EFFICACY OF COMMERCIALLY AVAILABLE SANITIZERS IN PREVENTING CROSS-CONTAMINATION DURING SIMULATED POSTHARVEST WASHING OF CUCUMBERS

by

RUCHA SANDEEP BORALKAR

(Under the Direction of Faith Critzer)

ABSTRACT

The effectiveness of different sanitizer concentrations, chemical oxygen demand (COD) levels, and sanitizers in preventing cross-contamination and reducing bacterial load during postharvest produce washing was examined. Cucumbers were immersed in recirculated water with two COD levels (300 and 2500 ppm) and treated with chlorine or peroxyacetic acid at foursanitizer concentrations (0, 20, 40, and 80 ppm). The reduction of *Escherichia coli* and *Salmonella*, as well as their transfer to cucumbers and water, were evaluated. The results revealed that sanitizer concentration had the most significant impact on reducing bacterial populations, with treatments containing sanitizers exhibiting substantial reductions compared tothose without sanitizers. Sanitizer concentrations of 20 ppm, 40 ppm, and 80 ppm achieved an average reduction of 3.01-2.32 log CFU/cucumber, while no sanitizer resulted in a lower reduction of 0.75 log CFU/cucumber. Sanitizers at 20-80 ppm effectively limited bacterial transfer to cucumbers and water, with minimal percentages observed.

INDEX WORDS: Postharvest washing, sanitizer efficacy, cross-contamination.

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RUCHA SANDEEP BORALKAR

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RUCHA SANDEEP BORALKAR

Major Professor:	Faith Critzer
Committee:	Govindaraj Dev Kumar
	Laurel Dunn

Electronic Version Approved:

Ron Walcott
Dean of the Graduate School
The University of Georgia
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DEDICATION

To Ajjee, Ajoba, Aai, Baba, Dada, and my beloved cat Metu - your unwavering love and support have shaped me into the person I am today.

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CHAPTER 1

INTRODUCTION

The United States has witnessed a significant increase in the consumption of fruits and vegetables as part of a growing emphasis on healthy lifestyles (Hadjilouka & Tsaltas, 2020; Waskow et al., 2018). However, this surge in consumption has also been accompanied by a highrate of foodborne illnesses associated with the consumption of fresh produce, posing a substantial public health and economic burden (Ahn et al., 2022; Aslam et al., 2020).

Between 1998 and 2007, the proportion of foodborne outbreaks linked to fresh produce inthe United States rose from 14.8% to 22.8% (Wijnands et al., 2014). The study examining the period from 2004 to 2012 found that Norovirus was the primary pathogen responsible for produce-related outbreaks in the country, followed by *Salmonella*. *Salmonella* emerged as the leading cause of multistate produce outbreaks. These outbreaks highlight the diverse range of pathogens and food vehicles involved in fresh produce contamination in the United States.

Notable outbreaks in recent years have brought attention to the issue(Faour-Klingbeil et al., 2016; Warriner, 2005). *Salmonella* outbreaks have been attributed to various fresh produce, including cucumbers and pre-packaged lettuce. *Listeria monocytogenes*, a pathogen associated with high mortality rates, has also caused outbreaks related to fresh produce consumption, suchas mung bean sprouts, caramel apples, and packaged salads (Garner & Kathariou, 2016).

Contamination of fresh produce can occur at any stage, from farm to plate. Factors contributing to the presence of hazards include postharvest practices, water quality, environmental conditions, worker hygiene, and consumer habits (Gombas et al., 2017; Lynch etal., 2009; Yi et al.,

2020). As fresh produce is often consumed raw or minimally processed, it is essential to maintain a low microbial load to prevent foodborne illnesses (Chelaghma et al.,

2021). Efforts to improve the microbiological safety of fresh produce in the United States are crucial. Implementing and enforcing strict food safety regulations, promoting good agricultural and manufacturing practices, enhancing surveillance, and testing procedures, and educating both producers and consumers about proper handling and preparation techniques can all contribute to reducing the risk of foodborne outbreaks associated with fresh produce consumption. Continued research and monitoring of the patterns and cause of contamination are necessary to develop targeted interventions and ensure the safety of fresh produce.

CHAPTER 2

LITERATURE REVIEW

2.1. Importance of fresh produce

Fresh produce is an essential component of the American diet, providing essential nutrients and supporting good health. Despite the numerous benefits of fresh produce consumption, access to fresh produce, perception of fresh produce, and food safety concerns present significant challenges to increased consumption (Gustat et al., 2015). Initiatives aimed atpromoting increased consumption and improving access to fresh produce, combined with effortsto improve food safety, are essential to ensuring that Americans have access to the fresh producethey need to maintain good health.

Fresh produce provides a range of nutrients that are essential for good health. Fruits and vegetables are rich in vitamins, minerals, fiber, and antioxidants, which can help to prevent chronic diseases such as heart disease, stroke, and certain types of cancer. Consuming a diet richin fruits and vegetables can help to lower the risk of chronic diseases and improve overall health.In addition to the health benefits, fresh produce consumption also has economic benefits (Guthrieet al., 2005). The fresh produce industry is a significant contributor to the US economy, generating over $200 billion in sales and supporting over two million jobs. Increased consumption of fresh produce can lead to increased demand, which can in turn create more job opportunities in the industry (Baker et al., 2015; Fernandez-Fenaroli, 2015).

Food safety is a major concern for fresh produce consumption. Fresh produce can become contaminated with harmful bacteria such as *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes*, which can cause foodborne illness (Lin etal., 2008). Outbreaks

of foodborne illness linked to fresh produce have been reported in recent

years, leading to increased concerns about the safety of commodities implicated. To address these concerns, the US government has implemented several regulations and guidelines aimed at improving the safety of fresh produce. The Food Safety Modernization Act (FSMA) of 2011, for example, requires farms and other producers to implement preventive measures to reduce the risk of foodborne illness. The guidelines cover topics such as water quality, worker hygiene, and soil amendments, and are intended to prevent contamination of fresh produce throughout the production process (Weinroth et al., 2018).

2.2. Fresh produce outbreaks

Over the past few decades, there has been an increase in fresh produce outbreaks. These outbreaks have been associated with various pathogens, including bacteria, viruses, and parasites. The causes of these outbreaks are complex, but they are largely due to changes in our food system, including changes in production, distribution, and consumption practices (Bennett et al., 2018). One of the main factors contributing to the increase in fresh produce outbreaks is globalization. As global trade has increased, fresh produce is being transported across continents and oceans, increasing the risk of contamination and widening the distribution network (Crandall et al., 2017; Dhankher & Foyer, 2018; Eapen et al., 2022; Machado Nardi et al., 2020; Martinović et al., 2022). Fresh produce is also being produced and harvested in countries with different food safety standards, increasing the risk of contamination from pathogens such as *Salmonella*, STEC, and *Listeria monocytogenes* (Carstens et al., 2019; Waltner-Toews, 2019).

Another factor contributing to the increase in fresh produce outbreaks is the shift towards larger-scale farming operations. Large-scale farms use advanced technologies to increase productivity, but these technologies can also lead to the contamination of crops. For example, the

use of irrigation systems, fertilizers, and can contaminate the soil and water sources used for irrigation, leading to the contamination of crops with harmful pathogens (Jung et al., 2014).

Changes in consumer behavior are also contributing to the increase in fresh produce outbreaks. As consumers demand fresh produce year-round, the industry has had to adapt by importing produce from around the world. This increases the risk of contamination during transportation and distribution (Balali et al., 2020a; Jung et al., 2014). Additionally, consumers are demanding more convenient, ready-to-eat produce, which has led to an increase in pre-packaged salads and other products that are more prone to contamination (Collins, 1997; Rahkovsky et al., 2018). Climate change is another factor contributing to the increase in fresh produce outbreaks. Changes in weather patterns, including extreme heat and rainfall, can create ideal conditions for the growth and spread of pathogens. Additionally, climate change can impact the soil and water quality, leading to the contamination of crops with harmful pathogens. The rise in temperature, water temperature, and precipitation associated with climate change affects the abundance, growth, and survival of pathogens in crops, livestock, and the environment. Warmer temperatures promote the rapid multiplication of pathogens, leading to higher contamination levels in food sources. Moreover, climate change impacts wildlife vectors, such as rodents, insects, and marine organisms, which can transmit pathogens to food sources, increasing contamination (Smith & Fazil, 2019) .

2.3. Routes of contamination

Fresh produce contamination can occur at various stages of the supply chain, from pre-harvest to postharvest (Dandie et al., 2020; Farouk et al., 2022; Gurtler & Gibson, 2022; Zhu et al., 2017). Pre-harvest contamination refers to the contamination of crops with harmful pathogens before they are harvested from the field (Wei & Kniel, 2010), while postharvest

contamination refers to the contamination that occurs after the crops have been harvested, during processing, transportation, and preparation (Varalakshmi, 2021). Pre-harvest contamination can occur due to various factors, including the use of contaminated soil or water, the presence of animals or pests in the growing area, and the use of contaminated fertilizers or pesticides (Fig. 1; Jagannathan et al., 2022; Wei & Kniel, 2010; Yemmireddy et al., 2022).

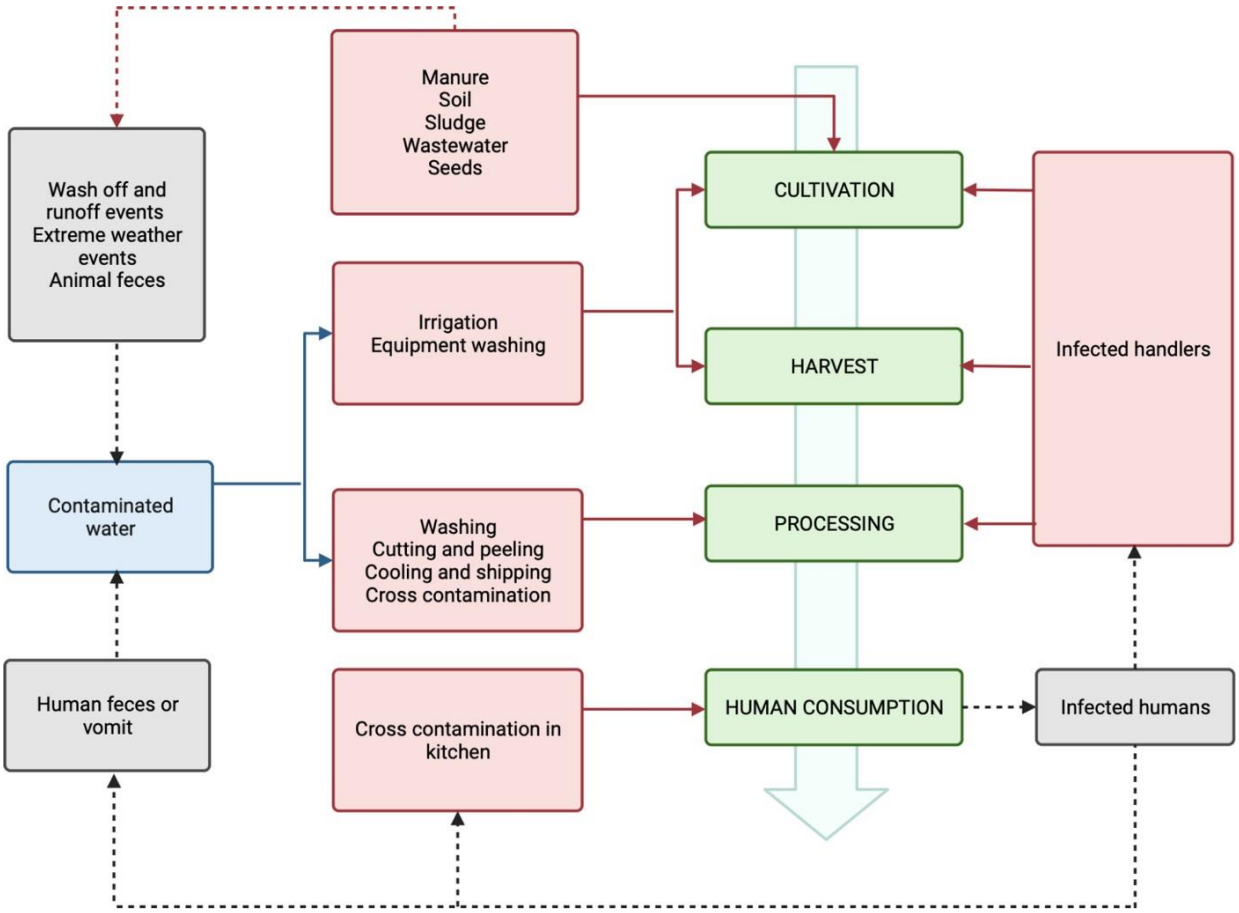

Figure 1. Routes of Contamination of Fresh Produce (Machado-Moreira et al., 2019)

Pathogens such as *Salmonella*, STEC, and *Listeria monocytogenes* can survive in soil for long periods and can be transferred to the surface of crops during harvesting or handling (Miceli & Settanni, 2019). The use of contaminated water for irrigation or washing crops can introduce harmful pathogens to the surface of crops, which can then be transferred to consumers. Animals

can also be a source of pre-harvest contamination of food. Wild animals, such as deer or rodents, can carry harmful pathogens and contaminate crops with their feces or other bodily fluids, while domestic animals, such as cows or pigs, can contaminate crops with their manure or other bodily fluids (Wei & Kniel, 2010). Contaminated fertilizers or pesticides can also introduce harmful pathogens to crops, which can then be transferred to consumers (Jagannathan et al., 2022; Miceli & Settanni, 2019; Wei & Kniel, 2010).

Postharvest contamination can occur during processing, transportation, and preparation. During processing, fruits and vegetables are washed, sorted, and packaged. If the processing equipment or the water used for washing is contaminated, the pathogens can be transferred to the surface of the produce. During transportation, produce can be exposed to various sources of contamination, such as unclean vehicles or storage facilities, which can introduce pathogens to the surface of the produce. Finally, during preparation, produce can be contaminated if it comes into contact with contaminated surfaces or utensils, or if it is not cooked to the appropriate temperature to kill any pathogens that may be present (Alegbeleye et al., 2018; Balali et al., 2020b; Iwu & Okoh, 2019; Possas & Pérez-Rodríguez, 2023; Rahman et al., 2021).

2.4. Foodborne illnesses in the US

Foodborne illnesses are common, costly, preventable and have been of major concern in the United States for many years now. The Centers for Disease Control and Prevention (CDC) estimates that each year one in six people get sick from consuming contaminated food or beverages. Roughly 48 million people are affected, 128,000 hospitalized and 3,000 die because of foodborne illnesses (Scallan, Griffin, et al., 2011; Scallan, Hoekstra, et al., 2011) The recently published Foodborne Diseases Active Surveillance Network (FoodNet) report mentions an 8%

decrease of enteric infections in the year 2021 compared to 2016-2018. COVID-19 pandemic and the interventions put in place to control its spread was cited as a possible reason for this decrease in reported infections (CDC, 2022).

The major pathogens of interest in the past decade have been *Salmonella*, STEC, *Listeria. monocytogenes*, *Campylobacter*, *Shigella*, *Clostridium perfringens* and *Staphylococcus aureus*. The incidence of *Salmonella* infections decreased in 2021, while *Campylobacter*, *Listeria*, STEC and *Shigella* infections did not change as compared to 2016-2018 (CDC, 2022). The consequences of foodborne illnesses go far beyond the health of the consumer. Financial costs to the company, the country, and the negative impact that it has on the company's reputation are examples of consequences faced (Robertson et al., 2016). The United States Department of Agriculture Economic Research Service estimated that foodborne illnesses cost the country $17.6 billion out of which about $10 billion was due to the eight pathogens mentioned above (CDC 2023) . Foods commonly associated with outbreaks are seeded vegetables, sprouts, herbs, fish, chicken, beef, eggs, turkey, and pork (Fig. 2; Richardson et al., 2021). The CDC estimated about 17% outbreak-associated illnesses from 2009-2018 were caused by the consumption of vegetables, while 39% were due to meats (chicken, beef, pork, and turkey combined; *CDC* 2023)

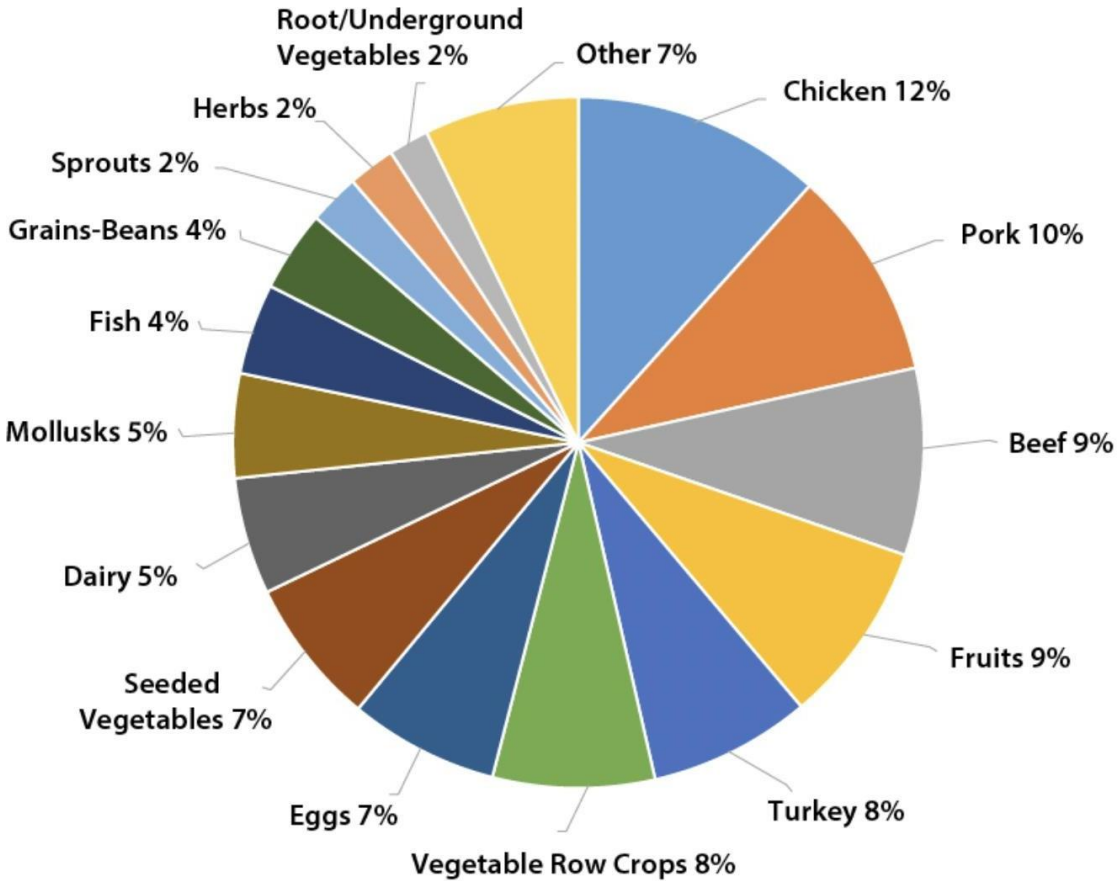

Figure 2. Top 15 foods that caused outbreak-associated illnesses, 2009-2018 (CDC 2023)

2.5. Salmonella

Salmonella enterica subsp. *enterica* causers salmonellosis in humans. It is estimated to be responsible for approximately 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths in the United States each year. *Salmonella* is found in the intestinal tract of animals, including humans, and can be shed in their feces. There are over 2,500 serotypes of *Salmonella*, but the most common serotypes that cause human illness are *Salmonella* Enteritidis, Typhimurium,

Newport, Heidelberg, and Javiana. *Salmonella* can be found in a variety of foods, including meat, poultry, eggs, dairy products, and fresh produce (Dickson, 2000; Patterson et al., 2014). Contamination of these foods can occur at any point during the production process, including on the farm, during processing, and during distribution.

Meat and poultry products are a common source of *Salmonella* contamination. The bacteria can be present in the intestines of animals and can contaminate the meat during slaughter and processing. Proper cooking and handling of these products can help prevent illness. Fresh produce, including fruits and vegetables, can become contaminated with *Salmonella* if they come into contact with contaminated soil or water, or if they are handled by an infected worker (Jacobsen & Bech, 2012; Wadamori et al., 2017a). Proper washing and handling of these products can help prevent illness. *Salmonella* can also be present in the intestines of chickens and can contaminate eggs before the shells are formed. Proper cooking and handling of eggs can help prevent illness (Patterson et al., 2014).

2.5.1. Salmonellosis

The severity of the infection depends on the specific strain of *Salmonella* and the health status of the infected individual. Certain groups, such as children under the age of 5, the elderly, and immunocompromised individuals, are more susceptible to severe cases of salmonellosis (Kurtz et al., 2017). The symptoms of salmonellosis include stomach flu-like symptoms such as nausea, vomiting, abdominal cramps, and bloody diarrhea. Other associated symptoms may include headache, fever, muscle pain, and dehydration, particularly in infants and the elderly. Most cases of salmonellosis are self-limiting and resolve within a week, but fatalities can occur, especially among vulnerable populations (CFSPH 2013). In some cases, salmonellosis can

progress to enteric fever, also known as typhoid fever, particularly when caused by *Salmonella* Typhi. Enteric fever is characterized by symptoms such as fever, headache, anorexia, lethargy, constipation, and other non-specific manifestations. It can be fatal if it leads to septicemia or meningitis (Kurtz et al., 2017).

Another condition associated with *Salmonella* infection is reactive arthritis or Reiter's syndrome. This is an inflammation of the joints that occurs following a gastrointestinal or genitourinary infection. The exact cause of Reiter's syndrome is not fully understood, but it has been linked to Salmonella infection. Symptoms may include painful joint inflammation, eye inflammation, discomfort during urination, swollen toes and fingers, lower back pain, and rash on the soles and palms (Ajene et al., 2013; Schempp et al., 2019).

Globally, salmonellosis is a significant public health concern. It affects millions of people each year, causing a substantial burden of disease and mortality. Malnourished children, the elderly, immunocompromised individuals, and those with pre-existing health conditions are at higher risk. Salmonella infections can lead to various complications, including bacteremia, meningitis, and infections in the tonsils. In 2017, salmonellosis affected 95.1 million people, causing 50,771 fatalities and 3.1 million disability-adjusted life-years (Stanaway et al., 2019).

2.5.2. *Salmonella* outbreaks linked to cucumbers

From 2010 to 2021, there were 32 outbreaks of *Salmonella* associated with cucumbers, resulting in 1,899 reported illnesses, 317 hospitalizations, and 7 deaths according to the NORS dashboard (*National Outbreak Reporting System (NORS)*, 2022). In 2013, there was a *Salmonella* Saintpaul outbreak associated with cucumbers. The cucumbers were grown in Mexico by Miracle Greenhouse of Culiacan and distributed by Tracer Sales Inc. of Rio Rico,

Arizona. This outbreak resulted in 84 reported infections across 18 states (*Salmonella Saintpaul Infections Linked to Imported Cucumbers, 2013*, 2013). In August 2014, a *Salmonella* Newport outbreak initially linked to tomatoes harvested in Virginia's Eastern Shore was found to be caused by contaminated cucumbers grown in the Delmarva region. The contamination was traced back to the application of contaminated poultry litter before harvest. This outbreak affected 275 people in 29 states (Angelo et al., 2015). From July 2015 to February 2016, a Salmonella Poona outbreak occurred, infecting 907 people in 40 states. The source of contamination was unknown, but cucumbers distributed by Andrew & Williamson Fresh Produce and grown in Mexico were implicated (Laughlin et al., 2019). This outbreak resulted in 204 hospitalizations and 6 deaths. In March and April 2016, a *Salmonella* Oslo outbreak affected 14 people across 8 states. The outbreak was linked to the consumption of Persian cucumbers (Bottichio et al., 2016).

2.6. Shiga toxin-producing *Escherichia coli*

Escherichia coli is a bacterium that is commonly found in the intestines of humans and animals. While most strains of *E. coli* are harmless, some can cause severe illness, particularly in people with weakened immune systems or children. The process of Shiga toxin-producing *Escherichia coli* (STEC) infection involves several steps. First, the bacteria, particularly strains like *E. coli* O157:H7, which are commonly found in the intestines of cattle, can contaminate meat, milk, and fresh produce through fecal matter or contaminated water sources. Once ingested, the bacteria reach the intestines and use specialized proteins called adhesins to adhere to the intestinal lining, allowing them to colonize and establish themselves in the gut. STEC strains possess genes that enable them to produce Shiga toxins, which are cytotoxigenic. These toxins are released by the bacteria and directly damage the lining of the intestines. This leads to symptoms such as severe diarrhea (often bloody), abdominal cramps, and vomiting. In some

cases, the Shiga toxins produced by STEC bacteria can enter the bloodstream, causing systemic effects. They can damage blood vessels and lead to complications like hemolytic uremic syndrome (HUS), a severe condition characterized by kidney failure, anemia, and low platelet count. STEC infection can also be transmitted from person to person through the fecal-oral route, especially in settings with poor hygiene or contaminated environments (Scallan, Griffin, et al., 2011; Scallan, Hoekstra, et al., 2011).

2.6.1. STEC outbreaks linked to fresh produce

STEC outbreaks have been linked to contaminated food products, with fresh produce being a common source of contamination. STEC can be found in various food products, including meat, poultry, and fresh produce. In meat and poultry products, contamination usually occurs during slaughter and processing (Abakpa et al., 2015; Djaja, 2008; Kundu et al., 2018). In fresh produce, contamination can occur at any stage of production, from the farm to the consumer. This can happen through contaminated irrigation water, manure used as fertilizer, and poor hygiene practices during harvesting, packing, and transportation (Feng & Reddy, 2014; Wadamori et al., 2017b; Yang et al., 2017).

STEC outbreaks associated with fresh produce have become a growing concern in recent years. According to the Centers for Disease Control and Prevention (CDC) (*National Outbreak Reporting System (NORS)*, 2022), between 2009 and 2018, there were 40 STEC outbreaks linked to fresh produce in the United States, resulting in 1,212 illnesses, 292 hospitalizations, and 8 deaths. One of the most significant STEC outbreaks in recent years was the 2018 outbreak linked to Romaine lettuce. The outbreak, which was traced back to lettuce grown in the Yuma, Arizona, region, resulted in 210 illnesses, 96 hospitalizations, and 5 deaths across 36 states Click or tap

here to enter text.. Another STEC outbreak linked to fresh produce occurred in 2021, when more than 100 people in 23 states were infected with STEC after consuming pre-cut melon products. The melon products were traced back to a facility in Indianapolis, Indiana, and were sold at various retailers, including Walmart, Kroger, and Whole Foods Market (Chan et al., 2023)

In 2011, an outbreak of *E. coli* infections occurred in the US that was linked to Romaine lettuce. The outbreak resulted in 60 reported cases of illness in 10 states, including 31 hospitalizations. The source of the contamination was believed to be contaminated irrigation water. The investigation into this outbreak revealed that the irrigation water used on the farm where the lettuce was grown was contaminated with *E. coli* O157:H7. The farm voluntarily ceased production and the affected product was recalled (*CDC*, 2012).

In 2015, an outbreak of STEC infections occurred in the US that was linked to Chipotle Mexican Grill restaurants. The outbreak resulted in 60 reported cases of illness in 14 states, including 22 hospitalizations. The source of the contamination was believed to be contaminated produce. The investigation into this outbreak revealed that the source of the contamination was likely a fresh produce item, such as lettuce or tomatoes, that was served at the Chipotle restaurants. The company temporarily closed all of its restaurants in the affected states and implemented a comprehensive food safety program to prevent future outbreaks (*CDC*, 2015).

2.7. Unit operations in a packinghouse

A produce packinghouse is a facility where fruits and vegetables are sorted, graded, washed, and packed for distribution and sale (Fallik et al., 2001). These facilities play a crucial role in the fresh produce supply chain by ensuring that produce is properly handled and packaged to maintain its quality and safety (Fallik et al., 2001; López-Gálvez et al., 2020). The layout and design of a produce packinghouse can vary depending on the size and type of operation. However, most packinghouses include several key areas, such as receiving, washing, and cleaning, sorting, and grading, packaging, and shipping (Fig. 3).

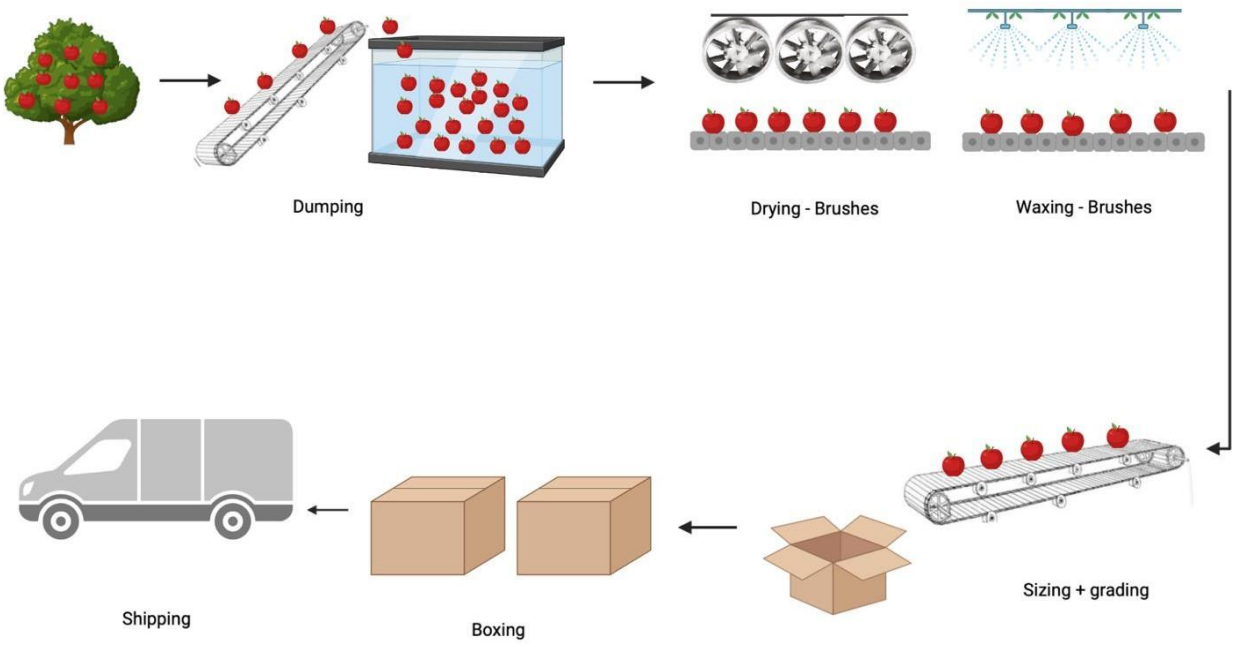

Figure 3: Unit operations in a produce packinghouse (Lisa Kitinoja, 2004)

The receiving area is where the produce is brought into the packinghouse from the field. In this area, the produce is unloaded and inspected to ensure that it meets the quality and safety standards set by the packinghouse. Any produce that does not meet the standards is rejected or set aside for further inspection. After the produce is received, it is washed and cleaned to remove any dirt, debris, or contaminants that may be on the surface of the produce. This process is

critical to ensuring that the produce is safe to eat and that it maintains its quality during storage and transport.

Depending on the type of produce and the packinghouse's processing methods, the washing and cleaning process can involve different techniques, such as brushing, scrubbing, or spraying with water or food grade detergents. Once the produce has been washed and cleaned, it is sorted and graded based on its quality, size, and other characteristics. This is typically done using specialized equipment, such as conveyor belts, sorting machines, or hand-grading tables. The sorting and grading process is important to ensure that only the best quality produce is packaged and sold, while lower quality or damaged produce is either discarded or used for other purposes, such as processing or animal feed.

After the produce has been sorted and graded, it is packaged for distribution and sale. Packaging can vary depending on the type of produce and the requirements of the customer, but commonly used packaging materials include cardboard boxes, plastic crates, and bags. Packaging may also include labels and other information about the produce, such as its origin, grade, and size. Finally, the packaged produce is shipped to its final destination, which may be a local grocery store, a regional distributor, or a national or international market. The shipping process involves careful handling and transport to ensure that the produce arrives at its destination in the best possible condition.

Produce packinghouses play an important role in ensuring the safety and quality of fresh fruits and vegetables. They are subject to strict regulations and standards set by government agencies and industry organizations to ensure that produce is handled and processed safely and efficiently. In addition to regulatory compliance, many packinghouses also implement their own quality control measures to ensure that the produce they handle meets their own high standards

for quality and safety. In addition to regulatory compliance and internal quality control measures, many packinghouses also face the requirement of third-party audits mandated by buyers. These audits are conducted by independent organizations or certification bodies to assess the packinghouse's adherence to specific standards and requirements set by the buyers themselves. These audits provide an additional layer of assurance to buyers that the packinghouse operates in accordance with their expectations and meets the desired quality and safety standards.

Third-party audits typically involve comprehensive evaluations of various aspects of the packinghouse's operations, including facility cleanliness, employee hygiene practices, equipment maintenance, pest control measures, record-keeping, and traceability systems. The auditors assess whether the packinghouse meets the specific criteria set by the buyers, which may include aspects such as organic certification, food safety management systems (such as HACCP), Good Manufacturing Practices (GMP), and adherence to industry-specific guidelines.

The results of these audits play a crucial role in maintaining business relationships between packinghouses and buyers. Buyers often rely on the audit outcomes to make informed decisions about which packinghouses to source their produce from. A favorable audit report can enhance the packinghouse's reputation and increase its marketability, while repeated non-compliance or unsatisfactory audit results can lead to loss of business opportunities.

Therefore, packinghouses recognize the significance of third-party audits and strive to meet the requirements set by buyers. By consistently implementing strong quality control measures, adhering to industry standards, and passing third-party audits, packinghouses can establish themselves as reliable partners in the supply chain, ensuring the safety and quality of fresh fruits and vegetables for consumers (*FDA* 2020).

This may include regular testing of water and equipment for contaminants, employee training on proper hygiene and sanitation practices, and audits of suppliers and vendors to ensure that they meet the packinghouse's standards (Estrada et al., 2020; Warriner & Namvar, 2014).

2.8. Methods of cleaning fresh produce

The cleaning process for fresh produce in packinghouses is critical to ensuring the safety and quality of the food. The process typically involves a combination of physical and chemical methods to remove dirt, debris, and pathogens that may be present on the surface of the produce.

2.8.1. Washing fresh produce with water

Washing fresh produce with water is one of the most common methods used in packinghouses to remove dirt and debris from the surface of the produce. Depending on the type of produce being washed, water may be sprayed onto the produce or the produce may be immersed into water. The temperature of the water can also vary depending on the produce being washed. For example, leafy greens may be washed in cold water to avoid wilting, while root vegetables may be washed in warm water to help loosen dirt. The temperature differential during the washing of tomatoes, or the difference in temperature between the tomatoes and the water they are submerged in, is important because it can influence the internalization of *Salmonella* bacteria into the tomatoes. When warm tomatoes are submerged into colder water, it creates a temperature differential. This temperature difference can cause a phenomenon known as thermal shock. Thermal shock occurs when there is a rapid change in temperature, which can lead to changes in the structure and permeability of the tomato's skin and tissues. This change in permeability can potentially allow bacteria, such as *Salmonella*, to enter the tomato pulp (Turner et al., 2016). In some cases, packinghouses may need to use additional cleaning methods, such as brushing or sanitizing, to ensure that the produce is safe for consumption (Delibato et al., 2018).

Hydrocooling can be used to cool down the temperature of produce to slow respiration, preserve quality attributes, and reduce microbial growth. This process involves immersing the produce in cold water for a short period of time. The cold water helps to reduce the temperature of the produce, which helps to slow down the growth of microorganisms. Hydrocooling is particularly effective for leafy greens, cherries, and berries, which are more sensitive to temperature changes. Hydrocooling can be an effective way to reduce the temperature of produce and slow down the growth of microorganisms. However, if the water used for hydrocooling is not properly treated or maintained, it can introduce contaminants to the produce. In addition, if the produce is not properly dried after hydrocooling, residual moisture can provide a breeding ground for bacteria (Ferreira et al., 2006).

Brushing or scrubbing is another method of cleaning produce that is particularly effective for removing stubborn dirt and debris. This method is often used for produce with a rough or uneven surface, such as potatoes, carrots, and beets. Packinghouses may use a variety of brushes or scrubbers to gently remove dirt and debris without damaging the produce. However, while brushing or scrubbing can be effective at removing dirt, it may not be sufficient for removing pathogens that may be present on the surface of the produce. Additional cleaning methods, such as sanitizing may be needed to ensure that the produce is safe for consumption. While brushing can help to physically remove dirt and debris from the surface of produce, it can also cause damage to the skin or surface of the produce. This can create entry points for microorganisms to penetrate the produce and cause contamination. Additionally, if the brushes are not cleaned and sanitized regularly, they can transfer bacteria from one batch of produce to another (Delibato et al., 2018; Hopkins et al., 2021).

2.8.2. Chlorine and PAA use as sanitizers in postharvest washing

Chlorine and other sanitizers are commonly used in packinghouses to reduce the risk of pathogens on produce (Bland et al., 2022; Mishra et al., 2018). These chemicals are added to the wash water and work by inactivating bacteria and other microorganisms on the surface of the produce. However, it is important to use the correct concentration of sanitizer in order to maintain water so that it is of safe and adequate sanitary quality and does not exceed use levels for postharvest washing. Using sanitizing solution can help to reduce the levels of microorganisms on the surface of the produce, but generally this is where the least efficacy is observed. Instead, sanitizers are added to batch and recirculated postharvest washing systems in order to inactivate foodborne pathogens that may be transferred to the water before they can cross-contaminate comingled crops. However, if the solution is not properly diluted or mixed, it can damage the produce or cause discoloration. In addition, if the produce is not properly rinsed after, residual sanitizing solution can remain on the surface of the produce and potentially cause health problems for consumers. Additionally, if the water is not properly filtered or treated, it can actually introduce contaminants to the produce (Mathew et al., 2018a, 2018b).

CHAPTER 3

MATERIALS AND METHODS

3.1. Bacterial cocktail preparation. A single strain of rifampicin resistant non- pathogenic surrogate *Escherichia coli* TVS 353 and a five-strain cocktail of rifampicin resistant *Salmonella* was used in this study which was obtained from Virginia Tech (Blacksburg, VA). Thestrains were *Salmonella* Saintpaul, *Salmonella* Newport, *Salmonella* Montevideo, *Salmonella* Agona, and *Salmonella* Enteritidis were used in this study which was obtained from Virginia Tech (Blacksburg, VA). The strains were adapted to 80 ppm rifampicin and to maintain their viability, the strains were stored at a temperature of -80 °C in 20% glycerol stocks.

Prior to use, 10 µL of each strain was transferred and grown individually in tryptic soy broth with rifampicin (TSBR; Becton, Dikinson & Company, Sparks, MD; Fisher Scientific, Fair Lawn, NJ, USA) for 24 hrs. at 37 °C with three consecutive transfers, thereby facilitating the adaptation and acclimation of the strain to the growth medium. After the third transfer, 250 µL ofthe strain was inoculated onto plates of tryptic soy agar with rifampicin (TSAR; Becton, Dikinson & Company, Sparks, MD) and incubated at 37 °C for 24 hrs. to create a bacterial lawn.Bacterial cells were harvested by flooding each plate with 10 ml buffered peptone water (BPW; Becton, Dikinson & Company, Sparks, MD) and dislodging cells with a cell spreader. Followingthe dislodgement of the *E. coli* cells, the resulting suspension contained a heterogeneous mixture of the harvested strains. For *Salmonella,* 10 mL of each strain was combined in a 50 mL Eppendorf tube(Thermo Scientific, Pittsburgh, PA) and vortexed to create a resulting cocktail, which was then used for inoculating cucumbers.

3.2. Model wash water preparation. To mimic the water used in cucumber packinghouses, model wash water was meticulously prepared for each experiment. The aim was to replicate the conditions present in the packinghouse environment. The model wash water consisted of two different levels of Chemical Oxygen Demand (COD): 300 ppm and 2500 ppm. Autoclaved silt loam soil (Athens, GA) was added to 2 L of deionized water (Table 1). This mixture was thoroughly mixed to ensure uniform distribution of the soil particles. To eliminate large debris and impurities from the water, the solution was carefully filtered through eight layers of grade-90 cheesecloth (Lion Service Inc., Charlotte, NC) along with cotton balls (Target Inc. in Minneapolis, MN). The filtration process effectively removed particulate typically removed by crude filtration present in the flume systems. The filtered water was then added to autoclaved bins obtained (Thermo Scientific, Pittsburgh, PA). To achieve a total volume of 10 liters of model wash water, an additional 8 liters of deionized water was introduced into the bins. The entire preparation process was conducted using aseptic techniques to ensure the sterility of the model wash water. After its preparation, the water was stored at room temperature for one day before commencing the experiment.

Table 1. Percentage by weight of silt loam soil added to 2 L of deionized water to achieve the desired Chemical Oxygen Demand (COD).

COD (ppm)	**Slit loam soil % (w/v)**
300	2.4
2500	27.3

3.3. Produce inoculation. Unwaxed slicing cucumbers, sourced locally from Lewis Taylor Farms in Tifton, GA, were selected for the purpose of produce inoculation. Each treatment combination involved the inoculation of a single cucumber, while an additional cucumber remained unwashed to serve as a control for determining the initial bacterial load on the inoculated cucumbers. The determination of bacterial load before washing was crucial for calculating the subsequent log reduction on the inoculated cucumbers. The harvested bacteria, with an estimated starting population of approximately 10 log colony-forming units per milliliter (CFU/mL), we3re homogenized by vortexing. Subsequently, spot inoculation was performed on each cucumber using ten spots of 10 µL each. Following inoculation, the cucumbers were allowed to dry in a biosafety cabinet for a period of two hours.

3.4. Sanitizer loading. Two commonly used sanitizers were used in this study, namely sodium hypochlorite solution (Pac-Chlor 12.5%, Pace International, Wapato, WA) and peracetic acid (PAA; Shield-Brite PAA 15.0, Pace International, Wapato, WA). Appropriate amounts of hypochlorite solution or PAA were added to each model wash water to reach 0, 20, 40 and 80 ppm of free chlorine and PAA concentration respectively per COD level. The pH of chlorine treatments was adjusted to 6.5 by adding 10% (v/v) phosphoric acid solution (Thomas Scientific, Swedesboro, NJ).

3.5. Physicochemical water parameter measurements. Samples taken from each bin were tested for water quality parameters such as COD, oxidation reduction potential (ORP), conductivity, pH, temperature, turbidity, and amount of antimicrobial present.

The COD of each sample was measured by dispensing 2 ml of sample into a high-range COD vial (HACH digestion solution for 20-1500 mg/L range, Hach company, Loveland, CO). A 10-fold dilution of the 2500 ppm COD samples was prepared prior to dilution. The vial was inverted several times to mix the contents and placed into a dry thermostat digital reactor (HACH DRB2000, Hach company, Loveland, CO). After the digestion process was completed in the reactor, the vial was taken out and allowed to cool down to room temperature. COD was determined by placing the vial in a multiparameter portable colorimeter (HACH DR900, Hach company, Loveland, CO). Turbidity was also recorded using the multiparameter portable colorimeter.

ORP, conductivity, pH and temperature were measured using a portable multi-parameter meter (HACH HQ4300, Hach company, Loveland, CO). The amount of antimicrobial present for each sample was measured by titration using either a combined free and total chlorine (LaMotte Ferrous Ammonium Sulfate-N,N-Diethyl-p-Phenylenediamine Chlorine kit, Chestertown, Maryland) or PAA (AquaPhoenix scientific, Hanover, PA) titration kit.

3.6. Contact of produce with water. Each bin represented a treatment combination. Nine uninoculated cucumbers and one inoculated cucumber were placed in each bin. After one minute of contact time, each cucumber was placed into separate stomacher bags containing a solution of 100 ml BPW containing 0.2% tween 80 (ICN Biomedical Inc., Aurora, OH), and 2% 0.1 N sodium thiosulfate (RICCA chemical company, Arlington, TX). Tween 80 was used to aid the recovery of bacterial cells from the surface of the cucumber while the 0.1 N sodium thiosulfate was used to arrest sanitizer activity.

3.7. Determination of bacteria on produce. For *E. coli,* the cucumbers were placed in individual stomacher bags, were each hand massaged for 30 s to help dislodge the bacterial cells from the surface. To determine the survival of bacterial populations on inoculated cucumbers and the transfer of bacteria in 0 ppm sanitizer treatments, 3 mL aliquots were taken from each stomacher bag and serial dilution were prepared in 0.1% peptone. Using spiral plater, dilutions 10^0 and 10^{-3} were spiral plated (100 µL) in duplicates on TSAR plates. The plates were incubated at 37 °C for 24 h.

To determine the transfer of bacteria on uninoculated cucumbers, a most probable number (MPN) method for the enumeration of *E. coli* was used. Colilert (IDEXX Laboratories, Inc., Westbrook, Maine) reagent was added to each stomacher bag and mixed until it completely dissolved. The liquid was poured into Quanti-trays (Quanti-Tray 2000, IDEXX Laboratories, Inc., Westbrook, Maine) and the trays were sealed using a Quanti-sealer (Quanti-Tray sealer Plus, IDEXX Laboratories, Inc., Westbrook, Maine). The trays were incubated at 37 °C for 24 hrs.

For *Salmonella,* following hand massaging, for assessing bacterial population survival on the inoculated cucumbers and bacterial transfer in 0 ppm sanitizer treatments, 3 mL aliquots were extracted from each stomacher bag and subjected to serial dilution in 0.1% peptone. Using a spiral plater, dilutions of 10^0 and 10^{-3} were spiral plated in duplicate (100 µL) on Xylose lysine tergitol 4 agar (XLT4; Becton, Dickinson and Company, Sparks, MD) + rifampicin plates. The plates were incubated at 37 °C for 24 hours.

To determine bacterial transfer on uninoculated cucumbers, 100 µL of rinsate was directly spiral plated in duplicate from a 10^0 dilution onto XLT4 + rifampicin plates and the most probable number (MPN) method was employed for the enumeration of *Salmonell*a. Columns B,

D, and F of 48-well MPN blocks (Thomas Scientific, Swedesboro, NJ) were filled with 4.5 mL of TSB. Subsequently, 5 mL of rinsate from one cucumber was added to column A, and 0.5 mL to column B. This process was repeated for other cucumbers in rows C-D and E-F. The MPN blocks were covered with a breathable film (Thermo Fisher Scientific, Suwanee, GA) and incubated at 37 °C for 20 hours. A volume of 50 µL from each well was then transferred using a multichannel pipettor to a second 48-well block pre-filled with 5 mL of RV broth (Becton, Dickinson & Company, Sparks, MD). The blocks were covered with a breathable film and incubated at 42 °C for 48 hours. Following incubation, the liquid was streaked along the channels onto XLT4 + rifampicin media and incubated at 37 °C for 48 hours. The plates were examined for the appearance of black colonies along the channels, indicating positive results. If no black colony was observed, the well was considered negative.

3.8. Determination of bacteria in water. To determine the transfer and survival of bacteria in wash water, a 100 mL aliquot was taken from each bin and mixed with a solution of 2% 0.1 N sodium thiosulfate was added to the sample and vortexed for 5 s. In the case of 0 ppm sanitizer treatments, 1:10 serial dilution was prepared in 0.1% peptone. 10^0 and 10^{-3} dilutions were spiral plated in duplicates on TSAR plates for *E. coli* and XLT4 + rifampicin plates for *Salmonella*. The plates were incubated at 37 °C for 24 hrs. In 20, 40, and 80 ppm sanitizer treatments, MPN method was used as previously described.

Statistical analysis of survival and transfer of bacteria. Six biological reps were analyzed per treatment combination in a full factorial design. Percent transfer to produce, percent transfer to water and log reduction on inoculated produce was calculated for each treatment

combination as shown in Figure 4 , 5 and 6. Data was reported as means ± standard deviation mean averaged from six independent experiments. Each analysis involved fitting a least squares model to evaluate the effects of sanitizers, sanitizer concentration, and COD, as well as their interactions on the respective outcomes with a significance level of $p \leq 0.05$ in JMP (SAS Institute Inc., Cary, NC).

For the significant effects identified in the inactivation analysis, a post hoc Tukey's HSD (Honestly Significant Difference) test was performed. This post-hoc test aimed to assess the significant differences between different treatments. By comparing means, the test provided insights into which treatment combinations yielded significantly distinct results in terms of log reduction on inoculated produce. This helped to comprehend the impact of various factors on the effectiveness of the sanitization process.

For the analysis of percent transfer to produce and water, a nonparametric test was performed using the same dependent and fixed effects in a reverse rank analysis. The model effects remained consistent with the previous analysis, including the sanitizer, sanitizer concentration, COD, and their interactions. After fitting the model, it was executed to obtain coefficient estimates and p-values. Effects with p-values less than 0.05 in the percent transfer to produce analysis underwent the same subsequent step as the inactivation analysis. The Tukey's HSD test was utilized to explore the significant differences between treatments, allowing researchers to understand the variations in percent transfer to produce across different combinations of sanitizers, sanitizer concentrations, and COD levels.

$$Percent\ transfer\ to\ produce = \left(\frac{CFU\ on\ uninoculated\ fruit\ after\ washing}{CFU\ on\ inoculated\ fruit\ prior\ to\ washing}\right) x100$$

*Population MUST NOT be log-transformed

Figure 4. Formula for percent transfer to produce calculation

$$Percent\ transfer\ to\ water = \left(\frac{MPN\ per\ 100\,ml\ x\ total\ volume\ of\ simulated\ wash\ water}{CFU\ on\ inoculated\ fruit\ prior\ to\ washing}\right) x100$$

*Population MUST NOT be log-transformed

Figure 5. Formula for percent transfer to water calculation

$$Log\ reduction\ on\ inoculated\ cucumber$$

$$= (Log\ CFU\ per\ ml\ on\ inoculated\ cucumber\ prior\ to\ washing)$$

$$- (Log\ CFU\ per\ ml\ on\ inoculated\ cucumber\ after\ washing)$$

Figure 6. Formula for log reduction on inoculated produce calculation

CHAPTER 4

RESULTS

4.1. Log reduction of *E. coli* on inoculated cucumbers

The model considered the factors of sanitizer (PAA or chlorine), sanitizer concentration(0, 20, 40, 80 ppm), and COD (300 or 2,500 ppm), along with their two- and three-way interactions. Sanitizer concentration was the most influential factor in predicting inactivation of *E. coli* on contaminated cucumbers $p<0.00001$. Treatments which had sanitizer present (20, 40,and 80 ppm) had significantly lower were populations of *E. coli* recovered when compared to treatments with no sanitizer ($p<0.00001$). Treatments which contained 20, 40, or 80 ppm had anaverage reduction of 3.01-2.32 log CFU/cucumber compared to 0.75 log CFU/cucumber for when washed in water alone.

The interaction of COD (300 or 2500 ppm) and sanitizer concentration was the next most influential factor ($p=0.00025$). The most pronounced difference was observed between treatments without a sanitizer present and those with a sanitizer present, regardless of concentration (Fig. 7). The results indicate that the combination of higher COD levels (2500 ppm) and higher sanitizer concentrations (80 ppm) leads to significantly higher log reduction in inoculated produce 3.79 and 2.2 log CFU/cucumber at 2500 and 300 ppm COD, respectively ($p=0.00025$). No significant differences were noted between different COD levels at 20 and 40 ppm sanitizer concentration ($p>0.05$).

No other factors or their interactions were found to be significant factors with respect tothe reduction of *E. coli* on inoculated cucumbers. This demonstrates that chlorine and PAA performed

similarly at the concentrations and COD levels evaluated. This is helpful to demonstrate that once a residual concentration is maintained, efficacy in this regard is similar

between the two compounds. Beyond inactivation of *E. coli* on contaminated cucumbers, transfer from the contaminated produce to water and adjacent uninoculated cucumbers was also evaluated.

4.2. Transfer of *E. coli* to cucumbers and water

4.2.1. Percent transfer to produce

As described above, the model effects included the variables related to the sanitizer used, sanitizer concentration, COD, and the two- and three-way interactions between these factors. Sanitizer concentration was the most influential factor with a $p<0.000001$. For all treatment combinations percent transfer was below 0.07% to uninoculated cucumbers (Fig. 8). The largest percent transfer was seen in samples lacking sanitizer ($p<0.000001$), which ranged from 0.066-0.03%.

The three-way interaction of sanitizer, sanitizer concentration, and COD was the next most influential factor ($p<0.000001$). The percent transfer of *E. coli* to uninoculated cucumbers for all variables is shown in Figure 2. The higher level of COD, 2,500 ppm, with 20 ppm free chlorine allowed for transfer to uninoculated cucumbers (0.045%) compared to 40-80 ppm (<0.002%) for both sanitizers and levels of COD evaluated. This demonstrates that transfer was significantly more likely with high COD and 20 ppm free chlorine compared to 20 ppm PAA or either sanitizer at 40 and 80 ppm ($p<0.000001$). While some significant differences were noted with the nonparametric reverse rank analysis, from a practical standpoint, the percent transfer to cucumbers is quite low ($2x10^{-8}$-$3x10^{-3}$%) in 20 ppm PAA or either sanitizer at 40 and 80 ppm.

4.2.2. Percent transfer to water

The statistical analysis of the percent transfer to water was conducted following the same approach as the analysis of percent transfer to produce. Sanitizer concentration was found to be the only significant variable ($p<0.000001$). No sanitizer present allowed for significantly greater transfer of *E. coli* compared to water with 20-80 ppm free chlorine or PAA (Fig. 9; $p<0.000001$). Under these conditions, 31% of *E. coli* were transferred to water when a sanitizer was not used (Fig. 3) and no more than 1.5% was transferred when at least 20 ppm free chlorine or PAA was used. It is notable that COD level and sanitizer type did not play a significant role in transfer of *E. coli* to water.

4.3. Log reduction of *Salmonella* on inoculated cucumbers

The model considered the factors of sanitizer (PAA or chlorine), sanitizer concentration (0, 20, 40, 80 ppm), and COD (300 or 2,500 ppm), along with their two- and three-way interactions. Sanitizer concentration was the most significant factor ($p<0.000001$) influencing the log reduction of *Salmonella* on inoculated cucumber. Using sanitizer concentrations of 20 ppm, 40 ppm, and 80 ppm does not lead to significant differences in the log reduction of *Salmonella* on inoculated produce (average log reduction 3.2 – 2.6 log CFU/cucumber). However, not using any sanitizer (0 ppm) results in a significantly lower log reduction of *Salmonella* (Fig. 10); 0.68 log CFU/cucumber).

The interaction between sanitizer concentration and COD significantly influenced the log reduction of *Salmonella* on cucumbers. The treatment with 20 ppm sanitizer and 300 ppm COD achieved the highest reduction (3.9 log CFU/cucumber), which was significantly different from

20 ppm sanitizer at 2,500 ppm COD and 40 ppm sanitizer at 300 ppm COD. 2.51 and 2.44 log CFU/cucumber, respectively ($p=0.013$).

Conversely, treatments with 0 ppm sanitizer demonstrated the lowest log reduction (0.8-0.5 log CFU/cucumber).

No other factors or their interactions were found to be significant in the reduction of *Salmonella* on inoculated cucumber ($p>0.05$). The study suggests that both PAA and chlorine sanitizers performed similarly in terms of log reduction of *Salmonella* on inoculated cucumbers. The analysis did not reveal any significant differences between the two sanitizers in their ability to reduce *Salmonella* contamination. These findings highlight the importance of selecting appropriate sanitizer concentrations.

4.4. Transfer of *Salmonella* to cucumbers and water

4.4.1. Percent transfer to produce

As described above, the model included variables like COD, sanitizer used, sanitizer concentration and the two – and three-way interactions between these factors. Sanitizer concentration was the most influential factor with a $p<0.000001$. For all treatment combinations with a sanitizer present, percent transfer was below 0.00006% to uninoculated cucumbers (Fig.11). The largest percent transfer was seen in samples lacking sanitizer ($p<0.000001$), which was an average of 0.02%.

The statistical analysis revealed a significant interaction effect between sanitizer concentration and COD on *Salmonella* transfer during washing ($p = 0.00003$). The treatment combination of 80 ppm sanitizer concentration with a COD level of 300 resulted in the lowest percent transfer of *Salmonella* ($2.3x10^{-7}$- $6.3x10^{-8}$%). However, no significant differences were

observed among the other treatments with sanitizers at different COD levels in terms of *Salmonella* transfer to uninoculated cucumbers. Treatments without any sanitizer displayed the highest percent transfer of *Salmonella* regardless of the COD level (0.01-0.02%).

The next most influential factor was the interaction between sanitizer used and sanitizer concentration ($p=0.00033$). PAA at 80 ppm concentration showed the least transfer; absence of either chlorine and PAA showed the greatest transfer. All the other treatments with either PAA or chlorine present at any concentration (20,40, or 80) showed no significant difference as compared to PAA at 80 ppm.

The three-way interaction between sanitizer, sanitizer concentration, and COD was found to have a significant effect on the percent transfer of *Salmonella* to produce ($p=0.01892$). Specifically, at a COD level of 300 ppm, there were significant differences observed between PAA treatments at 80 ppm and 40 ppm. Similarly, significant differences were observed between chlorine treatments at 40 ppm and 20 ppm, as well as between chlorine treatments at 80 ppm and 20 ppm. At a COD level of 2500 ppm, significant differences were observed between Chlorine treatments at 80 ppm and 20 ppm, as well as between Chlorine treatments at 40 ppm and 80 ppm. Additionally, a significant difference was observed between Chlorine treatment at 40 ppm (2500 ppm COD level) and PAA treatment at 80 ppm (2500 ppm COD level). Furthermore, treatments without any sanitizer present at both COD levels showed significant differences from all other treatments, regardless of the COD level.

4.4.2. Percent transfer to water

The statistical analysis of percent transfer to water was conducted, employing a similar approach to the analysis of percent transfer to produce. The results indicated that sanitizer

concentration was the sole significant variable ($p<0.000001$). Notably, the absence of sanitizer exhibited a significantly higher transfer of *Salmonella* to water compared to water treated with free chlorine or PAA at concentrations ranging from 20 ppm to 80 ppm. The transfer of *Salmonella* to water in the absence of sanitizer was quantified at 11.34% (Fig. 12), while its presence at a minimum concentration of 20 ppm free chlorine or PAA reduced the transfer to no more than 0.0007%. Intriguingly, neither the level of chemical oxygen demand (COD) nor the type of sanitizer demonstrated a significant influence on the transfer of *Salmonella* to water.

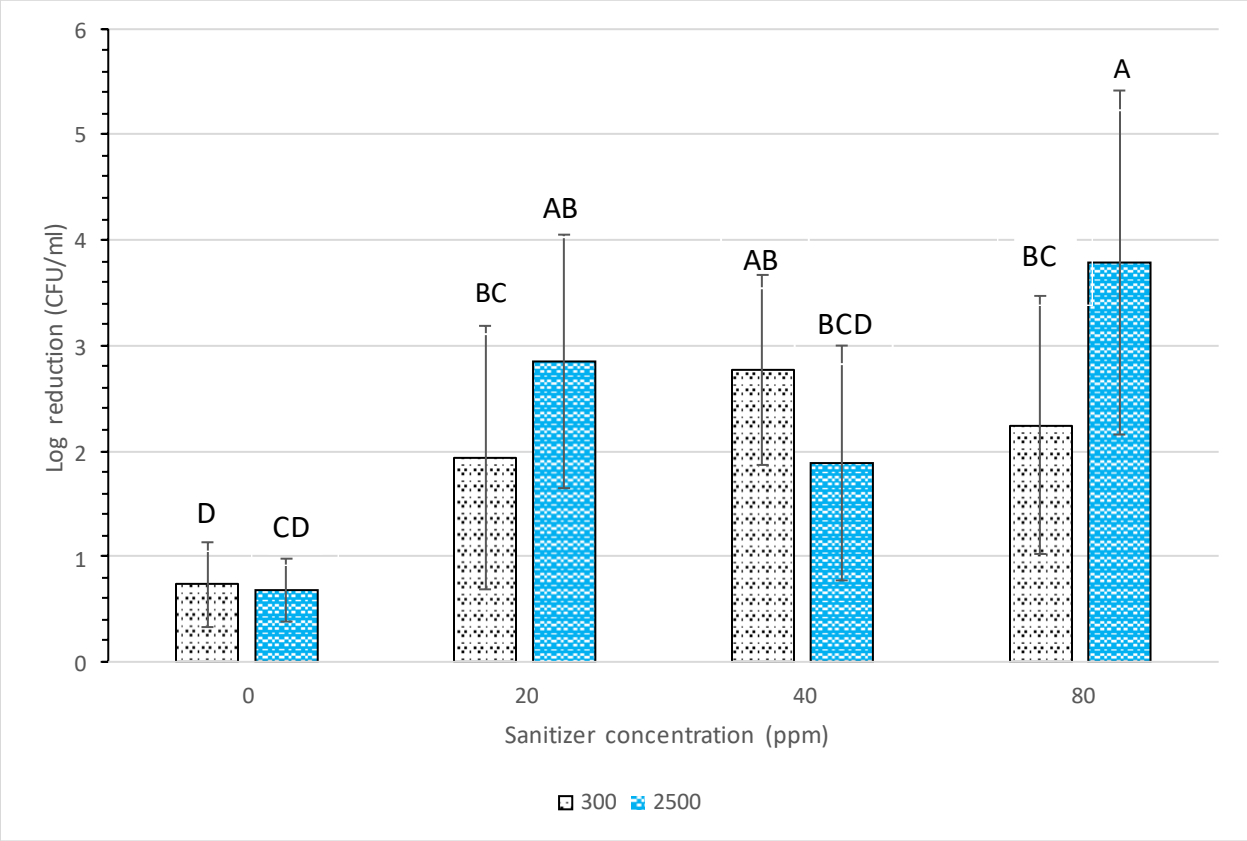

Figure 7. Log reduction of *E. coli* on inoculated produce (log CFU/cucumber) at four different sanitizer concentrations and two COD levels. Means with different letters are significantly different (p=0.00025)

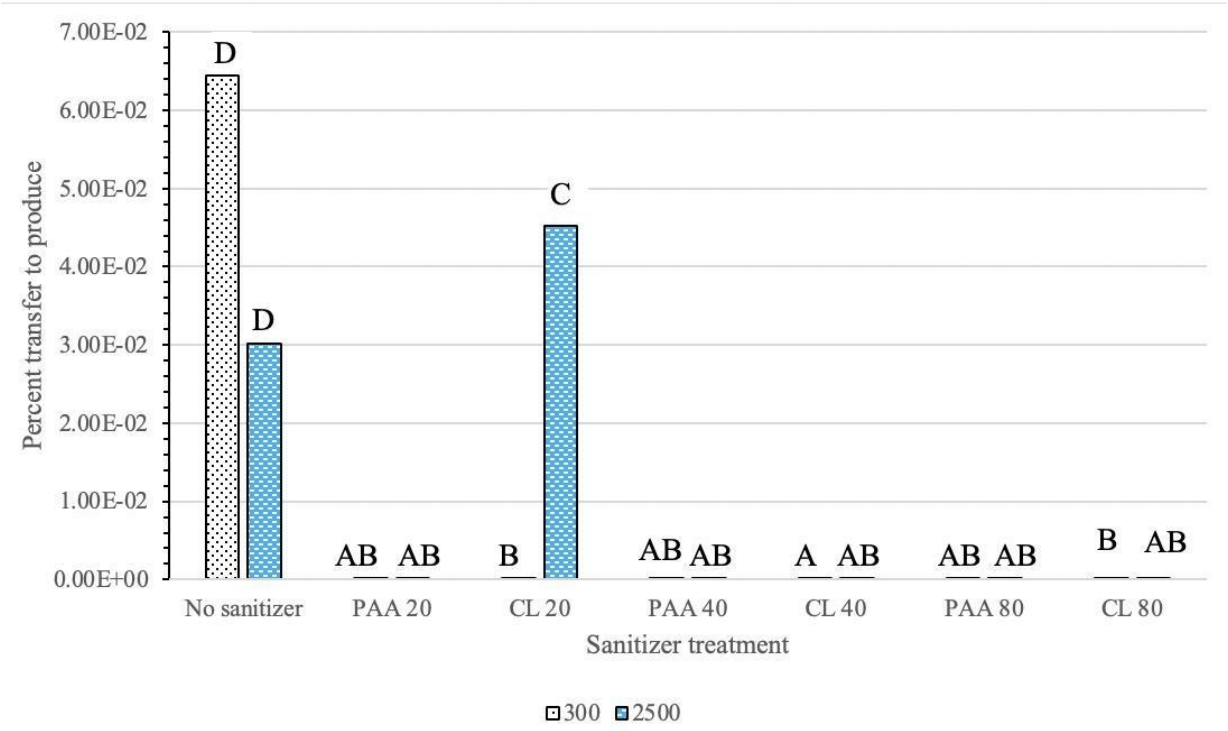

Figure 8. Percent transfer of *E. coli* to uninoculated cucumbers when washed in water with peracetic acid (PAA) or chlorine (CL), at a concentration of 0, 20, 40, or 80 ppm and a chemical oxygen demand of 300 or 2,500 ppm. Means with different letters are significantly different ($p<0.05$; limit of detection= 1 MPN/cucumber).

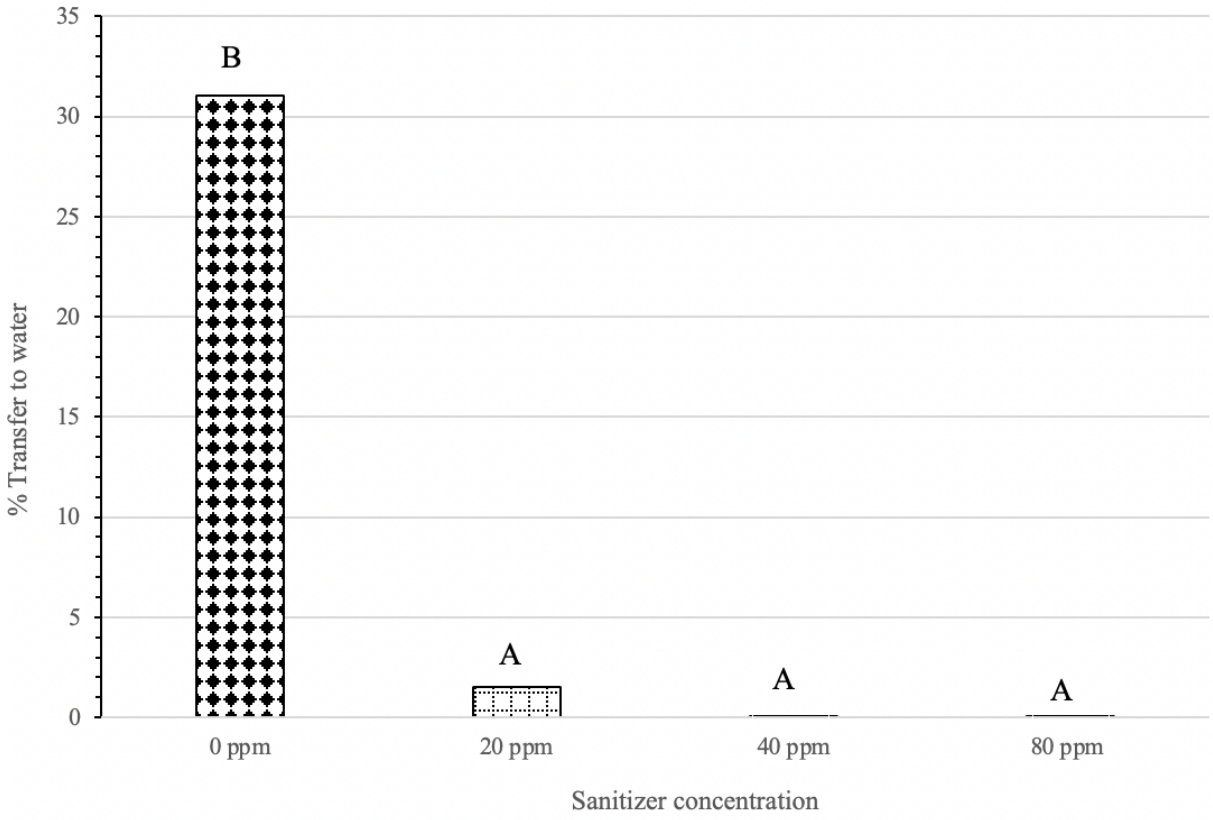

Figure 9. Percent transfer of *E. coli* to water at four different sanitizer concentrations 0, 20, 40, or 80 ppm in water treated with chlorine or peracetic acid. Means with different letters are significantly different ($p<0.05$; limit of detection= 1 MPN/ 100 ml).

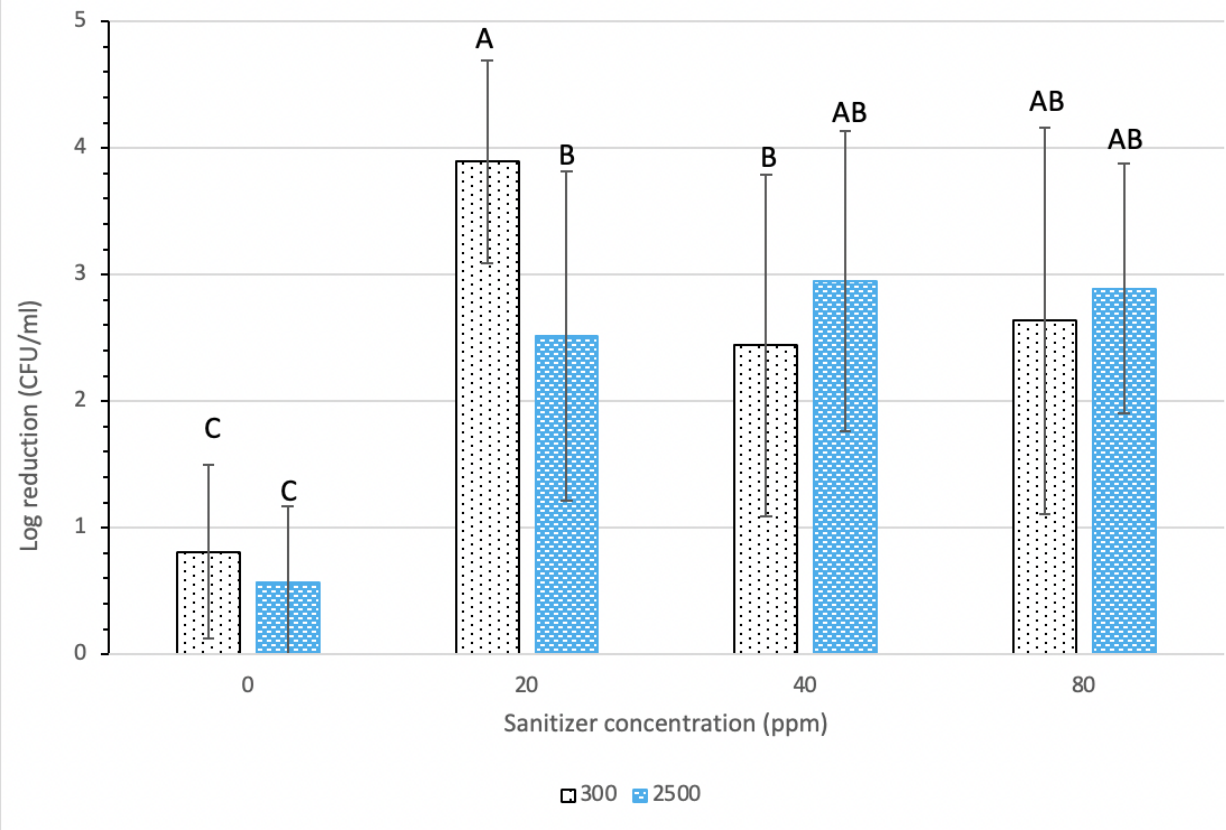

Figure 10. Log reduction of *Salmonella* on inoculated produce (log CFU/cucumber) at four different sanitizer concentrations and two COD levels. Means with different letters are significantly different ($p<0.00001$)

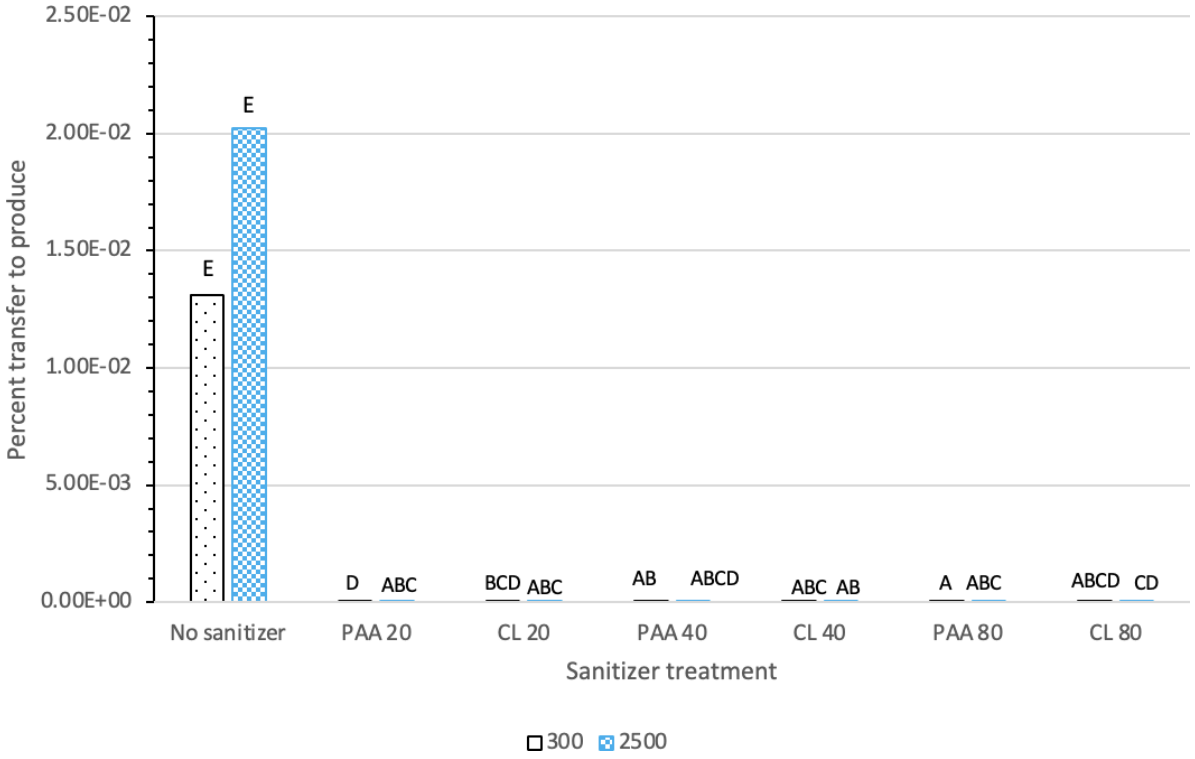

Figure 11. Percent transfer of *Salmonella* to uninoculated cucumbers when washed in water with peracetic acid (PAA) or chlorine (CL), at a concentration of 0, 20, 40, or 80 ppm and a chemical oxygen demand of 300 or 2,500 ppm. Means with different letters are significantly different ($p<0.05$; limit of detection= 1 MPN/cucumber).

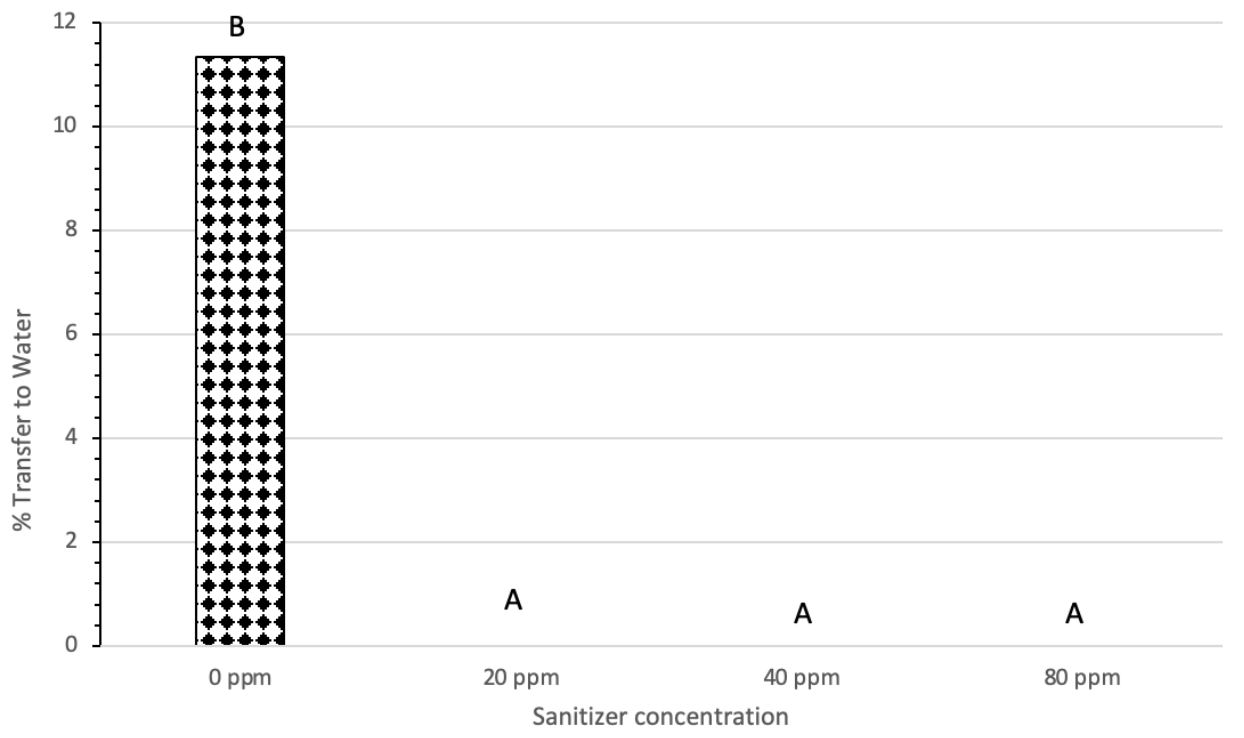

Figure 12. Percent transfer of *Salmonella* to water at four different sanitizer concentrations 0, 20, 40, or 80 ppm in water treated with chlorine or peracetic acid. Means with different letters are significantly different ($p<0.05$; limit of detection= 1 MPN/ 100 ml).

CHAPTER 5

DISCUSSION

Ensuring the safety and quality of produce is crucial in the food industry, particularly in packinghouses. These facilities employ various sanitization methods, and two popular choices are chlorine-based sanitizers and PAA (Petri et al., 2021). Chlorine-based sanitizers, such as chlorine gas, sodium hypochlorite, or calcium hypochlorite, are well-known for their effectiveness in killing or reducing microbial contamination (Luo et al., 2011, 2012; Shen et al.,2013; Sreedharan et al., 2017). They release free chlorine, which acts as a potent disinfectant, targeting a wide range of pathogens on produce surfaces. Chlorine-based sanitizers are easily accessible, cost-effective, and can quickly inactivate harmful microorganisms, reducing the risk of cross-contamination. However, chlorine-based sanitizers have limitations in effectively reducing populations on produce surfaces due to breakdown when in contact with organic matter
(Allende et al., 2008; Gonzalez et al., 2004; Oh et al., 2005; Zhang et al., 2009). Monitoring ORP and pH of the process water can improve treatment efficacy (Banach et al., 2015; Petri et al.,2015).

PAA has gained attention as a potent alternative. It is formed by the reaction of acetic acid and hydrogen peroxide and exhibits strong antimicrobial properties against a wide spectrumof microorganisms. PAA works well even in the presence of organic matter and decomposes into non-toxic byproducts, minimizing environmental impact (Buchholz & Matthews, 2010; Chang & Schneider, 2012; Neo et al., 2013; Oh et al., 2005). PAA offers advantages such as fast-acting nature and effectiveness against various pathogens, making it suitable for treating wash water

contaminated with soil or organic materials(Banach et al., 2015, 2020; Hilgren & Salverda, 2000; Petri et al., 2021).

Laboratory studies have demonstrated that sanitizers can effectively prevent the transfer of microbial pathogens in water when used at adequate levels. However, the presence of soil and other debris in flume tanks can quickly deplete sanitizers and lead to a decline in water quality, adding difficulty to make science-based recommendations to growers for what concentrations of sanitizers may be necessary to maintain water quality with increasing amounts of organic load. Several studies have investigated the efficacy of PAA and sodium hypochlorite sanitizers on different types of produce (Castro-Rosas et al., 2010; Gereffi et al., 2015; López-Velasco et al., 2012; Luo et al., 2011). Baert et al. (2009) found that both PAA and sodium hypochlorite were effective in preventing cross-contamination during the washing of shredded iceberg lettuce. Although they didn't significantly reduce the number of *E. coli* on the lettuce, using 200 ppm sodium hypochlorite or 80 ppm PAA in the wash water resulted in a significant reduction compared to washing with tap water alone. Pahariya et al., (2022) compared different sanitizing solutions for reducing *E. coli* populations on lettuce. Both PAA and sodium hypochlorite were effective, with PAA exhibiting a higher microbial reduction. Soaking the lettuce samples in PAA for 5 minutes resulted in the greatest reduction.

Cuggino et al. (2023) studied the effects of chlorine and PAA wash treatments on the growth of *Salmonella* in fresh-cut lettuce. They found that incorporating 25 mg/L of sodium hypochlorite or 80 mg/L of PAA in the wash water led to a greater decrease in *Salmonella* contamination compared to water alone. Wang & Ryser (2014) evaluated the efficacy of various sanitizers against *Salmonella* during the packing of tomatoes. Both PAA and chlorine treatments were effective in reducing *Salmonella* populations, with chlorine plus citric acid treatment

yielding the greatest reduction. Pabst, (2020) investigated the efficacy of PAA in preventing cross-contamination of *Salmonella* on tomatoes in a flume system. The study showed that higher concentrations of PAA were effective in reducing cross-contamination in cucumber washing systems, but the presence of organic load reduced its effectiveness.

The results from our study of inactivation and transfer of *E. coli* aligns with the studies previously done in terms of the reduction of *E. coli* populations through the use of sanitizers. They mention that the presence of sanitizers, specifically chlorine and PAA, resulted in significantly lower populations of *E. coli* compared to treatments without sanitizer and highlight the influence of sanitizer concentration, with higher concentrations leading to greater reductions in *E. coli* (<1 log reduction seen in treatments lacking sanitizers whereas 1.9 – 4.9 log reduction seen in treatments with sanitizer present irrespective of the concentration.)

Upon comparing the *Salmonella* results of our study and others previously conducted, it can be observed that in terms of log reduction, they emphasize the significance of sanitizer concentration, which was also a significant factor (Wang & Ryser, 2014). They indicate that using sanitizer concentrations of 20 ppm, 40 ppm, and 80 ppm leads to significant reductions in *Salmonella*, while the absence of sanitizer results in lower reduction (Pabst, 2020). The absence of sanitizer results in a higher percent transfer, while the presence of sanitizer significantly reduces the transfer (Pabst, 2020). Studies done earlier show that 80 ppm sanitizer concentration is effective at 300 ppm COD level in reducing cross-contamination of *Salmonella*, which was consistent with the results found in our study (Cuggino et al., 2023).

These studies demonstrate the effectiveness of PAA and sodium hypochlorite sanitizers in reducing microbial contamination on produce and transfer to wash water. The efficacy can vary depending on the type of produce, concentration of sanitizer, contact time, and the presence of

organic load, temperature of wash water. Further studies need to be conducted on the effect temperature, pH and depletion of sanitizer concentration due to organic matter present in the water have on the efficacy of sanitizers in the inactivation and prevention of cross-contamination of pathogens during the washing process of produce.

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