa* L.) (24 heads per case) were obtained from a local wholesaler (Stan Setas Produce Co., Lansing, MI), with the product originating from California or Arizona depending on the growing season. All lettuce was stored in a 4°C walk-in-cooler and used within 5 days of delivery.

2.3.3 Bacterial strains. Four non-toxigenic (stx_1^- and stx_2^-) strains of *E. coli* O157:H7 (ATCC 43888, CV2b7, 6980-2, and 6982-2) were obtained from Dr. Michael Doyle at the Center for Food Safety, University of Georgia, Griffin, GA. All strains had previously been transformed with a pGFPuv plasmid containing a GFP gene and an amplicillin-resistance gene. All four strains were stored at -80°C in tryptic soy broth (Difco, BD, Sparks, MD) containing 0.6% (w/v) yeast extract (TSBYE; Difco, BD), and 10% (v/v) glycerol (Sigma Chemical Co., St. Louis, MO) until needed. Working cultures were prepared by streaking each stock culture on Tryptic soy agar plates (Difco, BD) containing 0.6% (w/v) yeast extract and 100 ppm ampicillin (ampicillin sodium salt, Sigma Life Science, St. Louis, MO) (TSAYE plus amp). After 18 – 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 similarly incubated.

2.3.4 Lettuce inoculation. A 0.2 ml aliquot of each non-toxigenic *E. coli* O157:H7 strain was transferred to 200 ml of TSBYE with amp and incubated for 18 – 20 h at 37°C. Assuming similar growth rates, as determined previously *(22)*, the four strains were combined in equal volumes to obtain an 800-ml cocktail, which was added to 80 L of municipal tap water (~15^{o}C) in a 121 L plastic container (Rubbermaid, Wooster, OH) to a achieve a level of ~10^{7} CFU/ml. Hand-cored heads of iceberg lettuce (~12 heads) were immersed in the *E. coli* suspension for 15 min and then drained/air-dried for 1 h at 22°C before being spun in a dewatering centrifuge (described below) to remove residual inoculum from the interior of the heads. Duplicate 25-g samples were then aseptically collected to determine the initial inoculation level at the time of processing.

2.3.5 Lettuce processing line. A small-scale commercial leafy green processing line was assembled that consisted of a lettuce shredder, step conveyer, flume tank, shaker table and dewatering centrifuge. The commercial lettuce shredder (model TRS 2500 Urschel TranSlicer, Valparaiso, IN) was operated at a feed belt speed of 198 m/min and a slicing wheel speed of 905 RPM to obtain a shred size of approximately 5 x 5 cm. The polyurethane step conveyer belt (ThermoDrive, Mol Industries, Grand Rapids, MI) was mounted on a motorized conveyor (Dorner model 736018 mc series, Dorner Manufacturing, Hartland, WI) that operated at 0.11 m/s. The stainless steel water recirculation tank (~1000 L capacity) containing 890 L of tap water (~15^{o}C) was connected to a 3.6 m long stainless steel flume tank (Heinzen Manufacturing, Inc., Gilroy, CA) - equipped with two overhead spray jets (1 m from the start), by a 4.14 m long, 10 cm-diameter hard plastic discharge hose and a centrifugal pump (Model XB754FHA, Sterling Electric, Inc., Irvine, CA) that circulated the water at a rate of ~10 L/s. A custom-made stainless steel screen containing pores with a diameter of 1.25 cm spaced 0.65 cm apart (Heinzen

Manufacturing, Inc.) was affixed to the end of the flume tank to retain the product during washing. The stainless steel shaker table for partial dewatering was operated by a 1 HP Baldor washdown duty motor (Baldor Electric Co., Ft. Smith, AR) at 1760 RPM. Water removed from the leafy greens during mechanical shaking passed through a fine mesh screen and was fed into the water holding tank by a water recirculation spout underneath the shaker table. A 110 kg (50 lb) capacity centrifugal Spin Dryer (Model SD50-LT, Heinzen Manufacturing, Inc.) with three internally timed spin cycles totaling 80 s was used for centrifugal drying.

2.3.6 Wash water. Iceberg lettuce (0.5 kg) was homogenized in 500 ml of Michigan State University (MSU) tap water using a mechanical blender (Model BLC10650MB, Black & Decker, New Britain, CT) and then added to 890 L of processing water at 12 to 15^{o}C to achieve a low-level organic load of ~0.0006% (w/v lettuce solids). The following five commercial produce sanitizer treatments were assessed: 30 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 30 ppm mixed peracid (Tsunami 200, Ecolab), 30 ppm available chlorine (XY-12, Ecolab) at pH 7.85, 30 ppm available chlorine (XY-12) acidified to pH 6.50 with citric acid (Sigma-Aldrich, St. Louis, MO), and 30 ppm available chlorine (XY-12) acidified to pH 6.50 with T-128 (SmartWash Solutions) as measured with a pH probe (pHTestr 30, Oakton, Vernon Hills, IL). Peroxyacetic acid test kit 311 (Ecolab) was used to confirm the peroxyacetic acid and mixed peracid sanitizer concentrations while chlorine test kit 321 (Ecolab) was used to measure available chlorine. Sanitizer-free MSU tap water (< 0.05 ppm free chlorine) served as the control.

2.3.7 Leafy green processing. Inoculated heads of cored iceberg lettuce (5.4 kg) were hand-fed into the shredder at a rate of ~0.5 kg per second, with the shredded product then step-conveyed to the top of the conveyor. Processing was then halted for ~10 min to aseptically

collect and bag five 25-g lettuce samples in red mesh produce bags (5 lb Header Bag, Pacon Inc., Baldwin Park, CA) for subsequent sampling. Thereafter, processing was resumed with the iceberg lettuce conveyed to the flume tank, washed in 890 L of recirculating wash water with or without a sanitizer for 90 s, partially dewatered on the shaker table, collected in a single centrifugation basket and centrifugally dried.

2.3.8 Sample collection. During the 90 s of flume washing, three pre-bagged iceberg lettuce samples (25 g each) were retrieved at the flume gate at 30 s intervals, and immediately added to 100 ml of sterile Difco Neutralizing Buffer (BD, Franklin Lakes, NJ) in a Whirl-Pak™ filter bag (Nasco, Fort Atkinson, WI). In addition, nine 50-ml water samples were also collected at 10-s intervals in 50 ml centrifuge tubes containing 38x concentrated Difco Neutralizing Buffer (BD). After shaker table dewatering, product in the basket was dried in the pre-set 110 kg (50 lb) capacity Spin Dryer (Model SD50-LT, Heinzen Manufacturing). During centrifugal drying, four water samples (50 ml each) were similarly collected from the centrifuge drain at 10 s intervals for the first 40 s of the 80 s cycle. After centrifugation, two bagged lettuce samples (25 g each) were also retrieved from the centrifugation basket. Nine product contact areas on the equipment (3 flume tank (Figure 2.1), 3 shaker table (Figure 2.2) and 3 dewatering centrifuge (Figure 2.3)), previously described in detail by Buchholz et al. *(22)*, measuring 100 cm^2 as previously identified using Glo Germ™ were sampled immediately after processing as described by Vorst et al. *(130)* using 1-ply composite tissues moistened with 1 ml of sterile Difco Neutralizing Buffer (BD).

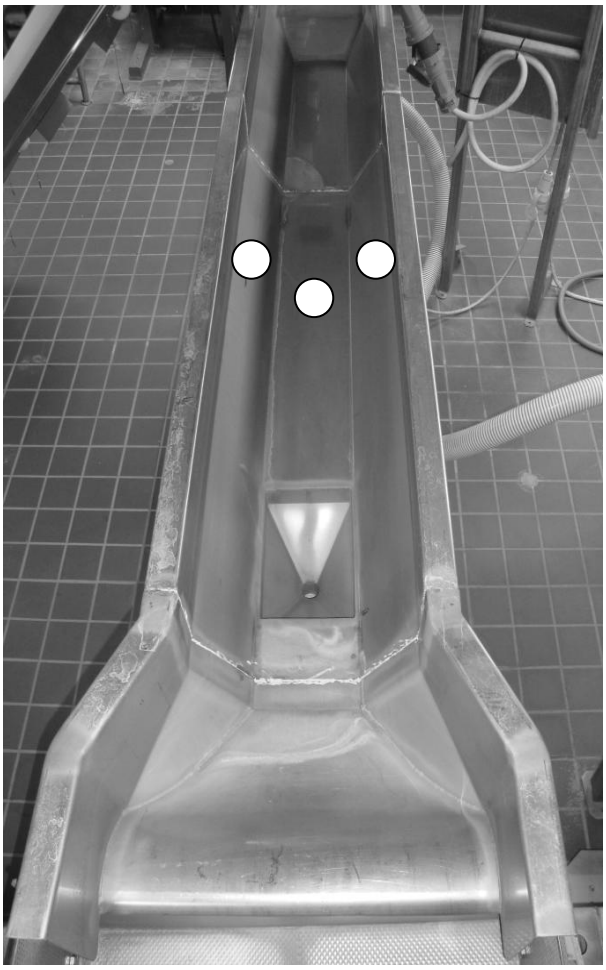


Figure 2.1*: Flume tank sampling locations*

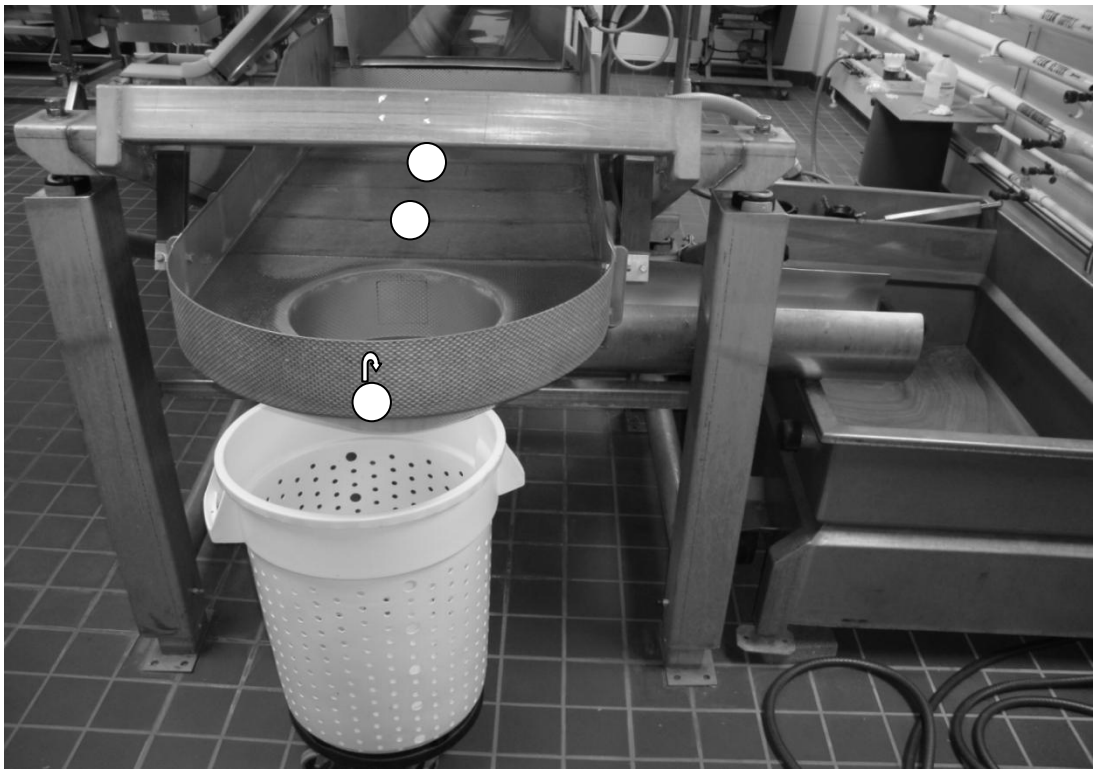


Figure 2.2*: Shaker table sampling locations*

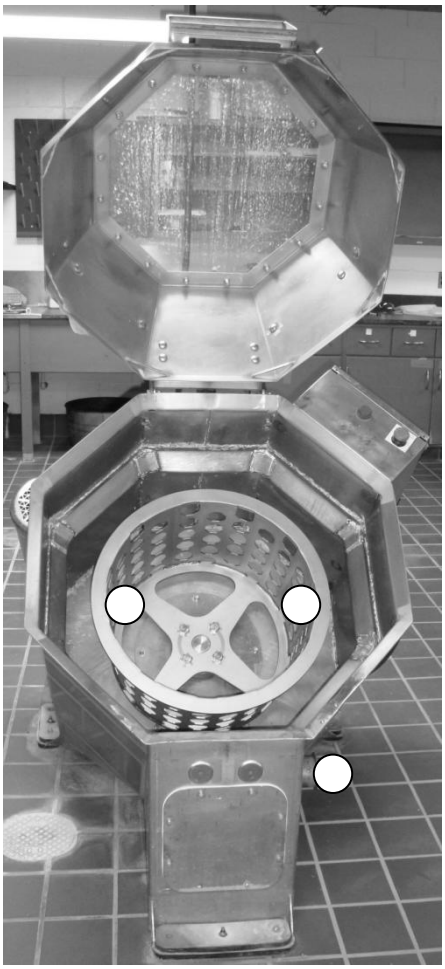


Figure 2.3*: Dewatering centrifuge sampling locations*

2.3.9 Microbiological analyses. All lettuce samples (25 g) were homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 260 rpm and then either appropriately diluted in sterile 1% (w/v) phosphate buffer (8.5 g/L NaCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4, J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ) and plated on TSAYE with amp (calculated minimum detection limit of 40 CFU/g) or processed using 0.45 μm membrane filters (Millipore, Millipore Corporation, Billerica, MA) (calculated minimum detection limit of 0.04 CFU/g), which were placed on 60-mm dia. petri plates containing TSAYE with amp to quantify *E. coli* O157:H7. The 1-ply composite tissue samples were added to 15 ml of sterile Difco Neutralizing Buffer in a Whirl-Pak™ bag, homogenized for 1 min at 260 rpm, and then plated identically to the lettuce samples, giving a calculated lower detection limit of 1 CFU/100 cm^2. The 50 ml water samples were either appropriately diluted in sterile 1% phosphate buffer and plated on TSAYE with amp or processed by membrane filtration, which gave a calculated minimum detection limit of 0.02 CFU/ml. Following 20 – 24 h of incubation at 37°C, all green fluorescing colonies as seen under ultraviolet light (365 nm, Blak-Ray, Ultra-violet Product Inc. San Gabriel, CA) were counted as *E. coli* O157:H7.

2.3.10 Sanitizer neutralization confirmation. Triplicate 1 L water samples containing 30 ppm available chlorine (XY-12, Ecolab), 30 ppm peroxyacetic acid (Tsunami 100, Ecolab) or 30 ppm mixed peracid (Tsunami 200 ppm, Ecolab) were prepared and confirmed with Chlorine test kit 321 (Ecolab) or Peroxyacetic acid test kit 311 (Ecolab). Citric acid (Sigma-Aldrich) and T-128 (SmartWash Solutions) were used to acidify the chlorine-based sanitizer solution to pH 6.5. A 50 ml centrifuge tube containing 3 ml of 38x concentrated Neutralizing Buffer (BD) was filled with the water sample containing sanitizer, agitated for 5 s and then immediately assessed

for neutralization of the sanitizer as previously described using the appropriate test kit. Preliminary experiments found a 38x concentration would neutralize various concentrations of the active component of each sanitizing agent used in this study without impacting *E. coli* O157:H7 counts.

2.3.11 Statistical analysis. *E. coli* O157:H7 counts were converted to log CFU per g, ml or 100 cm^2 and subjected to an ANOVA using JMP 9.0 (SAS Institute Inc., Cary, NC). Values equaling half the limit of detection were used for samples without *E. coli* O157:H7 counts. The three equipment surface samples from each respective piece of equipment were averaged. A *P* value of ≤ 0.05 was considered significant for all tests. The Tukey-Kramer HSD test was used to identify significant differences in *E. coli* O157:H7 populations for individual lettuce, water, and equipment surface samples.

2.4 RESULTS

2.4.1 Lettuce. Iceberg lettuce contained an average *E. coli* O157:H7 inoculum of 5.93 log CFU/g at the time of processing (Figure 2.4). After shredding, conveying, 90 s of washing, shaker table dewatering and centrifugal drying, no significant difference ($P > 0.05$) was seen in populations of *E. coli* O157:H7 recovered from the finished product, regardless of sanitizer treatment. Using mixed peracid, *E. coli* O157:H7 populations decreased 1.4 log CFU/g; however, this decrease was not significantly different ($P > 0.05$) than the 0.8 log CFU/g reduction seen for water alone. Processing significantly reduced ($P \leq 0.05$) *E. coli* O157:H7 populations on lettuce when mixed peracid, chlorine, or chlorine + CA were used, with reductions of 1.4, 0.8, and 0.9 log CFU/g, respectively. The reductions of 0.8, 0.9, and 1 log CFU/g seen for water alone, peroxyacetic acid, and chlorine + T-128, respectively, were not significant ($P > 0.05$) (Figure 2.4).

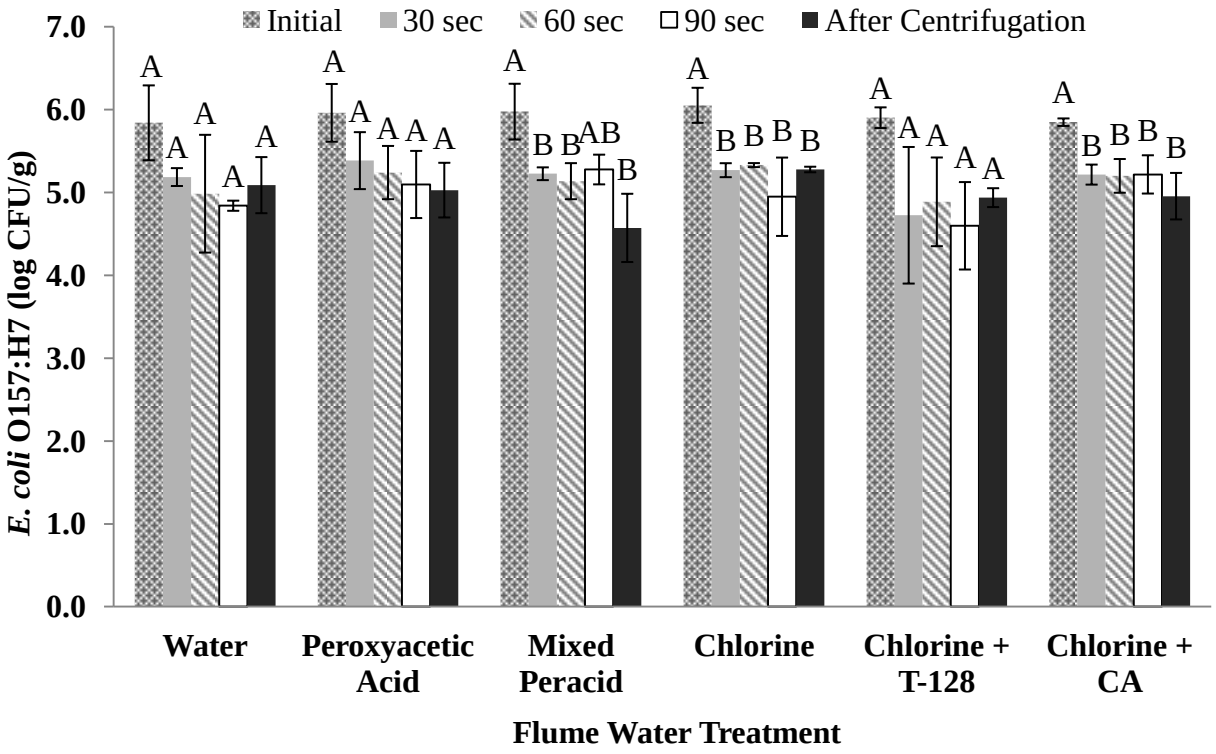


Figure 2.4*: Mean (± SD)* E. coli *O157:H7 populations on the iceberg lettuce inoculated at ~6 log CFU/g during and after processing (n=3). Means of the same wash water treatment with different letters are significantly different (*P ≤ *0.05).*

2.4.2 Flume water. *E. coli* O157:H7 populations in the flume water were homogenous after 10 s of processing, with no significant difference ($P > 0.05$) seen between 10 – 90 s in the water control at the flow rate of 9.3 L/s. Wash water containing chlorine, chlorine + T-128, and chlorine + CA had significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations at all sampling times (maximum of 1 log CFU/ml) compared to 4.6 log CFU/ml in water alone. Using chlorine + CA and chlorine + T-128, *E. coli* O157:H7 levels were below the limit of detection of 0.02 log CFU/ml by the end of processing. *E. coli* O157:H7 populations were similar ($P > 0.05$) using water alone and peroxyacetic acid, with respective populations of 3.5 and 3.0 log CFU/ml recovered after 90 s of processing. Similar *E. coli* O157:H7 populations were obtained using mixed peracid ($P > 0.05$) and peroxyacetic acid, with these populations rarely lower ($P \leq 0.05$) than those in water alone (Figure 2.5).

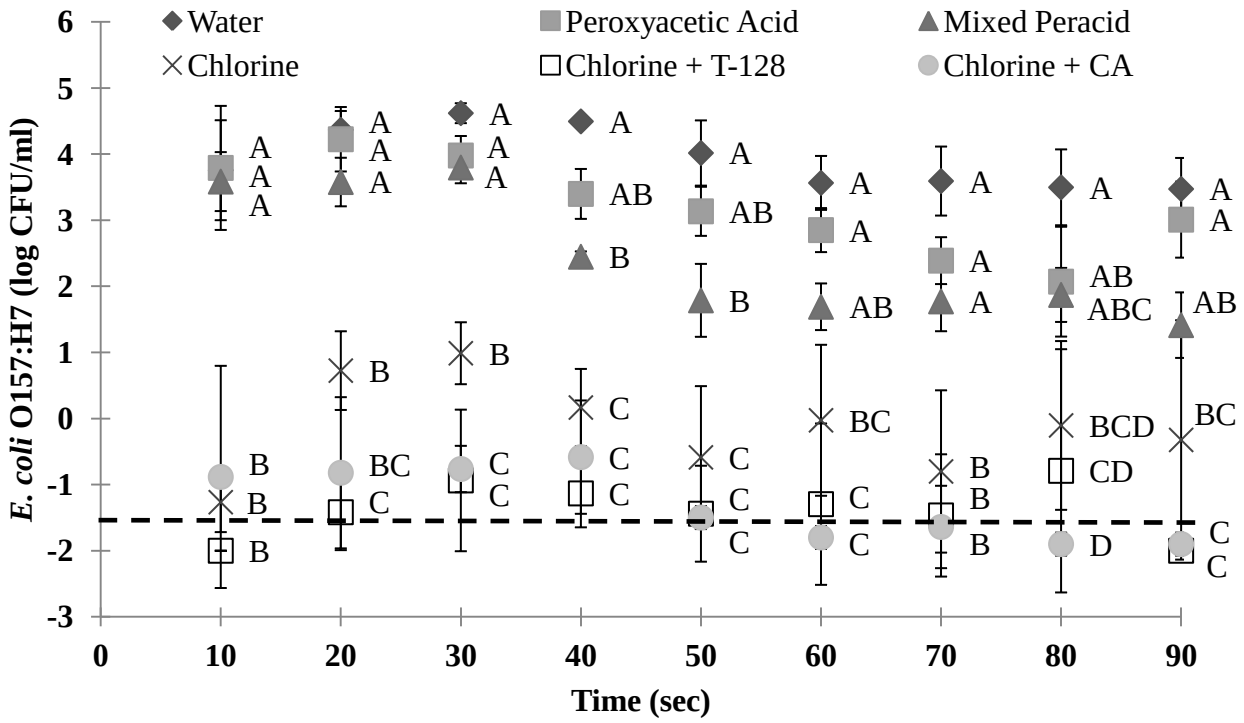


Figure 2.5*: Mean (± SD)* E. coli *O157:H7 populations in flume water during processing iceberg lettuce inoculated at ~6 log CFU/g (n=3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (*P ≤ *0.05).*

2.4.3 Centrifugation water. Using peroxyacetic acid, mixed peracid, or chlorine, wash water exiting the centrifuge drain after spin-drying yielded maximum *E. coli* O157:H7 populations of 4.5, 4.4, and 5.5 log CFU/ml, respectively, which were not significantly different ($P > 0.05$) from those in water alone (maximum population of 5.6 log CFU/ml) during the 40 s sampling period. However, chlorine + CA and chlorine + T-128 resulted in *E. coli* O157:H7 populations that were lower than those in water alone ($P \leq 0.05$) during the first 20 s of centrifugation. Water samples collected after 40 s of centrifugation yielded *E. coli* O157:H7 populations that were not significantly different among any of the treatments (Figure 2.6).

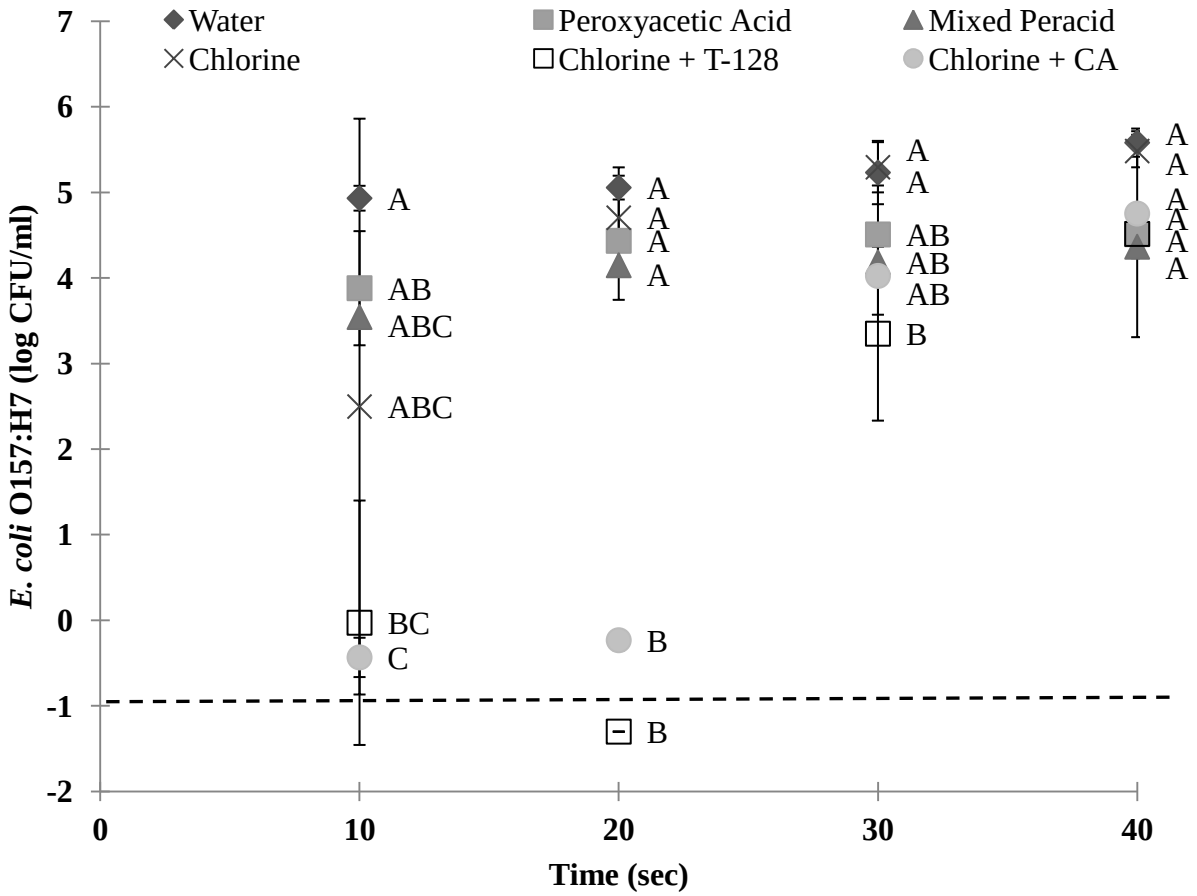


Figure 2.6*: Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from iceberg lettuce inoculated at ~6 log CFU/g (n=3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (*P ≤ *0.05).*

2.4.4 Processing equipment surfaces. After processing iceberg lettuce, all five sanitizer treatments yielded significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations remaining on the flume tank and shaker table as compared to the water control. Significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations were recovered on the centrifugal dryer using peroxyacetic acid (3.6 log CFU/100 cm^2) and mixed peracid (3.5 log CFU/100cm^2) compared to the other treatments, with the highest level (5 log CFU/100 cm^2) seen when water alone was used for washing (Figure 2.7).

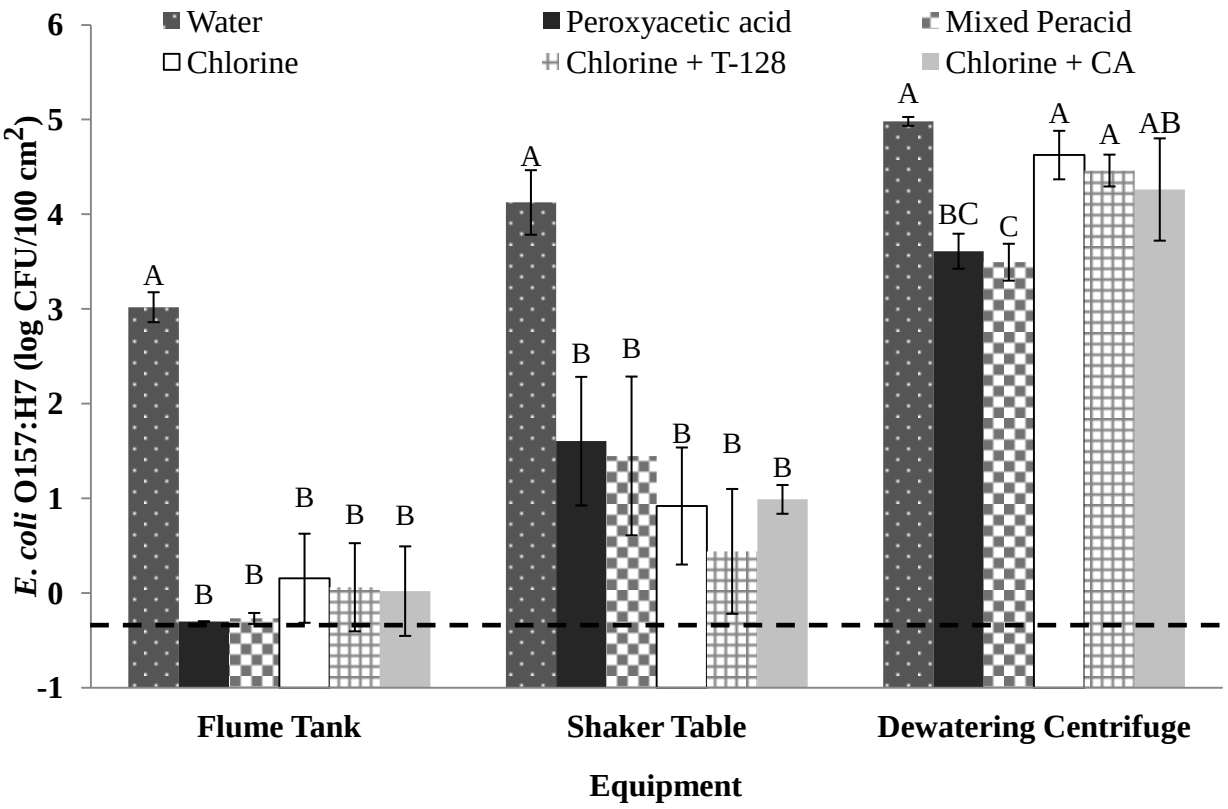


Figure 2.7*: Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing iceberg lettuce inoculated at ~6 log CFU/g (n=3). Half the limit of detection was used to calculate the mean log value when a sample did not yield any colonies by direct plating. Means of the same product type with different letters are significantly different (*P ≤ *0.05).*

2.5 DISCUSSION

Due to the potential production of infectious aerosols during lettuce processing, the same four non-toxigenic strains of *E. coli* O157:H7 were used as in earlier transfer studies *(22, 23).* The growth and adherence rates for these four non-toxigenic strains were previously shown to be similar to three strains from the 2006 leafy green outbreaks *(22).* As previously reported, GFP-labeling also allowed for easy differentiation of the inoculum from background bacteria *(22, 23, 131).*

Dip-inoculation of the lettuce to contain 6 log CFU/g was crucial to ensure uniform distribution of *E. coli* O157:H7 throughout the heads as well as quantifiable results for subsequent mathematical modeling, with this work to be reported elsewhere. While this inoculation level clearly exceeds levels thought to occur on field-grown lettuce, feces from "super-shedding" cows can potentially contain *E. coli* O157:H7 at levels of 6 log CFU/g *(42)*, with such fecal material potentially coming in contact with lettuce through irrigation water. Preliminary experiments completed using a mixture of Glo-Germ™ and water showed uniform fluorescence in dipped heads of iceberg lettuce. Additionally, Buchholz and others *(22)* found that *E. coli* O157:H7 populations were statistically similar prior to processing and after shredding (before washing), indicating that the inoculation was homogenous throughout the heads of iceberg lettuce. Dip-inoculation of cored heads of lettuce may have allowed for the internalization of the *E. coli* O157:H7 into lettuce through the damaged tissues, where it would remain protected from the sanitizing agents *(98).* Since all lettuce samples were processed by stomaching, any internalized cells would have gone undetected, with only the cells on the surface of the leaves recovered.

Commercial producers of fresh-cut leafy greens use different sanitizers, sanitizer concentrations, and contact times, depending on the design of the processing line. In this study, six different wash treatments were assessed during 90 s of flume washing. Processing inoculated iceberg lettuce resulted in *E. coli* O157:H7 reductions of 0.8 to 1.4 log CFU/g on the finished product. Both during and after processing, no significant differences in sanitizer efficacy ($P > 0.05$) were seen against *E. coli* O157:H7 on iceberg lettuce for any of the treatments, including water alone. However, three wash treatments- mixed peracid, chlorine and chlorine + CA- significantly reduced ($P \leq 0.05$) *E. coli* O157:H7 populations after washing. Numerous small-scale laboratory studies have shown similar pathogen reductions (~1 log CFU/g) during washing of various fruits and vegetables with or without sanitizers *(17, 21, 25, 134).* Using a pilot-scale leafy green processing line, Luo et al. *(81)* also reported an *E. coli* O157:H7 reduction of < 1 log after processing inoculated baby spinach *(81)*. Consequently, produce sanitizers cannot be relied upon to ensure end-product safety. Chemical sanitizers are routinely added to recirculating wash water to minimize the spread of microbial contaminants during flume washing *(78)*. Regarding their use, peroxyacetic acid-based sanitizers are limited to a maximum of 80 ppm peroxyacetic acid *(43, 62)*, while free chlorine concentrations typically range from 10 to a maximum of 200 ppm *(55, 91, 122)*. However, soil, debris, and vegetable latexes released during shredding of leafy greens will accumulate in the flume water over time *(86)*, decreasing the efficacy of many sanitizers, most notably chlorine *(5, 77, 100, 139).* The wash water used in this study contained an organic load of ~0.0006% blended iceberg lettuce (w/v) to simulate wash water quality during the early stages of processing. Hence, higher *E. coli* O157:H7 populations would have been expected after 90 s of processing if the organic load in the wash water had been higher, especially for the chlorine-based sanitizer. *E. coli* O157:H7 populations recovered from the wash

water were consistently lower ($P \leq 0.05$) using chlorine, chlorine + CA and chlorine + T-128, as compared to water alone, peroxyacetic acid and mixed peracid. Both chlorine + CA and chlorine + T-128 treatments yielded *E. coli* O157:H7 levels that were below the limit of detection, which is similar to the findings of Lopez-Galvez et al *(78)* using 40 ppm chlorine.

This study was designed to assess the efficacy of sanitizers during processing - not long-term pathogen persistence in the wash water. Produce sanitizers are primarily used to minimize cross-contamination during flume washing, with their effectiveness dependent on the type of sanitizer, concentration, temperature, and organic load in the wash water. The pilot-scale processing line used in this study was not equipped with a chiller. Therefore, all processing needed to be conducted at our incoming tap water temperature of 12 to 15°C rather than at the targeted commercial temperature of 4°C. Since sanitizer efficacy against *E. coli* O157:H7 is enhanced at temperatures above 4^{o}C *(140)*, our *E. coli* O157:H7 reductions likely exceed those that would be expected in commercial operations.

Levels of *E. coli* O157:H7 recovered from spent centrifugation water containing sanitizers were rarely lower than those seen in sanitizer-free water. Similar *E. coli* O157:H7 populations were recovered from centrifugation water containing peroxyacetic acid, mixed peracid, chlorine or no sanitizer at all four sampling times. The combination of chlorine and citric acid or T-128 was significantly more effective than the other sanitizers ($P \leq 0.05$) against *E. coli* O157:H7 in centrifugation water collected during the first 20 s; however, after 40 s no significant difference was seen compared to the water control ($P > 0.05$). These results indicate that while populations of *E. coli* O157:H7 may be close to or below the limit of detection in flume water, populations in the centrifugation water were not significantly different than the

water control by the end of sample collection. Therefore, spent centrifugation water would be best suited for pathogen testing.

E. coli O157:H7 cells recovered from equipment surfaces after processing reflect those that were present in the film of water on the equipment surface. During processing, the flume tank was in continuous contact with the recirculating wash water, with water contact decreasing during shaker table dewatering and centrifugal drying. Numbers of *E. coli* O157:H7 recovered from surfaces in the centrifugal dryer were not significantly different from the water control when any of the three chlorine-based sanitizer treatments were used, indicating that those surfaces may also be well suited for pathogen testing, depending on the particular sanitizer used.

This study was done to assess the efficacy of commercial produce sanitizers against *E. coli* O157:H7 on lettuce, in wash water, and on equipment surfaces during small-scale processing of iceberg lettuce. While none of the sanitizers were more effective than water alone against *E. coli* O157:H7 on leafy greens at any point during or after processing, it is important to reiterate that sanitizers are designed to reduce the microbial load in wash water rather than on the product. Overall, the populations of *E. coli* O157:H7 recovered in wash water containing peroxyacetic acid or mixed peracid were rarely significantly different from those seen in water alone. However, the three chlorine-based treatments were significantly more effective than water alone at reducing *E. coli* O157:H7 populations in wash water during processing. The wash water used in this study replicated a "best-case" scenario for processors due to the extremely low organic load and freshly added sanitizers. Similar studies using higher organic loads will be needed to assess sanitizer efficacy against *E. coli* O157:H7 under conditions that more closely simulate commercial processing (Chapters 3 – 5).

CHAPTER 3:

Impact of Organic Load on Sanitizer Efficacy against *Escherichia coli* O157:H7 in Simulated Leafy Green Processing Water

3.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 in wash water containing various organic loads in a novel and cost-effective bench-top system. Iceberg lettuce (25 g) was inoculated with a 4-strain cocktail of non-toxigenic, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 at10^6 CFU/g and stored for ~24 h at 4°C to simulate commercial storage conditions before processing. The lettuce was then placed in a red mesh produce bag and washed for 90 s using six different treatments (water alone, 50 ppm peroxyacetic acid, 50 ppm mixed peracid, or 50 ppm available chlorine either alone or acidified to pH 6.5 with citric acid (CA) or T-128) in 4 L of water containing organic loads of 0, 2.5, 5 or 10% (w/v blended iceberg lettuce) and immediately removed from the water. Water (50 ml) samples were collected and neutralized at 2 min intervals for 10 min, diluted in phosphate buffer and plated on tryptic soy agar containing 0.6% yeast extract and 100 ppm ampicillin with or without prior 0.45 μm membrane filtration to assess persistence of *E. coli* O157:H7. Various physicochemical parameters of the wash water were correlated to *E. coli* O157:H7 inactivation at each organic load. Organic load negatively impacted the efficacy of chlorine, chlorine + CA, and chlorine + T-128 ($P \leq 0.05$), with typical *E. coli* O157:H7 reductions of < 1 log CFU/ml after 10 min of exposure. However, the efficacy of peroxyacetic acid and mixed peracid was unaffected by organic load ($P > 0.05$) with average *E. coli* O157:H7 reductions of ~4.8 and ~5.5 log CFU/ml, respectively, after 10 min of exposure. Reduced sanitizer efficacy generally correlated to increased total solids, chemical oxygen demand, turbidity, and decreased maximum filterable volume, indicating that these tests may be effective alternatives to the industry standard of oxygen/reduction potential.

3.2 INTRODUCTION

Numerous small-scale laboratory studies have been conducted to assess sanitizer efficacy against pathogens on leafy greens *(4, 17, 66, 72, 78, 82, 88, 115, 139, 140).* However, the findings from these studies have not provided a means by which commercial processors can predict the efficacy of their wash water based upon water quality. Previous work completed by our group was performed without chemical sanitizers to quantify *E. coli* O157:H7 transfer during pilot-plant production of fresh-cut leafy greens *(22, 23),* or to assess the efficacy of commercial produce sanitizers against *E. coli* O157:H7 during pilot-plant production of iceberg lettuce using water without an organic load (Chapter 2). Since sanitizers are designed to minimize cross-contamination during washing, it is necessary to determine the extent of *E. coli* O157:H7 persistence in recirculating wash water containing various levels of organic solids that are generated during production. Consequently, this study aimed to 1) determine the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 in simulated processing water containing various organic loads in a novel bench-top model and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on *E. coli* O157:H7 inactivation.

3.3 MATERIALS AND METHODS

3.3.1 Experimental design. The impact of four different organic loads on five different sanitizing treatments against *E. coli* O157:H7 was assessed in triplicate by washing 25 g of iceberg lettuce inoculated at 10^6 CFU/g in a 4 L glass carboy, with sanitizer-free water serving as the control for each organic load. Thereafter, various water samples were collected and quantitatively examined for *E. coli* O157:H7 to determine persistence. Additional trials were done to assess the impact of water temperature (~5°C vs. 14^{o}C) on acidified sodium hypochlorite efficacy using the same experimental design. Log linear inactivation trend lines for *E. coli* O157:H7 inactivation were correlated to seven physicochemical parameters of the wash water - temperature, pH, oxidation/reduction potential, chemical oxygen demand, total solids, maximum filterable volume and turbidity.

3.3.2 Leafy greens. Identical to 2.3.2

3.3.3 Bacterial strains. Identical to 2.3.3

3.3.4 Lettuce inoculation. A 0.2 ml aliquot of each non-toxigenic *E. coli* O157:H7 culture was transferred to 9 ml of TSBYE with amp and incubated for 18 – 20 h at 37°C. Based on similar growth rates as determined previously *(22)*, the four strains were combined in equal volumes to obtain a 20-ml cocktail which was added to 2 L of municipal tap water (~15^{o}C, < 0.05 ppm free chlorine) in a sterile 2 L polypropylene container (Nalgene, Rochester, NY) to achieve a level of ~10^7 CFU/ml. Hand-torn pieces of iceberg lettuce (150 g) were immersed in the *E. coli* O157:H7 suspension for 15 min in a 28-cm long red mesh produce bag (5 lb header bag, Pacon Inc., Baldwin Park, CA). The lettuce was then drained/air-dried for 15 min at 22°C before being spun in a salad spinner (Model 32480V2, OXO, Chambersburg, PA) by hand-

pumping 5 times to remove residual inoculum, then transferred from the mesh bag into a Whirl-Pak^{TM} bag (Nasco, Fort Atkinson, WI) and stored for 20 – 24 h at 4°C before use. A 25 g sample was then aseptically collected to determine the initial inoculation level at the time of processing.

3.3.5 Processing equipment. A 4 L Kimax glass carboy with a 5 cm diameter opening at the top and a 0.64 cm diameter discharge spout at the bottom (Kimble Chase, Vineland, NJ) plugged by a cork was used. Before the start of each experiment, a 8-cm long magnetic stir bar was inserted into the carboy, which was placed on a stirring hotplate (Model LMS-100, Daihan Labtech Co., Ltd., Korea) at a stir speed of 6 (Figure 3.1).

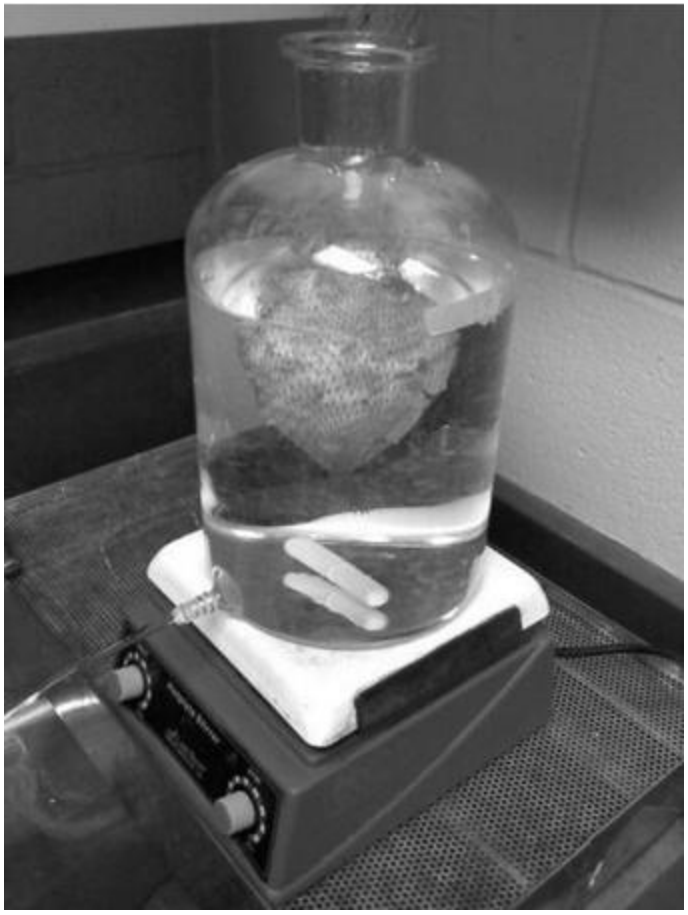


Figure 3.1: *Bench-top system developed to simulate commercial flume washing of iceberg lettuce.*

3.3.6 Wash water. Iceberg lettuce (0, 100 , 200, or 400 g) was blended for 2 min in 250 – 500 ml of tap water using a household blender (Model BLC10650MB, Black & Decker, New Britain, CT) and added to the wash water at ~14°C to achieve organic load levels of 0, 2.5, 5, or 10% (w/v), respectively, in 4 L. The following five commercial produce sanitizer treatments were assessed: 50 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 50 ppm mixed peracid (Tsunami 200, Ecolab), 50 ppm available chlorine (XY-12, Ecolab) (pH 7.09 – 8.03), 50 ppm available chlorine (XY-12) acidified to pH 6.5 with citric acid (Sigma-Aldrich, St. Louis, MO), and 50 ppm available chlorine (XY-12) acidified to pH 6.5 with T-128 (SmartWash Solutions) as measured with a pH probe (pHTestr 30, Oakton, Vernon Hills, IL). Peroxyacetic acid test kit 311 (Ecolab) was used to confirm the peroxyacetic acid and mixed peracid sanitizer concentrations while chlorine test kit 321 (Ecolab) was used to measure available chlorine. Free chlorine was measured immediately before exposing iceberg lettuce to the water using a Pocket Colorimeter (Model 46770-00, Hach Company, Loveland, CO). Sanitizer-free MSU tap water (< 0.05 ppm free chlorine) served as the control for each for each organic load. Chlorine + CA efficacy was also assessed at ~5°C by refrigerating MSU tap water ~12 h before use, with all other aspects of the experimental design remaining the same.

3.3.7 Leafy green washing and sample collection. Inoculated iceberg lettuce (25 g) was aseptically transferred to a 28-cm long red mesh produce bag that was knotted at one end. The lettuce was inserted into the carboy through the top after which the cork was removed from the spout. Following 90 s of exposure to the wash water with mechanical stirring, the spout was closed, after which the bag of lettuce was removed from the carboy and added to 100 ml of sterile Difco Neutralizing Buffer (Becton Dickson, Franklin Lakes, NJ) in a Whirl-Pak^{TM} filter bag (Nasco, Fort Atkinson, WI). After removing the lettuce, magnetic stirring of the wash water

continued for an additional 10 min, during which time water samples (50 ml) were collected at 2 min intervals for 10 min in 50 ml centrifuge tubes containing 38x concentrated Difco Neutralizing Buffer (Becton Dickinson).

3.3.8 Physiochemical parameters. Seven physicochemical parameters of the wash water were monitored before iceberg lettuce washing. Temperature and pH were measured with a probe (pHTestr 30, Oakton, Vernon Hills, IL). Oxidation/reduction potential (ORP) was monitored with a probe (ORPTestr 10, Oakton). Chemical oxygen demand (COD) was measured using Hach Digestion Reactor Method 8000 (Hach, Loveland, CO) *(59)*. Total solids was measured after drying 10 ml of wash water in a pre-heated and pre-weighed crucible in a drying oven (Model 625-A, Precision Scientific Inc, Chicago, IL) at 103 ± 2°C for 2 h *(14)*. Turbidity was assessed by pouring a 50 ml water sample through a 24-cm diameter Whatman Filter (Grade 113, Piscataway, NJ) to remove suspended solids followed by measuring absorbance of a 1 ml aliquot of the filtrate at 663 nm in a spectrophotometer (Model SB-100XR, Spectronics Corporation, Westbury, NY) *(133)*. Maximum filterable volume (MFV) was quantified as the volume of a 50 ml water sample pulled through a 0.45 µm membrane in 1 min at-80 kPa using a vacuum pump (Model WP6211560, Millipore, Billerica, MA). Free chlorine was measured using a pocket colorimeter (Model 46770, Hach).

3.3.9 Microbiological analyses. All lettuce samples (25 g) were homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 260 rpm, appropriately diluted in sterile 1% (w/v) phosphate buffer (8.5 g/L NaCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4, J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ) and plated on TSAYE with amp (calculated minimum detection limit of 40 CFU/g). The 50 ml water samples

were either appropriately diluted in sterile 1% phosphate buffer and plated on TSAYE with amp (calculated minimum detection limit of 10 CFU/ml) or processed using 0.45 μm membrane filters (Millipore, Millipore Corporation, Billerica, MA) (calculated minimum detection limits of 0.02 CFU/ml, 0.033 CFU/ml, 0.1 CFU/ml or 0.1 CFU/ml for organic loads of 0, 2.5, 5 and 10% (w/v), respectively), which were placed on 60-mm dia. petri plates containing TSAYE with amp. Following 40 – 48 h of incubation at 37°C, all green fluorescing colonies as seen under ultraviolet light (365 nm, Blak-Ray, Ultra-violet Product Inc. San Gabriel, CA) were counted as *E. coli* O157:H7.

3.3.10 Sanitizer neutralization confirmation. One liter aliquots of tap water containing 50 ppm chlorine (XY-12, Ecolab), 50 ppm chlorine + CA, 50 ppm chlorine + T-128, 50 ppm peroxyacetic acid (Tsunami 100, Ecolab) or 50 ppm mixed peracid (Tsunami 200 ppm, Ecolab) with organic loads of 0, 2.5, 5 or 10% were prepared using a mechanical blender (Model BLC10650MB, Black & Decker, New Britain, CT), after which the concentration of available chlorine or peroxyacetic acid was confirmed using a commercial test kit (Chlorine test kit 321, Ecolab and Peroxyacetic acid test kit 311, Ecolab). A 50 ml centrifuge tube containing 3 ml of 38x concentrated neutralizing buffer (BD) was filled with these prepared solutions, agitated for 5 s and then similarly assessed for chlorine or peroxyacetic acid neutralization. In preliminary experiments, a 38x concentration neutralized all sanitizer concentrations without impacting *E. coli* O157:H7 recovery.

3.3.11 Statistical analysis. *E. coli* O157:H7 counts were converted to log CFU per g or ml, and individual physicochemical parameter values were subjected to an ANOVA using JMP 9.0 (SAS Institute Inc., Cary, NC). For samples without *E. coli* O157:H7 counts, values equaling half the limit of detection were used. A P value of ≤ 0.05 was considered significant for all tests.

The Tukey-Kramer HSD test was used to identify significant differences in *E. coli* O157:H7 populations for individual lettuce and water samples as well as individual physicochemical parameter values. Microsoft Excel software was also used to conduct a log linear regression analysis and calculate 95% confidence intervals for the *E. coli* O157:H7 reductions in water over time. The following log linear equation was used:

$$\text{Log}(N/N_0) = -k*t$$

Where $\text{Log}(N/N_0)$ is the microbial reduction, t is the time in minutes, and k is the inactivation rate. Excel was used to determine the P and R^2 values, and JMP 9.0 software was used to determine the RMSE (log CFU/ml) by running the curves for each organic load used and sanitizer combination.

3.4 RESULTS

3.4.1 Lettuce. In general, *E. coli* O157:H7 population decreased 0.5 to 1.0 log CFU/g regardless of the sanitizer treatment or organic load. Organic load did not impact the efficacy of peroxyacetic acid or mixed peracid, resulting in *E. coli* O157:H7 reductions that were not significantly different ($P > 0.05$) on iceberg lettuce regardless of organic load (Table 3.1). Organic load significantly decreased ($P \leq 0.05$) the efficacy of chlorine, chlorine + CA, and chlorine + T-128, with reductions of ~1 log CFU/g achieved without an organic load while organic loads of 2.5, 5 and 10% yielded reductions of ~0.5 log CFU/g. No significant difference in *E. coli* O157:H7 reduction was observed for any of the organic loads using chlorine + CA at 5°C.

Table 3.1: E. coli *O157:H7 reduction on iceberg lettuce inoculated at ~6 log CFU/g after processing*[a]

	Mean ± SD *E. coli* O157:H7 reduction (log CFU/g)					
% Organic load (w/v)	Peroxyacetic acid	Mixed Peracid	Chlorine	Chlorine + CA (pH 6.5)	Chlorine + T-128 (pH 6.5)	Chlorine + CA (5°C) (pH 6.5)
0	1.1 ± 0.1 A	1.2 ± 0.2 A	1.0 ± 0.1 A	1.0 ± 0.1 A	0.9 ± 0.1 A	0.9 ± 0.3 A
2.5	1.0 ± 0.0 A	0.9 ± 0.3 A	0.5 ± 0.1 B	0.4 ± 0.1 B	0.5 ± 0.0 B	0.5 ± 0.2 A
5	0.9 ± 0.2 A	0.9 ± 0.3 A	0.5 ± 0.3 B	0.6 ± 0.2 B	0.5 ± 0.1 B	0.5 ± 0.2 A
10	1.0 ± 0.1 A	1.0 ± 0.2 A	0.4 ± 0.1 B	0.5 ± 0.0 B	0.5 ± 0.1 B	0.5 ± 0.1 A

[a] Means with different capital letters designate *E. coli* O157:H7 reductions that differ significantly in terms of sanitizing treatment ($P \leq 0.05$).

3.4.2 Wash water. Average *E. coli* O157:H7 populations in sanitizer-free wash water were ~3.53 log CFU/ml at all time points (Figure 3.2). Minimal *E. coli* O157:H7 persistence was seen in wash water for both peroxyacetic acid and mixed peracid, regardless of organic load, with most populations recovered after 4 min being at or below the limit of detection (0.02 to 0.1 log CFU/ml) (Table 3.2). Efficacy of peroxyacetic acid was not significantly influenced by organic load ($P > 0.05$), with an average reduction of 4.8 log CFU/ml seen at the end of sampling. Organic load negatively impacted the efficacy of mixed peracid, with greater ($P \leq 0.05$) *E. coli* O157:H7 reductions seen after 10 min at organic loads of 0 and 2.5% (5.2 log CFU/ml) as compared to 5 and 10% (5.8 log CFU/ml). Organic load also decreased the efficacy of chlorine, with greater *E. coli* O157:H7 reductions ($P \leq 0.05$) seen with a 0% organic load than all other organic loads after 0 (5.2 log CFU/ml), 4 (5.4 log CFU/ml), and 10 min (5.1 log CFU/ml) (Table 3.2). Similar results were obtained for chlorine + CA and chlorine + T-128, with significantly greater *E. coli* O157:H7 reductions (4.9 – 5.4 log CFU/ml) seen at all times points using a 0% organic load.

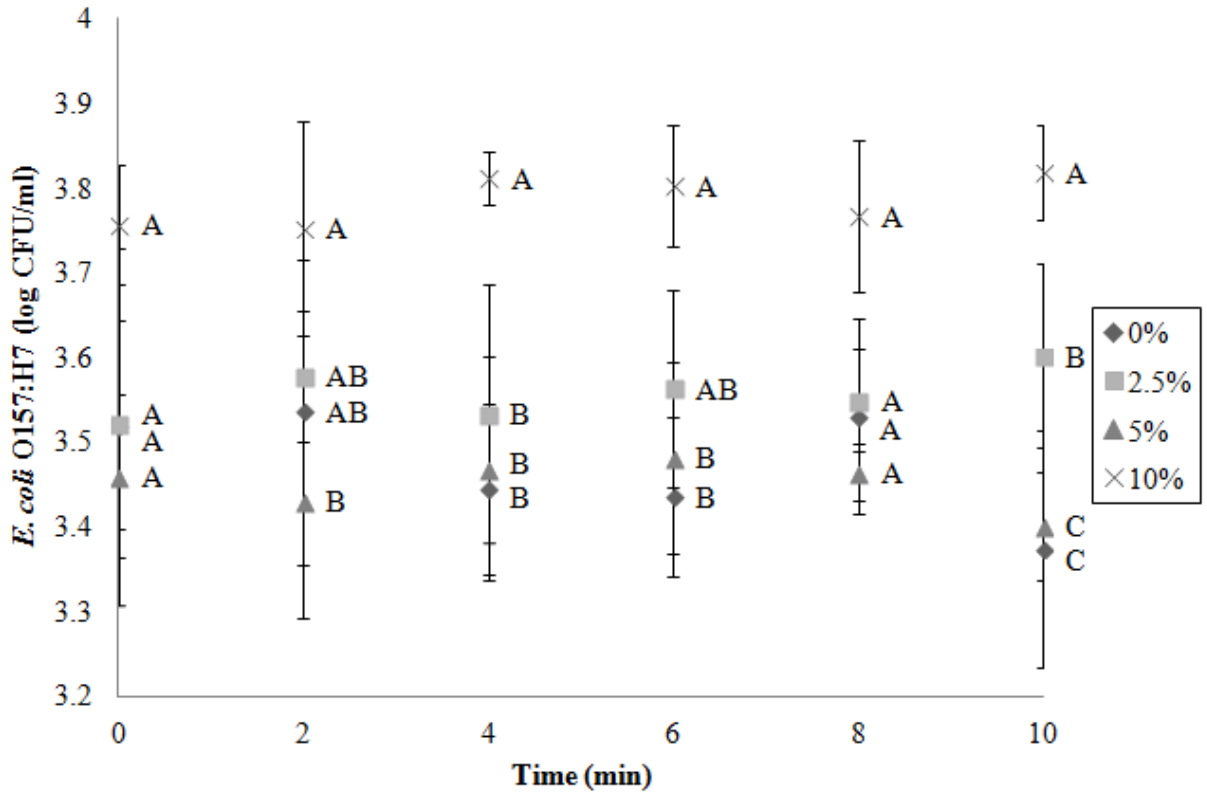


Figure 3.2: *Mean (± SD)* E. coli *O157:H7 populations (log CFU/ml) in water containing various organic loads (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Means of the same product type with different letters are significantly different (*P ≤ *0.05).*

Table 3.2: E. coli *O157:H7 reduction in the wash water at ~14°C* [a]

Sanitizing Treatment	% Organic Load (w/v)	Mean ± SD *E. coli* O157:H7 reduction in wash water (log CFU/ml) [b]		
		0 min	4 min	10 min
Peroxyacetic Acid	0	2.1 ± 0.2 A	5.2 ± 0.4 A	5.4 ± 0.0 A
	2.5	1.5 ± 0.7 A	4.2 ± 1.2 A	4.2 ± 1.1 A
	5	1.9 ± 0.5 A	4.6 ± 0.2 A	4.5 ± 0.1 A
	10	2.3 ± 0.3 A	4.9 ± 0.2 A	4.9 ± 0.2 A
Mixed Peracid	0	4.3 ± 0.9 A	5.1 ± 0.5 A	5.3 ± 0.2 A
	2.5	3.7 ± 0.3 A	5.1 ± 0.1 A	5.2 ± 0.1 A
	5	3.2 ± 0.2 A	4.8 ± 0.0 A	4.7 ± 0.1 B
	10	3.2 ± 0.6 A	4.8 ± 0.1 A	4.8 ± 0.1 B
Chlorine	0	5.2 ± 0.6 A	5.4 ± 0.1 A	5.1 ± 0.5 A
	2.5	-0.2 ± 0.1 B	-0.1 ± 0.1 B	0.8 ± 0.3 B
	5	-0.2 ± 0.2 B	-0.2 ± 0.2 B	-0.2 ± 0.1 C
	10	0.0 ± 0.1 B	0.1 ± 0.2 B	0.1 ± 0.1 BC
Chlorine + CA	0	4.9 ± 0.8 A	5.2 ± 0.3 A	5.4 ± 0.0 A
	2.5	-0.1 ± 0.2 B	0.3 ± 0.3 B	1.7 ± 1.0 B
	5	-0.2 ± 0.2 B	-0.1 ± 0.0 B	-0.0 ± 0.2 C
	10	0.0 ± 0.1 B	0.2 ± 0.1 B	0.2 ± 0.1 C

Table 3.2 (cont'd)

Chlorine + T-128	0	5.4 ± 0.2 A	5.1 ± 0.4 A	5.0 ± 0.6 A
	2.5	-0.3 ± 0.2 B	0.1 ± 0.2 B	1.2 ± 0.5 B
	5	-0.2 ± 0.2 B	-0.2 ± 0.1 B	-0.3 ± 0.3 C
	10	0.1 ± 0.1 B	0.1 ± 0.1 B	0.2 ± 0.1 BC

[a] Means with different capital letters designate *E. coli* O157:H7 reductions that differ significantly in terms of sanitizing treatment and time ($P \leq 0.05$).

[b] Time in minutes of *E. coli* exposure to the wash water after removal of the inoculated lettuce from the system.

3.4.3 Impact of temperature on sanitizer efficacy. Temperature rarely impacted chlorine + CA efficacy against *E. coli* O157:H7 in wash water, regardless of exposure time or organic load. Regardless of temperature, consistently greater *E. coli* O157:H7 reductions were seen for an organic load of 0% (4.9 – 5.4 log CFU/ml) as opposed to 2.5, 5 and 10% (Table 3.3). The only instance in which temperature influenced chlorine + CA efficacy against *E. coli* O157:H7 occurred with a 10% organic load after 10 min of exposure, where a greater reduction ($P \leq 0.05$) was observed using water at 5°C (0.4 log CFU/ml) as opposed to 14°C (0.2 log CFU/ml). However, neither of these reductions was significantly different from those seen for both temperatures at 2.5 and 5% organic loads at the same time point.

Table 3.3: E. coli *O157:H7 reduction in the wash water containing chlorine + CA at two temperatures* [a]

% Organic Load (w/v)	Temperature (°C)	Mean ± SD *E. coli* O157:H7 reduction in wash water (log CFU/ml) [b]		
		0 min	4 min	10 min
0	13	4.9 ± 0.8 A a	5.2 ± 0.3 A a	5.4 ± 0.0 A a
	5	5.3 ± 0.1 A a	5.4 ± 0.2 A a	5.3 ± 0.1 A a
2.5	14	-0.1 ± 0.2 B a	0.3 ± 0.3 B a	1.7 ± 1.0 B a
	5	0.4 ± 0.4 B a	0.9 ± 1.9 B a	1.5 ± 1.8 B a
5	14	-0.2 ± 0.2 B a	-0.1 ± 0.0 B a	0.0 ± 0.2 B a
	5	0.0 ± 0.1 B a	-0.1 ± 0.2 B a	0.0 ± 0.1 B a
10	14	0.0 ± 0.1 B a	0.2 ± 0.1 B a	0.2 ± 0.1 B b
	5	0.3 ± 0.1 B a	0.4 ± 0.2 B a	0.4 ± 0.1 B a

[a] Means with different same capital letters designate *E. coli* O157:H7 reductions that differ significantly in terms of time, while means with different lowercase letters differ significantly in terms of temperature for each organic load ($P \leq 0.05$).

[b] Time in minutes of *E. coli* exposure to the wash water after removal of the inoculated lettuce from the system.

3.4.4 Linear regression of *E. coli* O157:H7 reduction in wash water. *E. coli* O157:H7 populations were typically below the lower limit of detection for all organic loads when peroxyacetic-acid based sanitizers were used, resulting in intercepts ranging from -2.8 to -3.7 log CFU/ml for peroxyacetic acid and -3.9 to -4.8 log CFU/ml for mixed peracid. Slopes were similar for the different organic loads. Root-mean-square errors were ~1 log CFU/ml for peroxyacetic acid and ~0.5 log CFU/ml for mixed peracid. R^2 values for both peroxyacetic acid and mixed peracid were typically between 0.4 – 0.5 (Figures 3.3 and 3.4, Table 3.4). *E. coli* O157:H7 populations were typically near, at, or below the lower limit of detection for all chlorine variations with a 0% organic load in the wash water, resulting in intercepts of -5.4, -5.1, -5.5 and -5.5 for chlorine, chlorine + CA, chlorine + T-128 and chlorine + CA at 5°C, respectively. Using organic loads of 2.5, 5 and 10%, intercepts were close to 0, with inactivation rates at organic loads of 5 and 10% being close to 0, while a 2.5% organic load yielded inactivation rates of -0.1, -0.2, -0.1 and -0.1 log CFU/min for chlorine, chlorine + CA, chlorine + T-128, and chlorine + CA at 5°C, respectively. Root-mean-square errors ranged from 0 to 0.3 log CFU/ml for all chlorine treatments. R^2 values for all chlorine treatments ranged from 0 to 1, with the higher values attributed to the 2.5% organic load. (Figures 3.5 – 3.8, Table 3.4).

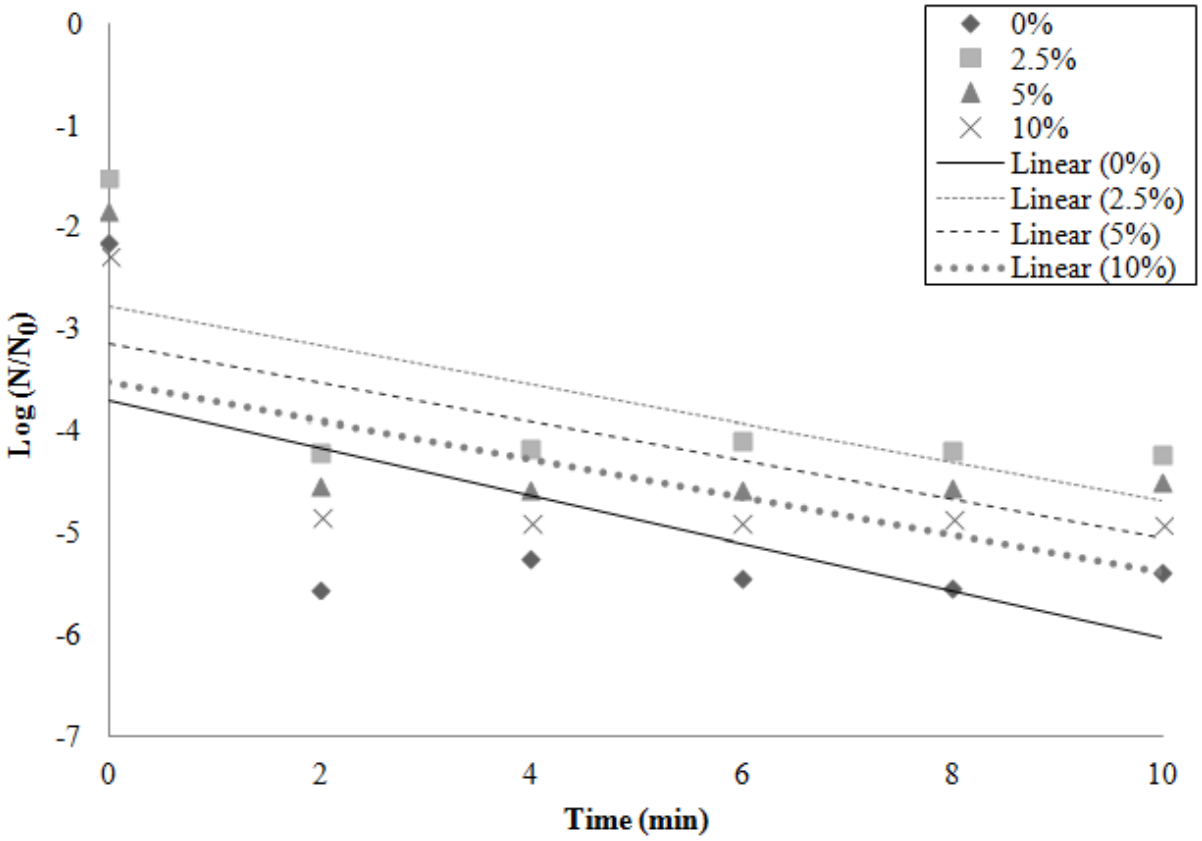


Figure 3.3: E. coli *O157:H7 inactivation (log CFU/ml) in water containing various organic loads and 50 ppm peroxyacetic acid (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

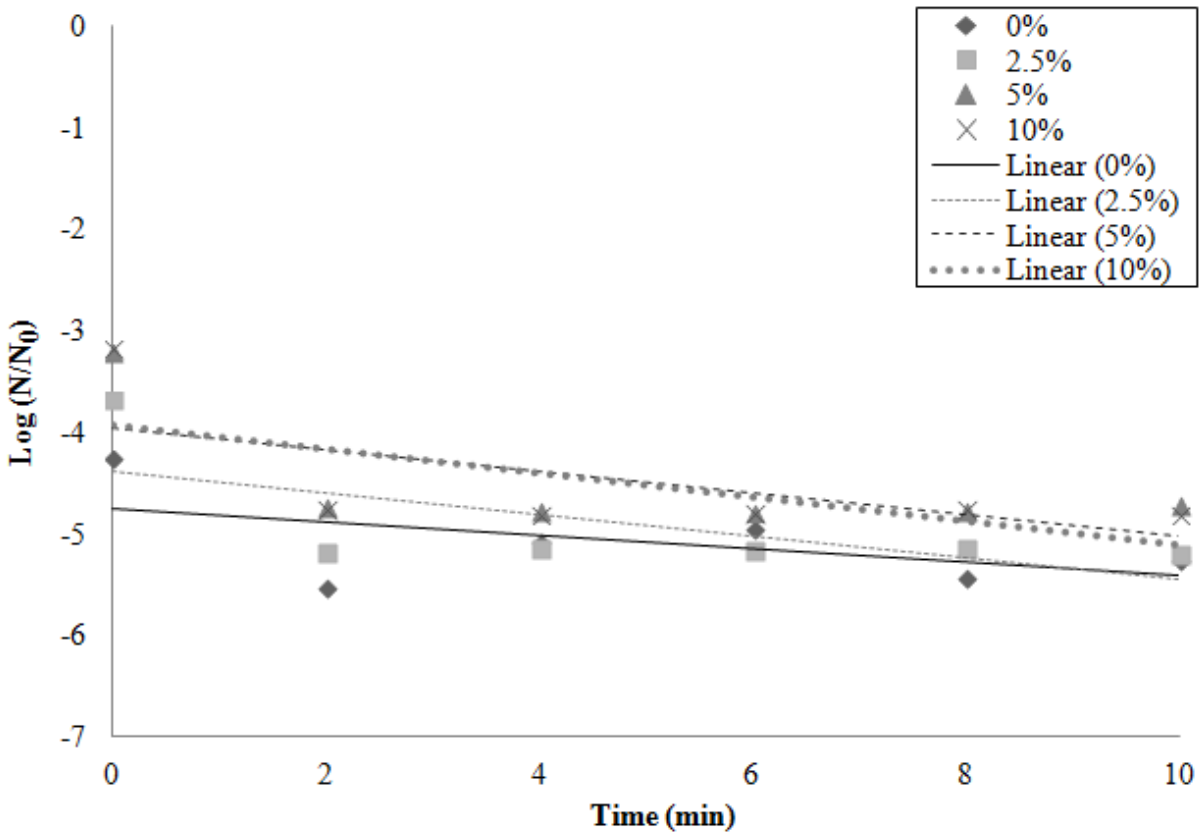


Figure 3.4: E. coli *O157:H7 inactivation (log CFU/ml) in water containing various organic loads and 50 ppm mixed peracid (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

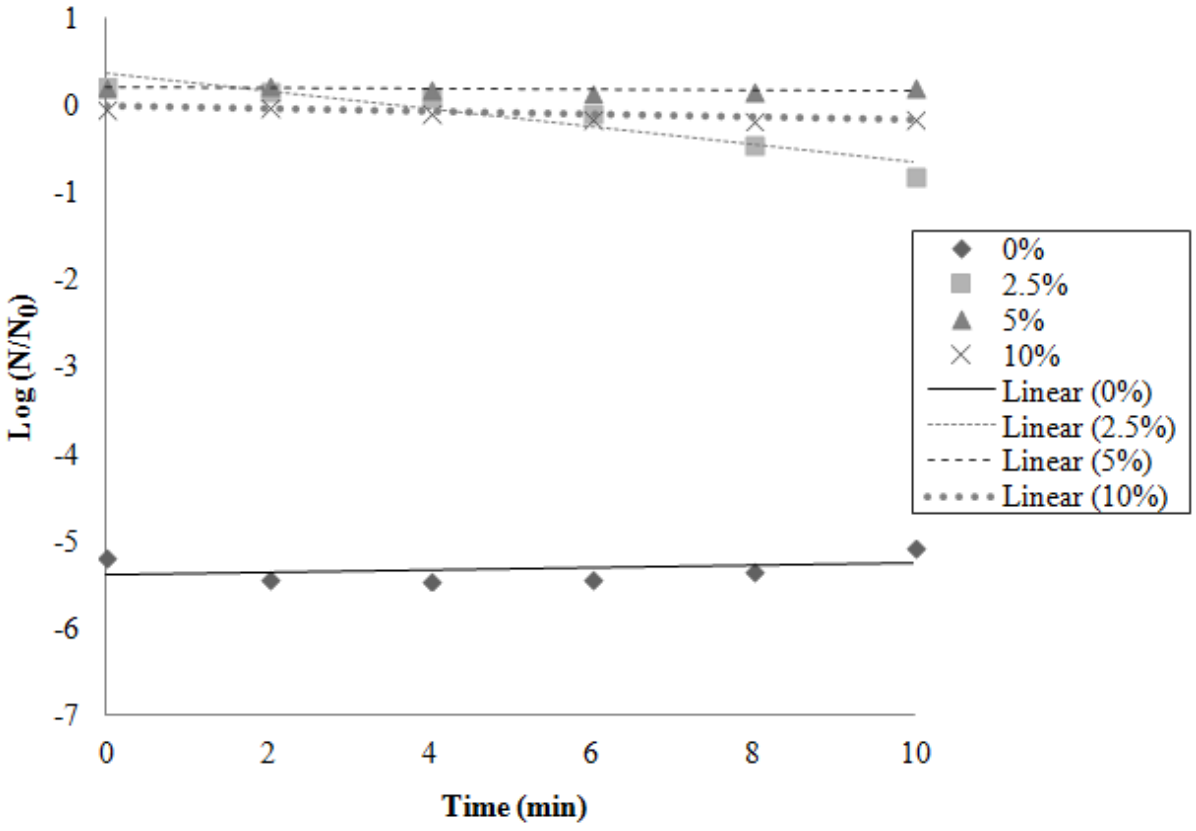


Figure 3.5: E. coli *O157:H7 inactivation (log CFU/ml) in water containing various organic loads and 50 ppm available chlorine (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

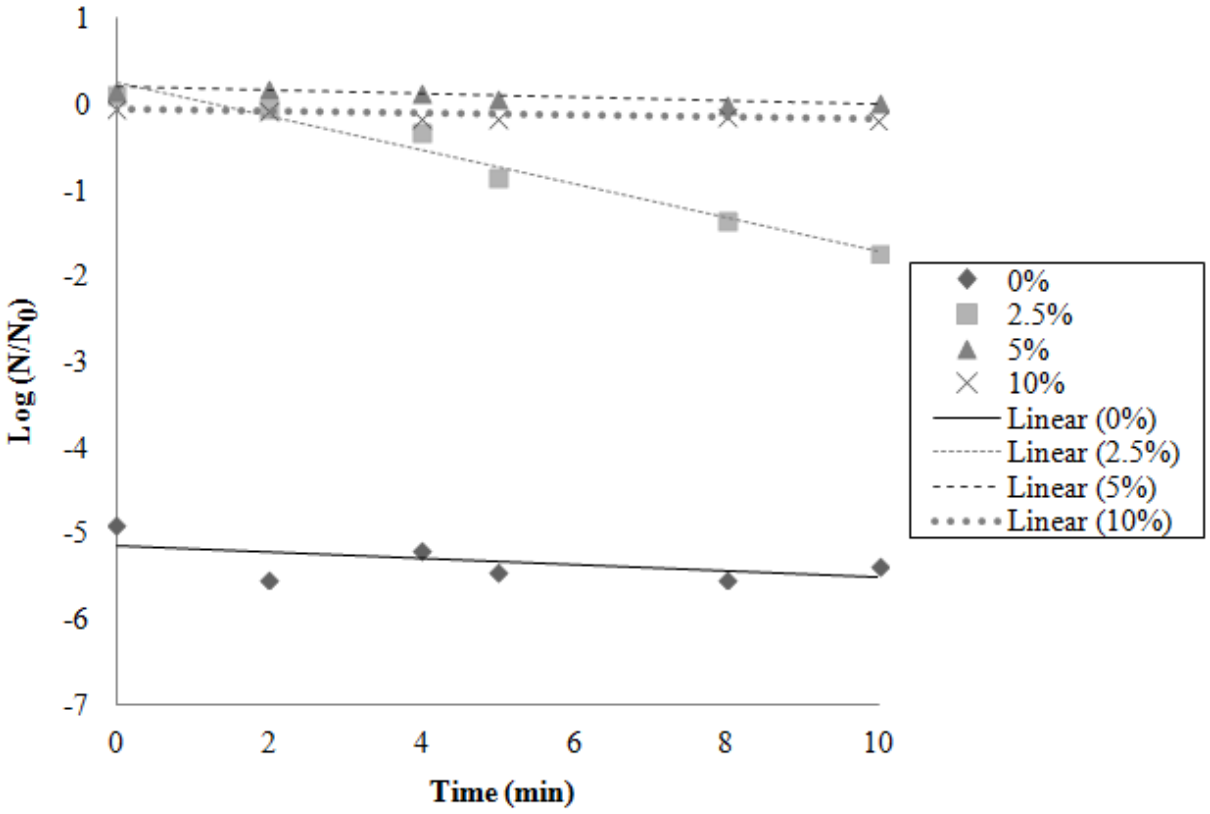


Figure 3.6: E. coli *O157:H7 inactivation (log CFU/ml) in water containing various organic loads and 50 ppm available chlorine + CA (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

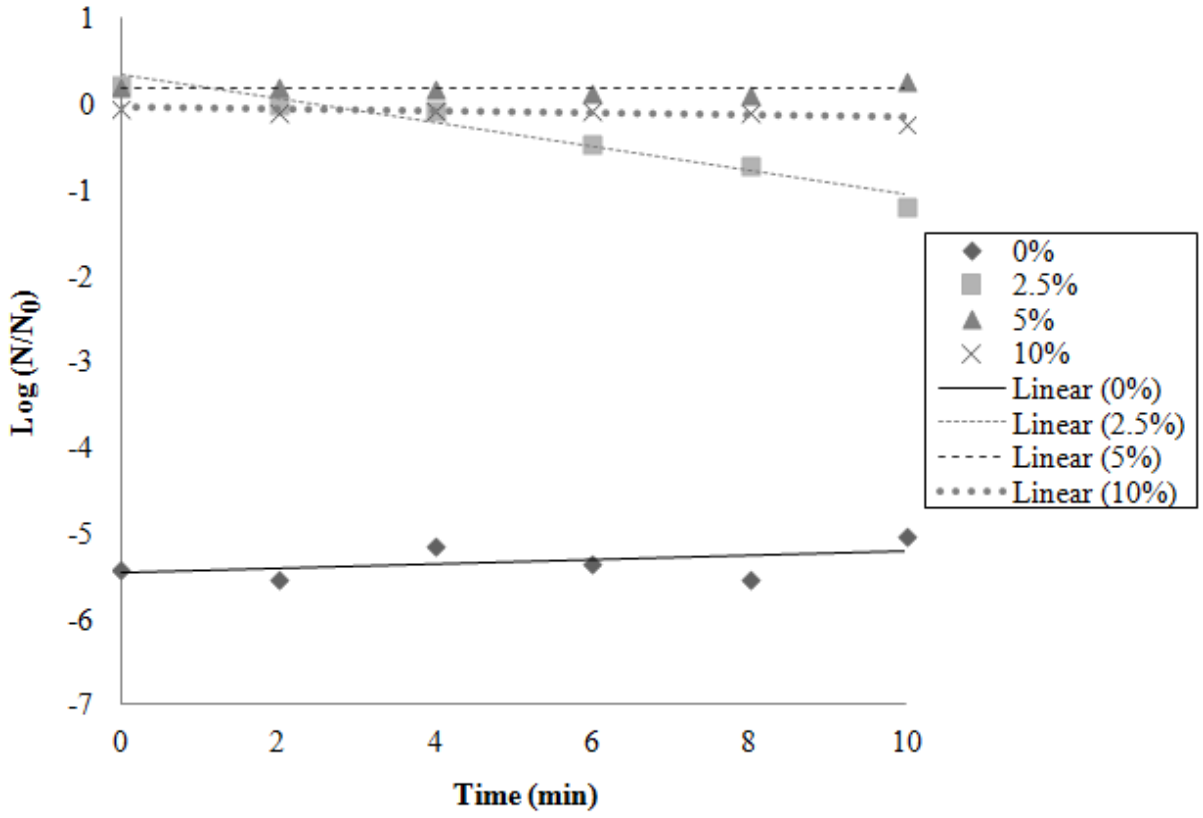


Figure 3.7: E. coli *O157:H7 inactivation (log CFU/ml) in water containing various organic loads and 50 ppm available chlorine + T-128 (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

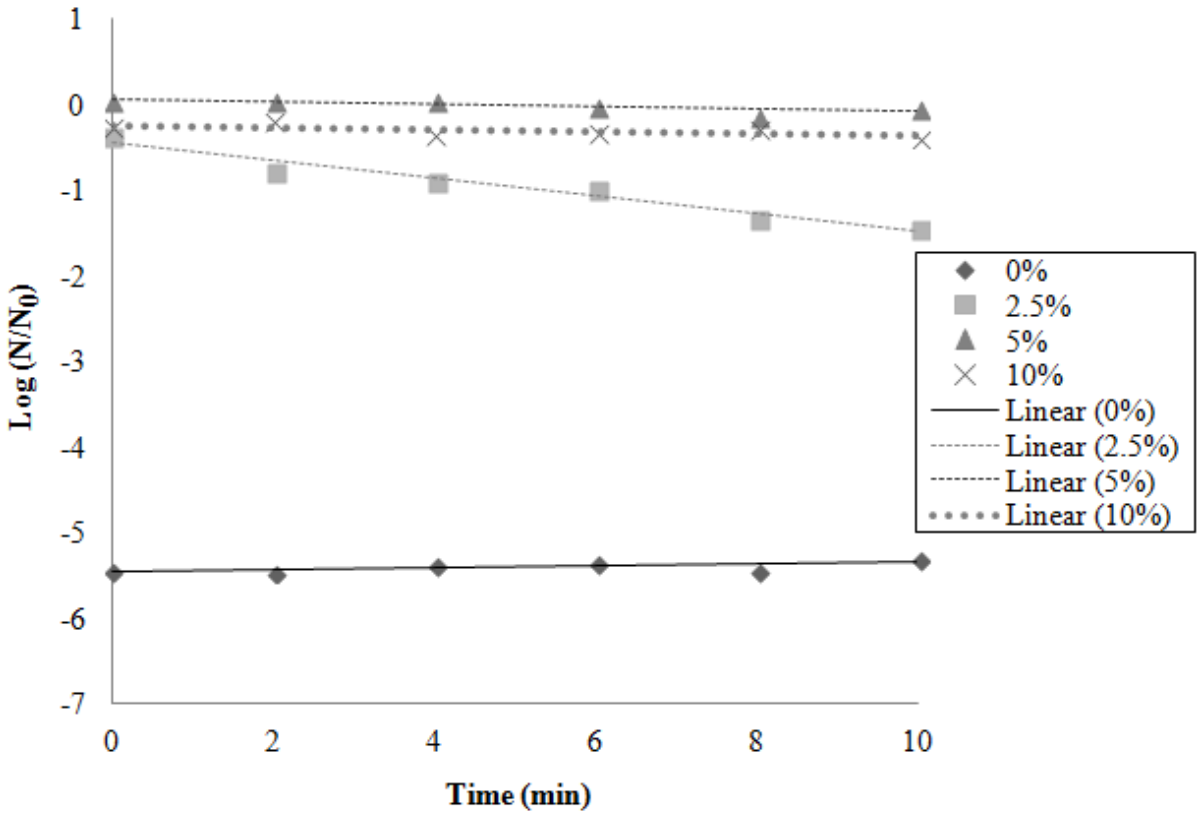


Figure 3.8: E. coli *O157:H7 inactivation (log CFU/ml) in 5°C water containing various organic loads and 50 ppm available chlorine + CA (n=3 per organic load) after washing iceberg lettuce inoculated at ~6 log CFU/g. Half the limit of detection was used when a sample did not yield any colonies by direct plating.*

Table 3.4: E. coli *inactivation trend lines in wash water containing various organic loads*[a]

Sanitizing Agent	% Org. Load (w/v)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
Peroxyacetic acid	0	$Log(N/N_0) = -0.2t - 3.7$	0.42	1.1	0.16
	2.5	$Log(N/N_0) = -0.2t - 2.8$	0.44	1.1	0.15
	5	$Log(N/N_0) = -0.2t - 3.1$	0.42	1.1	0.16
	10	$Log(N/N_0) = -0.2t - 3.5$	0.44	1.1	0.15
Mixed Peracid	0	$Log(N/N_0) = -0.1t - 4.8$	0.29	0.5	0.27
	2.5	$Log(N/N_0) = -0.1t - 4.4$	0.44	0.5	0.15
	5	$Log(N/N_0) = -0.1t – 4.0$	0.41	0.6	0.17
	10	$Log(N/N_0) = -0.1t - 3.9$	0.45	0.6	0.15
Chlorine	0	$Log(N/N_0) = -5.4$ [b]	0.09	0.2	0.56
	2.5	$Log(N/N_0) = -0.1t + 0.4$	0.89	0.2	< 0.001
	5	$Log(N/N_0) = 0.2$ [b]	0.32	0.0	0.24
	10	$Log(N/N_0) = 0.0$	0.80	0.0	0.02

Table 3.4 (cont'd)

Chlorine + CA	0	$\text{Log}(N/N_0) = -5.1$	0.33	0.3	0.24
	2.5	$\text{Log}(N/N_0) = -0.2t + 0.3$	0.98	0.1	< 0.001
	5	$\text{Log}(N/N_0) = +0.2$	0.82	0.0	0.01
	10	$\text{Log}(N/N_0) = -0.1$	0.68	0.1	0.04
Chlorine + T-128	0	$\text{Log}(N/N_0) = -5.5$	0.22	0.21	0.35
	2.5	$\text{Log}(N/N_0) = -0.1t + 0.3$	0.96	0.12	< 0.001
	5	$\text{Log}(N/N_0) = 0.2$	0.00	0.07	0.95
	10	$\text{Log}(N/N_0) = 0.0$	0.56	0.0	0.09
Chlorine + CA (5°C)	0	$\text{Log}(N/N_0) = -5.45$	0.37	0.1	0.20
	2.5	$\text{Log}(N/N_0) = -0.1t - 0.4$	0.95	0.1	< 0.001
	5	$\text{Log}(N/N_0) = +0.1$	0.53	0.1	0.10
	10	$\text{Log}(N/N_0) = -0.2$	0.50	0.1	0.12

[a] Inactivation rates were determined by using Microsoft Excel to create the linear regression trend lines for *E. coli* O157:H7 log reduction over time (t) in minutes.

[b] The slope (k) was < 0.1 and was therefore not included.

3.4.5 Physicochemical parameters of wash water. Organic load had no significant effect ($P > 0.05$) on the average pH values for peroxyacetic acid (6.79) mixed peracid (5.32) (Table 3.5). A significant reduction ($P \leq 0.05$) in pH was seen for each increase in organic load when chlorine was not acidified, with pH values decreasing from 8.03 (0%) to 7.09 (10%) (Table 3.6). MSU tap water was ~14°C, with some significant differences ($P \leq 0.05$) observed between different organic loads for the same sanitizing treatment, with the lower temperature often observed at the lower organic loads. For all three chlorine treatments, free chlorine was significantly higher ($P \leq 0.05$) at a 0% organic load (50 – 53 ppm) compared to the other organic loads (0.77 – 1.9 ppm), with available chlorine having stabilized at 50 ppm before the inoculated lettuce was exposed to the water (Tables 3.6 – 3.8). Total solids, COD and turbidity significantly increased ($P \leq 0.05$) with increasing organic load, whereas MFV decreased significantly ($P \leq 0.05$) as organic load increased for each sanitizing treatment. As the organic load increased with peroxyacetic acid or mixed peracid, ORP increased significantly ($P \leq 0.05$), likely due to the increase in peroxyacetic acid sanitizer added to the water to achieve a concentration of 50 ppm. Conversely, ORP decreased significantly ($P \leq 0.05$) as organic load increased for the chlorine treatments.

Table 3.5: *Physicochemical parameters of wash water containing various organic loads and 50 ppm peroxyacetic acid or 50 ppm mixed peracid* [a]

Controlled Physicochemical Parameters		Mean ± SD Dependent Physicochemical Parameters						
Sanitizing agent	% Org. Load (w/v)	pH	T (°C)	Total Solids (g)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
Peroxyacetic acid	0	6.89 ± 0.08 A	13±0 B	0.0061±0.0012 D	282±18 D	0±0 C	50±0 A	355±5 B
	2.5	6.79 ± 0.08 A	14±0 A	0.0190±0.0034 C	1703±41 C	0.082±0.033 BC	24±7 B	362±4 B
	5	6.79 ± 0.01 A	15±0 A	0.0288±0.0020 B	3787±160 B	0.142±0.029 B	15±4 BC	371±5 B
	10	6.69 ± 0.16 A	15±0 A	0.0502±0.0009 A	6890±301 A	0.275±0.050 A	10±2 C	404±18 A
Mixed Peracid	0	5.40 ± 0.05 A	14±0 A	0.0087±0.0006 D	874±21 D	0.000±0.000 D	50±0 A	479±4 B
	2.5	5.35 ± 0.06 A	15±1 A	0.0180±0.0009 C	2917±513 C	0.100±0.005 C	27±6 B	488±4 B
	5	5.33 ± 0.12 A	15±1 A	0.0312±0.0010 B	5100±304 B	0.199±0.013 B	17±5 BC	497±5 AB
	10	5.19 ± 0.12 A	15±1 A	0.0522±0.0041 A	7783±455 A	0.396±0.014 A	14±5 C	515±19 A

[a] Means with different capital letters designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

Table 3.6: *Physicochemical parameters of wash water containing various organic loads and 50 ppm available chlorine* [a]

Controlled Physicochemical Parameters	Mean ± SD Dependent Physicochemical Parameters							
% Org. Load (w/v)	pH	T (°C)	Free chlorine (ppm)	Total solids (g)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
0	8.03 ± 0.04 A	13±0 C	53.33±2.08 A	0.0061±0.0002 D	15±1 D	0.001±0.006 C	50±0 A	788±9 A
2.5	7.52 ± 0.02 B	15±1 AB	1.17±0.64 B	0.0173±0.0026 C	1208±286 C	0.081±0.018 BC	18±7 B	437±29 B
5	7.41 ± 0.01 C	14±0 BC	1.27±0.47 B	0.0257±0.0010 B	2340±60 B	0.133±0.005 B	14±2 BC	413±7 B
10	7.09 ± 0.04 D	15±1 A	1.00±0.44 B	0.0492±0.0017 A	5465±420 A	0.300±0.065 A	8±1 C	360±70 B

[a] Means with different capital letter designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

Table 3.7: *Physicochemical parameters of wash water containing various organic loads and 50 ppm chlorine + CA or 50 ppm chlorine + T-128, both used to acidify to pH 6.5* [a]

Controlled Physicochemical Parameters		Mean ± SD Dependent Physicochemical Parameters						
Supplement	% Org. Load (w/v)	T (°C)	Free chlorine (ppm)	Total solids (g)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
CA	0	13±0 B	50.67±1.53 A	0.0078±0.0003 D	244±32 B	0.001±0.001 C	50±0 A	903±12 A
	2.5	14±1 AB	0.77±0.38 B	0.0188±0.0026 C	1426±318 B	0.079±0.023 B	17±1 B	492±14 B
	5	14±0 A	0.90±0.20 B	0.0296±0.0040 B	2243±91 B	0.123±0.005 B	14±5 BC	491±3 B
	10	14±0 A	1.27±0.57 B	0.0492±0.0031 A	5815±306 A	0.255±0.032 A	10±1 C	425±32 C
T-128	0	13±0 B	51.67±1.53 A	0.0086±0.0002 D	278±17 D	0.001±0.001 D	50±0 A	902±6 A
	2.5	14±1 AB	0.27±0.12 B	0.0181±0.0014 C	1323±160 C	0.081±0.010 C	17±4 B	482±1 B
	5	14±0 AB	0.47±0.12 B	0.0275±0.0010 B	2073±64 B	0.128±0.013 B	17±6 B	495±23 B
	10	15±1 A	0.67±0.15 B	0.0454±0.0060 A	5733±329 A	0.282±0.026 A	9±1 B	427±60 B

[a] Means with different capital letters designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

Table 3.8: *Physicochemical parameters of wash water containing various organic loads and 50 ppm available chlorine* + CA at ~5°C [a]

Controlled Physicochemical Parameters		Mean ± SD Dependent Physicochemical Parameters					
% Org. Load (w/v)	T (°C)	Free chlorine (ppm)	Total solids (g)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
0	5 ± 0 A	50 ± 1.5 A	0.0090 ± 0.0075 D	296 ± 13 C	0 ± 0 C	50 ± 0 A	881 ± 10 A
2.5	6 ± 0 A	0.97 ± 0.57 B	0.0194 ± 0.0009 C	1518 ± 64 C	0.077 ± 0.060 BC	18 ± 5 B	563 ± 51 B
5	6 ± 0 A	0.77 ± 0.25 B	0.0306 ± 0.0010 B	3717 ± 990 B	0.178 ± 0.050 AB	19 ± 2 B	488 ± 72 B
10	5 ± 0 A	1.9 ± 0.1 B	0.0494 ± 0.0006 A	6557 ± 549 A	0.236 ± 0.041 A	17 ± 1 B	482 ± 97 B

[a] Means with different capital letters designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

3.5 DISCUSSION

The impact of organic load on sanitizer efficacy has been assessed previously in other small-scale laboratory studies *(57, 87, 107, 139)*. However, this is the first study to correlate wash water physicochemical parameters to *E. coli* O157:H7 inactivation trend lines for a variety of commercial produce sanitizers, organic loads and temperatures, with such efforts identified as a priority by the Center for Produce Safety *(33)*. This novel bench-top system was designed to simulate the wash phase of a pilot-scale or commercial leafy green processing line. The simple and economical carboy system presented here could be used to easily assess a range of sanitizers, pathogens, processing conditions, and produce products.

The same four non-toxigenic strains of *E. coli* O157:H7 were used as in our earlier transfer studies *(22, 23)* as well as in our pilot-scale studies (Chapters 2, and 4). Shen and others *(107)* found that inactivation of *E. coli* O157:H7, *Salmonella,* and non-O157:H7 Shiga toxin-producing *E. coli* in chlorinated water was a function of different pathogenic strains. However, most strains decreased 4.5 log CFU/ml (below the limit of detection) after 30 s of exposure when free chlorine concentrations exceeded 0.5 ppm, indicating that while there may be some differences in how strains of the same pathogen respond to sanitizing agents under various conditions, these differences are likely to be noticeable only initially and are likely negligible in experiments of longer duration. Preliminary work was completed to assess the extent of injury for each individual strain in 30 ppm peroxyacetic acid after 90 s of exposure. Results indicated immense variability after incubation at 37°C for 24 h, with far more consistent results obtained after 48 h of incubation at the same temperature. Extending the incubation time no doubt allowed for recovery of additional injured cells as previously reported *(68, 135)*.

Dip inoculating lettuce to contain 6 log CFU/g was crucial to ensure a consistent inoculation level. While this inoculation level clearly exceeds expected levels on field-grown lettuce, feces from "super-shedding" cows can contain *E. coli* O157:H7 at levels greater than 6 log CFU/g *(42)*, with such fecal material potentially coming in contact with lettuce through irrigation water.

Lettuce was stored for 24 h at 4°C after inoculation to simulate industrial storage conditions that occur before processing, which can promote further attachment of *E. coli* O157:H7 to the leaf surface. A previous study completed by Buchholz and others *(22)* found that 42.2% of the original inoculum remained on product that was held for 24 h at 4^{o}C before washing and centrifugal drying, significantly higher than the 8% retention rate seen for product that had been identically processed 1 h after inoculation.

The inoculated lettuce was in a mesh produce when exposed to 4 L of water to allow for quick removal from the water, simulating lettuce leaving a commercial flume system. *E. coli* O157:H7 reductions on the lettuce ranged from 0.4 – 1.2 log CFU/g. The fact that these reductions were on the lower end of the spectrum when compared to other small-scale laboratory studies (1 – 3 log CFU/g) *(17, 47, 55, 91, 100)*can be attributed to the lettuce being bagged, which likely hindered exposure to higher shear forces as would have been encountered by loose pieces. *(62, 132, 141)*. Organic load had a significant impact ($P \leq 0.05$) on the efficacy of chlorine, chlorine + CA and chlorine + T-128, with greater *E. coli* O157:H7 reductions seen with an organic load of 0% (~ 1 log CFU/g) as opposed to 2.5, 5 or 10% (~0.5 log CFU/g). However, both peroxyacetic acid and mixed peracid reduced *E. coli* O157:H7 populations ~1 log, with no significant difference ($P > 0.05$) seen between any of the organic loads. This emphasizes the

importance of monitoring water quality, especially if a chlorine-based sanitizer is being used, in order to maximize the removal/inactivation of pathogens from the leaf surface.

Chemical sanitizers are routinely added to recirculating wash water to minimize the spread of microbial contaminants during flume washing *(78)*. Regarding their use, peroxyacetic acid-based sanitizers are limited to a maximum of 80 ppm peroxyacetic acid *(43, 62)*, while free chlorine concentrations typically range from 10 to a maximum of 200 ppm *(55, 91, 122)*. However, soil, debris, and vegetable latexes released during shredding of leafy greens will accumulate in the flume water over time *(86)*, decreasing the efficacy of many sanitizers, most notably chlorine *(5, 77, 100, 139)*. The wash water used in this study contained organic loads of 0, 2.5, 5 and 10% blended iceberg lettuce (w/v) to simulate wash water quality at various stages of processing. Hence, *E. coli* O157:H7 reductions in wash water would likely decrease with increasing organic loads especially when using chlorine. *E. coli* O157:H7 reductions were consistently higher ($P \leq 0.05$) in water without an organic load for chlorine, chlorine + CA and chlorine + T-128. In contrast, organic load rarely impacted the efficacy of peroxyacetic acid and mixed peracid, with similar results seen by Zhang and others *(139)*.

This study was designed to assess the efficacy of sanitizers against *E. coli* O157:H7 in wash water after lettuce has been washed, with the assumption that pathogen persistence in the wash water will result in contamination of subsequently washed product. Produce sanitizers are primarily used to minimize cross-contamination during flume washing, with their effectiveness dependent on the type of sanitizer, concentration, temperature, and organic load in the wash water. The pilot-scale processing line used in the studies described in Chapters 2, 4 was not equipped with a chiller. The work described here was intended to be a model for the pilot-scale processing line, which is why most of this work was conducted at a water temperature of ~14°C.

However, subsequent analysis showed significant differences ($P < 0.05$) in *E. coli* O157:H7 reduction using similar water conditions and exposure times to those in Chapters 4 and 5. While the results were not always similar to the pilot-scale processing line, this inexpensive bench-top model is still a good indicator of the results that can be expected in larger pilot plant-scale processing studies. Additionally, the bench-top model allows easy and rapid testing of far more diverse water conditions, such as the work completed at a lower temperature (~5°C), in order to simulate targeted commercial processing conditions. Zhang and Farber *(140)* reported a decrease in sanitizer efficacy at a water temperature of 4°C as compared to of 22°C. In this study, no significant differences ($P > 0.05$) in *E. coli* O157:H7 reduction were seen between 5 and 14°C when chlorine + CA was used, indicating that temperature may not affect sanitizer efficacy to the extent previously reported *(62)*.

Chlorine, chlorine + CA and chlorine + T-128 were all significantly impacted ($P \leq 0.05$) by organic load in the wash water. In the absence of an organic load, the *E. coli* O157:H7 inactivation trend lines illustrate a > 5 log reduction in wash water for all three chlorine treatments. However, organic loads of 2.5, 5 and 10% resulted in intercepts that were close to 0, with organic loads of 5 and 10% having predicted slopes at or near 0 for the log linear trend lines. The dependent physicochemical parameters of free chlorine, MFV, and ORP were all significantly higher ($P \leq 0.05$) at a 0% organic load compared to organic loads of 2.5, 5, or 10%, while turbidity, COD and total solids significantly increased ($P \leq 0.05$) with organic load. The dependent physicochemical parameters for each chlorine treatment were correlated to the *E. coli* O157:H7 inactivation rates with the RMSE, R^2 and *P* values for each linear trend line shown in Table 3.4. Considering that *E. coli* O157:H7 populations decreased > 5 logs for all chlorine treatments without an organic load, commercial lettuce processors should maintain their wash

water physicochemical parameters within the standard deviations for an organic load of 0% when using chlorine-based sanitizers to minimize the spread of *E. coli* O157:H7 and other contaminants from the wash water to the product. Most of the dependent physicochemical parameters of the wash water are not critical to the prediction of peroxyacetic acid or mixed peracid efficacy, because water quality rarely had a significant impact on their respective performance; however, pH and ORP could be used to predict efficacy because both are dependent on sanitizer concentration more than organic load. Regardless of the physicochemical wash water parameters measured, the slopes from the inactivation rate equations were similar for each respective sanitizer, with minor differences predicted for the intercepts. The efficacy of both peroxyacetic acid-based sanitizers can best be predicted based on the controlled parameters of sanitizing agent and sanitizer concentration. The numerous physicochemical parameters monitored in this study offer alternatives to ORP which is the most used to assess sanitizer efficacy *(112, 125)*. Inactivation trend lines with *P* values > 0.05 are likely not ideal models for the data.

Commercial produce sanitizers are primarily designed to minimize cross-contamination from the wash water to the product during processing. This study was done to assess the efficacy of commercial produce sanitizers against *E. coli* O157:H7 in wash water using a new and novel bench-top system that is both customizable and a cost-effective alternative to pilot-scale processing. While *E. coli* O157:H7 reductions on the lettuce surface ranged from 0.4 – 1.2 log CFU/g, this lettuce was only used as a means of inoculating the water in order to assess pathogen persistence at different organic loads with different sanitizing agents. Those physicochemical parameters dependent on organic load for chlorine, chlorine + CA, or chlorine + T-128 are excellent predictors of *E. coli* O157:H7 persistence in wash water. Independent parameters, such

as sanitizer concentration or sanitizing agent, are the best predictors of peroxyacetic acid or mixed peracid efficacy, considering that sanitizer efficacy against *E. coli* O157:H7 was rarely dependent on organic load. While unable to accurately model the pilot-scale processing work presented in Chapter 4, this bench-top model is a good indicator of the *E. coli* O157:H7 reductions that can be expected at different organic loads with different sanitizing agents. Additionally, the bench-top system described here is a simple and cost-effective model that can be customized to assess various sanitizers and organic loads at different temperatures.

CHAPTER 4:

Impact of Organic Load on *Escherichia coli* O157:H7 Survival during Pilot-Scale Processing of Iceberg Lettuce with Commercial Produce Sanitizers

4.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 on iceberg lettuce, in wash water, and on surfaces of a pilot-scale processing line using flume water containing various organic loads. Iceberg lettuce (5.4 kg) was inoculated to contain 10^6 CFU/g of a 4-strain cocktail of non-toxigenic, GFP-labeled, ampicillin-resistant *E. coli* O157:H7 and held for ~24 h at 4°C to simulate commercial storage conditions before processing. Lettuce was shredded using a Urschel TransSlicer, step-conveyed to a flume tank, washed for 90 s using water alone or five different sanitizing treatments (50 ppm peroxyacetic acid, 50 ppm mixed peracid, or 50 ppm available chlorine either alone or acidified to pH 6.5 with citric acid or T-128) in water containing organic loads of 0, 2.5, 5 or 10% (w/v) blended iceberg lettuce, and then dried using a shaker table and centrifugal dryer. Thereafter, three 5.4-kg batches of uninoculated iceberg lettuce were identically processed. Various product (25 g), water (50 ml) and equipment samples (100 cm^2) were homogenized in neutralizing buffer, serially diluted and plated on tryptic soy agar containing 0.6% (w/v) yeast extract and 100 ppm ampicillin with or without prior 0.45 μm membrane filtration for quantification of *E. coli* O157:H7. Various physicochemical parameters of the wash water were correlated to *E. coli* O157:H7 inactivation trend lines at each organic load. Organic load negatively impacted the efficacy of all three chlorine treatments ($P \leq 0.05$), with typical *E. coli* O157:H7 reductions of > 5 log CFU/ml by the end of processing with no organic load in the wash water and 0.9 – 3.7 log CFU/ml with a 10% organic load. Conversely, organic load rarely impacted peroxyacetic or mixed peracid efficacy against *E. coli* O157:H7,

with typical reductions of > 5 log CFU/ml in flume water throughout processing for all organic loads. Organic load rarely impacted sanitizer efficacy against *E. coli* O157:H7 on lettuce. Reduced chlorine efficacy generally correlated to ($P \leq 0.05$) to increased total solids, chemical oxygen demand, and turbidity, indicating that these tests may be effective alternatives to the industry standard of oxygen/reduction potential. However, *E. coli* O157:H7 persisted on all previously uninoculated lettuce following the inoculated batch, emphasizing the need for improved intervention strategies that can better ensure end-product safety.

4.2 INTRODUCTION

Numerous small-scale laboratory studies have assessed sanitizer efficacy against pathogens on leafy greens *(4, 66, 72, 78, 82, 88, 115, 139, 140)*. However, much work remains in predicting sanitizer efficacy in commercial flume systems that contain various organic loads. Previous sanitizer work completed by our group was performed without an organic load in order to determine the immediate effects of sanitizers in flume water during processing of *E. coli* O157:H7-inoculated iceberg lettuce (Chapter 2), or with an organic load in a 4 L bench-top model in an attempt to predict sanitizer efficacy during pilot-plant processing of leafy greens (Chapter 3). Since chemical sanitizers remain the sole intervention strategy to prevent cross-contamination during commercial production of fresh-cut leafy greens, it is imperative that sanitizing agents be evaluated under conditions that more closely resemble commercial operations. Consequently, the two aims of this study were to 1) determine the efficacy of five commercial sanitizer treatments against *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce using water containing various organic loads and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on *E. coli* O157:H7 inactivation.

4.3 MATERIALS AND METHODS

4.3.1 Experimental design. The efficacy of five different sanitizing treatments against *E. coli* O157:H7 was assessed in triplicate by processing 5.4 kg of iceberg lettuce inoculated at 10^6 CFU/g followed by three consecutive 5.4 kg batches of uninoculated iceberg lettuce in flume water containing various organic loads. All lettuce was processed by shredding, conveying, fluming, shaker table dewatering and/or centrifugal drying, during and/or after which various product, water and equipment surface samples were collected and quantitatively examined for *E. coli* O157:H7. Pathogen persistence in wash water was then correlated to six physicochemical parameters of thewater: pH, oxidation/reduction potential, chemical oxygen demand, total solids, maximum filterable volume and turbidity.

4.3.2 Leafy greens. Identical to 2.3.2.

4.3.3 Bacterial strains. Identical to 2.3.3.

4.3.4 Lettuce inoculation. A 0.2 ml aliquot of each non-toxigenic *E. coli* O157:H7 strain was transferred to 200 ml of TSBYE with amp and incubated for 18 – 20 h at 37°C. Based on similar growth rates as determined previously *(22)*, the four strains were combined in equal volumes to obtain an 800-ml cocktail which was added to 80 L of municipal tap water (~15^{o}C, < 0.05 ppm free chlorine) in a 121 L plastic container (Rubbermaid, Wooster, OH) to achieve a level of ~10^7 CFU/ml. Hand-cored heads of iceberg lettuce (~12 heads) were immersed in the *E. coli* O157:H7 suspension for 15 min and then drained/air-dried for 1 h at 22°C before being spun in a dewatering centrifuge (described below) to remove residual inoculum from the interior of the heads and stored at 4°C for 24 h before processing. Duplicate 25-g samples were then aseptically collected to determine the initial inoculation level at the time of processing.

4.3.5 Lettuce processing line. A small-scale commercial leafy green processing line was assembled that consisted of a lettuce shredder, step conveyer, flume tank, shaker table and dewatering centrifuge. The commercial lettuce shredder (Model TRS 2500 Urschel TranSlicer, Valparaiso, IN) was operated at a feed belt/slicing wheel speed of 198 m/min and 905 RPM, respectively, to obtain a shred size of approximately 5 x 5 cm. The polyurethane step conveyer belt (ThermoDrive, Mol Industries, Grand Rapids, MI) on the conveyor (Dorner model 736018 mc series, Dorner Manufacturing, Hartland, WI) was operated at 0.11 m/s. A stainless steel water recirculation tank (~1000 L capacity) was filled with 890 L of tap water. This water tank was connected to a 3.6-m long stainless steel flume tank (Heinzen Manufacturing, Inc., Gilroy, CA) - equipped with two overhead spray jets (1 m from the start), by a 4.14 m-long, 10 cm-diameter hard plastic discharge hose and a centrifugal pump (Model XB754FHA, Sterling Electric, Inc., Irvine, CA) that circulated the water at a rate of ~10 L/s. A custom-made stainless steel gate with 1.25 cm-diameter holes spaced 0.65 cm apart (Heinzen Manufacturing, Inc.) was secured to the end of the flume tank to prevent product flow while allowing continuous circulation of the wash water. The stainless steel shaker table for partial dewatering was operated by a 1 HP Baldor washdown duty motor (Baldor Electric Co., Ft. Smith, AR) at 1760 RPM. Water removed from the leafy greens during mechanical shaking passed through a mesh screen and was fed into the water holding tank by a water recirculation spout underneath the shaker table. A 50-lb capacity centrifugal Spin Dryer (Model SD50-LT, Heinzen Manufacturing, Inc.) with three internally timed spin cycles totaling 80 s was used for centrifugal drying. The pilot-scale processing line was not equipped with a chiller to maintain the processing water at a target commercial temperature of 4°C *(62)*. However, the average flume water temperature of 13.5°C did not likely have a significant effect on chlorine efficacy based on results from the temperature

study discussed in Chapter 3 and a previous study showing that peroxyacetic acid maintained its efficacy over a range of temperatures *(64)*.

4.3.6 Wash water. Iceberg lettuce (0, 22.5, 45, or 90 kg) was blended in 11 – 18 kg batches in a 55 L cylindrical polypropylene tank (Nalgene, Rochester, NY) containing 14 L of tap water for 10 min using a Rotostat blender (Model XP-02, Admix, Manchester, NH). All batches were combined in the recirculation tank with additional water at 12 to 15^{o}C to achieve organic load levels of 0, 2.5, 5, and 10% (w/v), respectively, in a total volume of 890 L. The following five commercial produce sanitizer treatments were assessed: 50 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 50 ppm mixed peracid (Tsunami 200, Ecolab), 50 ppm available chlorine (XY-12, Ecolab) at pH 7.85, 30 ppm available chlorine (XY-12) acidified to pH 6.5 with citric acid (Sigma-Aldrich, St. Louis, MO), and 50 ppm available chlorine (XY-12) acidified to pH 6.5 with T-128 (SmartWash Solutions) as measured with a pH probe (pHTestr 30, Oakton, Vernon Hills, IL). Peroxyacetic acid test kit 311 (Ecolab) was used to confirm the peroxyacetic acid and mixed peracid sanitizer concentrations while chlorine test kit 321 (Ecolab) was used to measure available chlorine. Sanitizer-free MSU tap water (< 0.05 ppm free chlorine) served as the control. The sanitizer concentration and pH were maintained throughout processing, with necessary adjustments made during the 10 min pauses between lettuce processing.

4.3.7 Lettuce processing. To prime the system, 5.4 kg of uninoculated iceberg lettuce was hand-fed into the shredder at a rate of about 0.5 kg per second, with the shredded product then step-conveyed to the flume tank and washed in 890 L of recirculating sanitizing wash water for 90 s. The product was then released from the flume tank and partially dewatered on the shaker table, collected in a single centrifugation basket and centrifugally dried. Following a 10

min pause in processing to adjust the sanitizer concentration, the 5.4 kg batch of inoculated lettuce was identically processed followed by three uninoculated 5.4 kg batches.

4.3.8 Sample collection. For each batch of lettuce processed after the original uninoculated priming batch, a 50 ml water sample was collected in a 50 ml centrifuge tube containing 38x concentrated Difco Neutralizing Buffer (Becton Dickinson) at the fixed flume screen after 90 s of flume washing. Additional 50-ml water samples were collected every 2 min during the 10 min pause in processing between each batch of lettuce. During centrifugal drying, a 50 ml water sample was collected from the centrifuge drain after 15 s of the 90 s cycle. After centrifugation, a 25 g lettuce sample was collected from the centrifugation basket. After processing all five batches of lettuce, nine product contact areas on the equipment (3 flume tank (Figure 2.1), 3 shaker table (Figure 2.2) and 3 dewatering centrifuge (Figure 2.3)) measuring 100 cm^2 as previously identified using Glo Germ**TM** were sampled as described by Vorst and others *(130)* using 1-ply composite tissues moistened with 1 ml of sterile Difco Neutralizing Buffer (Becton Dickinson).

4.3.9 Physiochemical parameters of flume water. Six physicochemical parameters of the wash water were monitored before processing each of the five batches of iceberg lettuce. The pH and oxidation/reduction potential (ORP) (mV) were monitored with probes pHTestr 30 and ORPTestr 10, respectively (Oakton, Vernon Hills, IL). Chemical oxygen demand (COD) (mg O_2 per L of solution) was quantified using Hach Digestion Reactor Method 8000 (Hach, Loveland, CO) *(59)*. Total solids was measured by drying 10 ml of wash water in a pre-heated and pre-weighed crucible in a drying oven (Model 625-A, Precision Scientific Inc, Chicago, IL) set at 103°C ± 2°C for 2 h to determine the mass of solids in suspension *(14)*. Turbidity was assessed

by pouring a 50 ml water sample through a 24-cm diameter Whatman Filter (Grade 113, Piscataway, NJ) to remove suspended solids, followed by measuring absorbance of a 1 ml aliquot of the filtrate at 663 nm in a spectrophotometer (Model SB-100XR, Spectronics Corporation, Westbury, NY) *(133)*. Maximum filterable volume (MFV) was quantified as the volume of a 50 ml water sample pulled through a 0.45 µm membrane in 1 min at-80 kPa using a vacuum pump (Model WP6211560, Millipore, Billerica, MA).

4.3.10 Microbiological analyses. All lettuce samples (25 g) were homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) for 1 min at 260 rpm and then either appropriately diluted in sterile 1% (w/v) phosphate buffer (8.5 g/L NaCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4, J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ) and plated on TSAYE with amp (calculated minimum detection limit of 40 CFU/g) or processed using 0.45 µm membrane filters (Millipore, Millipore Corporation, Billerica, MA) (calculated minimum detection limit of 0.04 CFU/g) which were placed on 60-mm dia. petri plates containing TSAYE with amp to quantify *E. coli* O157:H7. The 1-ply composite tissue samples were added to 15 ml of sterile Difco Neutralizing Buffer in a Whirl-Pak^{TM} bag, homogenized for 1 min at 260 rpm and then plated identically to the lettuce samples, giving a lower detection limit of 1 CFU/100 cm^2. The 50 ml water samples were either appropriately diluted in sterile 1% phosphate buffer and plated on TSAYE with amp or processed by membrane filtration, which gave a calculated minimum detection limit of 0.02, .033, 0.1 and 0.1 CFU/ml for organic loads of 0, 2.5, 5 and 10% (w/v), respectively. Following 40 – 48 h of incubation at 37°C, all green fluorescing colonies as seen under ultraviolet light (365 nm, Blak-Ray, Ultra-violet Product Inc. San Gabriel, CA) were counted as *E. coli* O157:H7.

4.3.11 Sanitizer neutralization confirmation. Identical to 3.3.10.

4.3.12 Physicochemical parameters of commercial flume water. Samples of commercial flume water containing chlorine acidified with citric acid were collected at a commercial iceberg lettuce processing facility on three separate occasions just before processing commenced and then at 30 min intervals over the next 5 hours. Water was assessed for temperature, pH, oxidation/reduction potential, available chlorine, and free chlorine as measured by a Pocket Colorimeter (Model 46770, Hach). Additional samples collected in 50 ml centrifuge tubes at the same 30 min intervals were packed in ice and shipped overnight to Michigan State University and assessed for chemical oxygen demand, maximum filterable volume, and total solids within 30 h of collection using the previously described methods.

Samples of flume water containing peroxyacetic acid were collected at a commercial iceberg lettuce processing facility on three separate occasions just before processing commenced and then at 30 min intervals over the next 5 hours. Water was assessed for temperature, pH, oxidation/reduction potential, and peroxyacetic acid content. Additional samples collected in 50 ml centrifuge tubes at the same 30 min intervals were packed in ice, transported to Michigan State University and then assessed for chemical oxygen demand, maximum filterable volume, turbidity and total solids within 8 h of their collection using the previously described methods.

4.3.13 Statistical analysis. *E. coli* O157:H7 counts were converted to log CFU per g, ml or 100 cm^2 and subjected to an ANOVA using JMP 9.0 (SAS Institute Inc., Cary, NC). Values equaling half the limit of detection were used for samples without *E. coli* O157:H7 counts. The three equipment surface samples from each respective piece of equipment were averaged. Physicochemical parameter data from the wash water were averaged for each processing replicate before averaging between the three replicates. A P value of ≤ 0.05 was

considered significant for all tests. The Tukey-Kramer HSD test was used to identify significant differences in *E. coli* O157:H7 populations for individual lettuce, water, and equipment surface samples as well as individual physicochemical parameter values. Microsoft Excel software was also used to conduct a linear regression analysis and calculate the 95% confidence intervals for the *E. coli* O157:H7 reductions in water over time. The following log linear equation was used:

$$\text{Log}(N/N_0) = -k*t$$

Where $\text{Log}(N/N_0)$ is the microbial reduction, t is the time in minutes, and k is the inactivation rate. Excel was used to determine the P and R^2 values, and JMP 9.0 software was used to determine the RMSE (log CFU/ml) by running the curves for each organic load and sanitizing agent combination.

4.4 RESULTS

4.4.1 Lettuce. In general, none of the organic loads significantly impacted sanitizer efficacy against *E. coli* O157:H7 on lettuce. Average *E. coli* O157:H7 reductions on inoculated lettuce were 0.9, 1.0 and 1.2 log CFU/g for chlorine (Figure 4.1), chlorine + CA (Figure 4.2) and chlorine + T-128 (Figure 4.3), respectively. For each of the sanitizer treatments, populations of *E. coli* O157:H7 were rarely significantly lower ($P \leq 0.05$) in the three uninoculated 5.4 kg batches that followed the inoculated batch of lettuce, regardless of the organic load in the wash water. None of the organic loads had a significant impact on chlorine alone for batches 1 and 2, with average *E. coli* O157:H7 populations of 2.6 and 2.0 log CFU/g recovered, respectively (Figure 4.1). In the third batch, populations were significantly higher ($P \leq 0.05$) for lettuce washed in water containing a 10% (2.4 log CFU/g) as compared to 0% (1.1 log CFU/g) organic load. Similar results were seen for chlorine + CA, with none of the organic load treatments having a significant impact ($P > 0.05$) on sanitizer efficacy (Figure 4.2). Average *E. coli* O157:H7 populations for batches 1 and 2 were 2.6 and 1.5 log CFU/g, respectively, while the only significant difference ($P \leq 0.05$) in batch 3 was seen at organic loads of 2.5% (0.6 CFU/g) and 5% (1.4 log CFU/g). None of the organic loads had a significant impact ($P > 0.05$) on chlorine + T-128 efficacy (Figure 4.3), with average *E. coli* O157:H7 populations of 2.4, 1.6 and 1.1 log CFU/g for batches 1, 2 and 3, respectively.

None of the organic loads significantly impacted peroxyacetic acid or mixed peracid efficacy against *E. coli* O157:H7 on lettuce. *E. coli* O157:H7 reductions on inoculated lettuce ranged from 1.0 to 1.7 log CFU/g as seen with peroxyacetic acid (Figure 4.4), with an average of 1.4 log CFU/g for mixed peracid (Figure 4.5). Regardless of the organic load in the wash water, neither sanitizer treatment significantly affected *E. coli* O157:H7 populations in the three

uninoculated 5.4 kg batches that were processed after the inoculated lettuce ($P > 0.05$). Using peroxyacetic acid, uninoculated batches 1, 2 and 3 yielded average *E. coli* O157:H7 counts of 1.8, 0.9 and 0.2 log CFU/g, respectively. Similar results were seen using mixed peracid, with average *E. coli* O157:H7 populations for batches 1, 2 and 3 of 1.7, 1.2 and 0.2 log CFU/g, respectively.

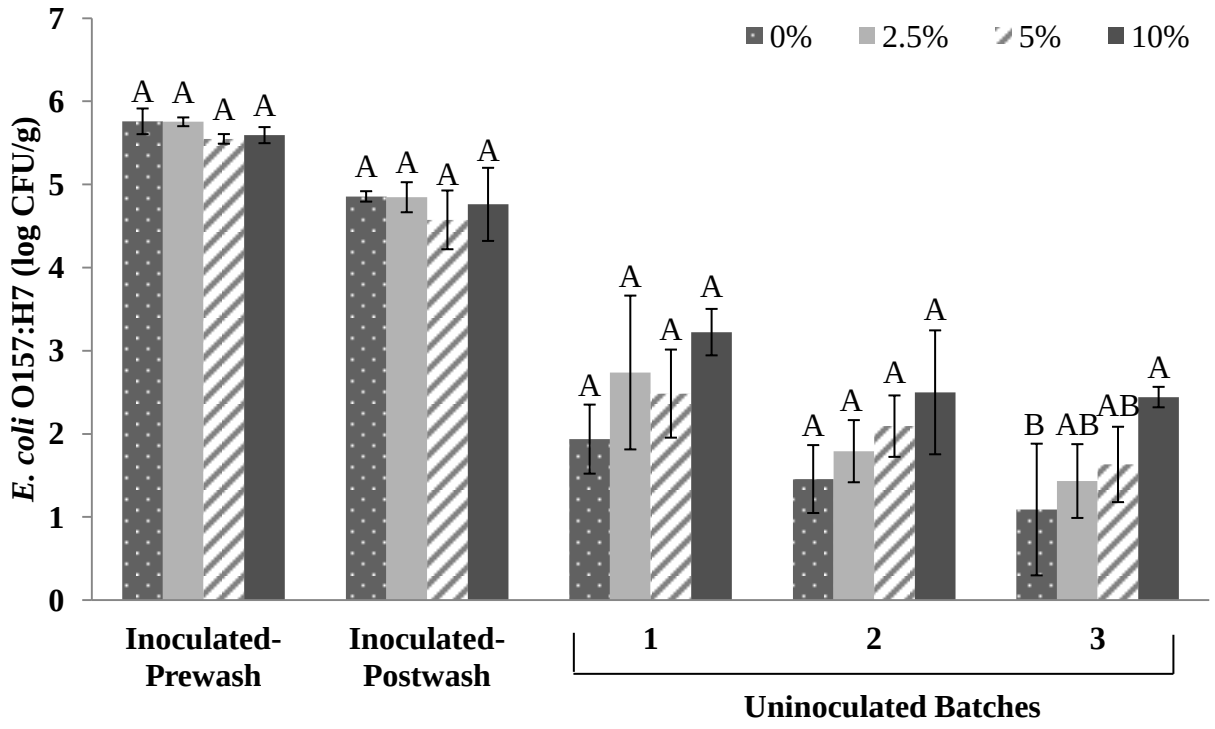


Figure 4.1: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after flume washing in water containing 50 ppm chlorine and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (P ≤ 0.05).*

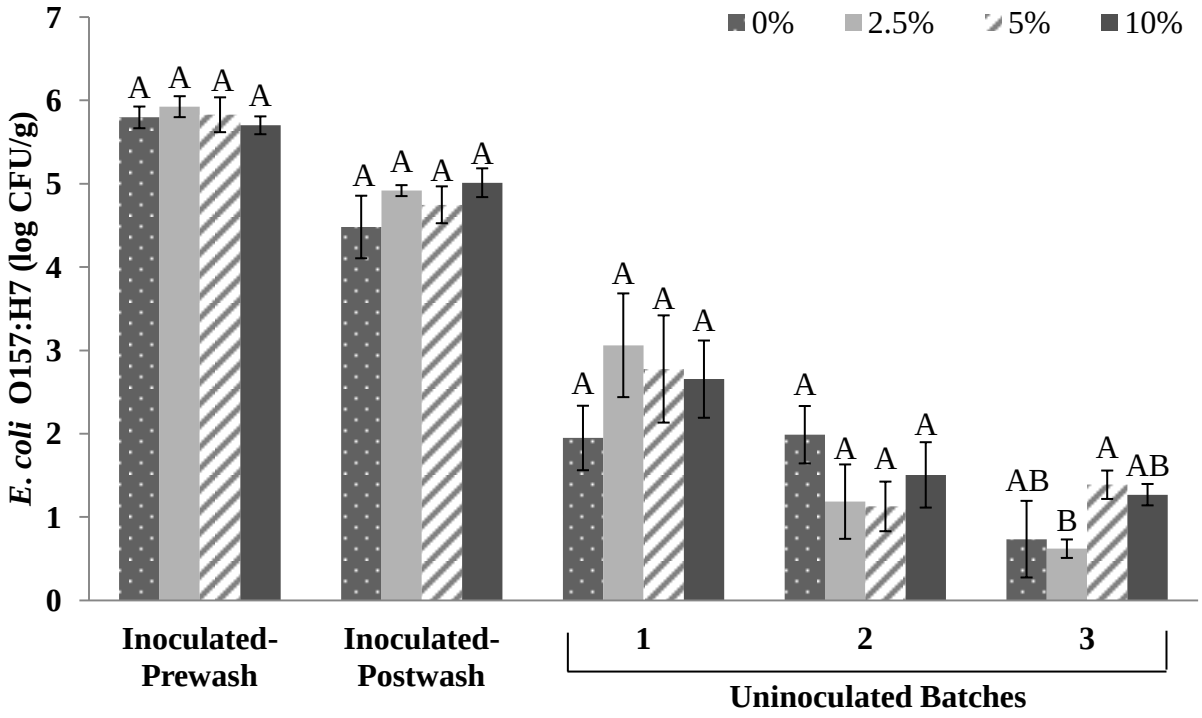


Figure 4.2: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after flume washing in water containing 50 ppm chlorine + CA and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*).*

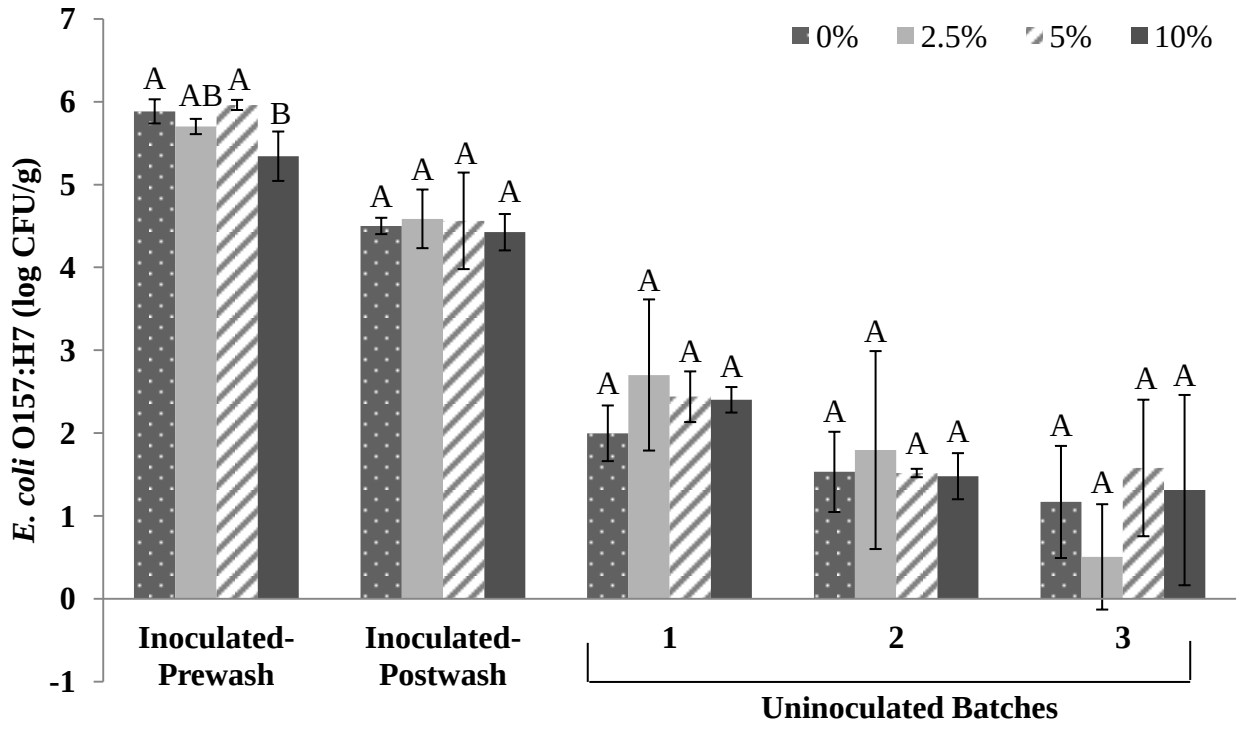


Figure 4.3: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after flume washing in water containing 50 ppm chlorine + T-128 and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*P ≤ *0.05).*

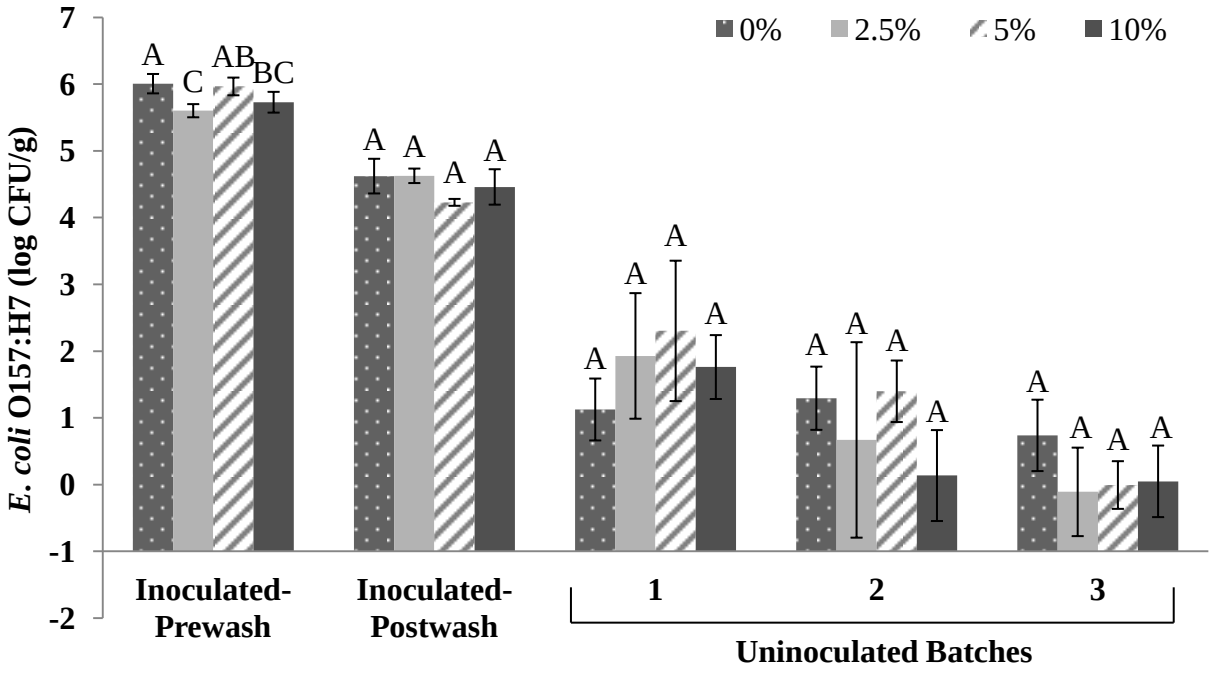


Figure 4.4: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after processing in flume water containing various organic loads and 50 ppm peroxyacetic acid (n=3 per organic load). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*).*

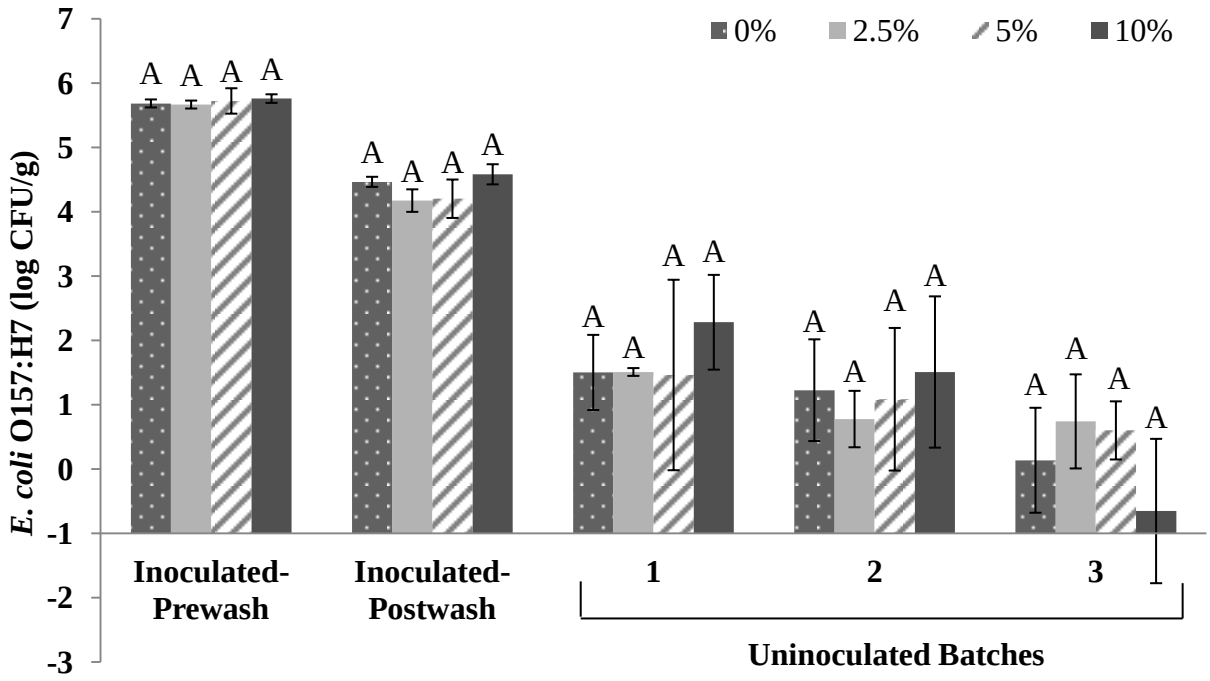


Figure 4.5: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after processing in flume water containing various organic loads and 50 ppm mixed peracid (n=3 per organic load). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*).*

4.4.2 Flume water. Average *E. coli* O157:H7 populations in sanitizer-free wash water were statistically similar ($P > 0.05$) for all organic loads, with an average of 3.9 log CFU/ml at all time points (Figure 4.6). Using organic loads of 5 and 10%, similar population reductions ($P > 0.05$) were seen for all three chlorine treatments. Average *E. coli* O157:H7 reductions at the start of processing (0 min) with organic loads of 5 and 10% were 0.2, 0.1 and 0.2 log CFU/ml for chlorine (Figure 4.7), chlorine + CA (Figure 4.8) and chlorine + T-128 (Figure 4.9), respectively. By the end of processing (44 min), average *E. coli* O157:H7 reductions were 0.9, 4.0 and 3.9 log CFU/ml for chlorine, chlorine + CA and chlorine + T-128, respectively, at a 10% organic load, with no significant difference ($P > 0.05$) seen at a 5% organic load (Table 4.1). Using chlorine alone, *E. coli* O157:H7 reductions were significantly higher ($P \leq 0.05$) after 21 min of processing (2.3 log CFU/ml) with an organic load of 2.5 than at 5 and 10% with the average final population being 3.9 log lower than the water control. Using chlorine + CA with a 2.5% organic load, the *E. coli* O157:H7 population initially decreased 0.2 log CFU/ml at the start of processing, with a final reduction of 5.2 log CFU/ml after 44 min, which was not significantly different ($P > 0.05$) from the *E. coli* O157:H7 reductions seen for the 0, 5 and 10% organic loads. Using chlorine + T-128 with a 2.5% organic load, *E. coli* O157:H7 populations decreased 0.0 and 4.8 log CFU/ml after 0 min and 44 min of processing, respectively, with these reductions statistically similar ($P > 0.05$) to those seen at organic loads of 5 and 10%. As expected, *E. coli* O157:H7 populations were typically at or below the lower limit of detection for all chlorine treatments containing a 0% organic load, with reductions of 5.2, 5.8 and 5.6 log CFU/ml seen for chlorine, chlorine + CA and chlorine + T-128, respectively, at the start of processing.

At the start of sample collection, as soon as the inoculated lettuce exited the flume tank (0 min), no significant difference ($P > 0.05$) in *E. coli* O157:H7 reductions was seen among the

different organic loads, with average reductions ranging from 2.9 – 3.6 log CFU/ml using peroxyacetic acid (Table 4.2). In addition, statistically similar ($P > 0.05$) *E. coli* O157:H7 reductions were observed at organic loads of 0 and 2.5% for peroxyacetic acid , with average reductions of 5.9 and 5.8 log CFU/ml after 21 min and 5.9 and 5.8 log CFU/ml after 44 min of processing, respectively. *E. coli* O157:H7 reductions using a 10% organic load with peroxyacetic acid after 21 (5.3 log CFU/ml) and 44 min (5.2 log CFU/ml) of processing were statistically similar ($P > 0.05$) to those seen at a 5% organic load. At the start of sample collection, organic load did not significantly impact ($P > 0.05$) mixed peracid efficacy, resulting in average *E. coli* O157:H7 reductions ranging from 3.9 – 4.9 log CFU/ml (Table 4.2). After 21 min of processing with mixed peracid, statistically similar *E. coli* O157:H7 reductions ($P > 0.05$) were observed at organic loads of 0 (5.9 log CFU/ml) and 2.5% (5.6 log CFU/ml) with no significant differences ($P > 0.05$) observed between organic loads of 5 (5.3 log CFU/ml) 10% (5.0 log CFU/ml). After 44 min of processing with mixed peracid, *E. coli* O157:H7 reductions were significantly different ($P \leq 0.05$) for each organic load, with reductions of 5.9, 5.6, 5.3 and 4.9 at organic loads of 0, 2.5, 5 and 10%, respectively.

E. coli O157:H7 populations were typically below the limit of detection in flume water containing a 0% organic load for all three chlorine treatments, resulting in y- intercepts of -5.4, -5.7 and -5.8 log CFU/ml and root-mean-square errors (RMSEs) of 0.2, 0.2 and 0.1 log CFU/ml for chlorine, chlorine + CA and chlorine + T-128, respectively (Table 4.3, Figures 4.7 – 4.9). Trend lines for chlorine alone with organic loads of 2.5, 5 and 10% had similar intercepts while the rate of inactivation differed, with 2.5% being 2.5 and 5 times greater than 5 and 10%, respectively. The y-intercept values increased with increasing organic load from -1.8, -0.7 and -0.5 log CFU/ml for 2.5, 5 and 10% organic loads, respectively, for chlorine + CA, while the

inactivation rates were similar for each of the three organic loads. Similar inactivation rates and intercepts were seen for chlorine + T-128, -2.0, -0.3 and 0.0 log CFU/ml seen for organic loads of 2.5, 5 and 10%, respectively. RMSE values decreased as organic load increased from 2.5 to 10% for each of the three chlorine treatments, with the inverse seen for the R^2 values.

Regional trend lines for chlorine, chlorine + CA and chlorine + T-128 developed for 0 – 10, 11.5 – 21, 23 – 32.5, and 34.5 – 44 min of processing showed higher R^2 values overall than the trend lines developed from 0 – 44 min of processing (Tables 4.4 – 4.7). However, the *P* values were typically higher in the regional trend lines than the 0 – 44 min trend lines.

E. coli O157:H7 populations were typically near the lower limit of detection in flume water for all organic loads, resulting in intercepts ranging from -4.6 to -5.4 log CFU/ml and -4.8 to -5.5 log CFU/ml for peroxyacetic acid mixed peracid, respectively (Table 4.8, Figures 4.10 and 4.11). Not surprisingly, the R^2 values for each linear regression were < 0.2 due to the near-horizontal slopes of the lines, indicating that exposure time is not a good predictor of *E. coli* O157:H7 inactivation in flume water containing peroxyacetic acid or mixed peracid.

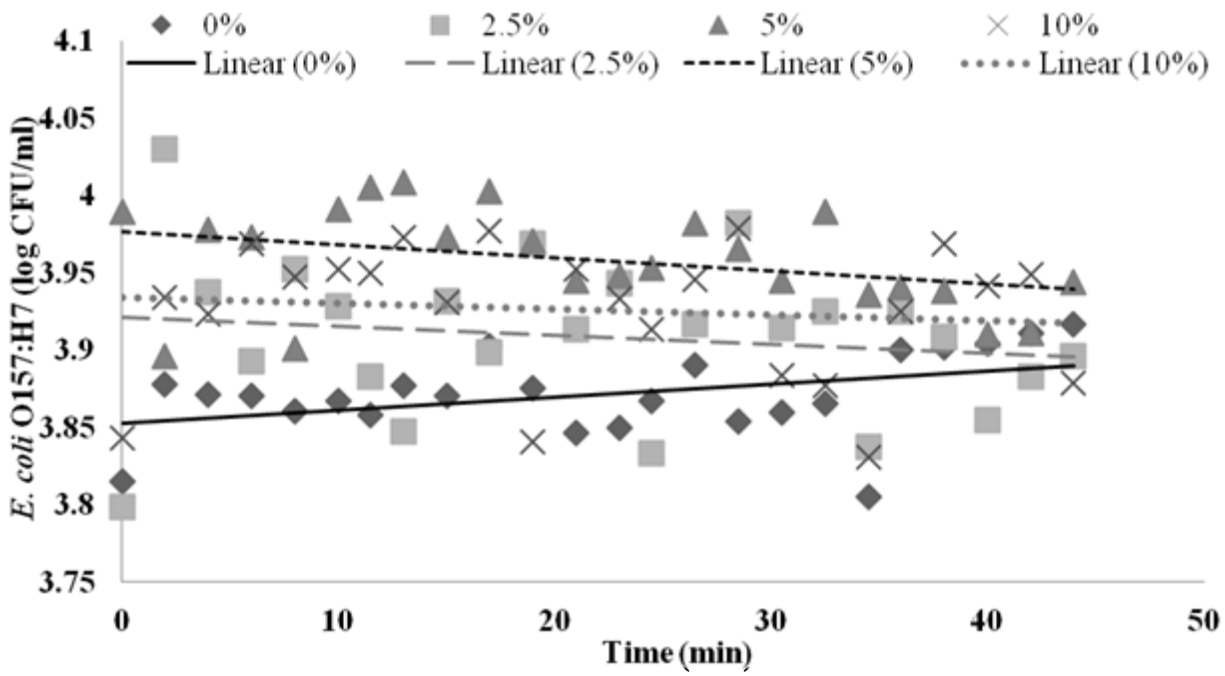


Figure 4.6: *Mean (± SD)* E. coli *O157:H7 populations in recirculating sanitizer-free flume water containing various organic loads (n=3 per organic load).*

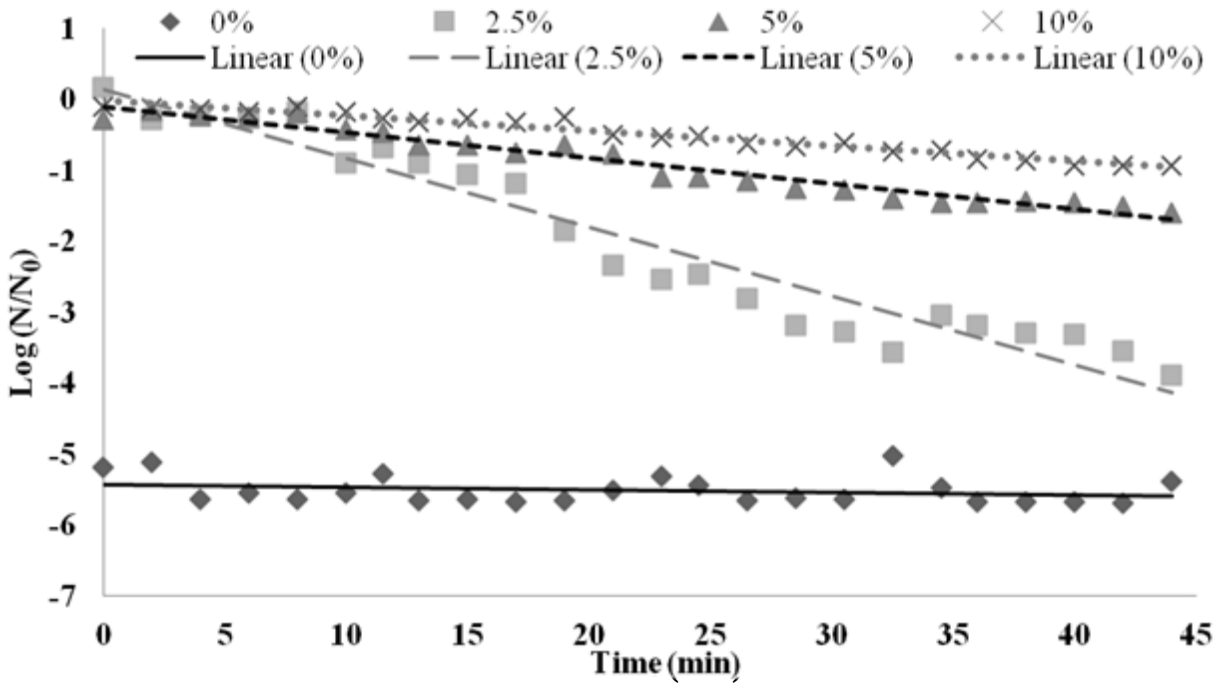


Figure 4.7: E. coli *O157:H7 inactivation in recirculating flume water containing various organic loads and chlorine compared to the sanitizer-free control (n=3 per organic load).*

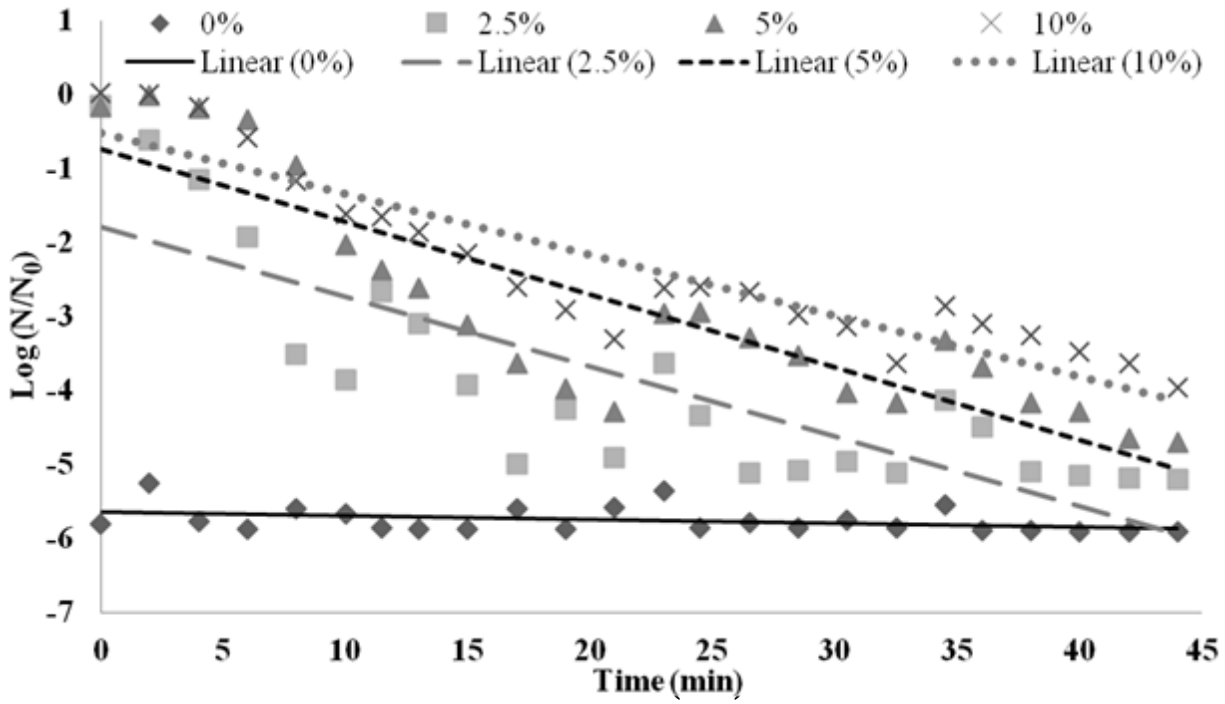


Figure 4.8: E. coli *O157:H7 inactivation in recirculating flume water containing various organic loads and chlorine + CA compared to the sanitizer-free control (n=3 per organic load).*

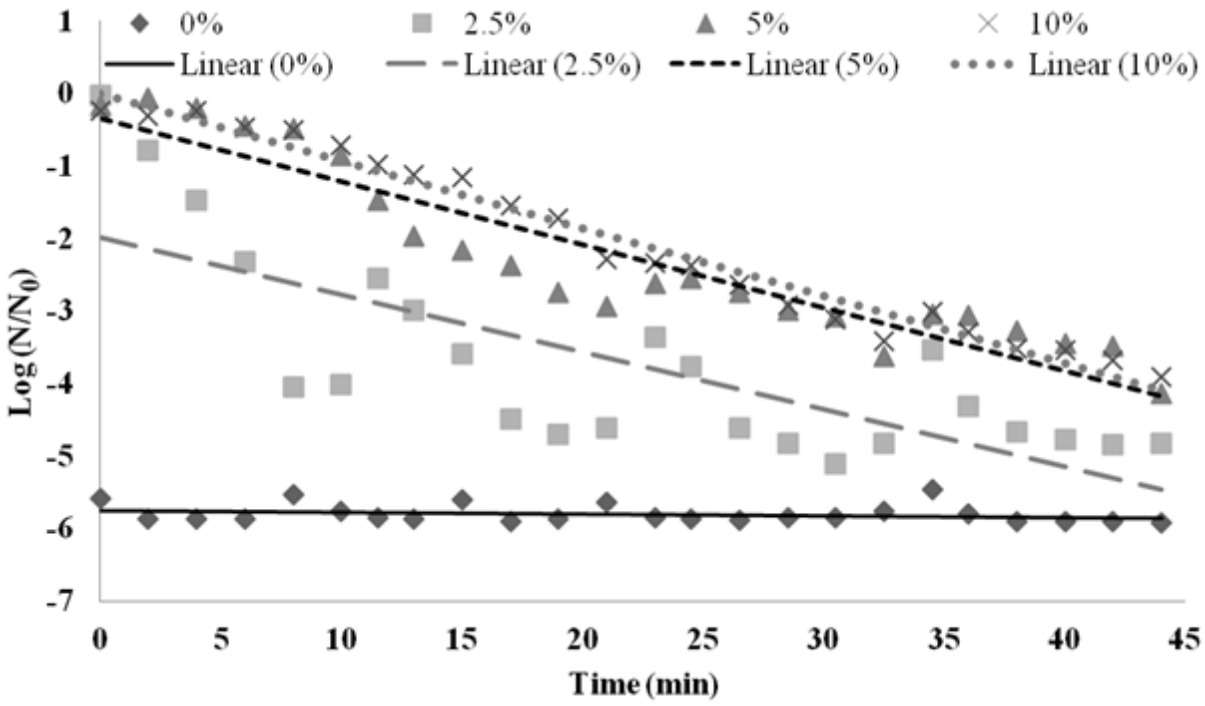


Figure 4.9: E. coli *O157:H7 inactivation in recirculating flume water containing various organic loads and chlorine + T-128 compared to the sanitizer-free control (n=3 per organic load).*

Table 4.1: E. coli *O157:H7 reductions in flume water containing chlorine, chlorine + CA and chlorine + T-128* [a]

Sanitizing Treatment	% Organic Load (w/v)	Mean ± SD *E. coli* O157:H7 reduction in flume water (log CFU/ml)		
		0 min	21 min	44 min
Chlorine	0	5.2 ± 1.1 A	5.5 ± 0.5 A	5.4 ± 0.5 A
	2.5	0.2 ± 0.3 B	2.3 ± 0.6 B	3.9 ± 0.9 A
	5	0.3 ± 0.2 B	0.8 ± 0.4 C	1.6 ± 0.7 B
	10	0.1 ± 0.2 B	0.5 ± 0.2 C	0.9 ± 0.3 B
Chlorine + CA	0	5.8 ± 0.1 A	5.6 ± 0.6 A	5.9 ± 0.0 A
	2.5	0.2 ± 0.0 B	4.9 ± 0.3 AB	5.2 ± 0.1 AB
	5	0.2 ± 0.4 B	4.3 ± 0.4 AB	4.7 ± 0.7 AB
	10	0.0 ± 0.1 B	3.3 ± 1.4 B	4.0 ± 1.2 B
Chlorine + T-128	0	5.6 ± 0.5 A	5.7 ± 0.5 A	5.9 ± 0.0 A
	2.5	0.0 ± 0.2 B	4.6 ± 0.7 A	4.8 ± 0.6 AB
	5	0.2 ± 0.1 B	2.9 ± 0.4 B	4.2 ± 1.1 AB
	10	0.2 ± 0.5 B	2.3 ± 0.2 B	3.9 ± 0.4 B

[a] Means with different capital letters within a given sanitizing treatment designate *E. coli* O157:H7 reductions that differ significantly in terms of sanitizing treatment and time ($P \leq 0.05$).

Table 4.2: E. coli *O157:H7 reduction in flume water containing peroxyacetic acid and mixed peracid* [a]

Sanitizing Treatment	% Organic Load (w/v)	Mean ± SD *E. coli* O157:H7 reduction in flume water (log CFU/ml)		
		0 min	21 min	44 min
Peroxyacetic Acid	0	3.6 ± 0.2 A	5.9 ± 0.2 A	5.9 ± 0.0 A
	2.5	3.6 ± 0.4 A	5.8 ± 0.1 A	5.8 ± 0.1 A
	5	2.9 ± 0.4 A	5.3 ± 0.0 B	5.3 ± 0.0 B
	10	3.5 ± 0.1 A	5.3 ± 0.0 B	5.2 ± 0.1 B
Mixed Peracid	0	4.1 ± 0.8 A	5.9 ± 0.2 A	5.9 ± 0.0 A
	2.5	5.0 ± 0.3 A	5.6 ± 0.2 A	5.6 ± 0.2 B
	5	3.9 ± 1.5 A	5.3 ± 0.0 B	5.3 ± 0.0 C
	10	4.0 ± 1.3 A	5.0 ± 0.0 B	4.9 ± 0.1 D

[a] Means with different capital letters within a given sanitizing treatment designate *E. coli* O157:H7 reductions that differ significantly in terms of sanitizing treatment and time ($P \leq 0.05$).

Table 4.3: E. coli *O157:H7 inactivation trend lines for flume water containing various organic loads and chlorine, chlorine + CA and chlorine + T-128* [a]

Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
-	0	50 ± 4	$Log(N/N_0) = -5.4$ [b]	0.06	0.2	0.26
	2.5	50 ± 4	$Log(N/N_0) = -0.1t + 0.1$	0.94	0.3	< 0.0001
	5	50 ± 6	$Log(N/N_0) = -0.1$	0.96	0.1	< 0.0001
	10	50 ± 3	$Log(N/N_0) = 0.0$	0.96	0.1	< 0.0001
CA	0	50 ± 4	$Log(N/N_0) = -5.7$	0.14	0.2	0.07
	2.5	50 ± 3	$Log(N/N_0) = -0.1t - 1.8$	0.70	0.9	< 0.0001
	5	50 ± 4	$Log(N/N_0) = -0.1t - 0.7$	0.78	0.7	< 0.0001
	10	50 ± 2	$Log(N/N_0) = -0.1t - 0.5$	0.85	0.5	< 0.0001
T-128	0	50 ± 6	$Log(N/N_0) = -5.8$ [b]	0.06	0.1	0.25
	2.5	50 ± 2	$Log(N/N_0) = -0.1t – 2.0$	0.60	0.6	< 0.0001
	5	50 ± 4	$Log(N/N_0) = -0.1t - 0.3$	0.89	0.4	< 0.0001
	10	50 ± 2	$Log(N/N_0) = -0.1t$	0.98	0.2	< 0.0001

Table 4.3 (cont'd)

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

[b] The slope (*k*) was < 0.1 and was therefore not included.

Table 4.4: E. coli *O157:H7 inactivation trend lines for flume water containing various organic loads and chlorine, chlorine + CA, or chlorine + T-128 (0 – 10 min)* [a]

Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
-	0	50 ± 4	$Log(N/N_0) = -5.2$ [b]	0.55	0.2	0.09
	2.5	50 ± 4	$Log(N/N_0) = -0.1t + 0.1$	0.61	0.3	0.07
	5	50 ± 6	$Log(N/N_0) = -0.2$	0.26	0.1	0.31
	10	50 ± 3	$Log(N/N_0) = -0.1$	0.24	0.0	0.32
CA	0	50 ± 4	$Log(N/N_0) = -5.6$	0.01	0.2	0.86
	2.5	50 ± 3	$Log(N/N_0) = -0.4t + 0.1$	0.96	1.5	< 0.001
	5	50 ± 4	$Log(N/N_0) = -0.2t + 0.3$	0.73	0.8	0.03
	10	50 ± 2	$Log(N/N_0) = -0.2t + 0.3$	0.91	0.7	< 0.001
T-128	0	50 ± 6	$Log(N/N_0) = -5.8$	0.00	0.2	0.93
	2.5	50 ± 2	$Log(N/N_0) = -0.4t + 0.1$	0.96	1.7	< 0.0001
	5	50 ± 4	$Log(N/N_0) = -0.1t$	0.83	0.3	0.01
	10	50 ± 2	$Log(N/N_0) = -0.1t - 0.2$	0.84	0.2	0.01

Table 4.4 (cont'd)

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (t) in minutes.

[b] The slope (k) was < 0.1 and was therefore not included.

Table 4.5: E. coli *O157:H7 inactivation trend lines for flume water containing various organic loads and chlorine, chlorine + CA, or chlorine + T-128 (11.5 – 21 min)* [a]

Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
-	0	50 ± 4	$Log(N/N_0) = -5.3$ [b]	0.16	0.2	0.43
	2.5	50 ± 4	$Log(N/N_0) = -0.2t + 1.4$	0.92	0.6	< 0.01
	5	50 ± 6	$Log(N/N_0) = -0.3$	0.60	0.1	0.07
	10	50 ± 3	$Log(N/N_0) = -0.1$	0.32	0.1	0.24
CA	0	50 ± 4	$Log(N/N_0) = -6.2$	0.38	0.1	0.19
	2.5	50 ± 3	$Log(N/N_0) = -0.2t - 0.3$	0.78	1.0	0.02
	5	50 ± 4	$Log(N/N_0) = -0.2t - 0.1$	0.99	0.8	< 0.0001
	10	50 ± 2	$Log(N/N_0) = -0.2t - 0.4$	0.99	0.6	< 0.0001
T-128	0	50 ± 6	$Log(N/N_0) = -6.0$	0.10	0.1	0.54
	2.5	50 ± 2	$Log(N/N_0) = -0.2t - 0.1$	0.90	0.9	< 0.01
	5	50 ± 4	$Log(N/N_0) = -0.2t - 0.1$	0.97	0.5	< 0.01
	10	50 ± 2	$Log(N/N_0) = -0.1t + 0.6$	0.92	0.5	< 0.01

Table 4.5 (cont'd)

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (t) in minutes.

[b] The slope (k) was < 0.1 and was therefore not included.

Table 4.6: E. coli *O157:H7 inactivation trend lines for flume water containing various organic loads and chlorine, chlorine + CA, or chlorine + T-128 (23 – 32.5 min)* [a]

Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
-	0	50 ± 4	$Log(N/N_0) = -5.9$ [b]	0.05	0.3	0.66
	2.5	50 ± 4	$Log(N/N_0) = -0.1t + 0.3$	0.95	0.4	< 0.001
	5	50 ± 6	$Log(N/N_0) = -0.4$	0.94	0.1	< 0.001
	10	50 ± 3	$Log(N/N_0) = -0.1$	0.76	0.1	< 0.001
CA	0	50 ± 4	$Log(N/N_0) = -4.9$	0.34	0.2	0.22
	2.5	50 ± 3	$Log(N/N_0) = -0.1t -1.0$	0.64	0.6	0.06
	5	50 ± 4	$Log(N/N_0) = -0.1t + 0.5$	0.96	0.5	< 0.001
	10	50 ± 2	$Log(N/N_0) = -0.1t$	0.88	0.4	0.01
T-128	0	50 ± 6	$Log(N/N_0) = -6.1$	0.39	0.0	0.19
	2.5	50 ± 2	$Log(N/N_0) = -0.2t + 0.3$	0.78	0.7	0.02
	5	50 ± 4	$Log(N/N_0) = -0.1t - 0.1$	0.88	0.4	0.01
	10	50 ± 2	$Log(N/N_0) = -0.1t + 0.4$	0.99	0.4	< 0.0001

Table 4.6 (cont'd)

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

[b] The slope (*k*) was < 0.1 and was therefore not included.

Table 4.7: E. coli *O157:H7 inactivation trend lines for flume water containing various organic loads and chlorine, chlorine + CA, or chlorine + T-128 (34.5 – 44 min)* [a]

Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
-	0	50 ± 4	$Log(N/N_0) = -5.9$ [b]	0.04	0.1	0.70
	2.5	50 ± 4	$Log(N/N_0) = -0.3$	0.91	0.2	< 0.01
	5	50 ± 6	$Log(N/N_0) = -0.9$	0.66	0.1	0.05
	10	50 ± 3	$Log(N/N_0) = -0.1$	0.77	0.1	0.02
CA	0	50 ± 4	$Log(N/N_0) = -0.48$	0.43	0.1	0.16
	2.5	50 ± 3	$Log(N/N_0) = -0.1t - 0.7$	0.74	0.5	0.03
	5	50 ± 4	$Log(N/N_0) = -0.2t + 1.6$	0.94	0.5	< 0.01
	10	50 ± 2	$Log(N/N_0) = -0.1t + 0.8$	0.99	0.4	< 0.0001
T-128	0	50 ± 6	$Log(N/N_0) = -4.4$	0.57	0.2	0.08
	2.5	50 ± 2	$Log(N/N_0) = -0.1t + 0.1$	0.71	0.5	0.04
	5	50 ± 4	$Log(N/N_0) = -0.1t + 0.7$	0.86	0.4	< 0.01
	10	50 ± 2	$Log(N/N_0) = -0.1t - 0.2$	0.94	0.3	< 0.01

Table 4.7 (cont'd)

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

[b] The slope (*k*) was < 0.1 and was therefore not included.

Table 4.8: E. coli *O157:H7 inactivation trend lines in flume water containing various organic loads and peroxyacetic acid and mixed peracid* [a]

Sanitizing Agent	% Org. Load (w/v)	POAA (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
Peroxyacetic Acid	0	48 ± 4	Log(N/N_0) = -5.4 [b]	0.13	0.5	0.09
	2.5	50 ± 4	Log(N/N_0) = -5.3	0.16	0.4	0.05
	5	51 ± 4	Log(N/N_0) = -4.6	0.19	0.5	0.03
	10	49 ± 2	Log(N/N_0) = -4.9	0.14	0.4	0.07
Mixed Peracid	0	49 ± 2	Log(N/N_0) = -5.5	0.16	0.4	0.05
	2.5	49 ± 2	Log(N/N_0) = -5.5	0.12	0.1	0.10
	5	49 ± 4	Log(N/N_0) = - 5.1	0.10	0.3	0.12
	10	48 ± 3	Log(N/N_0) = -4.8	0.08	0.2	0.18

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

[b] The slope (*k*) was < 0.1 and was therefore not included.

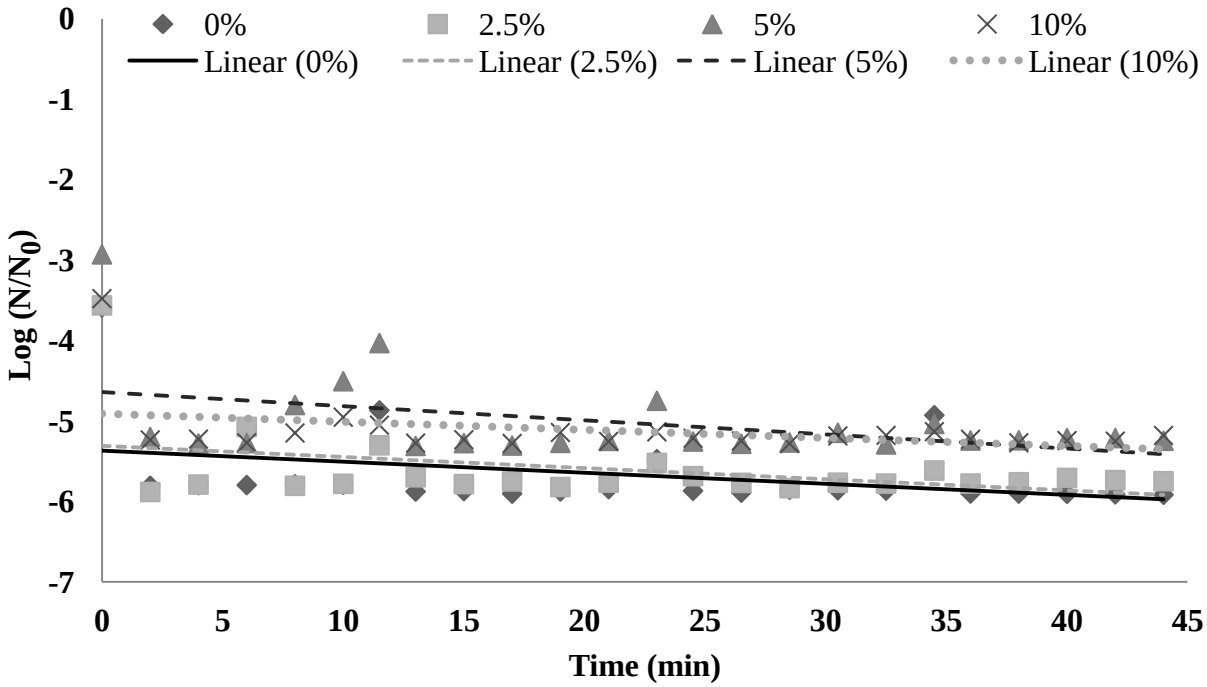


Figure 4.10: E. coli *O157:H7 inactivation in recirculating flume water containing various organic loads and peroxyacetic acid compared to the sanitizer-free control (n=3 per organic load).*

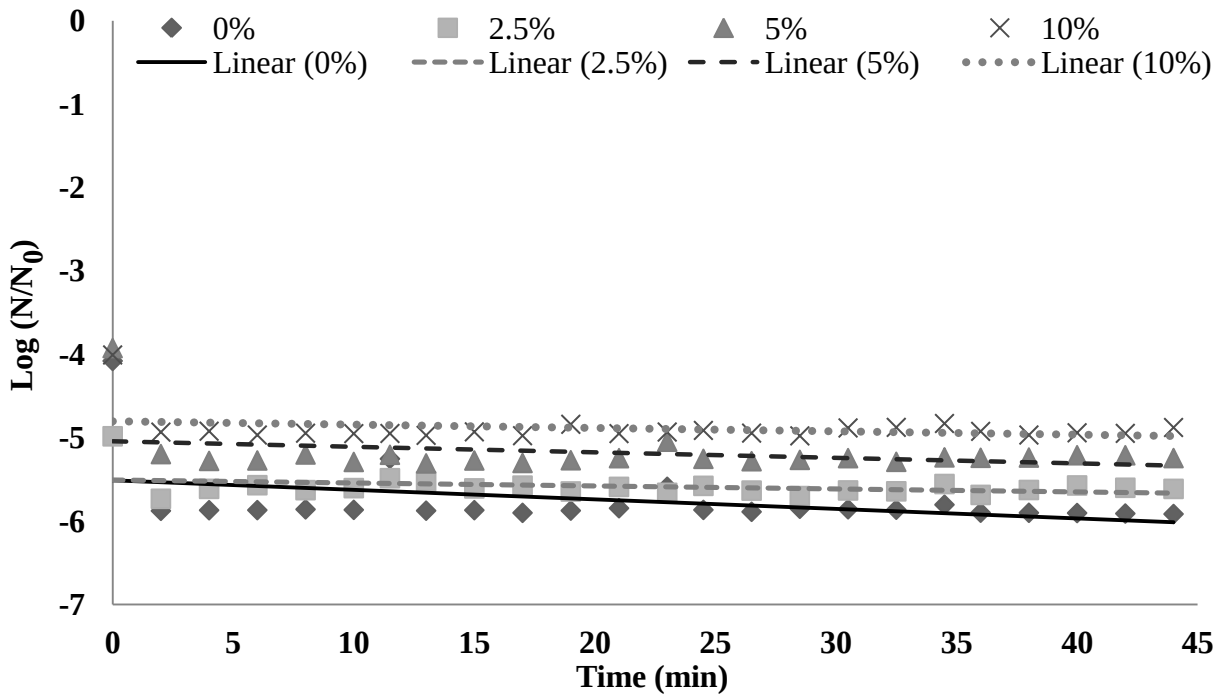


Figure 4.11: E. coli *O157:H7 inactivation in recirculating flume water containing various organic loads and mixed peracid compared to the sanitizer-free control (n=3 per organic load).*

4.4.3 Centrifugation water. Regardless of organic load, water exiting the centrifuge drain after spin-drying yielded similar *E. coli* O157:H7 populations in the absence of sanitizers ($P > 0.05$), with average *E. coli* O157:H7 populations of 4.8 log CFU/ml for the inoculated batch and 4.0 log CFU/ml for batches 1 – 3. Chlorine with an organic load of 10% resulted in *E. coli* O157:H7 populations of 4.6, 3.8, 3.3 and 3.1 log CFU/ml in the inoculated batch and batches 1, 2 and 3, respectively, none of which were significantly different ($P > 0.05$) than the populations recovered at a 5% organic load (Figure 4.12). Using chlorine alone *E. coli* O157:H7 populations were significantly lower ($P \leq 0.05$) at an organic load of 2.5 as compared to 10% for batches 2 (2.2 log CFU/ml) and 3 (1.8 log CFU/ml). At an organic load of 0% *E. coli* O157:H7 populations were significantly lower than the other organic loads, with -0.1, -0.7, 0.1, and -0.4 log CFU/ml seen in the inoculated batch and batches 1, 2 and 3, respectively. Using chlorine + CA, an organic load of 10% resulted in *E. coli* O157:H7 populations that were not significantly different ($P > 0.05$) from the 2.5 or 5% organic loads at any batch, with 4.8, 3.3, 2.1 and 1.8 log CFU/ml seen in the inoculated batch and batches 1, 2 and 3, respectively (Figure 4.13). Using chlorine + CA at an organic load of 0%, *E. coli* O157:H7 populations were significantly lower ($P \leq 0.05$) compared to the other organic loads, with populations of -1.9, -1.8, -1.8 and -1.2 log CFU/ml seen for the inoculated batch and batches 1, 2 and 3, respectively. Organic load treatments of 2.5, 5 and 10% yielded statistically similar ($P > 0.05$) *E. coli* O157:H7 populations for the inoculated batch and batches 2 and 3 when chlorine + T-128 was used, with 4.4, 2.1 and 1.6 log CFU/ml, respectively, recovered at a 10% organic load (Figure 4.14). Using chlorine + T-128 at an organic load of 0%, *E. coli* O157:H7 populations were significantly lower ($P \leq 0.05$) in all batches, with -1.1, -1.6, -1.9 and -2.0 log CFU/ml for the inoculated batch and batches 1, 2 and 3, respectively.

Organic load rarely affected peroxyacetic acid (Figure 4.15) or mixed peracid (Figure 4.16) efficacy, with statistically similar *E. coli* O157:H7 populations seen in centrifugation water for each batch of iceberg lettuce processed at the various organic loads. After washing the inoculated batch of lettuce with peroxyacetic acid in the presence of a 2.5% organic load, *E. coli* O157:H7 populations in the centrifugation water ranged from 2.3 – 2.9 log CFU/ml, with these populations significantly lower than those observed at organic loads of 5 and 10%. Regardless of the organic load, statistically similar *E. coli* O157:H7 populations ($P > 0.05$) were shed in the centrifugation water after peroxyacetic acid washing of lettuce, with average populations of 1.4 and 0.5 log CFU/ml, respectively. After washing in peroxyacetic acid, the third uninoculated batch of lettuce shed *E. coli* O157:H7 populations into the centrifugation water that were significantly lower ($P \leq 0.05$) at an organic load of 2.5 (-0.1 CFU/ml) as compared to 5% (0.8 CFU/ml). Organic load did not significantly impact ($P > 0.05$) the efficacy of mixed peracid, with average *E. coli* O157:H7 populations of 2.4, 1.0, 0.1, and -0.1 log CFU/ml for the inoculated batch and uninoculated batches 1, 2 and 3, respectively.

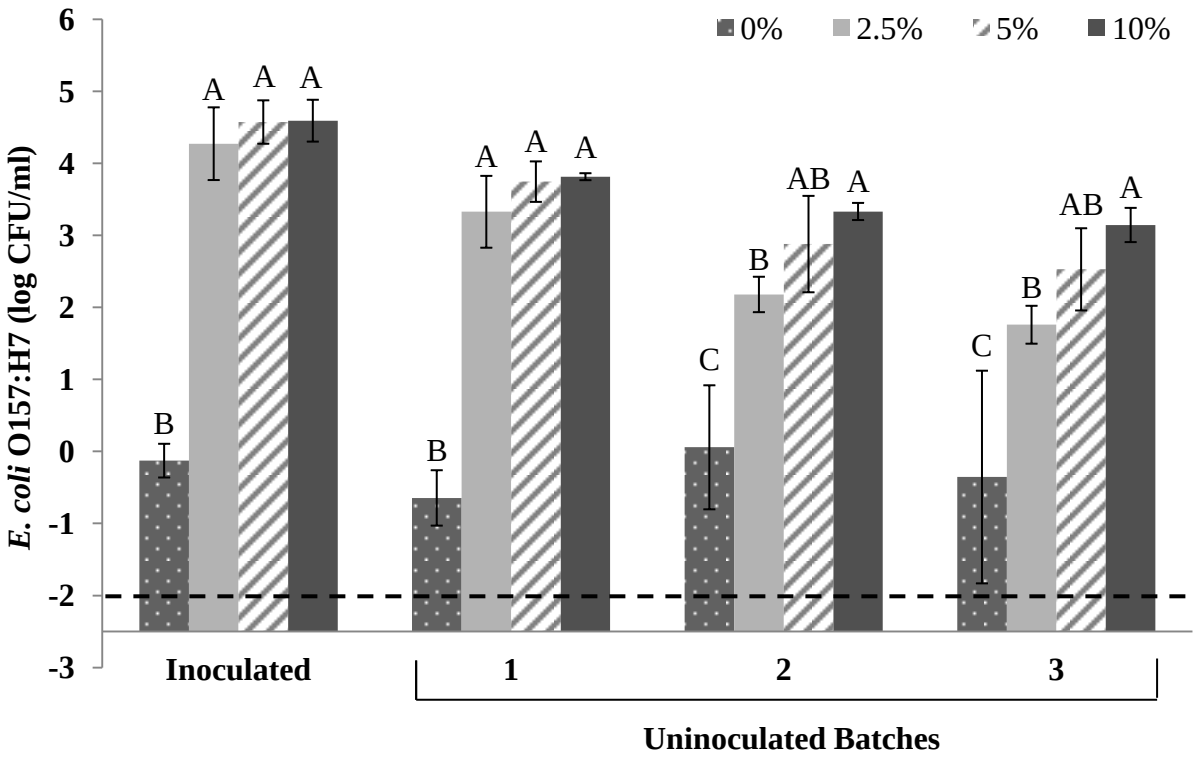


Figure 4.12: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing chlorine and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*P ≤ *0.05). Limit of detection = 0.02 CFU/ml.*

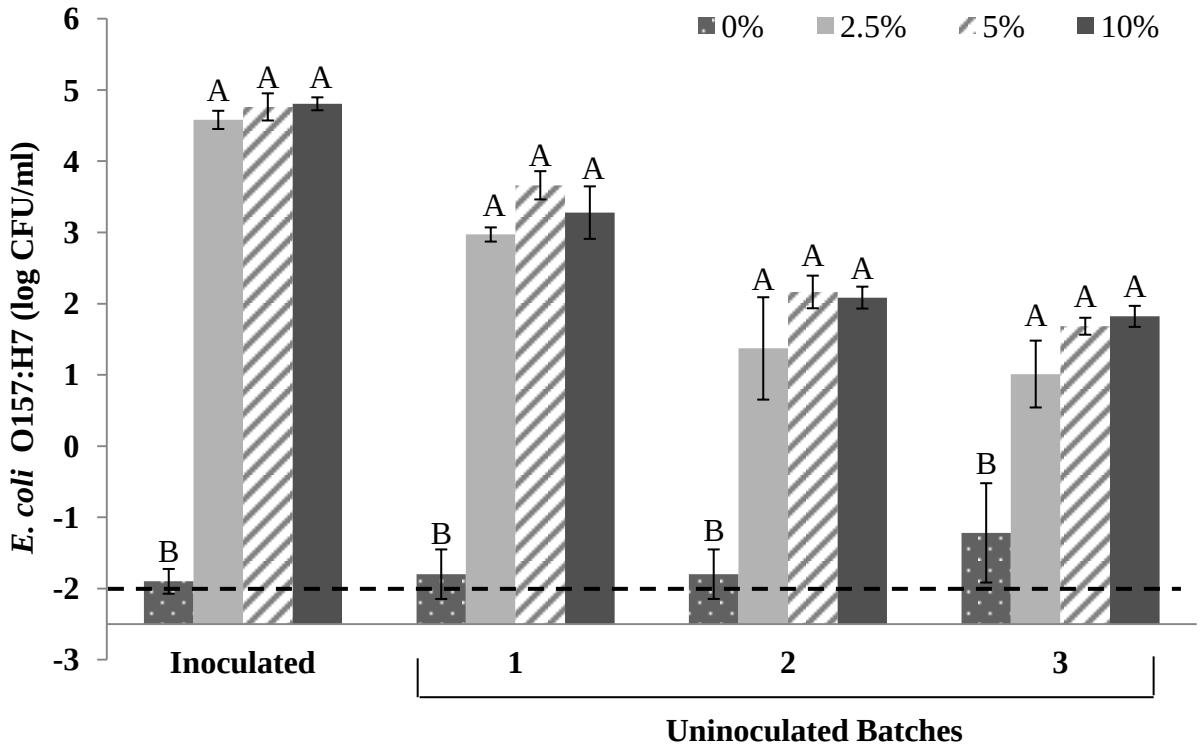


Figure 4.13: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing chlorine + CA and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (P ≤ 0.05). Limit of detection = 0.02 CFU/ml.*

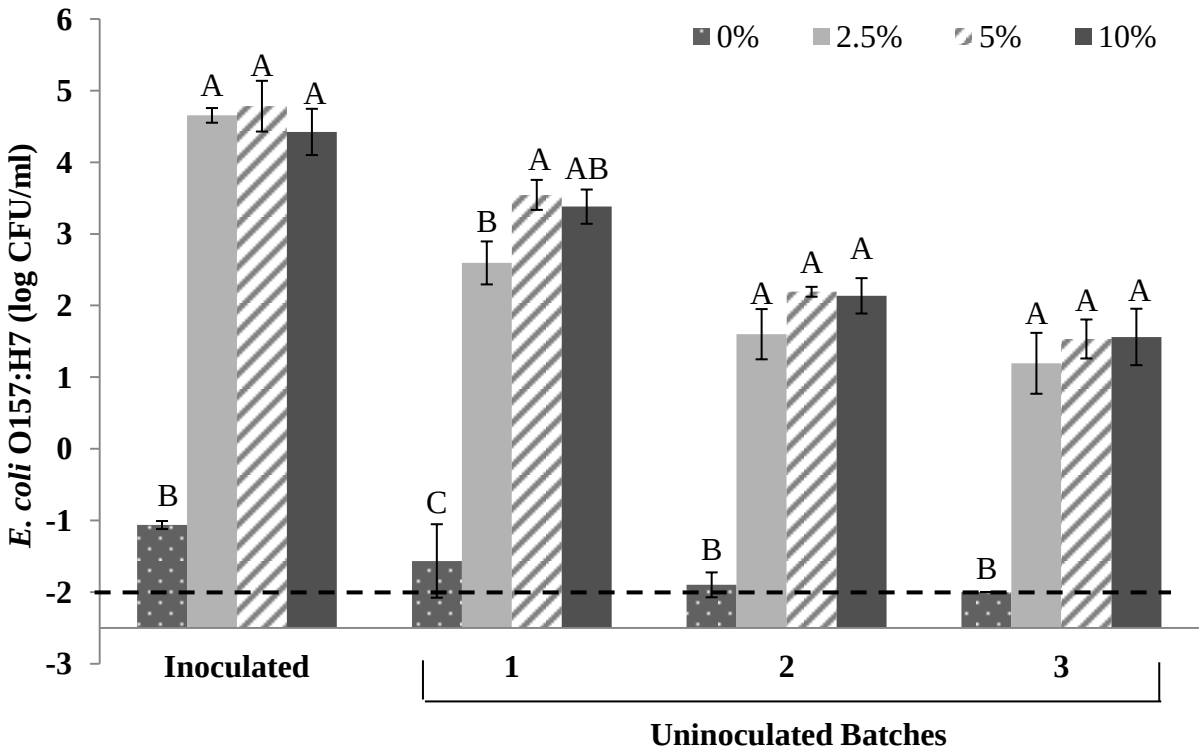


Figure 4.14: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing chlorine + T-128 and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*P ≤ *0.05). Limit of detection = 0.02 CFU/ml.*

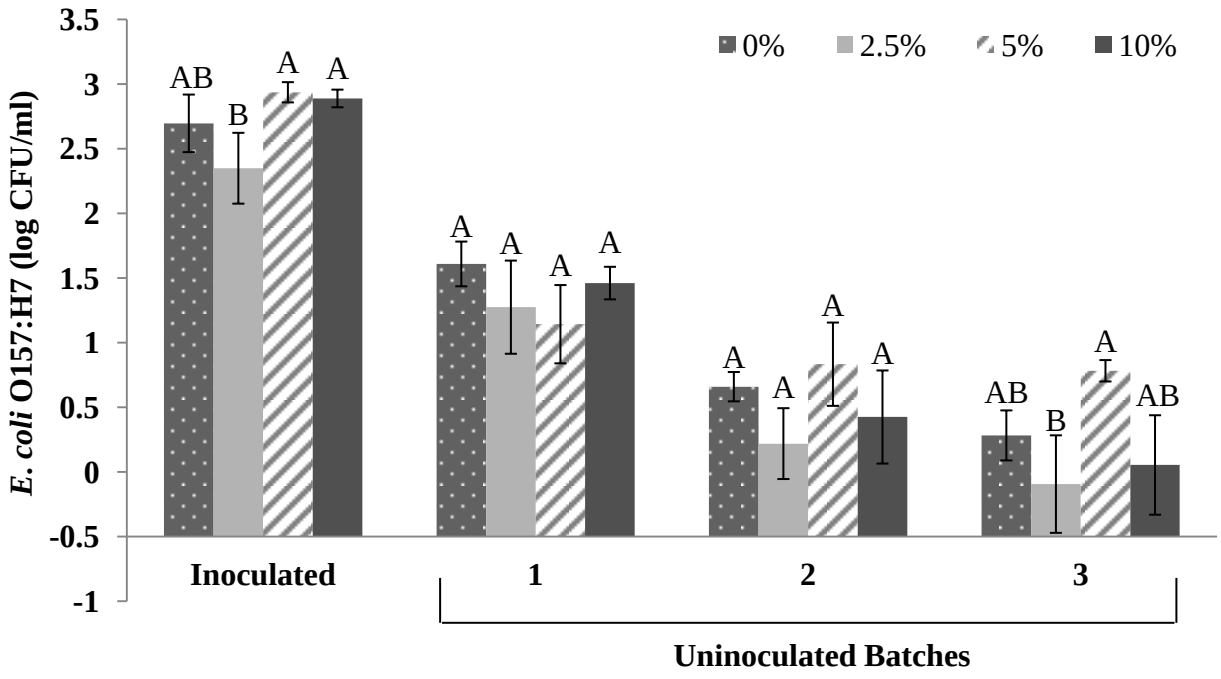


Figure 4.15: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing peroxyacetic acid and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*). Limit of detection = 0.02 CFU/ml.*

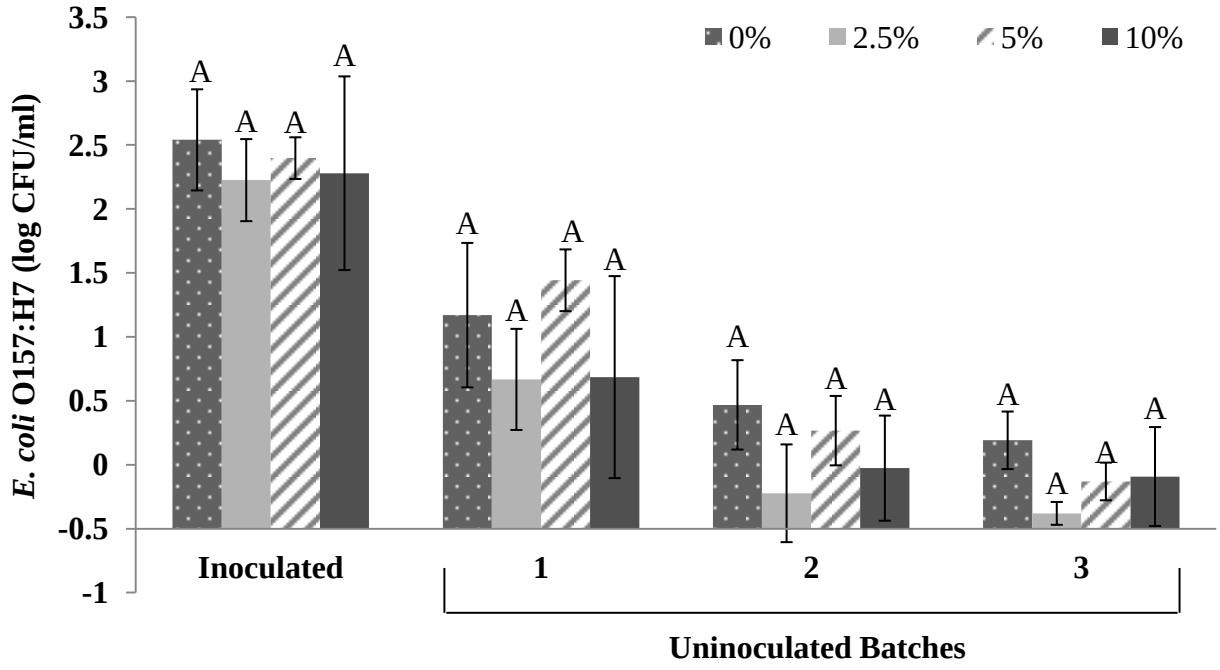


Figure 4.16: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing mixed peracid and various organic loads (n=3 per organic load). Means within the same batch with different letters are significantly different (*P ≤ *0.05). Limit of detection = 0.02 CFU/ml.*

4.4.4 Processing equipment surfaces. After iceberg lettuce was processed in chlorine-free water, statistically similar ($P > 0.05$) *E. coli* O157:H7 populations of 3.5, 3.6 and 3.3 log CFU/100 cm^2 were recovered from the flume tank, shaker table, and centrifugal dryer, respectively, regardless of organic load. When lettuce was washed with chlorine containing a 10% organic load, *E. coli* O157:H7 populations of 2.6, 2.9 and 3.0 log CFU/100 cm^2 were recovered from the flume tank, shaker table, and centrifugal dryer, respectively, with these populations statistically similar ($P > 0.05$) to those seen at a 5% organic load (Figure 4.17). Chlorine efficacy significantly decreased at organic loads above 2.5% ($P \leq 0.05$) for water samples collected from the flume tank and shaker table. However, *E. coli* O157:H7 populations recovered from the centrifugal dryer were not significantly different ($P > 0.05$) than those recovered after washing with a 5 or 10% organic load, with 2.3 log CFU/100 cm^2. Significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations were seen on the flume tank using chlorine without an organic load (-0.1 log CFU/100 cm^2), while *E. coli* O157:H7 populations on the shaker table (0.4 log CFU/100 cm^2) and centrifugal dryer (1.6 log CFU/100 cm^2) were not significantly different using a 2.5% organic load. Chlorine + CA containing an organic load of 10% yielded *E. coli* O157:H7 populations of 0.6, 1.2, and 2.4 log CFU/100 cm^2 on the flume tank, shaker table and centrifugal dryer, respectively, all of which were statistically similar to the populations seen at an organic load of 5% (Figure 4.18). In the absence of an organic load, *E. coli* O157:H7 populations were significantly lower ($P \leq 0.05$) on the flume tank (-0.1 log CFU/100 cm^2), shaker table (0.1 log CFU/100 cm^2), and centrifugal dryer (0.6 log CFU/100

cm^2) compared to a 10% organic load. Chlorine + T-128 containing a 10% organic load yielded *E. coli* O157:H7 populations of 0.4, 1.1 and 2.1 log CFU/100 cm^2 on the flume tank , shaker table and centrifugal dryer, respectively, all of which were statistically similar ($P > 0.05$) to those populations seen at organic loads of 2.5 and 5% (Figure 4.19). Significantly lower ($P \leq 0.05$) *E. coli* O157:H7 populations were also seen on the shaker table and centrifugal dryer using an organic load of 0 as compared to 10%.

Using peroxyacetic acid, *E. coli* O157:H7 populations from the flume tank were significantly lower ($P \leq 0.05$) at organic loads of 0 (-0.2 log CFU/100 cm^2) and 2.5% (-0.3 log CFU/100 cm^2) as compared to 5 (0.1 log CFU/100 cm^2) and 10% (0.4 log CFU/100 cm^2) (Figure 4.20). Regardless of organic load, statistically similar *E. coli* O157:H7 populations were recovered from the shaker table (0.1 to 0.6 log CFU/100 cm^2) and centrifugal dryer (-0.3 to 0.2 log CFU/100 cm^2) using peroxyacetic acid. In addition, no significant differences ($P > 0.05$) in *E. coli* O157:H7 populations were seen on the flume tank (-0.2 to -0.1 log CFU/100 cm^2), shaker table (-0.2 to -0.1 log CFU/100 cm^2), and centrifugal dryer (-0.3 to -0.1 log CFU/100 cm^2) between any of the organic loads when mixed peracid was used (Figure 4.21).

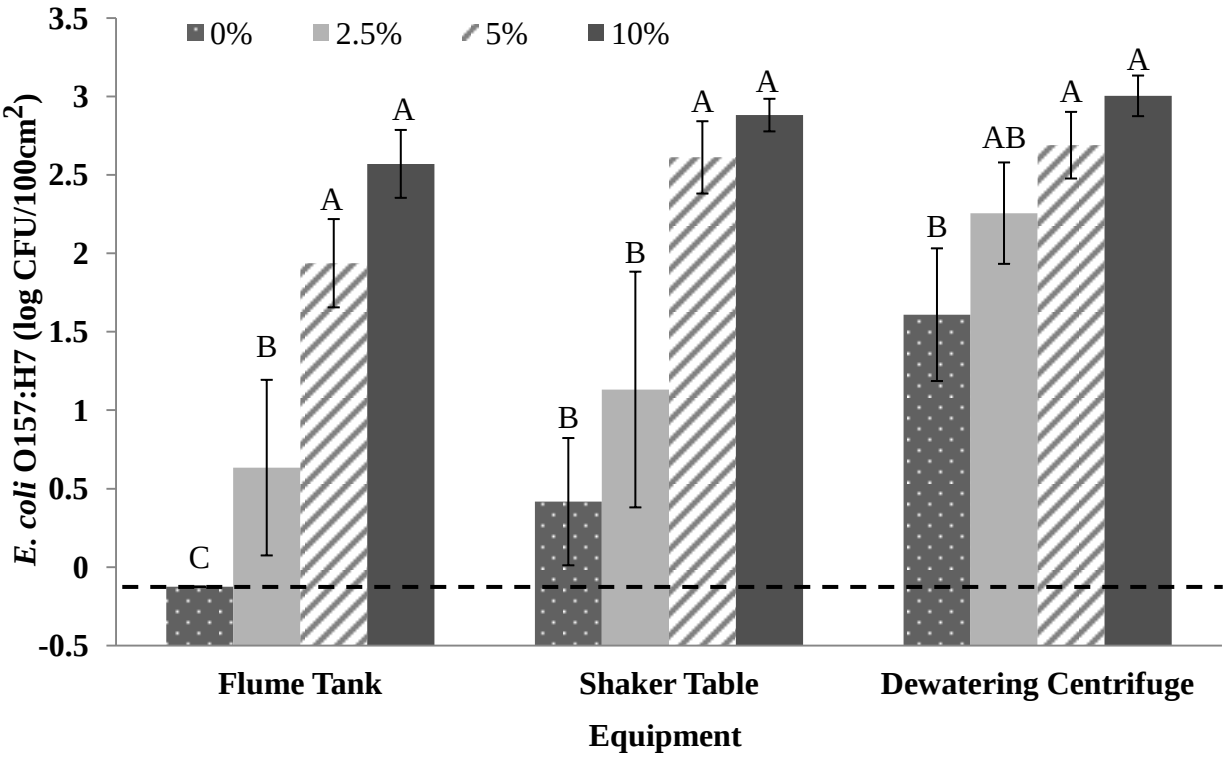


Figure 4.17: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing chlorine and various organic loads (n=3 per organic load). Means within the same equipment with different letters are significantly different (*$P \leq 0.05$*). Limit of detection = 1 CFU/100 cm*^{2}.

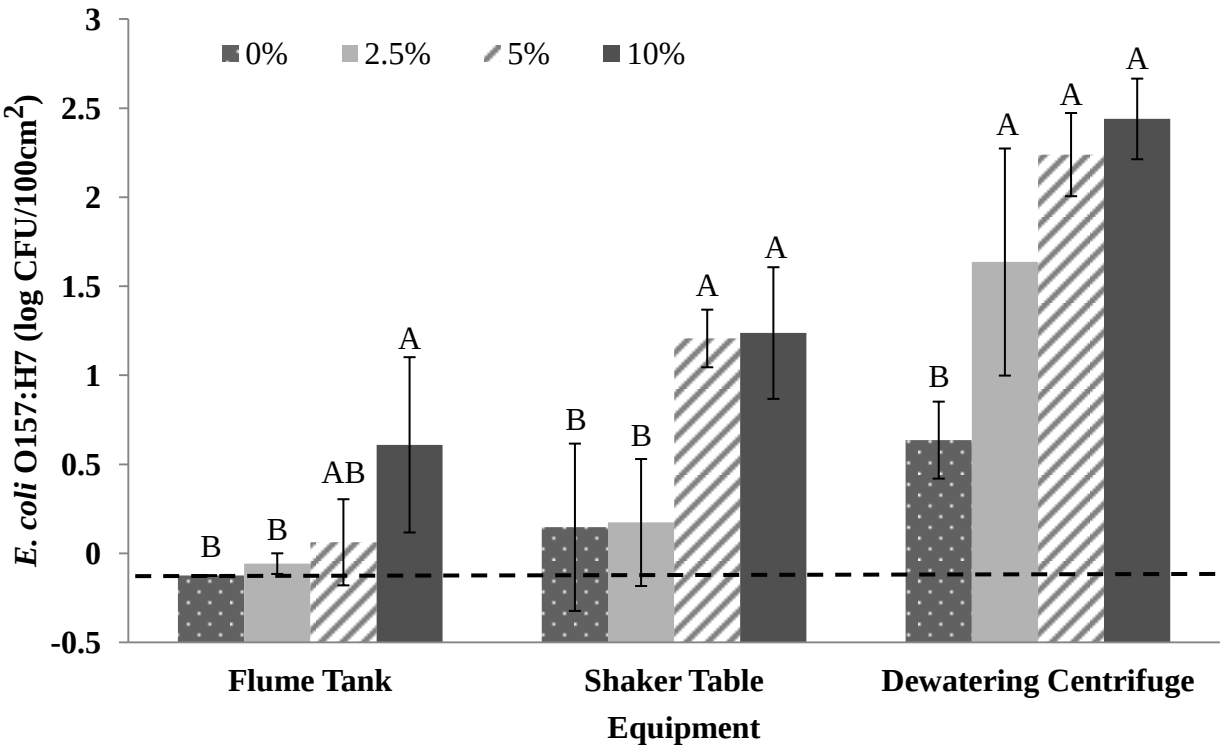


Figure 4.18: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing chlorine + CA and various organic loads (n=3 per organic load). Means within the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.

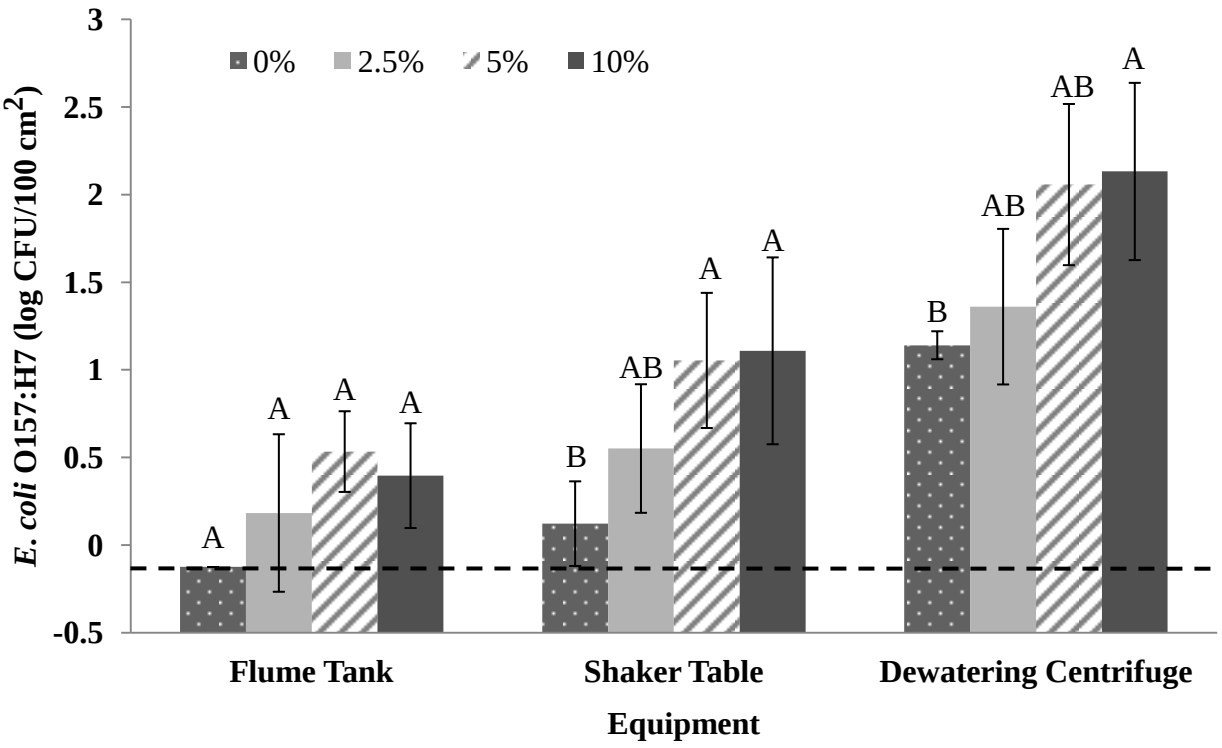


Figure 4.19: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing chlorine + T-128 and various organic loads (n=3 per organic load). Means within the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.

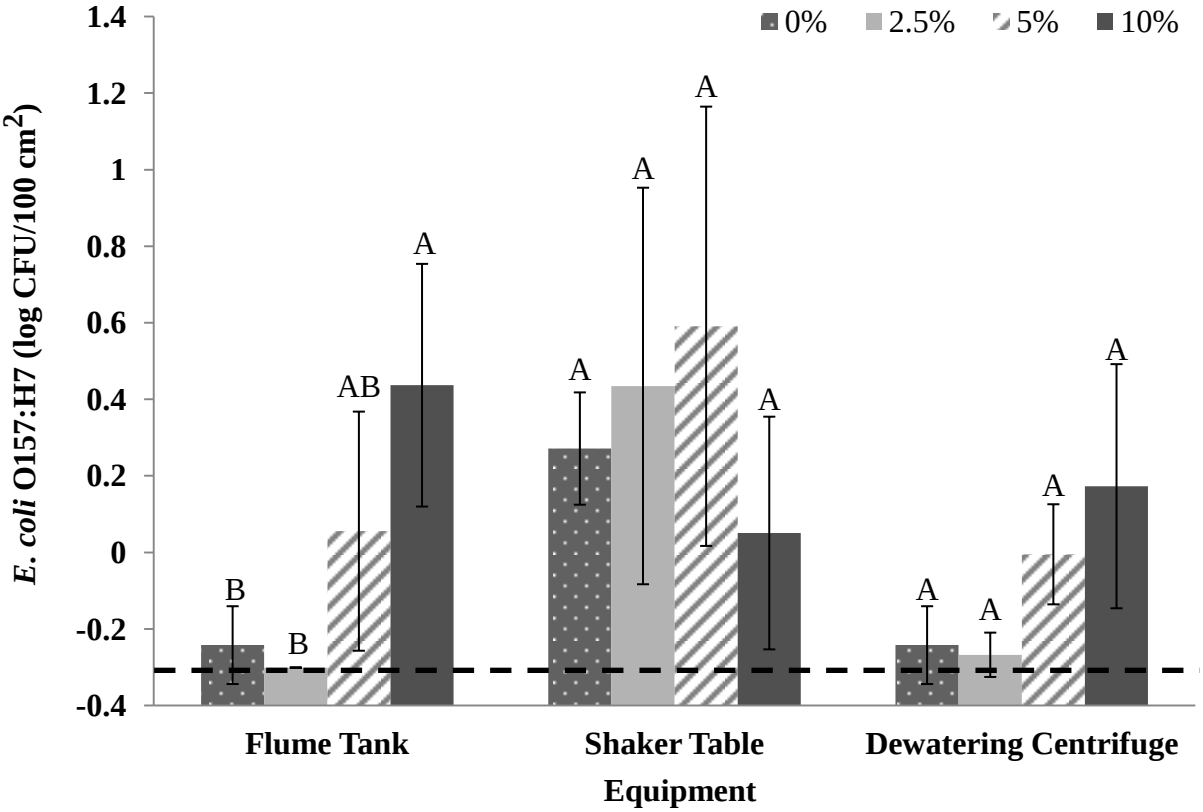


Figure 4.20: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing peroxyacetic acid and various organic loads (n=3 per organic load). Means within the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.

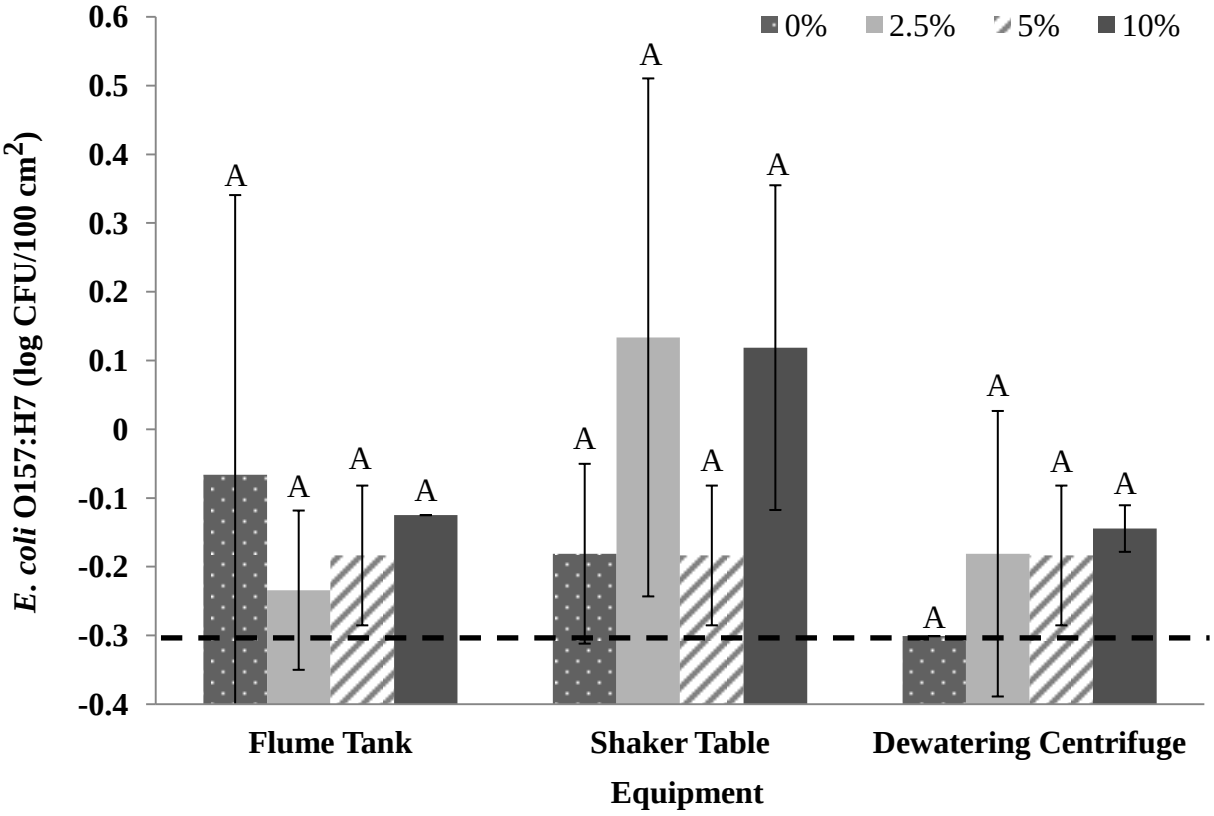


Figure 4.21: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing mixed peracid and various organic loads (n=3 per organic load). Means within the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.

4.4.5 Physicochemical parameters of flume water. All wash water physicochemical parameters were monitored throughout processing, with significant changes rarely seen for the different sanitizer treatments. Increasing the organic load significantly increased ($P \leq 0.05$) total solids, COD, and turbidity, whereas MFV and ORP significantly decreased ($P \leq 0.05$) for all three chlorine treatments (Tables 4.9 and 4.10). Using chlorine alone, pH significantly decreased ($P \leq 0.05$) from 8.52 to 8.10 as the organic load increased from 0 to 10%. A significant increase ($P \leq 0.05$) in total solids was seen for each increase in organic load for chlorine + CA (0.0112 to 0.0482 g) and chlorine + T-128 (0.0090 to 0.0533 g) (Table 4.10), while no significant difference ($P > 0.05$) in total solids was observed for organic loads of 2.5 and 5% when chlorine was used alone. COD increased significantly ($P \leq 0.05$) with organic load from 23 to 4058 mg O_2/L using chlorine alone and was also significantly higher for chlorine + CA and chlorine + T-128 containing a 10% as opposed to a 0 or 2.5% organic load. Turbidity increased ($P \leq 0.05$) between organic loads of 0, 5 and 10% for all chlorine treatments. MFV and ORP decreased as the organic load increased, with significantly higher ($P \leq 0.05$) values seen at 0 as compared to a 10% organic load for all three chlorine treatments (Tables 4.9 and 4.10). While free chlorine was not quantified during processing, it is likely that the concentrations were similar to those seen in the carboys discussed in Chapter 3- with ~50 ppm of free chlorine present in water containing a 0% organic load and < 1.5 ppm in water containing organic loads of 2.5 – 10%.

Increasing organic load correlated to significant increases ($P \leq 0.05$) in total solids, COD, ORP and turbidity for both peroxyacetic acid and mixed peracid. However, there was an inverse correlation between increasing organic load with significant decreases ($P \leq 0.05$) in MFV and pH for both sanitizing treatments (Table 4.11). As the organic load increased from 0 to 10%, a significant decrease ($P \leq 0.05$) in pH was observed for peroxyacetic acid (pH 7.00 to 5.98) and

mixed peracid (pH 6.66 to 4.94). When peroxyacetic acid was used, total solids increased significantly ($P \leq 0.05$) at each organic load (from 0.0065 to 0.0533 g/10 ml), while the only significant difference ($P \leq 0.05$) in total solids for mixed peracid was seen between organic loads of 0 (0.0069 g/10 ml) and 10% (0.0771 g/10ml). When peroxyacetic acid alone was used, COD increased significantly ($P < 0.05$) with increasing organic load (from 251 to 4977 mg O_2/L), while the COD at 10% (5444 mg O_2/L) was significantly higher ($P \leq 0.05$) than the 0 (548 mg O_2/L), 2.5 (1924 mg O_2/L) and 5% (2896 mg O_2/L) organic loads for mixed peracid. Significant differences ($P \leq 0.05$) in turbidity were seen between organic loads of 0, 5 and 10% for both peroxyacetic acid and mixed peracid with maximum turbidity values of 0.155 and 0.106, respectively. ORP increased significantly ($P \leq 0.05$) as organic load in the flume water increased for both peroxyacetic acid (311 – 604 mV) and mixed peracid (348 – 497 mV), likely due to the increased amount of sanitizer added to achieve 50 ppm peroxyacetic acid at the higher organic loads. As organic load levels increased, significant decreases in pH were also observed ($P \leq 0.05$) for both peroxyacetic acid (7.00 – 5.98) and mixed peracid (6.66 – 4.94), which were likely due to the additional amount of sanitizer needed to achieve the target concentration at the higher organic loads. As expected, MFV was inversely related to organic load, with significantly higher ($P \leq 0.05$) volumes seen at an organic load of 0 as compared to 10% for both peroxyacetic acid (50 and 11 ml) and mixed peracid (50 and 16 ml).

Table 4.9: *Physicochemical parameters of flume water containing chlorine and various organic loads* [a]

Controlled Physicochemical Parameters		Mean ± SD Dependent Physicochemical Parameters						
% Org. Load (w/v)	Available Chlorine (ppm)	Temp (°C)	pH	Total Solids (g/10 ml)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
0	50 ± 4	13±0 B	8.52±0.05 A	0.0070±0.0011 C	23±9 D	0.001±0.001 C	50±0 A	720±13 A
2.5	50 ± 4	13±0 B	8.38±0.06 AB	0.0247±0.0123 B	971±72 C	0.021±0.001 C	43±2 A	366±16 B
5	50 ± 6	13±0 B	8.21±0.08 BC	0.0289±0.0020 B	2067±271 B	0.055±0.009 B	23±6 B	391±15 B
10	50 ± 3	14±0 A	8.10±0.12 C	0.0552±0.0049 A	4058±499 A	0.124±0.017 A	11±1 C	368±19 B

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

Table 4.10: *Physicochemical parameters of flume water containing chlorine + CA or chlorine + T-128 with various organic loads* [a]

Controlled Physicochemical Parameters			Mean ± SD Dependent Physicochemical Parameters					
Supplement	% Org. Load (w/v)	Available Chlorine (ppm)	Temp (°C)	Total Solids (g/10 ml)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
CA	0	50±4	14±0 AB	0.0112±0.0015 D	467±162 C	0.001±0.000 C	50±0 A	887±4 A
	2.5	50±3	13±0 B	0.0210±0.0010 C	1330±45 BC	0.016±0.003 BC	45±2 A	545±4 B
	5	50±4	14±0 AB	0.0335±0.0040 B	2695±802 AB	0.039±0.012 B	40±10 A	519±6 BC
	10	50±2	14±0 A	0.0482±0.0026 A	4527±1446 A	0.103±0.013 A	12±0 B	502±22 C
T-128	0	50±6	13±0 B	0.0090±0.0006 D	345±9 B	0.001±0.000 D	50±0 A	887±3 A
	2.5	50±2	14±0 C	0.0190±0.0007 C	1230±133 B	0.023±0.003 C	36±9 B	564±22 B
	5	50±4	13±0 B	0.0326±0.0018 B	2163±654 AB	0.060±0.003 B	25±3 B	503±8 C
	10	50±2	14±0 A	0.0533±0.0019 A	4030±1527 A	0.127±0.006 A	12±2 C	502±8 C

[a] Means with different capital letters designate physicochemical parameters differ significantly in terms of organic load ($P \leq 0.05$).

Table 4.11: *Physicochemical parameters of flume water containing organic load and 50 ppm peroxyacetic acid or 50 ppm mixed peracid* [a]

Controlled Physicochemical Parameters		Mean ± SD Dependent Physicochemical Parameters						
Sanitizing Agent	% Org. Load (w/v)	Temp (°C)	pH	Total Solids (g/10 ml)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
	0	11±0 B	7.00±0.01 A	0.0065±0.0002 D	251±32 D	0.001±0.000 C	50±0 A	311±3 B
Peroxyacetic	2.5	12±0 B	6.98±0.08 A	0.0154±0.0006 C	1244±72 C	0.018±0.002 C	50±0 A	538±61 A
Acid	5	11±1 B	6.70±0.06 B	0.0272±0.0018 B	2866±208 B	0.074±0.010 B	30±5 B	619±7 A
	10	14±1 A	5.98±0.11 C	0.0533±0.0019 A	4977±298 A	0.155±0.015 A	11±2 C	604±45 A
	0	11±0 C	6.66±0.16 A	0.0069±0.0003 B	548±157 C	0.000±0.000 C	50±0 A	348±11 C
Mixed	2.5	13±1 B	5.28±0.11 A	0.0184±0.0008 AB	1924±94 BC	0.033±0.007 B	37±8 AB	418±7 BC
Peracid	5	11±0 C	6.16±0.04 B	0.0237±0.0017 AB	2896±480 B	0.047±0.008 B	32±10 BC	604±84 A
	10	15±0 A	4.94±0.11 C	0.0771±0.0502 A	5444±960 A	0.106±0.005 A	16±4 C	497±8 AB

Table 4.11 (cont'd)

[a] Means with different capital letters designate are physicochemical parameters differ significantly in terms of organic load ($P \leq 0.05$).

4.4.6 Physicochemical parameters of commercial flume water. The available chlorine concentration was 65 ppm with a pH of ~5 and an ORP of 868 mV for the commercial flume water containing chlorine + CA (Table 4.12). Total solids content averaged 0.0244 g with a maximum of 0.0329 g after 3 h of processing. COD averaged 2550 mg O_2/L, with a maximum of 3647 mg O_2/L after 3 h of processing. MFV was ~35 ml.

The peroxyacetic acid concentration was 44 ppm with a pH of 5.56 and an ORP of 459 mV for the commercial flume water (Table 4.13). Total solids content averaged 0.0229 g with a maximum of 0.0345 g seen after 4 h of processing. COD averaged 2130 mg O_2/L, with a maximum of 3216 mg O_2/L seen after 4 h of processing. The average turbidity was 0.033, with a maximum of 0.056 seen after 4 h of processing. MFV averaged ~45 ml.

Table 4.12: *Physicochemical parameters of commercial flume water containing chlorine + CA*

	Mean ± SD Physicochemical Parameters							
	Available Chlorine (ppm)	Free Chlorine (ppm)	Temp (°C)	pH	Total Solids (g)	COD (mg O_2/L)	MFV (ml)	ORP (mV)
Industry Flume Avg.	65 ± 12	0.31 ± 0.34	3 ± 1	5.06 ± 0.78	0.0244 ± 0.0066	2550 ± 891	35 ± 7	868 ± 74
Industry Flume Max.	90 ± 0	2.15 ± 1.15	3 ± 1	5.53 ± 0.95	0.0329 ± 0.0051	3647 ± 646	24 ± 2[a]	1000 ± 0

[a] *MFV inversely correlated with organic load. Minimum MFV is shown.*

Table 4.13: *Physicochemical parameters of commercial flume water containing peroxyacetic acid*

	Mean ± SD Physicochemical Parameters							
	POAA (ppm)	T (°C)	pH	Total Solids (g/10 ml)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
Industry Flume Avg.	44 ± 6	7 ± 2	5.56 ± 0.22	0.0229 ± 0.0127	2130 ± 1088	0.033 ± 0.0098	45 ± 4	459 ± 16
Industry Flume Max.	58 ± 13	11 ± 4	6.09 ± 0.38	0.0345 ± 0.0181	3216 ± 2124	0.056 ± 0.0070	35.5 ± 7[a]	486 ± 17

[a] *MFV inversely correlated with organic load. Minimum MFV is shown.*

4.5 DISCUSSION

The impact of organic load in simulated produce wash water on sodium hypochlorite efficacy has been previously assessed in numerous small-scale laboratory experiments *(57, 78, 80, 87, 107, 139).* Additional studies have assessed the efficacy of peroxyacetic acid in simulated processing water *(1, 17, 65, 78, 95, 129)* or peroxyacetic acid in the presence of an organic load. *(57, 64, 97, 139)*. However, relatively few studies have quantified the impact of various wastewater parameters, such as temperature, free and total chlorine, COD, pH, and ORP on the efficacy of chlorine dioxide *(10)* and electrolyzed water *(56, 79)* against *E. coli* O157:H7, with this study being the first to correlate *E. coli* O157:H7 inactivation to the physicochemical parameters of leafy green flume water in a processing line.

The same 4-strain cocktail of non-toxigenic, GFP-labeled *E. coli* O157:H7 from previous studies *(22, 23)* as well as in Chapters 2 and 3 was used for this work. As in Chapter 3, lettuce was held for 24 h at 4°C rather than 1 h at room temperature to better simulate industrial conditions and promote attachment of *E. coli* O157:H7. Additionally, recovery of sub-lethally injured *E. coli* O157:H7 cells was enhanced by counting the plates after 48 rather than 24 h of incubation in accordance with other sub-lethal injury studies *(68, 135)*.

Chlorine is the most widely used sanitizer in the fresh produce industry due to its low cost and minimal impact on product quality *(62).* Additionally, chlorine has been effective in previous studies with low organic load, resulting in *E. coli* O157:H7 populations in the wash water being below the limit of detection after exposure *(78, 139).* Increased concern over the impact of organic load on chlorine efficacy and the potential for noxious or carcinogenic off-gassing have lead to a heightened interest in methods to better monitor sanitizer efficacy against pathogens in wash water *(33)* and in alternatives to chlorine, such as peroxyacetic acid-based

sanitizers *(1, 62)*. Free chlorine concentrations in clean commercial flume water generally range from 10 to 200 ppm *(55, 91, 122)*. However, as processing continues, soil, debris, and vegetable latexes will accumulate in the flume water *(86)*, decreasing chlorine efficacy *(5, 77, 100, 139)*. This study was performed using organic loads of 0, 2.5, 5 and 10% in order to simulate flume water at different stages of processing. The rate at which the organic load increases in commercial flume systems will vary depending on the type and amount of product being washed, product shred size, the rate of processing, and the means by which the organic load is decreased in the water during processing (e.g., separating screens, foam removal, filters, clarifiers, precipitators). The maximum COD and total solids of commercial flume water containing chlorine + CA collected in this study correspond to organic loads of ~9% and 5.6% (w/v blended iceberg lettuce) based on values obtained from simulated flume water prepared for pilot-scale processing. These differences in physicochemical parameters between the commercial and pilot-scale processing line are likely due to variations in particle size and the nature of the organic load (latexes, soil, and cellular fluids).

Organic load rarely impacted sanitizer efficacy against *E. coli* O157:H7 on iceberg lettuce. *E. coli* O157:H7 persisted on the lettuce throughout processing, even with an organic load of 0% - where *E. coli* O157:H7 populations were typically near or below the lower limit of detection in flume water. When Buchholz and others *(22)* used the same leafy green processing line to quantify *E. coli* O157:H7 transfer during production, 0.20 and 0.67% of the total *E. coli* O157:H7 population was recovered from the shredder and step conveyor, respectively, after processing iceberg lettuce inoculated at 10^6 CFU/g. Given the contact between uninoculated lettuce and *E. coli* O157:H7-contaminated equipment during the processing of batches 1, 2 and 3, the shredder and conveyor also likely contributed to the numbers of *E. coli* O157:H7

recovered from the contaminated lettuce *(93).* A small-scale laboratory study completed by Zhang and others *(139)* found that *E. coli* O157:H7 transferred from inoculated to uninoculated lettuce leaves during washing while no *E. coli* O157:H7 cells were recovered from processing water containing 30 ppm mixed peracid and a 10% organic load. This phenomena was also observed in a pilot-scale study by Luo and others *(81)* while processing both *E. coli* O157:H7 inoculated and uninoculated spinach in wash water containing chlorine + T-128. However, inoculated lettuce in the study reported here was used to expose the flume water to *E. coli* O157:H7 in order to determine sanitizer efficacy against the pathogen in wash water, not to determine the effectiveness of washing against *E. coli* O157:H7 on the lettuce. The persistence of *E. coli* O157:H7 on finished product indicates that factors other than sanitizer efficacy of the wash water contribute to end-product safety.

In this study, after first processing 5.4 kg of inoculated iceberg lettuce, a total of only 16.4 kg of uninoculated lettuce was processed in three 5.4-kg batches at 10 min-intervals during a total processing period of 44 min. The pilot-scale processing line as used in this study is capable of processing ~216 kg/h or 158 kg during the 44 min of sample collection. The inoculated lettuce served as a vehicle to contaminate the flume water for sanitizer efficacy testing rather than a means to assess effectiveness of washing. The relatively small amount of lettuce processed in this study offers "snapshots" of what may have been seen had the maximum of 158 kg been processed.

A linear regression analysis was conducted to further assess *E. coli* O157:H7 reductions for each organic load and chlorine treatment. Using a 0% organic load, *E. coli* O157:H7 populations immediately decreased > 5 log CFU/ml for all three chlorine treatments, resulting in trend lines with minimal or no slope and intercepts ranging from -5.4 to -5.8 log CFU/ml. *E. coli*

O157:H7 inactivation by chlorine was inversely related to organic load. While chlorine + CA and chlorine + T-128 were also impacted by organic load, the inactivation rates at the higher organic loads of 5 and 10% (-0.1 and -0.1 log CFU/ml per min, respectively) were similar to those seen at a 2.5% organic load with chlorine alone (-0.1 log CFU/ml per min). This result illustrates the importance of acidifying chlorinated wash water to increase the hypochlorous acid content, thereby increasing the efficacy of chlorine at higher organic loads. The R^2 values for the chlorine + CA and chlorine + T-128 trend lines at an organic load of 2.5% were 0.70 and 0.60, respectively, which is attributable to the variable nature of the *E. coli* O157:H7 reduction in wash water. *P* values for the inactivation trend lines for chlorine, chlorine + CA and chlorine + T-128 at organic loads of 2.5, 5 and 10% were < 0.0001, indicating that the trend lines were accurate predictors of *E. coli* O157:H7 inactivation. As each batch of uninoculated lettuce entered the flume tank 10, 21.5, and 33 min after the initial batch of inoculated lettuce was processed, some *E. coli* O157:H7 cells that were likely transferred to the lettuce during shredding and conveying were released into the water during washing. Unsurprisingly, the R^2 values for the regional trend lines (0 – 10, 11.5 – 21, 23 – 32.5, and 34.5 – 44 min) were typically higher than the trend lines developed over the entirety of procession (0 – 44 min). However, the variable nature of a commercial processing line placed an emphasis on creating trend lines for the entire 44 min of exposure. Additionally, the regional trend lines typically had higher *P* values than the trend lines developed from all 44 min of processing- indicating a weaker fit to the inactivation of *E. coli* over shorter periods of time.

As discussed in Chapter 2, *E. coli* O157:H7 populations were consistently higher in flume water containing peroxyacetic acid or mixed peracid than in flume water containing

chlorine acidified with citric acid or T-128. Hence, *E. coli* O157:H7 populations were expected to persist in the flume water during processing, especially at higher organic loads when peroxyacetic acid-based sanitizers were used. However, using the bench-top system developed in Chapter 3, *E. coli* O157:H7 did not persist in wash water containing peroxyacetic acid or mixed peracid after removal of the inoculated product. These findings are similar to those from the current processing line study where *E. coli* O157:H7 populations typically decreased > 5 log CFU/ml in the wash water with little persistence occurring. While some significant differences in *E. coli* O157:H7 reductions were observed in the flume water based on organic load, notably after 21 and 44 min of processing, populations were often below the limit of detection which decreased with increasing organic load in the flume water.

The maximum total solids and COD of the commercial flume water containing peroxyacetic acid corresponded to organic loads of 6.3 and 6.2% (w/v blended iceberg lettuce), respectively, based on comparisons to the same values from flume water prepared in the pilot-scale processing line. At an organic load of ~6.25%, the commercial flume water likely provided a log linear inactivation of $\text{Log}(N/N_0) = -4.7$. However, minimal *E. coli* O157:H7 persistence was seen in the flume water during pilot-scale processing, regardless of the dependent physiochemical parameters of the water. Therefore, as in the bench-top carboy study, the dependent physicochemical parameters in Table 4.11 are likely not ideal for predicting *E. coli* O157:H7 inactivation rates in flume water containing different organic loads for either peroxyacetic acid or mixed peracid in Table 4.8. Monitoring the sanitizer concentration may be the most accurate predictor of *E. coli* O157:H7 inactivation. While the log linear inactivation trend lines derived from the *E. coli* O157:H7 reductions in flume water may not correlate well to

time considering the low R^2 values, the y-intercept can be used to predict the *E. coli* O157:H7 reductions in flume water for both peroxyacetic acid and mixed peracid.

Regardless of the chlorine treatment, total solids and COD were the best indicators of organic load in the flume water. The physicochemical parameters of the wash water in Tables 4.9 and 4.10 can be correlated to the inactivation equations in Table 4.3 to predict *E. coli* O157:H7 persistence. Based upon the persistence of *E. coli* O157:H7 at organic loads of 2.5, 5 and 10% with all three chlorine treatments, the physicochemical parameters of flume water should be maintained within the standard deviations for a 0% organic load to minimize the possibility of water becoming a carrier for *E. coli* O157:H7. The numerous physicochemical parameters monitored in this study offer alternatives to ORP which is most widely used to assess sanitizer efficacy in commercial flume systems *(112, 125).* However, since a minimum of 2 h is needed to determine COD and total solids, both of these tests are impractical for routine testing. In order for commercial processors to rapidly predict sanitizer efficacy of their flume water, studies specific to the commercial processing line in question must be completed to determine which rapid tests (e.g. turbidity or MFV) correlate to the more time-consuming but more accurate tests of COD and/or total solids.

One additional study in which a pilot-scale leafy green processing line was used to process inoculated spinach leaves reported *E. coli* O157:H7 reductions of < 1 log on the product when the flume water containing chlorine-based sanitizer was acidified to pH 5.0 with T-128. *(81)*. However, *E. coli* O157:H7 populations were significantly lower in the wash water *(81).* In this study, a pH of 6.5 was used to compare the efficacy of T-128 to the targeted pH when citric acid is used *(62, 81).* An additional study by the same group concluded that T-128 on its own had

weak bactericidal activity and did not play a significant role in pathogen inactivation *(87)*, which emphasizes the products role as a means to enhance the efficacy of chlorine.

A survey of commercial leafy greens was conducted by Barrera and others *(13)* to determine the impact of different processing parameters on the efficacy of various sanitizing agents during processing. Peroxyacetic acid concentration, pH, turbidity, and solids content of the wash water were correlated to aerobic and coliform counts on spinach before and after washing at one of the three facilities tested. An average peroxyacetic acid concentration of 6.1 ppm was maintained by manual dosing. Average aerobic and coliform counts were not significantly lower after washing, which is not surprising considering the concentration of peroxyacetic acid in the wash water. Additionally, average coliform counts in the wash water of 3.12 log CFU/100 ml were negatively correlated with the log reductions, which were attributed to the lower average sanitizer concentration. There was a positive correlation between the solids content of the wash water and the reductions of aerobic bacteria, emphasizing that organic load plays a critical role in sanitizer efficacy, at least with a low peroxyacetic acid concentration. This finding emphasizes the importance of monitoring sanitizer concentration in the wash water in addition to organic load.

Regardless of the chlorine treatment, *E. coli* O157:H7 populations were typically higher on all wet equipment surfaces when an organic load was present in the wash water, emphasizing the spread of *E. coli* O157:H7 by means other than washing. Populations on the flume tank, shaker table and dewatering centrifuge were rarely significantly different at any of the organic loads tested for peroxyacetic acid and never significantly different for mixed peracid. The fact that *E. coli* O157:H7 was quantifiable on most surfaces after 44 min of processing with either sanitizing agent likely contributed to persistence of the pathogen on the product.

Regardless of the chlorine treatment, higher *E. coli* O157:H7 populations were always seen in spent centrifugation water after washing each batch of lettuce in water containing an organic load. Levels of *E. coli* O157:H7 recovered from the spent centrifugation water were rarely significantly different at any of the organic loads for peroxyacetic acid and never significantly different with mixed peracid. While often below the limit of detection in the flume water, *E. coli* O157:H7 was consistently present at levels above the lower limit of detection in the centrifugation water (0.2 CFU/ml). Therefore, spent centrifugation appears to be an attractive target for pathogen testing during commercial processing.

This study was conducted to assess the impact of organic load on sodium hypochlorite and peroxyacetic acid efficacy against *E. coli* O157:H7 on lettuce, in wash water, and on equipment surfaces during small-scale commercial leafy green processing, with the pathogen recovered from all uninoculated batches of lettuce processed after the inoculated batch. Chlorine efficacy markedly decreased with increasing organic load organic load, resulting in increased *E. coli* O157:H7 persistence in the flume water. Total solids, COD and turbidity of the flume water were inversely related to linear trend lines of *E. coli* O157:H7 inactivation, with the opposite true for ORP and MFV. Since organic load rarely impacted the efficacy of either peroxyacetic acid or mixed peracid, the dependent physicochemical parameters that were assessed for flume water are not ideal predictors of their efficacy, with sanitizer concentration being a better predictor of *E. coli* O157:H7 inactivation. While the minimum effective concentration of peroxyacetic acid or mixed peracid was not determined in this study, concentrations of 50 ppm should result in minimal *E. coli* O157:H7 persistence, regardless of organic load. Based on these findings, commercial processors should consider maintaining the organic load in chlorinated flume water as close to 0% as possible to minimize microbial cross-contamination during processing.

However, given the ability of *E. coli* O157:H7 to persist on iceberg lettuce throughout processing, the incorporation other microbial intervention steps in addition to flume washing with a sanitizer may be advisable to better enhance end-product safety.

CONCLUSIONS

Flume washing remains a critical step during commercial processing of leafy greens in order to remove soil and debris, which decreases the microbial load, improves quality and appearance, and enhances product shelf life and safety. Due to the large amount of product that is regularly processed, flume water is recirculated to reduce waste and operational costs. Various sanitizing agents are regularly added to the water in order to prevent the water from becoming a microbial carrier. The first research objective assessed the efficacy of five different commercial sanitizing treatments against *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce. *E. coli* O157:H7 populations decreased 0.8 – 1.4 log CFU/g on the lettuce, with no sanitizing agent being significantly more effective than water alone. When compared to flume water containing peroxyacetic acid or mixed peracid, significantly fewer *E. coli* O157:H7 were typically recovered in flume water containing chlorine, chlorine + citric acid, or chlorine + T-128 during processing of inoculated lettuce, with some populations falling below the lower limit of detection. However, this lettuce was processed in the absence of an organic load under a "best-case" scenario. Since produce sanitizers are designed to decrease the persistence of microbial contaminants during processing, it is critical that they remain effective throughout processing in order to prevent the amplification of an isolated contamination event.

Organic load, consisting of plant tissues and cellular fluids released during cutting in addition to soil, insects, and microbes, will accumulate in recirculating flume water as produce is washed, decreasing the ability of sanitizers to minimize cross-contamination from the water during processing. Chlorine is the most widely used sanitizing agent by commercial leafy greens processors due to its low cost. However, organic load and pH are well known to impact the efficacy of

chlorine, increasing the interest in chlorine supplements, such as T-128, or chlorine alternatives, such as peroxyacetic acid. Given the various commercial sanitizers available and the variable rates at which organic material accumulates in flume water during processing, commercial leafy green processors are clearly in need of better means to both predict and monitor the efficacy of sanitizers during processing.

The second research objective focused on the persistence of *E. coli* O157:H7 in wash water containing sanitizers and different organic loads and the correlation of various physicochemical parameters of the wash water to sanitizer efficacy. A novel carboy system was developed to simulate leafy green washing in a pilot-scale processing line. Organic load impacted the efficacy of all three chlorine treatments, with typical *E. coli* O157:H7 reductions of < 1 log CFU/ml after 10 min of exposure. In contrast, efficacy of peroxyacetic acid and mixed peracid was largely unaffected by organic load, with average *E. coli* O157:H7 reductions of ~4.8 and ~5.5 log CFU/ml, respectively, after 10 min of exposure. Reduced sanitizer efficacy generally correlated to increased total solids, chemical oxygen demand, turbidity, and decreased maximum filterable volume, indicating that these tests may be effective alternatives to the industry standard of oxygen/reduction potential.

The research goals of the third study were to 1) quantify the persistence of *E. coli* O157:H7 in wash water and on lettuce during pilot-scale processing using flume water containing five different sanitizers and various organic loads and 2) assess the relationship between various physicochemical parameters and organic load of the wash water on *E. coli* O157:H7 inactivation. *E. coli* O157:H7 was recovered from all lettuce that was processed after the inoculated batch of iceberg lettuce, regardless of organic load in the wash water. Organic load negatively impacted the efficacy of all three chlorine treatments, with typical *E. coli*

O157:H7 reductions of > 5 log by the end of processing with no organic load in the wash water and 0.9 – 3.7 log CFU/ml with a 10% organic load. Much like the second study, reduced sanitizer efficacy generally correlated to significant differences in total solids, chemical oxygen demand, turbidity, and maximum filterable volume, indicating that these tests may be effective alternatives to the industry standard of oxygen/reduction potential. Additionally, the numerous physicochemical parameters of the flume water correlated to *E. coli* O157:H7 inactivation throughout processing. These findings emphasize the importance of monitoring flume water quality as a means to predict chlorine efficacy. However, organic load rarely impacted the efficacy of either peroxyacetic acid or mixed peracid, with typical reductions of > 5 log CFU/ml in wash water throughout processing for all organic loads. Additionally, *E. coli* O157:H7 persisted on all previously uninoculated lettuce following the inoculated batch, emphasizing the need for improved intervention strategies that can better ensure end-product safety.

The two major limitations to this research include the use of 1) only one sanitizer concentration for each sanitizing agent used in each individual study and 2) and a processing water temperature that was higher than the targeted industry standard of 4°C. Chemical sanitizer efficacy is concentration dependent; however, these studies did not assess the extent to which sanitizer concentration impacted *E. coli* O157:H7 inactivation. Due to the lack of a chilling system in the pilot-scale leafy green processing line used in Chapters 2 and 4, the average water temperature was ~14°C. The impact of water temperature on chlorine + CA efficacy was described in Chapter 3; however, the impact of temperature on any of the sanitizing agents during pilot-scale processing was not assessed. Additionally, the organic load concentrations used in the studies described in Chapter 4 were fixed at specific concentrations of 0 – 10% (w/v blended lettuce). Organic load levels would naturally accumulate over an entire processing shift

in a commercial operation, theoretically resulting in variable sanitizer efficacy throughout processing.

Overall, this research showed that sanitizing agents in flume water are effective against *E. coli* O157:H7 in wash water when no organic load was present. However, chlorine efficacy was significantly impacted by the presence of organic load in the wash water, allowing *E. coli* O157:H7 to persist in the wash water and on finished product. Conversely, both peroxyacetic acid and mixed peracid remained largely unaffected by the presence of organic load in the wash water. With sanitizing agents in wash water remaining the sole microbial mitigation strategy during commercial processing of leafy greens, it is imperative that growers and harvesters follow GAPs and GHPs in order to minimize product contamination before processing because conventional flume washing will only provide a 1 – 3 log reduction of microbial contaminants. Considering that chlorine is the most used sanitizing agent in the leafy green industry and that its efficacy is impacted by organic load, monitoring the physicochemical parameters of the water is critical to ensure that the flume water does not promote the amplification of an isolated contamination event.

FUTURE RESEARCH RECOMMENDATIONS

The studies presented in Chapters 3 and 4 showed that both peroxyacetic and mixed peracid maintained their efficacies in the presence of organic load, resulting in *E. coli* O157:H7 populations that were typically below the lower limit of detection in the wash water. Chlorine-based sanitizers are more cost-effective than peroxyacetic acid-based sanitizers; however the results of Chapters 3 and 4 showed that their efficacy is significantly impacted in the presence of organic load. Considering that a concentration of 50 ppm for both peroxyacetic acid and mixed peracid consistently provided *E. coli* O157:H7 reductions of > 5 log CFU/ml, it is likely that lower concentrations may be just as effective.

A study could be completed using the carboy system described in Chapter 3 in which various concentrations of peroxyacetic acid and mixed peracid are assessed in wash water containing organic load. Even though both POAA-based sanitizers maintained their efficacy at 50 ppm in the presence of organic load, it would be critical to examine the impact of organic load on lower POAA concentrations in order to verify that organic load immunity was not dependent on sanitizer concentration. Once a minimum effective dose was determined in the carboy model, a study similar to that presented in Chapter 4 could be conducted in order to determine if the lower sanitizer concentration prevented *E. coli* O157:H7 persistence in flume water throughout processing of numerous batches of leafy greens. Determination of the minimum effective dose of peroxyacetic-acid based sanitizers would give commercial leafy green processors a target concentration thereby preventing the over-use of sanitizing agents. Maintenance of a lower peroxyacetic acid concentration would reduce operational costs and

corrosion to processing equipment. It is likely that commercial processors would be more likely to adopt the use of peroxyacetic acid if it was more cost-effective.

The studies presented in Chapters 3 – 4 focused on the impact of organic load (blended iceberg lettuce) on sanitizer efficacy against *E. coli* O157:H7. In a commercial processing facility, organic load may be comprised of soil and debris in addition to cellular fluids and latexes and may not have a similar impact on sanitizer efficacy. Additionally, other fresh produce products (e.g., baby spinach, tomatoes, celery, carrots, and onions) are also frequently subjected to flume washing after cutting or dicing, resulting in the inevitable accumulation of cellular fluids in the wash water. Due to the differences between the compositions of products in regards to pH, latexes, and cellular fluids, no conclusions can be made regarding the impact of other types of organic load on sanitizer efficacy based upon the series of experiments performed with iceberg lettuce in Chapters 3 – 4.

The carboy system described in Chapter 3 could be modified to examine the impact of various types of organic load (e.g., soil, various blended produce products) on both chlorine and peroxyacetic acid-based sanitizers. Physicochemical parameters of the wash water could be correlated to the inactivation of native microflora or other pathogens of concern, such as *Listeria monocytogenes* or *Salmonella.* Such research would benefit a much larger group of produce processors and may help to prevent future product recalls and outbreaks as demand for convenient, value-added fresh-cut produce increases.

The lettuce used in these studies was dip-inoculated to contain 10^6 CFU/g of *E. coli* O157:H7 in order to provide quantifiable results. The levels of *E. coli* O157:H7 on leafy greens contaminated during pre- or postharvest operations, while unknown, are thought to be lower than the inoculation level used in the studies discussed in Chapters 2 – 4. Inoculated lettuce that was

used in the studies discussed in Chapters 3 – 4 was held for 24 h at 4°C after dip inoculation in order to simulate industrial practice as well as promote the attachment of *E. coli* O157:H7 to the leaf surface.

A study could be conducted to assess the impact of low-level field inoculation (by spraying or flooding) of heads of iceberg lettuce on *E. coli* O157:H7 persistence during subsequent pilot-scale processing. It is assumed that the greater attachment and potentially even biofilm formation by *E. coli* O157:H7 on the leaf surface would occur in the field- grown as compared to dip-inoculated lettuce as used in the previously described studies. Due to greater attachment and lower levels of *E. coli* O157:H7 on the leafy greens, quantification or even detection would likely be difficult with typical methods. In order to detect the pathogen, a Portable Multi-use Automated Concentration System (PMACS) would likely need to be used. The PMACS rapidly and efficiently collects microbial pathogens and other particulates from large volumes of water and uses a back-flush protocol to recover the pathogens in a concentrated sample for subsequent analysis. The results of a study conducted with field inoculated lettuce would determine what difference, if any, inoculation method has on *E. coli* O157:H7 persistence in wash water and on finished product during subsequent pilot-scale processing.

This dissertation primarily focused on the efficacy of various chemical sanitizers against *E. coli* O157:H7 in wash water, with lettuce essentially used as a means to inoculate the water. However, inactivation of *E. coli* O157:H7 on lettuce using wash water containing sanitizing agents was rarely impacted by organic load. While it was not surprising, it is still a troubling result of how fresh-cut leafy greens are processed and reinforces countless previous claims that pathogens cannot be removed from product by conventional washing once it is contaminated. Considering the results of Chapter 4, where *E. coli* O157:H7 was recovered from all previously

uninoculated lettuce that was processed after the inoculated batch, it is clear that microbial intervention steps other than having effective sanitizing agents in the wash water are critical to ensure end-product safety. Considering that the amount of uninoculated lettuce (16.2 kg) that was processed after the inoculated batch was relatively small in comparison to the throughput of a commercial processor, the extent to which *E. coli* O157:H7 can spread to finished product under more realistic processing conditions remains unclear. A previous study by our group showed that *E. coli* O157:H7 transferred to all 907 kg of iceberg lettuce that was processed subsequent to *E. coli* O157:H7-inoculated product. However, that study was performed without sanitizing agents in the wash water. In order to quantify the persistence of *E. coli* O157:H7 on a larger amount of product, a similar large-scale study could be conducted with sanitizing agents in the wash water.

Since it has been established that conventional washing procedures will only reduce microbial populations 1 – 3 log CFU from the surfaces of leafy greens, it is critical that contamination of the product is prevented. However, it is important that steps are taken to prevent the amplification of an isolated contamination event during processing of leafy greens because contamination of product will undoubtedly continue. Considering that *E. coli* O157:H7 was found throughout the previously uninoculated product after processing in the study conducted in Chapter 4, regardless of the efficacy of the sanitization agents, it is clear that contamination is being amplified in processing steps that occur during washing- namely shredding and conveying. Visual inspection of the shredder and conveyor after processing showed that some product remained on the shredder and conveyor, some of it likely the contaminated product. Modifications to the pilot-scale processing line could be made where sanitizing sprayers are installed inside the shredder and on the conveyor to minimize the

accumulation of product on equipment surfaces. Another could assess the persistence of *E. coli* O157:H7 in wash water and on finished product after processing in the modified pilot-scale leafy green processing line. It is expected that the reductions on the inoculated product would be similar to the studies conducted previously and the amplification contamination would be inhibited, thereby improving end-product safety.

APPENDICES

APPENDIX I:

Impact of Organic Load and Sanitizer Concentration on Inactivation of a Four-Strain Cocktail of *Escherichia coli* O157:H7 in Simulated Leafy Green Processing Water

AI.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of various concentrations of chlorine, peroxyacetic acid, and mixed peracid against *E. coli* O157:H7 in simulated wash water containing various organic loads. Four liters of water containing organic loads of 0, 1, 5 and 10% (w/v blended lettuce) and chlorine (30, 50 and 100 ppm), peroxyacetic acid (10, 50 and 80 ppm), or mixed peracid (10, 50 and 80 ppm) were used to assess *E. coli* O157:H7 survival over 90 sec of exposure. Organic load negatively impacted the efficacy of all three sanitizing agents at each concentration, resulting in significantly ($P \leq 0.05$) higher *E. coli* O157:H7 populations. Increasing organic load correlated to significant increases in COD, turbidity, and total solids for all sanitizing treatments.

AI.2 INTRODUCTION

Wash water quality has been a major focus of the produce industry for many years with these concern heightened in response to three nationwide outbreaks in 2006 traced to fresh-cut lettuce and baby spinach. Despite the widespread use of chemical sanitizers in produce wash water, efficacy of these sanitizers remains problematic due to the presence of organic material in the water that decreases antibacterial activity.

The two aims of this study were to 1) determine the efficacy of three concentrations of three different sanitizing agents against *E. coli* O157:H7 in water containing organic loads of 0, 1, 5 and 10% (w/v blended lettuce solids) and 2) assess various physicochemical parameters of the wash water.

AI.3 MATERIALS AND METHODS

AI.3.1 Experimental design. The impact of four different organic loads on three concentrations of three different sanitizing treatments against a 4-strain cocktail of *E. coli* O157:H7 at 10^8 CFU/ml was assessed in triplicate in a 4 L glass carboy, with sanitizer-free water serving as the control for each organic load. Various water samples were collected and quantitatively examined for *E. coli* O157:H7 to determine persistence during 90 sec of exposure. Temperature, pH, oxidation/reduction potential, chemical oxygen demand, total solids, maximum filterable volume and turbidity of the wash water were also assessed.

AI.3.2 Leafy greens. Identical to 2.3.2.

AI.3.3 Bacterial strains. Identical to 2.3.3.

AI.3.4 *E. coli* O157:H7 cocktail. The four *E. coli* O157:H7 cultures were combined in equal volumes in a 50 ml centrifuge tube to create a 36 ml cocktail.

AI.3.5 Processing equipment. Identical to 3.3.5.

AI.3.6 Wash water. Iceberg lettuce (0, 40, 200, or 400 g) was blended for 2 min in 250 – 500 ml of tap water using a household blender (Model BLC10650MB, Black & Decker, New Britain, CT) and added to the wash water at ~14°C to achieve organic loads of 0, 1, 5, or 10% (w/v), respectively, in 4 L. The following nine sanitizer treatments were assessed: 10, 50 and 80 ppm peroxyacetic acid (Tsunami 100, Ecolab, St. Paul, MN), 10, 50 and 80 ppm mixed peracid (Tsunami 200, Ecolab), and 30, 50 and 100 ppm available chlorine (XY-12, Ecolab). Peroxyacetic acid test kit 311 (Ecolab) was used to confirm the peroxyacetic acid and mixed peracid sanitizer concentrations while chlorine test kit 321 (Ecolab) was used to measure available chlorine. Sanitizer-free MSU tap water (< 0.05 ppm free chlorine) served as the control for each for each organic load.

AI.3.7 *E. coli* O157:H7 exposure to wash water and sample collection. The *E. coli* O157:H7 cocktail (4 ml) was inoculated directly into the carboy containing 4 L of wash water during mechanical stirring, immediately after which the cork was removed from the spout. Water samples (50 ml) were collected at 10 s intervals over 90 s of exposure in 50 ml centrifuge tubes containing 38x concentrated Difco Neutralizing Buffer (Becton Dickinson).

AI.3.8 Physiochemical parameters. Identical to 3.3.8.

AI.3.9 Microbiological analyses. Identical to 3.3.9.

AI.3.10 Sanitizer neutralization confirmation. One liter aliquots of tap water containing 30, 50 and 100 ppm chlorine (XY-12, Ecolab), 10, 50 and 80 ppm peroxyacetic acid (Tsunami 100, Ecolab) or 10, 50 and 80 ppm mixed peracid (Tsunami 200 ppm, Ecolab) with organic loads of 0, 1, 5 or 10% were prepared using a mechanical blender (Model BLC10650MB, Black & Decker, New Britain, CT), after which the concentration of available chlorine or peroxyacetic acid was confirmed using a commercial test kit (Chlorine test kit 321, Ecolab and Peroxyacetic acid test kit 311, Ecolab). A 50 ml centrifuge tube containing 3 ml of 38x concentrated neutralizing buffer (BD) was filled with these prepared solutions, agitated for 5 s and then similarly assessed for chlorine or peroxyacetic acid neutralization. In preliminary experiments, a 38x concentration neutralized all sanitizer concentrations without impacting *E. coli* O157:H7 recovery.

AI.3.11 Statistical analysis. *E. coli* O157:H7 counts were converted to log CFU/ml and individual physicochemical parameter values were subjected to an ANOVA using JMP 9.0 (SAS Institute Inc., Cary, NC). For samples without *E. coli* O157:H7 counts, values equaling half the limit of detection were used. A P value of ≤ 0.05 was considered significant for all tests. The Tukey-Kramer HSD test was used to identify significant differences in *E. coli* O157:H7

populations for individual lettuce and water samples as well as individual physicochemical parameter values.

AI.4 RESULTS

AI.4.1 Wash water. Average *E. coli* O157:H7 populations in the water controls ranged from 5.9 – 6.0 log CFU/ml across all organic loads (Table AI.1). A concentration of 10 ppm peroxyacetic acid was impacted by organic load, with significantly higher ($P \leq 0.05$) *E. coli* O157:H7 populations typically recovered in organic loads of 1, 5 and 10% (Table AI.2). Concentrations of 50 and 80 ppm peroxyacetic acid were also impacted by organic load, with average *E. coli* O157:H7 populations recovered after 50 s typically significantly lower with a 0% organic load (-2.0 to -1.0 log CFU/ml) than at a 10% organic load (0.0 to 2.9 log CFU/ml). All three concentrations of mixed peracid were impacted by organic load, with significantly fewer ($P \leq 0.05$) *E. coli* O157:H7 consistently recovered with a 0% organic load (-2.0 to 5.7 log CFU/ml) than at a 10% organic load (-1.0 to 6 log CFU/ml) after 50 s of exposure (Table AI.3). Organic load had a negative impact on all chlorine concentrations, with significantly ($P \leq 0.05$) fewer *E. coli* O157:H7 populations recovered at a 0% organic load (-2.0 to -1.8 log CFU/ml) than at a 10% organic load (5.7 – 6.1 log CFU/ml) at all times sampled (Table AI.4).

Table AI.1: E. coli *O157:H7 populations in wash water controls* [a]

% Organic Load	Mean ± SD *E. coli* O157:H7 (log CFU/ml)								
	10	20	30	40	50	60	70	80	90
0	5.5±0.2 B	5.8±0.1 A	5.8±0.1 B	5.9±0.1 A	5.9±0.1 A	6.0±0.1 AB	6.0±0.1 AB	6.1±0.1 A	6.0±0.1 AB
1	5.6±0.1 B	5.9±0.0 A	6.0±0.1 A	6.0±0.0 A	6.0±0.0 A	6.1±0.1 A	6.1±0.1 A	6.1±0.0 A	6.1±0.1 A
5	6.0±0.1 A	5.9±0.1 A	5.9±0.0 AB	5.9±0.1 A	5.9±0.1 A	5.9±0.1 AB	5.9±0.0 B	5.9±0.0 B	5.9±0.0 AB
10	6.0±0.1 A	5.9±0.0 A	5.9±0.1 AB	5.9±0.1 A	5.9±0.1 A	5.9±0.1 B	5.9±0.0 B	5.9±0.1 B	5.8±0.1 B

[a] Means with different capital letters designate *E. coli* O157:H7 populations that differ significantly in terms of time ($P \leq 0.05$).

Table AI.2: E. coli *O157:H7 populations in wash water containing peroxyacetic acid* [a]

POAA (ppm)	% Organic Load	Mean ± SD *E. coli* O157:H7 (log CFU/ml)								
		10	20	30	40	50	60	70	80	90
10	0	4.9±0.8 A	5.6±0.3 A	5.8±0.2 A	5.8±0.2 A	5.6±0.1 B	5.5±0.1 B	5.2±0.3 B	4.6±0.8 B	4.0±1.1 B
	1	5.7±0.4 A	6.0±0.2 A	6.0±0.0 A	6.0±0.1 A	6.0±0.1 A	5.9±0.2 A	5.8±0.1 A	5.6±0.1 AB	5.4±0.3 AB
	5	6.0±0.2 A	6.1±0.1 A	6.0±0.1 A	5.9±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.2 A	5.9±0.1 A
	10	6.0±0.1 A	6.0±0.1 A	6.0±0.0 A	6.0±0.0 A	5.9±0.1 A	5.9±0.0 A	5.9±0.1 A	5.8±0.2 A	5.8±0.3 A

Table AI.2 (cont'd)

		4.8±0.7								
	0	B	3.8±1.0 B	0.8±0.2 B	-0.4±0.8 C	-1.0±0.6 C	-1.8±0.2 B	-1.9±0.1 B	-1.3±1.2 A	-2.0±0.0 B
		5.2±0.1	4.8±0.3	1.8±1.1		0.7±1.3	0.3±1.8	0.2±1.9		0.7±2.4
	1	AB	AB	AB	0.8±1.5 BC	BC	AB	AB	0.2±2.0 A	AB
50		5.7±0.1				1.9±0.6		1.1±0.5		1.2±1.1
	5	A	5.4±0.3 A	3.5±0.8 A	1.9±0.2 AB	AB	1.6±0.3 A	AB	0.9±1.0 A	AB
		5.9±0.2	4.7±0.3							
	10	A	AB	3.3±0.3 A	3.1±0.1 A	2.9±0.5 A	2.3±1.2 A	2.0±1.4 A	2.1±0.9 A	2.0±0.6 A

Table AI.2 (cont'd)

80	0	4.2±0.8 A	1.7±0.7 A	1.0±0.6 A	-0.7±1.5 A	-1.9±0.2 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 C	-2.0±0.0 A
	1	4.5±0.4 A	1.3±0.6 A	0.3±0.9 A	-1.0±0.7 A	-1.4±0.6 B	-1.4±0.8 B	-0.8±1.4 B	-1.7±0.4 BC	-1.6±0.5 A
	5	5.1±0.3 A	1.8±0.3 A	-0.0±0.6 A	0.05±0.13 A	-0.8±0.9 B	-1.2±0.2 B	-0.9±0.5 B	-1.3±0.0 B	-0.2±1.0 A
	10	4.3±0.5 A	0.9±0.4 A	0.1±1.0 A	1.11±1.08 A	2.2±0.4 A	2.1±1.0 A	2.3±0.4 A	0.8±0.0 A	0.0±1.1 A

[a] Means with different capital letters designate *E. coli* O157:H7 populations that differ significantly in terms of each sanitizer concentration and time ($P \leq 0.05$).

Table AI.3: E. coli *O157:H7 populations in wash water containing mixed peracid*[a]

POAA (ppm)	% Organic Load	Mean ± SD *E. coli* O157:H7 (log CFU/ml)								
		10	20	30	40	50	60	70	80	90
10	0	5.7±0.2 A	5.9±0.1 A	5.9±0.1 A	5.9±0.1 A	5.7±0.1 C	4.8±0.7 B	4.9±0.0 C	4.2±0.2 C	3.3±0.2 C
	1	5.3±0.1 B	5.6±0.07 B	5.8±0.1 A	5.9±0.1 A	5.8±0.1 BC	5.7±0.1 AB	5.5±0.1 B	5.1±0.1 B	4.8±0.4 B
	5	5.4±0.1 AB	5.8±0.1 AB	5.9±0.0 A	5.9±0.0 A	6.0±0.1 A	5.9±0.0 A	5.9±0.1 A	5.9±0.1 A	5.8±0.2 A
	10	5.6±0.2 AB	5.8±0.1 AB	6.0±0.1 A	5.9±0.1 A	6.0±0.1 AB	6.0±0.1 A	6.0±0.0 A	6.0±0.1 A	6.0±0.0 A

Table AI.3 (cont'd)

	0	4.6±0.2 A	2.6±0.3 A	-1.0±1.1 B	-1.9±0.1 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B
	1	4.9±0.1 A	3.1±0.2 A	0.4±0.2 AB	-1.5±0.2 B	-1.5±0.2 AB	-1.5±0.2 B	-1.5±0.2 B	-1.5±0.2 AB	-1.5±0.2 AB
50	5	5.0±0.4 A	3.0±0.8 A	0.5±0.4 AB	-0.5±0.8 AB	0.1±1.2 A	-0.9±0.7 AB	-0.9±0.6 AB	-0.7±1.0 AB	-1.3±0.0 AB
	10	5.2±0.5 A	3.8±0.5 A	1.6±1.0 A	0.5±1.1 A	0.3±0.8 A	0.1±0.8 A	-0.1±0.7 A	0.1±0.8 A	0.4±1.5 A

Table AI.3 (cont'd)

					-2.0±0.0		-2.0±0.0	-2.0±0.0	-2.0±0.0	-2.0±0.0
	0	1.3±1.0 A	-1.9±0.1 B	-1.9±0.1 C	D	-2.0±0.0 C	D	D	D	D
				-1.6±0.0		-1.50±0.2				
	1	1.9±0.4 A	-1.6±0.0 B	BC	-1.6±0.0 C	B	-1.6±0.0 C	-1.6±0.0 C	-1.6±0.0 C	-1.6±0.0 C
80			-1.2±0.2	-1.3±0.0						
	5	1.0±0.7 A	AB	AB	-1.3±0.0 B	-1.3±0.0 B	-1.3±0.0 B	-1.3±0.0 B	-1.3±0.0 B	-1.3±0.0 B
			-0.3±0.9	-0.7±0.5	-1.0±0.0	-1.0±0.0	-1.0±0.0	-1.0±0.0	-1.0±0.0	-1.0±0.0
	10	0.8±1.8 A	A	A	A	A	A	A	A	A

[a] Means with different capital letters designate *E. coli* O157:H7 populations that differ significantly in terms of each sanitizer concentration and time ($P \leq 0.05$).

Table AI.4: E. coli *O157:H7 populations in wash water containing chlorine*[a]

Chlorine (ppm)	% Organic Load	Mean ± SD *E. coli* O157:H7 (log CFU/ml)								
		10	20	30	40	50	60	70	80	90
30	0	-1.8±0.3 B	-1.9±0.1 B	-1.9±0.1 B	-1.8±0.2 B	-1.9±0.1 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B
	1	5.9±0.2 A	6.0±0.0 A	6.0±0.0 A	5.9±0.1 A	5.9±0.1 A	5.9±0.0 A	5.9±0.1 A	5.9±0.0 A	6.0±0.1 A
	5	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.0 A
	10	5.9±0.1 A	5.9±0.0 A	5.9±0.0 A	6.0±0.1 A	6.0±0.1 A	5.94±0.01 A	6.0±0.0 A	6.0±0.1 A	6.0±0.0 A

Table AI.4 (cont'd)

		-1.9±0.1	-1.9±0.1	-1.9±0.1	-1.9±0.1	-1.9±0.1		-2.0±0.0	-2.0±0.0	-2.0±0.0
	0	C	C	C	B	B	-2.0±0.0 B	B	B	C
					-0.7±1.0	-0.6±0.9				
50	1	1.8±0.2 B	1.4±1.3 B	0.4±1.3 B	B	B	-0.3±1.7 B	0.1±1.9 B	0.4±2.1 B	1.4±2.5 B
	5	6.1±0.1 A	6.0±0.0 A	6.0±0.0 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A
	10	6.0±0.1 A	6.0±0.1 A	6.0±0.1 A	6.1±0.1 A	6.0±0.0 A	6.1±0.0 A	6.0±0.0 A	6.0±0.0 A	6.0±0.0 A

Table AI.4 (cont'd)

100	0	-1.9±0.1 B	-1.9±0.1 B	-1.9±0.1 B	-1.9±0.1 B	-1.9±0.1 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B	-2.0±0.0 B
	1	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B	-1.6±0.0 B
	5	6.0±0.1 A	5.8±0.2 A	5.9±0.2 A	5.7±0.3 A	5.7±0.4 A	5.6±0.4 A	5.6±0.5 A	5.7±0.3 A	5.7±0.4 A
	10	5.9±0.2 A	5.8±0.1 A	5.9±0.1 A	5.8±0.2 A	5.7±0.3 A	5.9±0.1 A	5.9±0.2 A	5.8±0.2 A	5.8±0.2 A

[a] Means with different capital letters designate *E. coli* O157:H7 populations that differ significantly in terms of each sanitizer concentration and time ($P \leq 0.05$).

AI.4.2 Physicochemical parameters of wash water. Increasing organic load correlated to significant increases ($P \leq 0.05$) in COD (10 – 5543 mg O_2/L), turbidity (0 – 0.293), and total solids (0.0064 – 0.0471 g/10 ml) in the water control (Table AI.5). Increasing organic load correlated to significant increases ($P \leq 0.05$) in COD (66 – 6020 mg O_2/L), turbidity (0 – 0.421), ORP (282 – 539 mV), and total solids (0.0057 – 0.0471 g/10 ml) for all three peroxyacetic acid treatments (Table AI.6). Increasing organic load correlated to significant increases ($P \leq 0.05$) in COD (78 – 6267 mg O_2/L), turbidity (0 – 0.431), ORP (431 – 590 mV), and total solids (0.0048 – 0.0505 g/10 ml) for all three mixed peracid treatments (Table AI.7). Increasing organic load correlated to significant increases ($P \leq 0.05$) in COD (1 – 5937 mg O_2/L), turbidity (0 – 0.289), and total solids (0.0053 – 0.0492 g/10 ml) for all three chlorine treatments (Table AI.8).

Table AI.5: *Physicochemical parameters of wash water controls containing various organic loads* [a]

% Organic Load (w/v blended lettuce)	Mean ± SD Dependent Physicochemical Paramters						
	COD (mg O2/L)	MFV (ml)	Turbidity (abs. @ 663 nm)	ORP (mV)	pH	Temperature (°C)	Total Solids (g/10 ml)
0	10±3 D	50±0 A	0±0 C	393±21 A	6.82±0.03 A	14±1 A	0.0064±0.0007 D
1	451±6 C	37±2 B	0.040±0.013 C	301±23 B	6.87±0.04 A	14±1 A	0.0132±0.0041 C
5	2330±159 B	9±2 C	0.150±0.021 B	238±2 C	7.05±0.06 A	15±0 A	0.0267±0.0025 B
10	5543±205 A	6±1 C	0.293±0.046 A	206±14 C	6.90±0.16 A	15±1 A	0.0471±0.0014 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of organic load ($P \leq 0.05$).

Table AI.6: *Physicochemical parameters of wash water containing various organic loads and peroxyacetic acid concentrations* [a]

POAA (ppm)	% Organic Load (w/v blended lettuce)	Mean ± SD Dependent Physicochemical Parameters						
		COD (mg O2/L)	MFV (ml)	Turbidity (abs. @ 663 nm)	ORP (mV)	pH	Temperature (°C)	Total Solids (g/10 ml)
10	0	66±13 C	50±0 A	0±0 C	410±30 A	6.00±1.59 A	20±4 A	0.0057±0.0012 C
	1	697±49 C	35±5 B	0.034±0.009 BC	373±2 A	6.73±0.07 A	14±1 A	0.0103±0.0009 C
	5	3243±352 B	14±3 C	0.119±0.012 B	282±46 B	6.54±0.10 A	16±1 A	0.0291±0.0025 B
	10	5317±1043 A	3±2 D	0.347±0.075 A	376±17 A	6.29±0.11 A	16±1 A	0.0449±0.0069 A
50	0	231±28 C	50±0 A	0±0 C	409±47 B	5.57±1.51 A	19±5 A	0.0060±0.0005 C
	1	885±52 C	36±6 B	0.040±0.005 C	429±2 B	6.31±0.04 A	14±0 A	0.0109±0.0008 C
	5	3477±337 B	15±3 C	0.146±0.026 B	451±35 AB	6.29±0.17 A	15±1 A	0.0280±0.0023 B
	10	5820±870 A	5±1 D	0.370±0.069 A	531±50 A	5.78±0.04 A	15±1 A	0.0471±0.0066 A

Table AI.6 (cont'd)

80	0	372±29 C	50±0 A	0±0 C	435±8 C	6.17±0.11 A	14±1 B	0.0066±0.0008 C
	1	959±65 C	41±6 A	0.039±0.006 BC	450±5 BC	5.92±0.09 A	14±0 B	0.0105±0.0010 C
	5	3827±679 B	13±4 B	0.146±0.022 B	464±4 B	5.88±0.16 A	15±1 AB	0.0269±0.0019 B
	10	6020±1054 A	6±1 B	0.421±0.092 A	539±10 A	5.44±0.14 B	16±1 A	0.0457±0.0077 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of organic load at each sanitizer concentration ($P \leq 0.05$).

Table AI.7: *Physicochemical parameters of wash water containing various organic loads and mixed peracid concentrations* [a]

Mixed POAA (ppm)	% Organic Load (w/v blended lettuce)	Mean ± SD Dependent Physicochemical Paramters						
		COD (mg O2/L)	MFV (ml)	Turbidity (abs. @ 663 nm)	ORP (mV)	pH	Temperature (°C)	Total Solids (g/10 ml)
10	0	78±15 B	50±0 A	0±0 C	443±12 A	6.68±0.18 A	14±1 C	0.0048±0.0003 D
	1	636±54 B	31±6 B	0.033±0.007 C	431±5 A	6.71±0.15 A	14±0 BC	0.0100±0.0017 C
	5	6863±3386 A	10±2 C	0.154±0.065 B	433±28 A	6.87±0.09 A	15±0 AB	0.0254±0.0013 B
	10	5233±445 A	5±0 C	0.276±0.032 A	356±30 B	6.77±0.04 A	15±0 A	0.0414±0.0018 A
50	0	359±9 B	50±0 A	0±0 B	482±7 C	6.36±0.06 A	13±0 C	0.0053±0.0009 D
	1	1039±217 B	24±7 B	0.048±0.017 B	483±8 BC	6.41±0.10 A	14±0 BC	0.0105±0.0006 C
	5	6070±3403 A	8±1 C	0.144±0.014 B	564±51 AB	6.25±0.01 A	14±1 AB	0.0266±0.0009 B
	10	5880±695 A	5±1 C	0.355±0.113 A	570±35 A	5.98±0.13 B	15±1 A	0.0461±0.0023 A

Table AI.7 (cont'd)

80	0	557±10 C	50±0 A	0±0 C	475±4 B	5.96±0.11 A	13±0 C	0.0051±0.0010 D
	1	1088±94 C	25±7 B	0.044±0.014 C	505±5 B	5.96±0.03 A	14±0 BC	0.0117±0.0015 C
	5	3807±890 B	8±0 C	0.171±0.019 B	558±34 A	5.72±0.08 A	14±0 B	0.0259±0.0004 B
	10	6267±521 A	5±1 C	0.431±0.077 A	590±18 A	5.33±0.23 B	15±1 A	0.0505±0.0046 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of organic load at each sanitizer concentration ($P \leq 0.05$).

Table AI.8: *Physicochemical parameters of wash water containing various organic loads and chlorine concentrations* [a]

Available Chlorine (ppm)	% Organic Load (w/v blended lettuce)	Mean ± SD Dependent Physicochemical Paramters						
		COD (mg O2/L)	MFV (ml)	Turbidity (abs. @ 663 nm)	ORP (mV)	pH	Temperature (°C)	Total Solids (g/10 ml)
30	0	1±1 C	50±0 C	0±0 C	724±8 A	7.54±0.04 A	14±0 A	0.0053±0.0006 D
	1	617±30 C	31±13 C	0.048±0.037 C	437±44 B	7.40±0.08 A	15±0 A	0.0113±0.0006 C
	5	3260±1474 B	9±2 B	0.181±0.054 B	403±35 B	7.16±0.13 A	15±1 A	0.0275±0.0027 B
	10	5937±900 A	6±1 A	0.283±0.009 A	361±12 B	7.24±0.32 A	15±A A	0.0492±0.0017 A
50	0	8±8 B	50±0 A	0±0 C	743±30 A	7.68±0.09 A	15±0 A	0.0054±0.0005 D
	1	514±85 B	34±7 B	0.030±0.003 C	758 ±42 A	7.57±0.10 A	15±0 A	0.0109±0.0009 C
	5	3517±1737 A	10±1 C	0.132±0.016 B	439±3 B	7.28±0.05 B	15±0 A	0.0264±0.0008 B
	10	5727±1141 A	7±2 C	0.289±0.052 A	363±13 C	7.10±0.06 B	16±1 A	0.0492±0.0031 A

Table AI.8 (cont'd)

100	0	3±3 C	50±0 A	0±0 C	761±16 A	7.84±0.09 A	15±1 A	0.065±0.0005 C
	1	649±69 C	20±4 B	O.034±0.005 C	788±16 A	7.91±0.16 A	14±1 A	0.0158±0.0047 C
	5	3330±734 B	10±1 C	0.124±0.037 B	454±12 B	7.38±0.15 B	15±1 A	0.0302±0.0027 B
	10	5880±630 A	6±1 C	0.281±0.039 A	441±27 B	7.18±0.09 B	16±1 A	0.0454±0.0060 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of organic load at each sanitizer concentration ($P \leq 0.05$).

AI.5 SUMMARY OF FINDINGS

The two aims of this study were to 1) determine the efficacy of three concentrations of three different sanitizing agents against *E. coli* O157:H7 in water containing organic loads of 0, 1, 5 and 10% (w/v blended lettuce solids) and 2) assess various physicochemical parameters of the wash water. This study was originally meant to serve as a model for the pilot-scale processing line used in Chapters 2, 4 and 5, however, comparisons of these results with the results from the processing line found that the methods presented here do not adequately predict the results from the processing line. Additionally, this study did not assess persistence of *E. coli* O157:H7 in wash water, simply the immediate effect of the sanitizing agents during a 90 s exposure period. The work presented here served as a framework for the study presented in Chapter 3, where lettuce was used to inoculate the wash water in the carboy and *E. coli* persistence was assessed over 10 min. Differences in results seen between the pilot-scale processing line, the carboy system in Chapter 3, and the carboy system here illustrate that the methods of exposing *E. coli* O157:H7 to the wash water in addition to the scale of the system used for the study are critical factors for consistency between various systems.

Increases in organic load correlated to significant increases ($P \leq 0.05$) in COD, turbidity, and total solids for all sanitizers and sanitizer concentrations. Future work can be done in order to determine the significance of different inoculation methods (e.g., lettuce vs. cocktail) and different systems (e.g., centrifuge tubes, 4 L carboy, and a pilot-scale leafy greens processing line).

Appendix II:

Efficacy of Multiple Chlorine Concentrations Acidified with T-128 against *Escherichia coli* O157:H7 during Pilot-Scale Processing of Iceberg Lettuce Using Wash Water Containing an Organic Load

AII.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the efficacy of three chlorine concentrations acidified with T-128 against *E. coli* O157:H7 on iceberg lettuce, in wash water, and on surfaces of a pilot-scale processing line using flume water containing an organic load. Methods were similar to those in Chapter 4 except that a single organic load (5% w/v blended lettuce) and three different chlorine concentrations (10, 30, and 100 ppm), all acidified to pH 6.0 with T-128 were used. Increasing chlorine concentrations correlated to significantly greater *E. coli* O157:H7 reductions ($P \leq 0.05$) in wash water and on lettuce. A chlorine concentration of 100 ppm prevented the persistence of *E. coli* O157:H7 on equipment surfaces and in water spun off during centrifugal drying. ORP correlated with increased chlorine concentration and antimicrobial activity.

AII.2 INTRODUCTION

Wash water quality has been a major focus of the produce industry for many years with these concern heightened in response to three nationwide outbreaks in 2006 traced to fresh-cut lettuce and baby spinach. Despite the widespread use of chemical sanitizers in produce wash water, efficacy of these sanitizers remains problematic due to the presence of organic material in the water that decreases antibacterial activity. Weak acids, such as citric acid, are routinely added to chlorinated wash water to acidify to a targeted pH 6.5 – 7.0, where the active component, HClO predominates. A new, generally recognized as safe (GRAS) acidifying agent comprised of phosphoric acid and propylene glycol, known as T-128 (SmartWash Solutions, Salinas, CA), has been developed to improve the stability of chlorine. T-128 was examined in Chapters 2 – 4; however, only one chlorine concentration was used for each study.

The two aims of this study were to 1) determine the efficacy of three different chlorine concentrations acidified with T-128 (SmartWash Solutions) against *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce using water containing an organic load of 5% (w/v) blended lettuce solids and 2) assess the relationship between various physicochemical parameters and chlorine concentration of the wash water on *E. coli* O157:H7 inactivation.

AII.3 MATERIALS AND METHODS

AII.3.1 Experimental design. The efficacy of three chlorine concentrations acidified to pH 6.0 using T-128 against *E. coli* O157:H7 was assessed in triplicate by processing a 5.4 kg batch of iceberg lettuce inoculated at 10^6 CFU/g followed by three consecutive 5.4 kg batches of uninoculated iceberg lettuce in flume water containing an organic load of 5% (w/v) blended lettuce solids. All lettuce was processed by shredding, conveying, fluming, shaker table dewatering and/or centrifugal drying, during and/or after which various product, water and equipment surface samples were collected and quantitatively examined for *E. coli* O157:H7. *E. coli* O157:H7 persistence was correlated to six physicochemical parameters of the wash water: temperature, oxidation/reduction potential, chemical oxygen demand, total solids, maximum filterable volume and turbidity.

AII.3.2 Leafy greens. Identical to 2.3.2.

AII.3.3 Bacterial strains. Identical to 2.3.3.

AII.3.4 Lettuce inoculation. Identical to 4.3.4.

AII.3.5 Lettuce processing line. Identical to 4.3.5.

AII.3.6 Wash water. Iceberg lettuce (45 kg) was blended for 10 min in tap water using a Rotostat blender (Model XP-02, Admix, Manchester, NH) and added to the recirculation tank where water was added to achieve an organic load level of 5% (w/v) in a total volume of 890 L at 12 to 15^{o}C. Three chlorine concentrations were used – 10, 30 and 100 ppm available chlorine (XY-12, Ecolab, St. Paul, MN) which were acidified with T-128 (SmartWash Solutions) to a pH of 6.0 as measured with a pH probe (pHTestr 30, Oakton, Vernon Hills, IL). The chlorine concentration and pH was maintained throughout processing, with necessary adjustments made

during the 10 min pauses in lettuce processing. Chlorine test kit 321 (Ecolab) was used to confirm the available chlorine concentration. Sanitizer-free MSU tap water served as the control.

AII.3.7 Leafy green processing. Identical to 4.3.7.

AII.3.8 Sample collection. Identical to 4.3.8.

AII.3.9 Physiochemical parameters of flume water. Identical to 4.3.9.

AII.3.10 Microbiological analyses. Identical to 4.3.10.

AII.3.11 Sanitizer neutralization confirmation. Chlorine concentrations of 10, 30 and 100 ppm (XY-12, Ecolab) acidified to pH 6.0 using T-128 (SmartWash Solutions) were prepared and confirmed with Chlorine test kit 321 (Ecolab) in triplicate 1 L water samples containing a 5% organic load prepared a mechanical blender (Model BLC10650MB, Black & Decker, New Britain, CT). A 50 ml centrifuge tube containing 3 ml of 38x concentrated neutralizing buffer (BD) was filled with the water sample containing sanitizer, agitated for 5 sec and then immediately assessed for neutralization of the sanitizer as previously described using the Chlorine test kit. Preliminary experiments found that a 38x concentration would neutralize various concentrations of the active component of each sanitizing agent used in this study without impacting *E. coli* O157:H7 counts.

AII.3.12 Statistical analysis. Identical to 4.3.13.

AII.4 RESULTS

AII.4.1 Lettuce. Average *E. coli* O157:H7 reductions on inoculated lettuce were 1.2, 1.1 and 1.3 log CFU/g as seen with 10, 30, and 100 ppm available chlorine acidified to pH 6.0 with T-128, respectively (Figure AII.1). None of the chlorine concentrations were significantly more effective ($P > 0.05$) against *E. coli* O157:H7 on the first uninoculated batch of lettuce, with populations ranging from 2.2 – 2.9 log CFU/g. One hundred ppm was significantly more effective ($P \leq 0.05$) than 10 and 30 ppm chlorine against *E. coli* O157:H7on uninoculated lettuce from batch 2, with populations of 2.3, 2.0, and 0.9 log CFU/g recovered, respectively. Similarly, 100 ppm was also significantly more effective than 10 ppm chlorine against *E. coli* O157:H7 on uninoculated lettuce batch 3, with populations of 2.1 and 0.1 log CFU/g recovered, respectively.

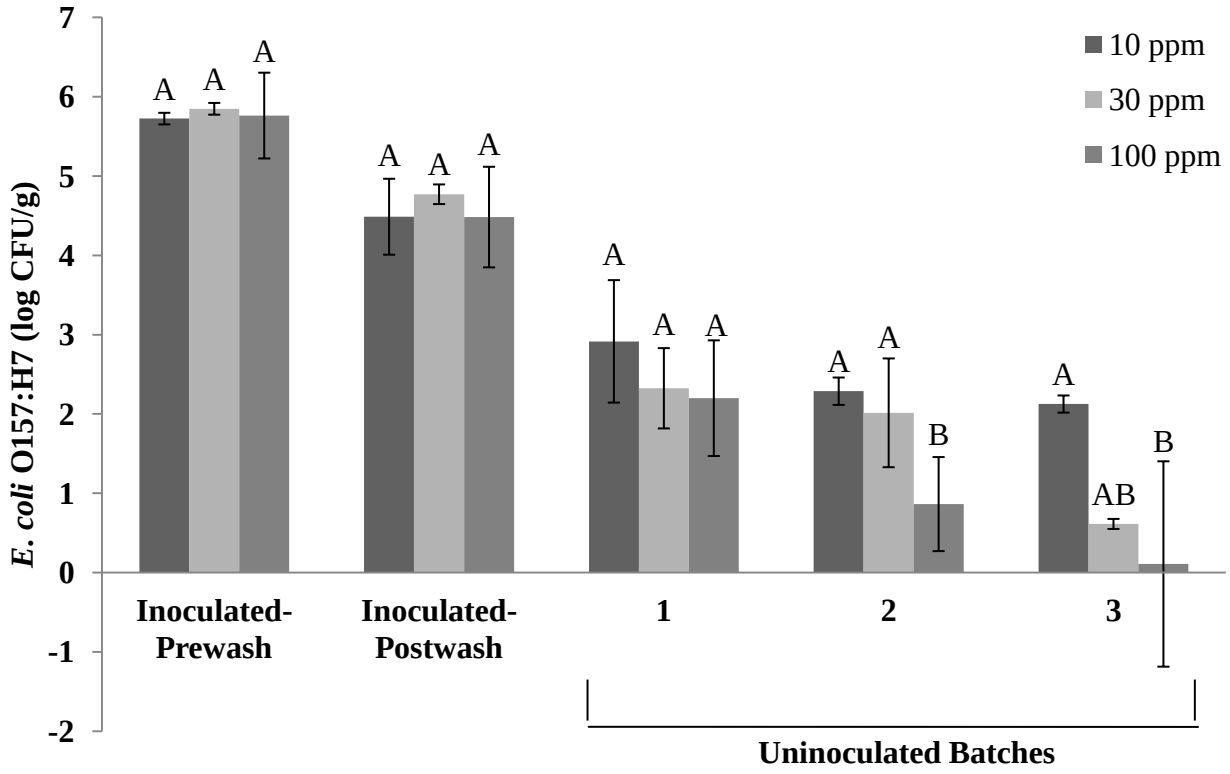


Figure AII.1: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after flume washing in water containing 5% organic load (w/v blended lettuce) and chlorine concentrations of 10, 30 and 100 ppm + T-128 (n=3 per chlorine concentration). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*).*

AII.4.2 Flume water. Average *E. coli* O157:H7 reductions typically increased with increasing chlorine concentration in the flume water. At the start of water sampling when the inoculated lettuce was first removed from the flume system (0 min), average *E. coli* O157:H7 reductions with 10 (0.3 log CFU/ml) and 30 ppm chlorine (0.2 log CFU/ml) were significantly lower ($P \leq 0.05$) than those seen with 100 ppm (5.3 log CFU/ml) (Table AII.1). After 21 min of processing, the average *E. coli* O157:H7 reduction with 10 ppm (0.5 log CFU/ml) was significantly lower ($P \leq 0.05$) than those seen for 30 (3.1 log CFU/ml) or 100 ppm chlorine (5.3 log CFU/ml). By the end of processing (44 min), average *E. coli* O157:H7 reductions with 10 ppm chlorine (0.8 log CFU/ml) were significantly lower ($P \leq 0.05$) than for 30 (4.4 log CFU/ml) and 100 ppm (5.3 log CFU/ml).

E. coli O157:H7 populations were typically below the limit of detection in flume water containing 100 ppm available chlorine, resulting in a y-intercept of -5.3 andno slope over time with an R^2 value of 0.12 and a RMSE of 0.03 CFU/ml) (Table AII.2, Figure AII.2). Trend lines for *E. coli* O157:H7 inactivation in flume water containing 10 and 30 ppm had similar y-intercepts of -0.3 and -0.3 log CFU/ml, however, the slope of the inactivation equation for 30 ppm (-0.1*t*) was 10x greater than the slope seen at 10 ppm chlorine (-0.01*t*). The R^2 values for 10 (0.92) and 30 ppm (0.93) show that *E. coli* O157:H7 inactivation is strongly correlated with chlorine concentration. The RMSE for 10 ppm was 0.2 log CFU/ml, with a higher RMSE seen for 30 ppm (1.4 log CFU/ml).

Table AII.1: E. coli *O157:H7 reductions in flume water* [a]

Available Chlorine (ppm)	Mean ± SD *E. coli* O157:H7 reduction in flume water (log CFU/ml)		
	0 min	21 min	44 min
10	0.3 ± 0.2 B	0.5 ± 01 C	0.8 ± 0.1 B
30	0.2 ± 0.1 B	3.1 ± 1.2 B	4.4 ± 0.8 A
100	5.3 ± 0.1 A	5.3 ± 0.0 A	5.3 ± 0.0 A

[a] Means with different capital letters designate *E. coli* O157:H7 reductions that differ significantly in terms of sanitizing treatment and time ($P \leq 0.05$).

Table AII.2: E. coli *O157:H7 inactivation trend lines for flume water containing various chlorine concentrations* [a]

	E. coli O157:H7 Inactivation			
Available Chlorine (ppm)	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
10	$Log(N/N_0) = -0.01t - 0.3$	0.92	0.2	1.38E-13
30	$Log(N/N_0) = -0.10t - 0.3$	0.93	1.4	6.08E-14
100	$Log(N/N_0) = -5.3$ [b]	0.12	0.0	0.09

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

[b] The slope (*k*) was < 0.1 and was therefore not included.

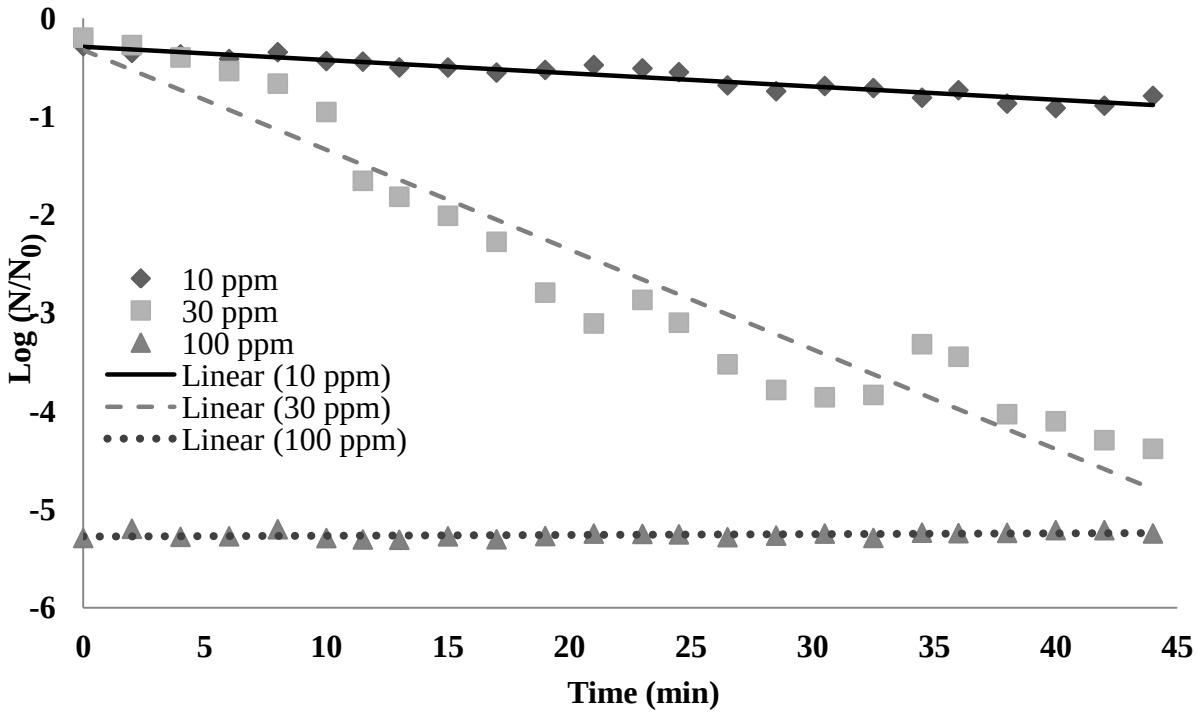


Figure AII.2: E. coli *O157:H7 reductions in recirculating flume water containing 5% organic load (w/v blended lettuce) and chlorine concentrations of 10, 30 and 100 ppm + T-128 compared to the sanitizer-free control (n=3 per chlorine concentration).*

AII.4.3 Centrifugation water. A chlorine concentrations of 100 ppm resulted in *E. coli* O157:H7 populations that were always significantly lower ($P \leq 0.05$) than those recovered at 10 or 30 ppm, with -1.3 log CFU/ml seen for the inoculated batch as well as batches 1 – 3 (Figure AII.3). Numbers of *E. coli* O157:H7 recovered from water spun off from the inoculated batch (4.6 log CFU/ml) and first uninoculated batch of lettuce (3.6 log CFU/ml) after washing in 10 ppm were not significantly different ($P > 0.05$) from populations recovered after washing in 30 ppm chlorine. *E. coli* O157:H7 populations recovered from centrifugation water removed from uninoculated batches 2 (2.1 log CFU/ml) and 3 (1.6 log CFU/ml) after product was washed in 30 ppm chlorine were significantly lower ($P \leq 0.05$) than recovered with 10 ppm.

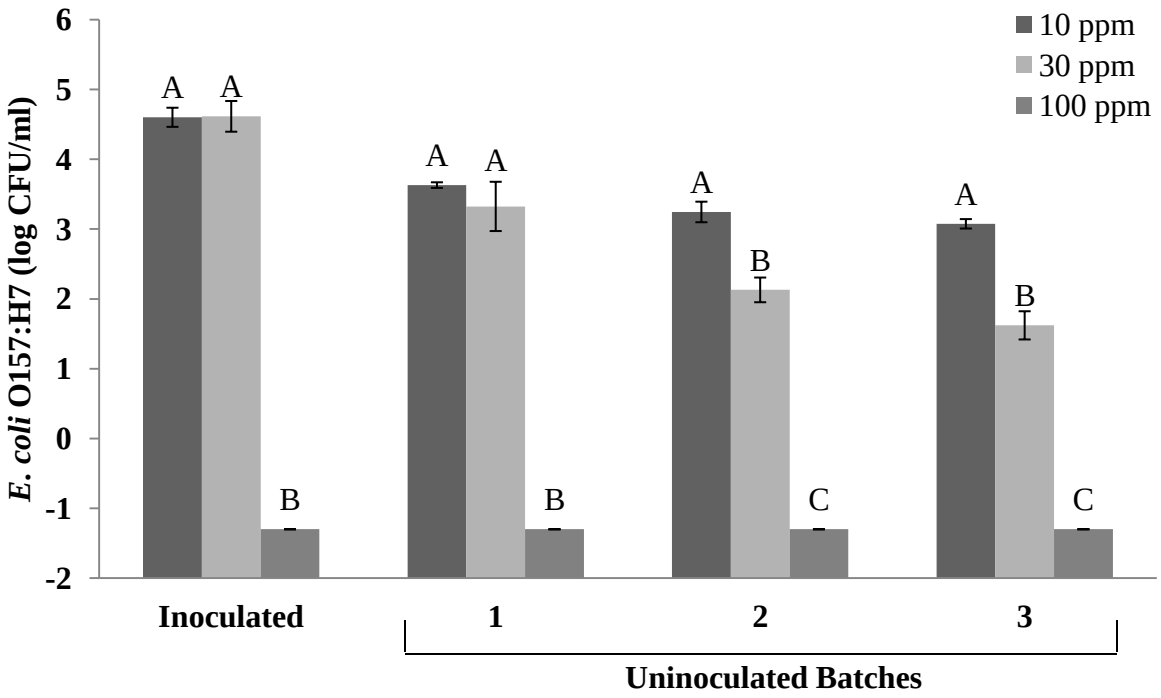
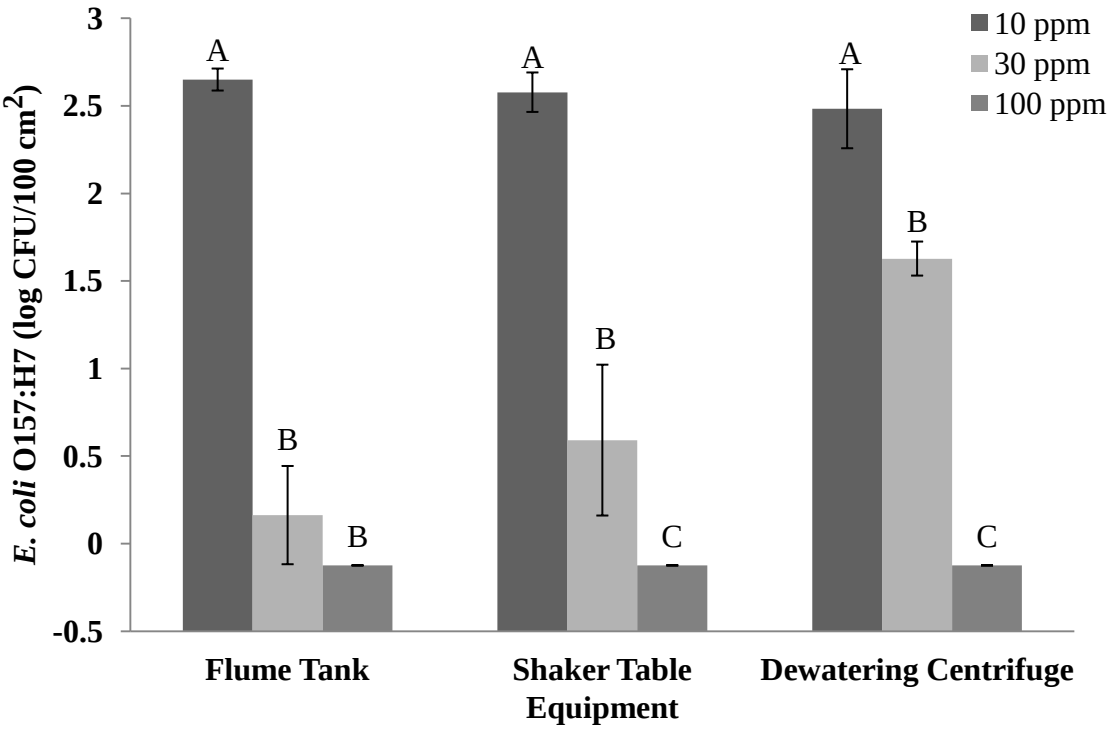


Figure AII.3: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing 5% organic load (w/v blended lettuce) and chlorine concentrations of 10, 30 and 100 ppm + T-128 (n=3 per chlorine concentration). Means within the same batch with different letters are significantly different (*P ≤ *0.05). Limit of detection = 0.02 CFU/ml.*

AII.4.4 Processing equipment surfaces. *E. coli* O157:H7 populations recovered from the flume tank, shaker table, and dewatering centrifuge after processing with 10 ppm chlorine were 2.7, 2.6 and 2.5 log CFU/100 cm^2, respectively, and were significantly higher ($P \leq 0.05$) than those seen using 30 or 100 ppm chlorine (Figure AII.4). Washing with 100 ppm chlorine resulted in *E. coli* populations that were consistently at or below the limit of detection for all equipment surfaces, resulting in -0.1 log CFU/100 cm^2 for all surfaces. *E. coli* O157:H7 populations recovered after washing with 30 ppm chlorine were significantly lower ($P \leq 0.05$) than those recovered after processing with 10 ppm, resulting in populations of 0.2, 0.6, and 1.6 log CFU/100 cm^2 for the flume tank, shaker table, and dewatering centrifuge, respectively.

Figure AII.4: *Mean (± SD),* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing 5% organic load (w/v blended lettuce) and chlorine concentrations of 10, 30 and 100 ppm + T-128 (n=3 per chlorine concentration). Means of the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.



AII.4.5 Physicochemical parameters of flume water. Increasing sanitizer concentration correlated to significant increases ($P \leq 0.05$) in ORP, with 416, 477 and 855 mV recorded for chlorine concentrations of 10, 30 and 100 ppm, respectively (Table AII.3). Considering that the organic load was 5% (w/v) blended lettuce for all three chlorine treatments, it is not surprising that no significant difference was seen in total solids, COD or turbidity between any of the treatments. Total solids ranged from 0.0289 to 0.0336 g/10 ml, COD ranged from 2870 to 3374 mg O2/L, and turbidity ranged from 0.043 to 0.056. MFV was significantly lower ($P \leq 0.05$) with 100 ppm (12 ml) than with 10 ppm (43 ml) or 30 ml (42 ml).

Table AII.3: *Physicochemical parameters of flume water containing organic load and various chlorine concentrations acidified with T-128* [a]

Independent Physicochemical Parameters	Mean ± SD Dependent Physicochemical Parameters					
Available Chlorine (ppm)	Temp (°C)	Total Solids (g)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
10	14 ± 0 A	0.0289 ± 0.0016 A	3374 ± 414 A	0.046 ± 0.003 A	43 ± 1 A	416 ± 13 C
30	14 ± 0 A	0.0326 ± 0.0035 A	3235 ± 1197 A	0.043 ± 0.011 A	42 ± 8 A	477 ± 17 B
100	14 ± 0 A	0.0336 ± 0.0019 A	2870 ± 676 A	0.056 ± 0.006 A	12 ± 0 B	855 ± 15 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of chlorine concentration ($P \leq 0.05$).

AII.5 SUMMARY OF FINDINGS

The two aims of this study were to 1) determine the efficacy of three different chlorine concentrations acidified with T-128 (SmartWash Solutions) against *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce using water containing an organic load of 5% (w/v) blended lettuce solids and 2) assess the relationship between various physicochemical parameters and chlorine concentration of the wash water on *E. coli* O157:H7 inactivation. Previous work presented in Chapter 4 of this dissertation focused on the impact of various organic load concentrations (0 – 10% w/v blended lettuce) using a single concentration of chlorine (50 ppm) either unacidified or acidified with citric acid or T-128. This study was completed in collaboration with a research group from the University of South Florida, with their study focusing on determining the efficacy of a water concentrator at quantifying *E. coli* O157:H7 populations in wash water containing an organic load.

Not surprisingly, *E. coli* O157:H7 persistence in wash water and on finished product was dependent on sanitizer concentration of the wash water with significantly fewer ($P \leq 0.05$) *E. coli* O157:H7 cells recovered from uninoculated batches 2 and 3 when 100 ppm available chlorine was present in the wash water. Chapter 4 of this dissertation showed that a concentration of 50 ppm chlorine was significantly impacted by an organic load of 5%. In this study, 100 ppm free chlorine reduced *E. coli* O157:H7 populations below the limit of detection in the flume water. ORP proved to be the best predictor of sanitizer efficacy.

Based on these findings, processors should consider acidifying their wash water using T-128 to pH 6.0 when using 100 ppm chlorine. This sanitizing treatment proved to be effective against *E. coli* O157:H7 on the product, equipment surfaces, and in wash water. However, proper ventilation will be needed due to off-gassing of the chlorine.

Appendix III:

Impact of In-Line Equipment Sanitation Processes on *Escherichia coli* O157:H7 Persistence during Pilot-Scale Processing of Iceberg Lettuce Using Flume Water Containing Sodium Hypochlorite and Organic Load

AIII.1 ABSTRACT

Chemical sanitizers are routinely used during commercial flume washing of fresh-cut leafy greens to minimize cross-contamination from the water. This study assessed the impact of sanitizing practices on *E. coli* O157:H7 persistence on finished product, in wash water, and on equipment using flume water containing 50 ppm chlorine. Methods were similar to those in Chapter 4 except that a single organic load (2.5% w/v blended lettuce) and chlorine concentration (50 ppm) were used. By sanitizing the shredder and conveyor after processing the inoculated lettuce, significantly greater *E. coli* O157:H7 reductions ($P \leq 0.05$) were seen in the wash water and on equipment surfaces. However, no significant differences ($P > 0.05$) in *E. coli* O157:H7 populations were observed on lettuce after processing between either operational condition. Physicochemical parameters of the wash water were rarely significantly different between either operating condition.

AIII.2 INTRODUCTION

Wash water quality has been a major focus of the produce industry for many years with these concern heightened in response to three nationwide outbreaks in 2006 traced to fresh-cut lettuce and baby spinach. Despite the widespread use of chemical sanitizers in produce wash water, efficacy of these sanitizers remains problematic due to the presence of organic material in the water that decreases antibacterial activity. While the focus of Chapter 4 was the impact of sanitizing deficiencies on *E. coli* O157:H7 in flume water, it was noted that *E. coli* O157:H7 persisted on all lettuce after processing, even if the pathogen was below the lower limit of detection in the flume water.

Consequently, the two aims of this study were to 1) determine the persistence of *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce using a shredder and conveyor that had been sanitized after processing the inoculated product and before the uninoculated product was then washed in flume water containing 50 ppm available chlorine and an organic load of 2.5% (w/v blended lettuce solids) and 2) assess the relationship between various physicochemical parameters and chlorine concentration of the wash water on *E. coli* O157:H7 inactivation.

AIII.3 MATERIALS AND METHODS

AIII.3.1 Experimental design. A 5.4 kg batch of iceberg lettuce inoculated with *E. coli* O157:H7 at 10^6 CFU/g was initially processed, after which the shredder and conveyor were sanitized before processing three additional 5.4 kg batches of uninoculated lettuce, thereby leaving the flume water and surfaces of the shaker table as only means to contaminate the product. All lettuce was processed by shredding, conveying, fluming in water containing 50 ppm chlorine and an organic load of 2.5% (w/v) blended lettuce solids, shaker table dewatering and/or centrifugal drying. During and/or after processing various product, water and equipment surface samples were collected and quantitatively examined for *E. coli* O157:H7. *E. coli* O157:H7 persistence was correlated to six physicochemical parameters of the wash water: temperature, oxidation/reduction potential, chemical oxygen demand, total solids, maximum filterable volume and turbidity. A water control (containing 2.5% organic load and no chlorine) was processed without sanitizing the shredder or conveyor.

AIII.3.2 Leafy greens. Identical to 2.3.2.

AIII.3.3 Bacterial strains. Identical to 2.3.3.

AIII.3.4 Lettuce inoculation. Identical to 4.3.4.

AIII.3.5 Lettuce processing line. Identical to 4.3.5.

AIII.3.6 Wash water. Iceberg lettuce (22.5 kg) was blended for 10 min in tap water using a Rotostat blender (Model XP-02, Admix, Manchester, NH) and added to the recirculation tank where water was added to achieve an organic load level of 2.5% (w/v) in a total volume of 890 L at 12 to 15^{o}C. A chlorine concentration of 50 ppm available chlorine (XY-12, Ecolab, St. Paul, MN) was maintained throughout processing, with necessary adjustments made during the

10 min pauses in lettuce processing. Chlorine test kit 321 (Ecolab) was used to confirm the available chlorine concentration. Sanitizer-free MSU tap water served as the control.

AIII.3.7 Leafy green processing. To prime the system, 5.4 kg of uninoculated iceberg lettuce was hand-fed into the shredder at a rate of about 0.5 kg per s, with the shredded product then step-conveyed to the flume tank and washed in 890 L of recirculating sanitizing wash water for 90 s. The product was then released from the flume tank and partially dewatered on the shaker table, collected in a single centrifugation basket and centrifugally dried. Following a 10 min pause in processing to adjust the sanitizer concentration, the 5.4 kg batch of inoculated lettuce was identically processed. The shredder and conveyor belt were then cleaned, sanitized, and rinsed to remove inoculated product and residual inoculum from the equipment surfaces. Three uninoculated 5.4 kg batches were then processed identically to the primer and inoculated batches of lettuce.

AIII.3.8 Sample collection. Identical to 4.3.8.

AIII.3.9 Physiochemical parameters of flume water. Identical to 4.3.9.

AIII.3.10 Microbiological analyses. Identical to 4.3.10.

AIII.3.11 Sanitizer neutralization confirmation. Identical to 4.3.11.

AIII.3.12 Statistical analysis. Identical to 4.3.13.

AIII.4 RESULTS

AIII.4.1 Lettuce. *E. coli* O157:H7 persisted on all iceberg lettuce after washing and centrifugation, regardless of operational conditions. Average *E. coli* O157:H7 reductions from inoculated lettuce were 1.0, 0.9 and 1.1 log CFU/g as seen with the water control, 50 ppm chlorine, and 50 ppm chlorine + clean shredder and conveyor (Figure AIII.1). No significant difference ($P > 0.05$) in *E. coli* O157:H7 populations were seen after processing inoculated lettuce or the first batch of uninoculated lettuce, with populations ranging from 2.2 – 2.8 log CFU/g for uninoculated batch 1. By the second uninoculated batch, *E. coli* O157:H7 populations on lettuce processed with a sanitized shredder and conveyor had significantly less ($P \leq 0.05$) *E. coli* O157:H7 than the water control, with 0.7 and 2.7 log CFU/g, respectively. *E. coli* O157:H7 populations on the third batch of uninoculated lettuce were significantly higher ($P \leq 0.05$) on lettuce washed with the water control (2.4 log CFU/g) than lettuce washed in chlorine (1.4 log CFU/g) or chlorine + a clean shredder and conveyor (0.6 log CFU/g). Additionally, no significant difference in *E. coli* O157:H7 populations recovered from batch 3 were seen for lettuce processed with chlorine or chlorine + a clean shredder and conveyor.

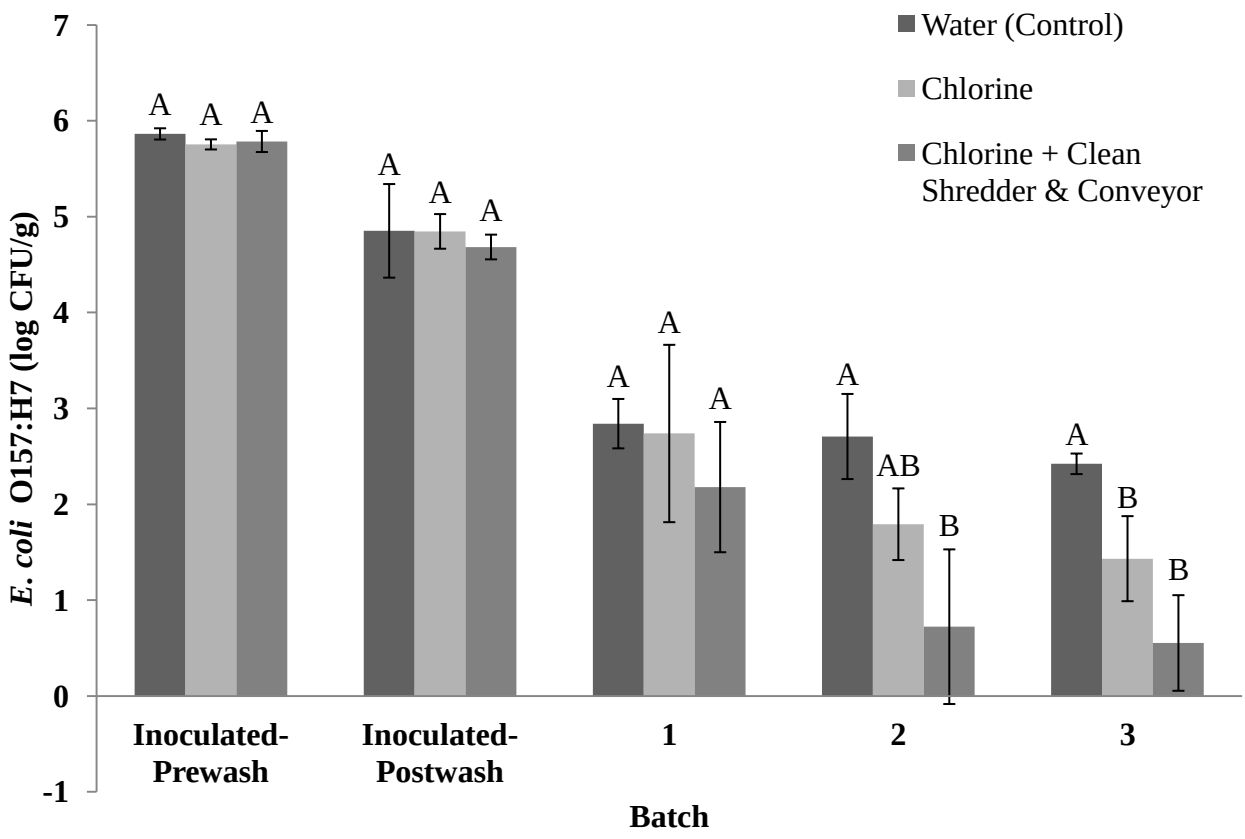


Figure AIII.1: *Mean (± SD)* E. coli *O157:H7 populations on lettuce after flume washing in water containing 2.5% organic load (w/v blended lettuce) and 50 ppm chlorine, with or without a sanitized shredder and conveyor (n=3 per operational condition). Means within the same batch with different letters are significantly different (*$P \leq 0.05$*).*

AIII.4.2 Flume water. Cleaning and sanitizing the shredder and conveyor reduced the populations of *E. coli* O157:H7 in the flume water. As soon as the inoculated lettuce left the flume tank (0 min), *E. coli* O157:H7 reductions in water with a clean shredder and conveyor were significantly higher ($P \leq 0.05$) (4.1 log CFU/ml) than seen with the normal processing operation (-0.2 log CFU/ml) (Table AIII.1). About halfway through processing (21 min), *E. coli* O157:H7 reductions were still significantly higher ($P \leq 0.05$) using the previously cleaned and sanitized shredder and conveyor (5.2 log CFU/ml) than seen with the normal processing operation (2.3 log CFU/ml). By the end of processing (44 min), no significant difference ($P > 0.05$) in *E. coli* O157:H7 reductions in flume water were observed between the normal processing (3.9 log CFU/ml) or the cleaned and sanitized shredder and conveyor (5.2 log CFU/ml).

E. coli O157:H7 populations were typically much closer to the lower limit of detection in flume water using the cleaned and sanitized shredder and conveyor, resulting in a y-intercept of -4.4, slope of -0.02*t*, R^2 value of 0. 7, and RMSE of 0.2 log CFU/ml (Table AIII.2, Figure AIII.2). In contrast, un-sanitized shredder and conveyor yielded a y-intercept of 0.1. While this y-intercept was much higher, the slope was five times higher at -0.1*t*, with a resulting R^2 value of 0.94 and a RMSE of 0.3 log CFU/ml. *P* values for both trend lines were < 0.0001.

Table AIII.1: E. coli *O157:H7 reductions in flume water* [a]

Operational Conditions	Mean ± SD *E. coli* O157:H7 reduction in flume water (log CFU/ml)		
	0 min	21 min	44 min
Chlorine	-0.2 ± 0.3 B	2.3 ± 0.6 B	3.9 ± 0.9 A
Chlorine + Clean Shredder & Conveyor	4.1 ± 1.0 A	5.2 ± 0.1 A	5.2 ± 0.1 A

[a] Means with different capital letters designate *E. coli* O157:H7 reductions that differ significantly in terms of operational condition and time ($P \leq 0.05$).

Table AIII.2: E. coli *O157:H7 inactivation trend lines for flume water after different processing conditions* [a]

Operational Conditions	*E. coli* O157:H7 Inactivation			
	Log Reduction	R^2	RMSE (log CFU/ml)	*P* Value
Chlorine	$Log(N/N_0) = -0.1t + 0.1$	0.94	0.3	< 0.0001
Chlorine + Clean Shredder & Conveyor	$Log(N/N_0) = -0.02t - 4.4$	0.67	0.2	< 0.0001

[a] Inactivation rates were determined by using Microsoft Excel to create linear regression trend lines for *E. coli* O157:H7 log reduction over time (*t*) in minutes.

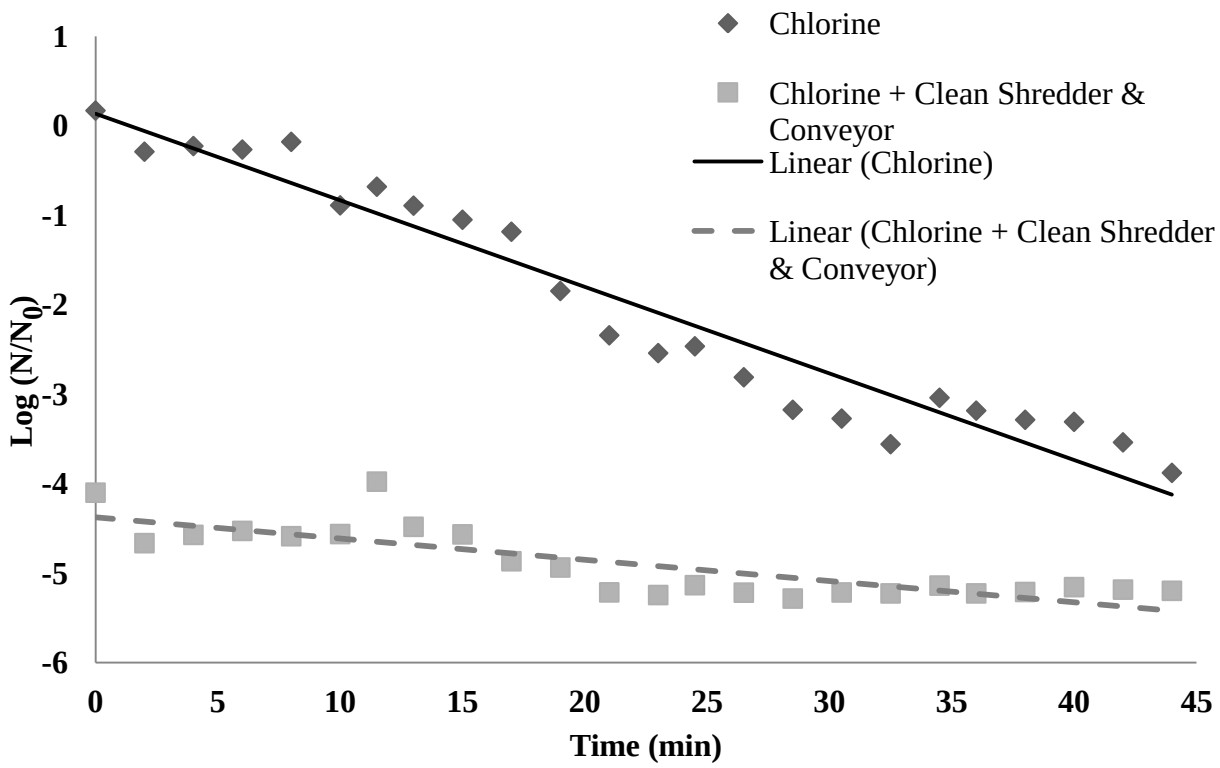


Figure AIII.2: E. coli *O157:H7 reductions in recirculating flume water containing 2.5% organic load (w/v blended lettuce) and 50 ppm chlorine, with or without a sanitized shredder and conveyor compared to the sanitizer-free control (n=3 per operational condition).*

AIII.4.3 Centrifugation water. Average *E. coli* O157:H7 populations in spent centrifugation from the water control were 4.7 log CFU/ml for the inoculated batch and 3.8 – 4.0 log CFU/ml for the three uninoculated batches (Figure AIII.3). Addition of chlorine resulted in *E. coli* O157:H7 populations that were not significantly lower ($P \leq 0.05$) than the water control for the inoculated batch or the first uninoculated batch, with 4.3 and 3.3 log CFU/ml recovered, respectively. *E. coli* O157:H7 populations in centrifugation water from uninoculated batches 2 (2.2 log CFU/ml) and 3 (1.8 log CFU/ml) after washing in chlorine were significantly lower than the water control. *E. coli* O157:H7 populations recovered from water spun off after processing with the cleaned and sanitized shredder and conveyor were consistently significantly lower ($P \leq 0.05$) than the water control from the inoculated batch of lettuce (3.3 log CFU/ml) and 1.4, 0.4 and 0.3 log CFU/ml for uninoculated batches 1, 2 and 3, respectively. Additionally, the *E. coli* O157:H7 populations recovered from the centrifugation water from uninoculated batches 1 – 3 after processing with the cleaned and sanitized shredder and conveyor were significantly lower than the populations recovered after processing under normal conditions using chlorine.

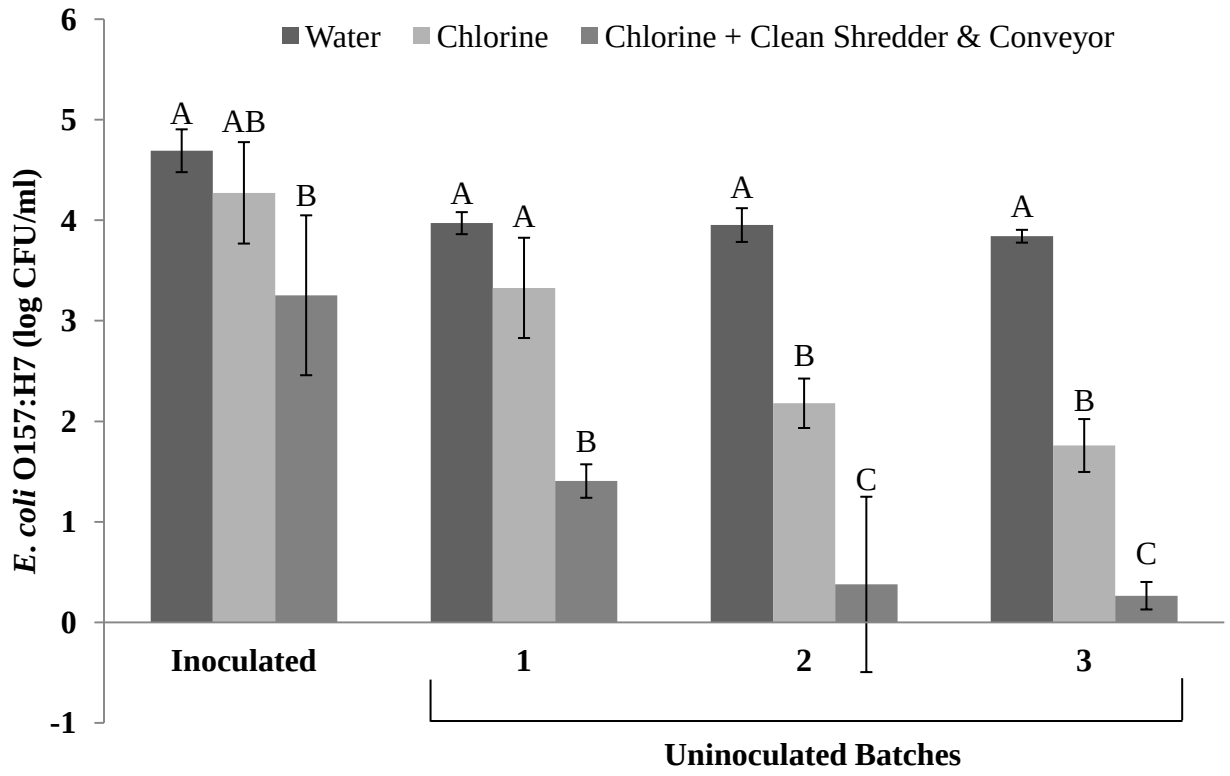


Figure AIII.3: *Mean (± SD)* E. coli *O157:H7 populations in spent centrifugation water from lettuce after washing in flume water containing 2.5% organic load (w/v blended lettuce) and 50 ppm chlorine, with or without a sanitized shredder and conveyor (n=3 per operational condition). Means within the same batch with different letters are significantly different (*P ≤ *0.05). Limit of detection = 0.02 CFU/ml.*

AIII.4.4 Processing equipment surfaces. *E. coli* O157:H7 populations recovered from the surfaces of the flume tank, shaker table, and dewatering centrifuge after processing with water were 3.4, 3.6 and 3.1 log CFU/100 cm^2, respectively, and were significantly higher ($P \leq 0.05$) than those seen on the flume tank and shaker table after processing with chlorine or chlorine + a clean shredder and conveyor (Figure AIII.4). *E. coli* O157:H7 populations were significantly higher ($P \leq 0.05$) on the flume tank (0.2 log CFU/100 cm^2) and shaker table (1.0 log CFU/100 cm^2) after processing with chlorine than seen with chlorine + a cleaned and sanitized shredder and conveyor (-1.3 and -0.6 log CFU/100 cm^2, respectively). Average *E. coli* O157:H7 populations on the centrifugal dryer were significantly lower ($P \leq 0.05$) than the water control after processing with a cleaned and sanitized shredder and conveyor, with 1.1 log CFU/100 cm^2, but statistically similar to the populations recovered after processing with chlorine (2.3 log CFU/100 cm^2).

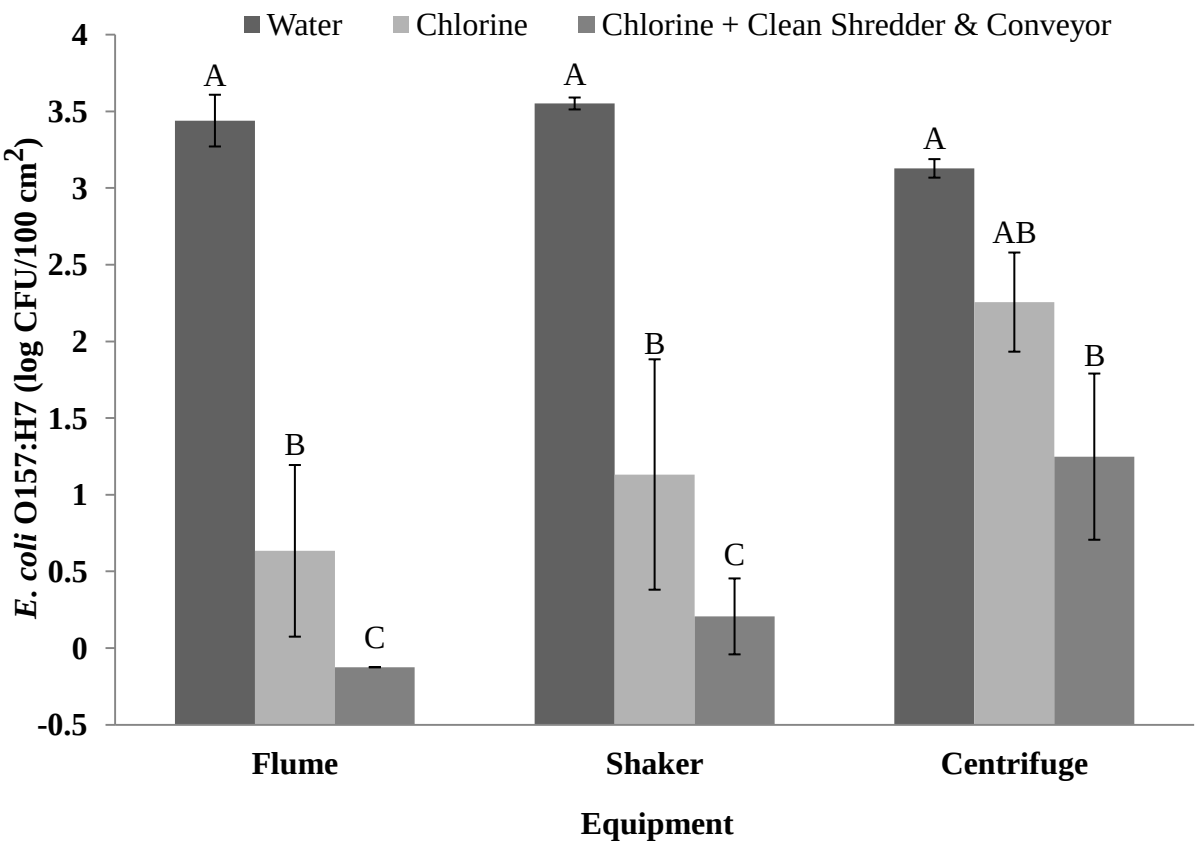


Figure AIII.4: *Mean (± SD)* E. coli *O157:H7 populations on equipment surfaces after processing lettuce in flume water containing 2.5% organic load (w/v blended lettuce) and 50 ppm chlorine, with or without a sanitized shredder and conveyor (n=3 per operational condition). Means within the same equipment with different letters are significantly different (*P ≤ *0.05). Limit of detection = 1 CFU/100 cm*^{2}.

AIII.4.5 Physicochemical parameters of flume water. Significant differences ($P \leq 0.05$) between the physicochemical parameters of the flume water were rarely seen between the two different operational conditions. Normal operation with chlorine resulted in significantly lower ($P \leq 0.05$) pH (8.38) and ORP (366 mV) than the pH (8.67) and ORP (464 mV) seen for the cleaned and sanitized shredder and conveyor (Table AIII.3). There were no significant differences ($P > 0.05$) between the other physiochemical parameters of the two operational conditions with total solids ranging from 0.0175 – 0.0178 g/10 ml, COD ranging from 956 – 971 mg O_2/L, turbidity ranging from 0.017 – 0.021, and MFV ranging from 38 – 43 ml.

Table AIII.3: *Physicochemical parameters of flume water 2.5% containing organic load and 50 ppm chlorine during normal operation and with a sanitized shredder and conveyor* [a]

Operational Conditions	Dependent Physicochemical Parameters						
	Temp (°C)	pH	Total Solids (g/10 ml)	COD (mg O_2/L)	Turbidity (abs @ 663 nm)	MFV (ml)	ORP (mV)
Chlorine	13±0 A	8.38±0.06 B	0.0178±0.0007 A	971±67 A	0.021±0.001 B	43±2 A	366±15 B
Chlorine + Clean Shredder & Conveyor	13±0 A	8.67±0.06 A	0.0175±0.0013 A	956±52 A	0.017±0.004 B	38±1 A	464±53 A

[a] Means labeled with different capital letters designate physicochemical parameters that differ significantly in terms of operation condition ($P \leq 0.05$).

AIII.5 SUMMARY OF FINDINGS

The two aims of this study were to 1) determine the persistence of *E. coli* O157:H7 during pilot-scale processing of iceberg lettuce using a shredder and conveyor that has been sanitized after processing the inoculated product and before the uninoculated product then washed in flume water containing 50 ppm available chlorine and an organic load of 2.5% (w/v blended lettuce solids) and 2) assess the relationship between various physicochemical parameters and chlorine concentration of the wash water on *E. coli* O157:H7 inactivation. Overall, physicochemical parameters of the flume water were rarely different between the two operational conditions, emphasizing that those differences in *E. coli* O157:H7 populations in wash water, on lettuce and equipment surfaces are attributable to the level of *E. coli* O157:H7 contamination on the product before washing. The studies in Chapters 4 and 5 assessed *E. coli* O157:H7 persistence while processing under a variety of water conditions. This study was completed in response to the persistence of *E. coli* O157:H7on all finished product throughout processing in the earlier studies.

There were no significant differences in *E. coli* O157:H7 populations on the finished product between the two operating conditions, emphasizing that cross-contamination of product during processing is a systemic issue and cannot be pin-pointed to a single processing step. However, by simulating a sanitizing step during shredding and conveying, it was noticed that average *E. coli* O157:H7 populations in the wash water were consistently significantly lower in the wash water when the shredder and conveyor were sanitized after processing the inoculated lettuce, resulting in a much lower y-intercept for the predictive *E. coli* O157:H7 inactivation trend line. Additional studies would need to be completed in order to determine if *E. coli* O157:H7 cross-contamination could be reduced by processing additional lettuce after the

uninoculated lettuce. *E. coli* O157:H7 contamination of the three uninoculated batches of lettuce processed after the inoculated batch likely occurred in the wash water and on the shaker table- where lettuce was seen to remain on equipment between batches.

Based on these results, commercial processors should consider including a sanitizing spray rinse on equipment that leafy greens contact before washing to reducing the likelihood of amplifying a previously isolated contamination event. Additional studies would need to be completed in order to determine if an *E. coli* O157:H7 contamination event would be more isolated if more uninoculated lettuce was processed than the 16.2 kg of lettuce in this study.

REFERENCES

REFERENCES

1. Abadias, M., I. Alegre, J. Usall, R. Torres, and I. Vinas. 2011. Evaluation of alternative sanitizers to chlorine disinfection for reducing foodborne pathogens in fresh-cut apple. *Postharvest Biol. Technol.* 59:289-297.

2. Ahmed, J., and T. Alam. 2011. Minimal processing and novel technologies applied to vegetables. p. 317-333. *In* N.K. Sinha (ed.). Handbook of Vegetables and Vegetable Vegetable Processing. Wiley-Blackwell, Ames, IA.

3. Allende, A., E. Aguayo, and F. Artes. 2003. Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf life. *Int. J. Food. Microbiol.* 91:109-117.

4. Allende, A., J. McEvoy, Y. Tao, and Y. Luo. 2009. Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. *Food Control*. 20:230-234.

5. Allende, A., M. V. Selma, F. Lopez-Galvez, R. Villaescusa, and M. I. Gil. 2008. Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *J. Food Prot.* 71:2514-2518.

6. Alvardo-Casillas, S., L. S. Ibarra-Sanchez, E. Martinez-Gonzalesn, M. O. Rodriguez-Garcia, and A. Castillo. 2010. Validation of a washing and sanitizing procedure for cantaloupes at a Mexican packing facility. *J. Food Prot.* 73:362–365.

7. Annous, B. A., P. M. Fratamico, and J. L. Smith. 2009. Quorum sensing in biofilms: why bacteria behave the way they do. *J. Food Sci.* 74:R24-R37.

8. Arnade, C., L. Calvin, and F. Kuchler. 2009. Consumer response to a food safety shock: the 2006 food-borne illness outbreak of *E.coli* O157: H7 linked to spinach. *Appl. Econ. Perspect. Policy*. 31:734-750.

9. Artés, F., P. Gómez, F. Artés-Hernández, E. Aguayo, and V. Escalona. 2007. Improved strategies for keeping overall quality of fresh-cut produce. *Acta Hort*. 746:245-258.

10. Ayyildiz, O., B. Ileri, and S. Sanik. 2009. Impacts of water organic load on chlorine dioxide disinfection efficacy. *J. Hazard. Mater.* 168:1092-1097.

11. Baert, L., I. Vandekinderen, F. Devlieghere, E. V. Coillie, J. Debevere, and M. Uyttendaele. 2009. Efficacy of sodium hypochlorite and peroxyacetic acid to reduce murine norovirus 1, B40-8, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 on shredded iceberg lettuce and in residual wash water. *J. Food Prot.* 72:1047-1054.

12. Baird, R. B. 2005. 5220 B. Open Reflux Method. *In* M.A.H. Franson (ed.). Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC.

13. Barrera, M. J., R. Blenkinsop, and K. Warriner. 2012. The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control*. 25:745-751.

14. Baxter, T. E., and D. J. Rexing. 2005. 2540 B. Total Solids Dried at 103-105°C. *In* M.A.H. Franson (ed.). Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC.

15. Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, and J. G. Wells. 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *JAMA.* 272:1349-1353.

16. Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Available at: http://www.who.int/foodsafety/publications/fs_management/en/surface_decon.pdf. Accessed 28 August 2012.

17. Beuchat, L. R., B. B. Adler, and M. M. Lang. 2004. Efficacy of chlorine and a peroxyacetic acid sanitizer in killing *Listeria monocytogenes* on iceberg and Romaine lettuce using simulated commercial processing conditions. *J. Food Prot.* 67:1238-1242.

18. Beuchat, L. R., D. A. Mann, and W. Q. Alali. 2013. Efficacy of sanitizers in reducing *Salmonella* on pecan nutmeats during cracking and shelling. *J. Food Prot.* 76:770-778.

19. Beuchat, L. R., and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg. Infect. Dis.* 3:459-465.

20. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Stockbine. 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1982-2002. *Journal of Infectious Diseases*. 192:1422-1429.

21. Buchanan, R. L., S. G. Edelson, R. L. Miller, and G. M. Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J. Food Prot.* 62:444-450.

22. Buchholz, A. L., G. R. Davidson, B. P. Marks, E. C. D. Todd, and E. T. Ryser. 2012. Quantitative transfer of *Escherichia coli* O157:H7 to equipment during small-scale production of fresh-cut leafy greens. *J. Food Prot.* 75:1184-1197.

23. Buchholz, A. L., G. R. Davidson, B. P. Marks, E. C. D. Todd, and E. T. Ryser. 2012. Transfer of *Escherichia coli* O157:H7 from equipment surfaces to fresh-cut leafy greens during

processing in a model pilot-plant production line with sanitizer-free water. *J. Food Prot.* 75:1920–1929.

24. Buchholz, A. L., G. R. Davidson, R. E. Sloup, B. P. Marks, E. C. D. Todd, and E. T. Ryser. 2010. Tracking an *Escherichia coli* O157:H7 contaminated batch of leafy greens through a commercial processing line, Abst. T4-02. *In*, Abst. Ann. Mtg. Int. Assoc. Food Prot., Anaheim, CA.

25. Burnett, A. B., M. H. Iturriaga, E. F. Escartin, C. A. Pettigrew, and L. R. Beuchat. 2004. Influence of variations in methodology on populations of *Listeria monocytogenes* recovered from lettuce treated with sanitizers. *J. Food Prot.* 67:742–750.

26. Burnett, S. L., and L. R. Beuchat. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *J. Indust. Microbiol. Biotechnol.* 27:104-110.

27. Butt, M. S., and M. T. Sultan. 2011. Nutritional profile of vegetables and its significance to human health. p. 107-123. *In* N.K. Sinha (ed.). Handbook of Vegetables and Vegetable Processing. Wiley-Blackwell, Ames, IA.

28. California Department of Public Health. 2007. Investigation of an *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach. Available at: http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20Spnch%20EC%20Dole032007wph.PDF. Accessed 3 October 2012.

29. California Leafy Green Products Handler Marketing Agreement. 2012. About us. Available at: http://www.caleafygreens.ca.gov/about-us. Accessed 12 January 2013.

30. Caprioli, A., S. Morabito, H. Brugere, and E. Oswald. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.* 36:289-311.

31. Carlin, F., and C. Nguyen-The. 1994. Fate of *Listeria monocytogenes* on four types of minimally processed green salads. *Lett. Appl. Microbiol.* 18:222-226.

32. Carmichael, I., I. S. Harper, M. J. Coventry, P. W. J. Taylor, J. Wan, and M. W. Hickey. 1999. Bacterial colonization and biofilm development on minimally processed vegetables. *J. Appl. Microbiol.* 85:45S-51S.

33. Center for Produce Safety. 2013. Grants program: Center for Produce Safety 2013 request for proposals. Available at: https://cps.ucdavis.edu/grant_opportunities.php. Accessed 6 February 2013.

34. Center for Science in the Public Interest (CSPI). 2008. CSPI Outbreak Alert Data: Info on Produce Outbreaks. Available at: http://www.cspinet.org/new/pdf/cspi_outbreak_alert.pdf. Accessed 12 December 2012.

35. Center for Science in the Public Interest (CSPI). 2008. Outbreak Alert! 2008. Available at: http://www.cspinet.org/new/pdf/outbreak_alert_2008_report_final.pdf. Accessed 13 October 2012.

36. Center for Science in the Public Interest (CSPI). 2009. The ten riskiest foods regulated by the US Food and Drug Administration. Available at: http://cspinet.org/new/pdf/cspi_top_10_fda.pdf. Accessed 2 October 2012.

37. Centers for Disease Contol and Prevention. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice -- British Columbia, California, Colorado, and Washington, October 1996. *MMWR*. 45:975.

38. Centers for Disease Contol and Prevention. 2006. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach --- United States, September 2006. *MMWR*. 55:1-2.

39. Centers for Disease Contol and Prevention. 2010. Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded Romaine lettuce from a single processing facility. Available at: http://www.cdc.gov/ecoli/2010/ecoli_o145/. Accessed 12 January 2013.

40. Centers for Disease Contol and Prevention. 2012. Investigation update: Multistate outbreak of *E. coli* O157:H7 infections linked to Romaine lettuce. Available at: http://www.cdc.gov/ecoli/2011/ecoliO157/romainelettuce/032312/index.html. Accessed 20 February 2013.

41. Centers for Disease Contol and Prevention. 2012. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to organic spinach and spring mix blend (final update). Available at: http://www.cdc.gov/ecoli/2012/O157H7-11-12/. Accessed 12 January 2013.

42. Chase-Topping, M. E., I. J. McKendrick, M. C. Pearce, P. MacDonald, L. Matthews, J. Halliday, L. Allison, D. Fenlon, J. C. Low, G. Gunn, and M. E. Woolhouse. 2007. Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. *J. Clin. Microbiol.* 45:1594-603.

43. Code of Federal Regulations. 2012. Title 21, Part 173.315. Chemicals used in washing or to assist in the peeling of fruits and vegetables. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.315. Accessed 16 September 2012.

44. Cody, S. H., M. K. Glynn, J. A. Farrar, K. L. Cairns, P. M. Griffin, J. Kobayashi, M. Fyfe, R. Hoffman, A. S. King, J. H. Lewis, B. Swaminathan, R. G. Bryant, and D. J. Vugia. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Annal. Intern. Med.* 130:202-209.

45. Cooley, M., D. Carychao, L. Crawford-Miksza, M. T. Jay, C. Myers, C. Rose, C. Keys, J. Farrar, and R. E. Mandrell. 2007. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PLoS ONE*. 2:e1159.

46. Ecolab. 2010. Acid antimicrobial additive: Tsunami 100. Available at: http://salustarim.com/uploads/Tsunami_orjinal_etiket.pdf. Accessed 15 October 2012.

47. Foleya, D. M., A. Dufourb, L. Rodriguezb, F. Caporasob, and A. Prakash. 2002. Reduction of *Escherichia coli* O157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. *Rad. Phys. Chem.* 63:391–396.

48. Food and Agriculture Organization and the World Health Organization. 2010. CODEX Alimentarius: Code of hygenic practice for fresh fruits and vegetables. Available at: http://www.codexalimentarius.org/standards/list-of-standards/en/. Accessed 20 February 2013.

49. Foodborne Illness Outbreak Database. 2013. Aunt Mid's Produce Company lettuce 2008. Available at: http://outbreakdatabase.com/details/aunt-mids-produce-company-lettuce-2008/?organism=E.+coli+O157%3AH7&vehicle=lettuce. Accessed 12 January 2013.

50. Foodborne Illness Outbreak Database. 2013. Church Brothers LLC/ Cafe Rio Restaurant/ Chipotle Restaurant Romaine lettuce 2009. Available at: http://outbreakdatabase.com/details/church-brothers-llc-cafe-rio-restaurant-chipotle-restaurant-romaine-lettuce-2009/?organism=E.+coli+O157%3AH7&vehicle=lettuce. Accessed 12 January 2013.

51. Foodborne Illness Outbreak Database. 2013. *E. coli* O157:H7 outbreak linked to Romaine lettuce - Canada and California, April 2012. Available at: http://www.outbreakdatabase.com/details/e.-coli-o157h7-outbreak-linked-to-romaine-lettuce-canada-and-california-april-2012/. Accessed 12 Janurary 2013.

52. Foodborne Illness Outbreak Database. 2013. Multistate pre-packaged lettuce 2009. Available at: http://outbreakdatabase.com/details/multistate-pre-packaged-lettuce-2009/?organism=E.+coli+O157%3AH7&vehicle=lettuce. Accessed 12 January 2013.

53. Foodborne Illness Outbreak Database. 2013. Multistate schnucks salad bars, college campuses Romaine lettuce 2011. Available at: http://outbreakdatabase.com/details/multistate-schnucks-salad-bars-college-campuses-romaine-lettuce-2011/?organism=E.+coli+O157%3AH7&vehicle=lettuce. Accessed 12 Janurary 2013.

54. Garg, N., J. J. Churney, and D. F. Splittstoesser. 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. *J. Food Prot.* 53:701-703.

55. Gil, M. I., M. V. Selma, F. López-Gálvez, and A. Allende. 2009. Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *Int. J. Food. Microbiol.* 134:37-45.

56. Gomez-Lopez, V. M., J. Gobet, M. V. Selma, M. I. Gil, and A. Allende. 2013. Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with *E. coli* O157:H7. *Food Control*. 29:42-48.

57. Gonzalez, R. J., Y. Luo, S. Ruiz-Cruz, and J. L. McEvoy. 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *J. Food Prot.* 67:2375–2380.

58. Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. *Epidem. Rev.* 13:60-98.

59. Hach. 2009. Chemical oxygen demand, Method 8000 for water, wastewater, and seawater. Available at: http://www.hach.com/quick.search-download.search.jsa?keywords=8000. Accessed 10 November 2012.

60. Harp, D. L. Date 2002. The current technology of chlorine analysis for water and wastewater. Technical information series- booklet No. 17 [Type of Medium].

61. Haute, S. V., I. Sampers, K. Holvoet, and M. Uyttendaele. 2013. Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Appl. Environ. Microbiol.* 79:2850-2861.

62. Herdt, J., and H. Feng. 2009. Aqueous antimicrobial treatments to improve fresh and fresh-cut produce safety. p. 169-190. *In* B.A.N. Xuetong Fan, Christopher J. Doona, Florence E. Feeherry, Robert B. Gravani (ed.). Microbial Safety of Fresh Produce. Wiley-Blackwell, Ames, Iowa.

63. Herman, K. M., T. L. Ayers, and M. Lynch. 2008. Foodborne disease outbreaks associated with leafy greens, 1973–2006. Available at: http://wwwnc.cdc.gov/eid/pdfs/ICEID2008.pdf. Accessed 10 October 2012.

64. Hilgren, J., K. M. J. Swanson, F. Diez-Gonzalez, and B. Cords. 2007. Inactivation of *Bacillus anthracis* spores by liquid biocides in the presence of food residue. *Appl. Environ. Microbiol.* 73:6370-6377.

65. Hilgren, J. D., and J. A. Salverda. 2000. Antimicrobial efficacy of a peroxyacetic/octanoic acid mixture in fresh-cut-vegetable process waters. *J. Food Sci.* 65:1376-1379.

66. Huang, Y., and H. Chen. 2011. Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. *Food Control*. 22:1178-1183.

67. Jay, M. T., M. Cooley, D. Carychao, G. W. Wiscomb, R. A. Sweitzer, L. Crawford-Miksza, J. A. Farrar, D. K. Lau, J. O'Connell, A. Millington, R. V. Asmundson, E. R. Atwill, and R. E. Mandrell. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. *Emerg. Infect. Dis.* 13:1908-1911.

68. Jordan, S. L., C. Pascual, E. Bracey, and B. M. Mackey. 2001. Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. *J. Appl. Microbiol.* 91:463-469.

69. Kaper, J. B., J. P. Nataro, and H. L. T. Mobley. 2004. Pathogenic *Escherichia coli. Nat. Rev. Microbiol.* 2:123-140.

70. Karmali, M. A., M. Petric, B. T. Steele, and C. Lim. 1983. Sporadic cases of haemolytic-urameic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet*. 321:619-620.

71. Keene, W. E., K. Hedberg, D. E. Herriott, D. D. Hancock, R. W. McKay, T. J. Barrett, and D. W. Fleming. 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. *J. Infect. Dis.* 176:815-818.

72. Keskinen, L. A., and B. A. Annous. 2011. Efficacy of adding detergents to sanitizer solutions for inactivation of *Escherichia coli* O157:H7 on Romaine lettuce. *Int. J. Food. Microbiol.* 147:157-61.

73. Kitis, M. 2004. Disinfection of wastewater with peracetic acid: a review. *Environ. Intern.* 30:47-55.

74. Koivunen, J., and H. Heinonen-Tanski. 2005. Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments. *Water Res.* 39:1519-1526.

75. Lang, M. M., L. J. Harris, and L. R. Beuchat. 2004. Survival and recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on lettuce and parsley as affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. *J. Food Prot.* 67:1092-1103.

76. Lemons, K. E. 2009. Antimicrobial compositions and methods of use thereof. U.S. patent 2009/0192231 A1.

77. Li, Y., R. E. Brackett, J. Chen, and L. R. Beuchat. 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15 C. *J. Food Prot.* 64:305-309.

78. López-Gálvez, F., A. Allende, M. V. Selma, and M. I. Gil. 2009. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *Int. J. Food. Microbiol.* 133:167-171.

79. Lopez-Galvez, F., G. D. Posada-Izquierdo, M. V. Selma, F. Perez-Rodriguez, J. Gobet, M. I. Gil, and A. Allende. 2012. Electrochemical disinfection: An efficient treatment to inactivate *Escherichia coli* O157:H7 in process wash water containing organic matter. *Food Microbiol.* 30:146-156.

80. Luo, Y. 2007. Fresh-cut produce wash water reuse affects water quality and packaged product quality and microbial growth in Romaine lettuce. *Hort. Sci.* 42:1413-1419.

81. Luo, Y., X. Nou, P. Millner, and B. Zhou. 2012. A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. *Int. J. Food. Microbiol.* 158:133–139.

82. Luo, Y., X. Nou, Y. Yang, I. Alegre, E. Turner, H. Feng, M. Abadias, and W. Conway. 2011. Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. *J. Food Prot.* 74:352-358.

83. Lynch, M. F., R. V. Tauxe, and C. W. Hedberg. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol. Infect.* 137:307-315.

84. Mandrell, R. E. 2009. Enteric human pathogens associated with fresh produce: sources, transport, and ecology. p. 5-41. *In* B.A.N. Xuetong Fan, Christopher J. Doona, Florence E. Feeherry, Robert B. Gravani (ed.). Microbial Safety of Fresh Produce. Wiley-Blackwell, Ames, IA.

85. Meng, J., M. P. Doyle, T. Zhao, and S. Zhao. 2007. Enterohemorrhagic *Escherichia coli.* p. 260. *In* M.P. Doyle, and L.R. Beuchat (ed.). Food Microbiology: Fundamentals and Frontiers, 3rd ed. ASM Press, Washington D.C.

86. Nou, X., and Y. Luo. 2010. Whole-leaf wash improves chlorine efficacy for microbial reduction and prevents pathogen cross-contamination during fresh-cut lettuce processing. *J. Food Sci.* 75:M283-M290.

87. Nou, X., Y. Luo, L. Hollar, Y. Yang, H. Feng, P. Millner, and D. Shelton. 2011. Chlorine stabilizer T-128 enhances efficacy of chlorine against cross-contamination by *E. coli* O157:H7 and *Salmonella* in fresh-cut lettuce processing. *J. Food Sci.* 76:218-224.

88. Ölmez, H. 2010. Effect of different sanitizing methods and incubation time and temperature on inactivation of *Escherichia coli* on lettuce. *J. Food Safety*. 30:288-299.

89. Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Branden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg. Infect. Dis.* 19:In press.

90. Palumbo, M. S., J. R. Gorny, D. E. Gombas, L. R. Beauchat, C. M. Bruhn, B. Cassens, P. Delaquis, J. M. Farber, L. J. Harris, K. Ito, M. T. Osterholm, M. Smith, and K. M. J. Swanson. 2007. Recommendations for handling fresh-cut leafy green salads by consumers and retail foodservice operators. *Food Prot. Trends*. 27:892-898.

91. Parish, M. E., L. R. Beuchat, T. V. Suslow, L. J. Harris, E. H. Garrett, J. N. Farber, and F. F. Busta. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Compr. Rev. Food Sci. F.* 2:161-173.

92. Paton, J. C., and A. W. Paton. 1998. Pathogenesis and diagnosis of Shiga-toxin producing *Escherichia coli* infections. *Clinical Microbiology Reviews*. 11:450-479.

93. Ren, L., G. D. Posada-Izquierdo, and E. T. Ryser. 2013. Dissemination of *Escherichia coli* O157:H7 from a contaminated shredder or conveyor belt to fresh-cut iceberg lettuce during simulated commercial production. *In*, Unpublished data.

94. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Engl. J. Med.* 308:681-685.

95. Rodgers, S. L., J. N. Cash, M. Siddiq, and E. T. Ryser. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J. Food Prot.* 67:721–731.

96. Rosen, H., B. R. Michel, D. R. V. Devanter, and J. P. Hughes. 1998. Differential effects of myeloperoxidase-derived oxidants on *Escherichia coli* DNA replication. *Infect. Immun.* 66:2655–2659.

97. Ruiz-Cruz, S., E. Acedo-Felix, M. Diaz-Cinco, M. A. Islas-Osuna, and G. A. Gonzalez-Aguilar. 2007. Efficacy of sanitizers in reducing *Escherichia coli* O157:H7, *Salmonella spp.* and *Listeria monocytogenes* populations on fresh-cut carrots. *Food Control*. 18:1383-1390.

98. Ryser, E. T., J. Hao, and Z. Yan. 2009. Internalization of pathogens in produce. p. 55-80. *In* B.A.N. Xuetong Fan, Christopher J. Doona, Florence E. Feeherry, Robert B. Gravani (ed.). Microbial Safety of Fresh Produce. Wiley-Blackwell, Ames, IA.

99. Ryu, J. H., and L. R. Beuchat. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. *Appl. Environ. Microbiol.* 71:247-254.

100. Sapers, G. M. 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technol. Biotech.* 39:305–311.

101. Sapers, G. M. 2006. Washing and sanitizing treatments for fruits and vegetables. *In* J.R.G. G. M. Sapers, and A. E. Yousef (ed.). Microbiology of Fruits and Vegetables. CRC Press, Boca Raton, FL.

102. Sapers, G. M. 2009. Disinfection of contaminated produce with conventional washing and sanitizing technology. p. 393-424. *In* G. Sapers, E. Solomon, and K.R. Matthews (ed.). The Produce Contamination Problem: Causes and Solutions. Elsevier, Burlington. MA.

103. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States- major pathogens. *Emerg. Infect. Dis.* 17:7-15.

104. Seo, K. H., and J. F. Frank. 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *J. Food Prot.* 62:3-9.

105. Shaw, R. K., C. N. Berger, B. Feys, S. Knutton, M. J. Pallen, and G. Frankel. 2008. Enterohemorrhagic *Escherichia coli* exploits EspA filaments for attachment to salad leaves. *Appl. Environ. Microbiol.* 74:2908-2914.

106. Shen, C., Y. Luo, X. Nou, G. Bauchan, B. Zhou, Q. Wang, and P. Millner. 2012. Enhanced inactivation of *Salmonella* and *Pseudomonas* biofilms on stainless steel by use of T-128, a fresh-produce washing aid, in chlorinated wash solutions. *Appl. Environ. Microbiol.* 78:6789-6798.

107. Shen, C., Y. Luo, X. Nou, Q. Wang, and P. Millner. 2013. Dynamic effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7, and non-O157 Shiga toxin–producing *E. coli. J. Food Prot.* 76:386-393.

108. Stafford, R. J., B. J. McCall, A. S. Neill, D. S. Leon, G. J. Dorricott, C. D. Towner, and G. R. Micalizzi. 2002. A statewide outbreak of *Salmonella* Bovismorbificans phage type 32 infection in Queensland. *Commun.Dis. Intell.* 26:568-573.

109. Suslow, T. V. 1997. Postharvest chlorination: basic properties and key points for effective disinfection. Publication 8003. Available at: http://anrcatalog.ucdavis.edu/pdf/8003.pdf. Accessed 15 September 2012.

110. Suslow, T. V. 2000. Postharvest handling for organic crops. University of California, Davis, Agriculture and Natural Resources. Publication 7254. Available at: http://anrcatalog.ucdavis.edu/pdf/7254.pdf. Accessed 4 February 2013.

111. Suslow, T. V. 2001. Water disinfection, a practical approach to calculating dose values for preharvest and postharvest applications. University of California, Davis, Agriculture and Natural Resources. Publication 7256. Available at: http://anrcatalog.ucdavis.edu/pdf/7256.pdf. Accessed 22 September 2012.

112. Suslow, T. V. 2004. Oxidation-reduction potential (ORP) for water disinfection monitoring, control, and documentation. University of California, Davis, Agriculture and Natural Resources. Publication 8149. Available at: http://anrcatalog.ucdavis.edu/pdf/8149.pdf. Accessed 13 September 2012.

113. Suslow, T. V. 2006. Making sense of rules governing chlorine contact in postharvest handling of organic produce. University of California, Davis, Agriculture and Natural Resources. Publication 8198. Available at: http://anrcatalog.ucdavis.edu/pdf/8198.pdf. Accessed 22 September 2012.

114. Swerdlow, D. L., B. A. Woodruff, R. C. Brady, P. M. Griffin, S. Tippen, H. D. Donnell, E. Geldreich, B. J. Payne, A. Meyer, J. G. Wells, K. D. Greene, M. Bright, N. H. Bean, and P. A.

Blake. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Annal. Intern. Med.* 117:812-819.

115. Szabo, E. A., L. Simons, M. J. Coventry, and M. B. Cole. 2003. Assessment of control measures to achieve a food safety objective of less than 100 CFU of *Listeria monocytogenes* per gram at the point of consumption for fresh precut iceberg lettuce. *J. Food Prot.* 66:256–264.

116. Tarr, P. I., C. A. Gordon, and W. L. Chandler. 2005. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet*. 365:1073-1086.

117. Tarver, T. 2009. Biofilms: a threat to food safety. *Food Technol.* 63:46-52.

118. Tirpanalan, Ö., M. Zunabovic, K. J. Domig, and W. Kneifel. 2011. Mini review: antimicrobial strategies in the production of fresh-cut lettuce products Available at: http://www.formatex.info/microbiology3/book/176-188.pdf. Accessed 17 Janurary 2013.

119. Todd, E. C. D., C. K. Harris, A. J. Knight, and M. R. Worosz. 2007. Spinach and the media: how we learn about a major outbreak. *Food Prot. Trends*. 27:314-322.

120. Tomas-Callejas, A., G. Lopez-Velasco, A. M. Valdez, A. Sbodio, F. Artes-Hernandez, M. D. Danyluk, and T. V. Suslow. 2012. Evaluation of current operating standards for chlorine dioxide in disinfection of dump tank and flume for fresh tomatoes. *J. Food Prot.* 75:304-313.

121. Turini, T., M. Cahn, M. Cantwell, L. Jackson, S. Koike, E. Natwick, R. Smith, K. Subbarao, and E. Takele. 2011. Iceberg lettuce production in California. University of California, Davis, Agriculture and Natural Resources. Publication 7215. Available at: http://anrcatalog.ucdavis.edu/pdf/7215.pdf. Accessed 15 January 2013.

122. U.S. Food and Drug Administration. 1998. Guidance for industry: guide to minimize microbial food safety hazards for fresh fruits and vegetables. Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm064574.htm#ii. Accessed 8 September 2012.

123. U.S. Food and Drug Administration. 2006. UPDATE: FDA narrows investigation of *E. coli* O157:H7 outbreak at Taco Bell restaurants. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2006/ucm108803.htm. Accessed 2 September 2012.

124. U.S. Food and Drug Administration. 2007. FDA and states close to identifying sources of *E. coli* contamination associated with illnesses at Taco John's restaurants. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108827.htm. Accessed 2 September 2012.

125. U.S. Food and Drug Administration. 2008. Commodity specific food safety guidelines for the fresh tomato supply. Available at: http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM171708.pdf. Accessed 18 February 2013.

126. U.S. Food and Drug Administration. 2009. Guidance for industry: guide to minimize microbial food safety hazards of leafy greens; draft guidance. Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm174200.htm#fresh. Accessed 7 September 2012.

127. U.S. Food and Drug Administration. 2013. Standards for the growing, harvesting, packing and holding of produce for human consumption, a proposed rule. Available at: https://www.federalregister.gov/articles/2013/01/16/2013-00123/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption. Accessed 20 February 2013.

128. U.S. Food and Drug Administration. 2013. Standards for the growing, harvesting, packing, and holding of produce for human consumption; current good manufacturing practice and hazard analysis and risk-based preventive controls for human food; draft qualitative risk assessment of risk of activity/food combinations for activities (outside the farm definition) conducted in a facility co-located on a farm; availability; proposed rules. 21 CFR Parts 1, 16, 106. Available at: http://www.gpo.gov/fdsys/pkg/FR-2013-01-16/pdf/2013-00123.pdf. Accessed 14 January 2013.

129. Vandekinderen, I., F. Devlieghere, B. De Meulenaer, P. Ragaert, and J. Van Camp. 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiol.* 26:882-888.

130. Vorst, K. L., E. C. D. Todd, and E. T. Ryser. 2004. Improved quantitative recovery of *Listeria monocytogenes* from stainless steel surfaces ssing a one-ply composite tissue. *J. Food Prot.* 67:2212–2217.

131. Wachtel, M. R., L. C. Whitehead, and R. E. Mandrell. 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *J. Food Prot.* 65:18–25.

132. Warning, A., and A. K. Datta. 2013. Interdisciplinary engineering approaches to study how pathogenic bacteria interact with fresh produce. *J. Food Eng.* 114:426–448.

133. Watanabe, M. M., Y. Takeda, T. Sasa, I. Inouye, S. Suda, T. Sawaguchi, and M. Chihara. 1987. A green dinoflagellate with chlorophylls and morphology, fine structure of the chloroplast and chlorophyll composition. *J. Phycol.* 23:382-389.

134. Weissinger, W. R., W. Chantarapanont, and L. R. Beuchat. 2000. Survival and growth of *Salmonella baildon* in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. *Int. J. Food. Microbiol.* 62:123-131.

135. Wuytack, E. Y., L. D. T. Phuong, A. Aertsen, K. M. F. Reyns, D. Marquenie, B. D. Ketelaere, B. Masschalck, I. V. Opstal, A. M. Diels, and C. W. Michiels. 2003. Comparison of sublethal injury induced in *Salmonella enterica* serovar Typhimurium by heat and by different nonthermal treatments. *J. Food Prot.* 66:31-37.

136. Xicohtencatl-Cortes, J., E. S. Chacon, Z. Saldana, E. Freer, and J. A. Giron. 2009. Interaction of *Escherichia coli* O157:H7 with leafy green produce. *J. Food Prot.* 72:1531-1537.

137. Zagory, D. 1999. Effects of post-processing handling and packaging on microbial populations. *Postharvest Biol. Technol.* 15:313-321.

138. Zeng, W., K. Vorst, W. Brown, B. Marks, F. Perez-Rodriguez, and E. Ryser. 2012. Monte Carlo simulation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* growth in bagged salad greens during commercial transport, retail storage and display, Abst T4-12. *In,* Ann. Mtg. Int. Assoc. Food Prot., Providence, Rhode Island.

139. Zhang, G., L. Ma, V. H. Phelan, and M. P. Doyle. 2009. Efficacy of antimicrobial agents in lettuce leaf processing water for control of *Escherichia coli* O157:H7. *J. Food Prot.* 72:1392–1397.

140. Zhang, S., and J. M. Farber. 1996. The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiol.* 13:311-321.

141. Zhou, B., H. Feng, and A. J. Pearlstein. 2012. Continuous-flow ultrasonic washing system for fresh produce surface decontamination. *Innov. Food Sci. Emerg.* 16:427-435.