COMPARISON OF THE EFFICACY OF SANITIZERS TO ELIMINATE *SALMONELLA*, *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* ON FRESH PRODUCE IN SMALL BATCH TREATMENTS

by

MIKAYLA LILLIE KATHERINE GOODMAN

(Under the Direction of Mark A. Harrison)

ABSTRACT

Effectiveness of chlorine dioxide gas generated in self-contained sachets suitable for use in retail operations to reduce pathogen contamination in mixed produce containers was explored. Kale, oranges, celery, and cucumbers inoculated with *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* were treated with either 20 mg ClO_2 gas/kg produce, 200 ppm hypochlorite, or 80 ppm peracetic acid (PAA) and compared to no gas and water controls. Pathogen numbers were reduced by 2.66 log CFU/ml of rinsate on produce treated with ClO_2 gas regardless of the produce type or condition, and this treatment was significantly different from the others and controls. PAA and chlorine treatments were not statistically different and produced reductions of 1.96 and 1.89 log CFU/ml of rinsate, respectively. ClO_2 gas generated in self-generating sachets may be a practical, convenient delivery method and superior to commonly used sanitizers in decontaminating mixed types of produce used in retail juicing operations.

INDEX WORDS: Chlorine dioxide gas, hypochlorite, peracetic acid, *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes,* fresh produce, juicing

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DEDICATION

I would like to dedicate this to my mom and stepdad. Without your support, I would not be where I am today. Thank you for lending an ear whenever I needed, and most importantly, thank you for always making me feel that I can conquer anything.

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CHAPTER 1

INTRODUCTION

The United States' Office of Disease Prevention and Health Promotion puts forth 10-year objectives to improve the well-being of the American population *(129)*. Increasing fruits and vegetable consumption for those over 2 years of age and reducing the incidence of foodborne illness are two objectives the Healthy People 2020 campaign has outlined *(128, 130)*. *Salmonella, Listeria monocytogenes,* and Shiga toxin-producing *Escherichia coli* O157 are all objective targets, and fresh fruits and vegetables have been implicated in outbreaks with each of these pathogens *(14)*. Improving produce safety is valuable in working toward meeting objectives in the Healthy People 2020 campaign.

Fresh-squeezed and cold-pressed mixed produce juices are being marketed to consumers as convenient and healthful snack options by specialty shops, grocery stores, and street vendors. There are arguments that unprocessed juices are more nutritious than their pasteurized counterparts since there is less vitamin degradation *(139)*. However, foodborne pathogens can contaminate these fresh juices and pose risk to consumers due to a lack of a kill step. Sanitizing produce destined for fresh juices is a measure that can be taken to decrease risk, and there are commercial antimicrobial rinses available for produce washing.

Chlorine dioxide gas as a produce sanitizer has been previously researched, and lethality has been shown to be better than commonly used chlorine and peracetic acid (PAA) rinses in several studies *(52)*. Gas as a sanitizer eliminates the need for water used

in produce dips. The majority of studies on ClO_2 gas for decontaminating produce did not use methods in which it was feasible to treat more than a few produce items at once. The goal of this study was to evaluate ClO_2 gas effectiveness in reducing *Salmonella, E. coli* O157:H7, and *L. monocytogenes* on several types of produce in a single treatment container that could easily be used in small juicing operations. Mixed juices commonly use produce such as kale, cucumbers, oranges, and celery to concoct delicious and nutritious juices; thus, these were chosen to be used in the study. Damaged, aged or wilted produce was also chosen to be included as juice producers may choose to use lower quality fruit that would not easily sell as a fresh item at retail. The effectiveness of ClO_2 gas was evaluated by comparing to more conventional immersion treatments of chlorine or PAA.

CHAPTER 2

LITERATURE REVIEW

Fresh fruit and vegetable juice associated outbreaks and contamination

An estimated 48 million people in the United States fall ill each year due to foodborne illness according to the Centers for Disease Control and Prevention. Of those 48 million illnesses, 128,000 are thought to be hospitalized, and approximately 3,000 people die *(32)*. Though eggs, dairy, meat, poultry, and other foods all contribute to these illnesses, fruits and vegetables are particularly concerning due to the risk of contamination in fields followed by raw consumption. An estimated 46% of illnesses and 23% of deaths are attributed to produce *(32)*. Though many outbreaks are associated with whole or cut raw produce, juice is also a possible vehicle for foodborne illness. Many believed juice's low pH protected consumers from pathogenic microorganisms; however, several outbreaks have occurred from juice consumption including those pasteurized and unpasteurized. Table 1 lists many of the outbreaks associated with juices from 1922-2015 (modified and updated from Beuchat, 1996) *(14)*.

A 2015 study on the consumption of produce and juice by the Produce for Better Health Foundation found that produce consumption declined approximately 7% from 2009 to 2014 *(106)*. Nevertheless, it is evident that recently popular fresh or cold-pressed juice bars have opened in cities across the United States *(60)*. Raw fresh juice can be purchased in some grocery stores (particularly health food stores), from street vendors, and from specialty juice bar shops. The Organic Trade Association studied the sales of

organic produce in 2015 and found that the fresh juice and drink category increased 33.5% in 2015 which is a testament to the popularity of current raw juice*(96)*. This increasing popularity may be attributed to perceived associated health benefits, fad juicing diets, or convenience *(60)*. In addition, the United States has developed initiatives for healthier eating which emphasizes increasing consumption of fresh fruits and vegetables *(29)*. To meet this goal of increasing produce consumption, mixing vegetable juice with fruit is useful as the nutritional value may be increased due to added vegetables whose flavors are masked by sweet fruits. Raw produce that is made into juice can become contaminated before processing leading to contamination in the bottle. Processors must be aware of risks and try to minimize contamination.

Contamination of fruits and vegetables can occur from preharvest or postharvest sources which include soil, feces, irrigation water, air, equipment, animals, workers and other means *(14)*. The major drawback of consuming fresh juice is the lack of a 5-log reduction that most pasteurized retail juices undergo. Retail establishments like street vendors and juice bars are not mandated to use an approved kill step *(134)*. However, a warning label reading "WARNING: This product has not been pasteurized and therefore may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems" is required for juices that have not been treated in a validated manner *(134)*. For pasteurized products that would presumably be free of viable pathogenic organisms, insufficient pasteurization, contaminated water, or subsequent worker handling may be a cause of contamination leading to outbreaks *(18, 28, 44, 45, 121)*. Fresh juice seemingly relies on pathogen-free produce or relatively low numbers that would not cause illness. Because more than one piece of produce likely

passes through juicing equipment before cleaning in many establishments, one contaminated piece can cause issues for juice successively made; therefore, it is critical that pathogens are not introduced onto the equipment. Additionally, juice processing like high pressure pasteurization or thermal pasteurization can be detrimental to some nutrients *(70, 104)*. Combatting a contamination problem on produce before juice is made is the best solution to lower disease risks associated with fresh juices.

Pathogenic microorganisms of concern

Numerous microorganisms are associated with fruits and vegetables. The natural microflora includes several different genera of bacteria, yeasts, and molds, and pathogens can contaminate produce *(14)*. There are recommendations such as following good agricultural practices (GAPs) which can minimize the contamination risks in the field, during harvest and post-harvest handling *(14)*. Methods to combat contamination start in the field and include using clean water for irrigation, using correctly composted materials, and being aware of historical use of land. Postharvest methods include using clean water for cooling, maintaining optimum temperatures, practicing equipment sanitation, and training for worker hygiene *(131)*. However, no intervention steps will completely eliminate the possibility for contamination unless fruits and vegetables are grown in a sterile environment without outside contact. Subsequent mishandling down the supply chain cannot be overcome with GAPs.

Pathogen attachment on and infiltration into fruits and vegetables has been investigated extensively to determine the manner in which they can persist and survive *(46, 145)*. Topology of the surface of fruits and vegetables affects how pathogens can attach; thus, topology also affects pathogen removal *(145)*. Rough, irregular surfaces

have been shown to allow better attachment of *E. coli* O157:H7 than smooth surfaces in the case of undamaged and damaged bell pepper *(58)*. The roughness of produce surfaces is thought to offer protection to pathogens against removal during washing *(144)*. Presumably due to the differences in parameters such as surface roughness and hydrophobicity, microorganisms attach to some produce types more successfully than others *(46)*. Pathogens have been found to possess different genes that allow them to vary in their ability for attachment, colonization, or even internalization *(46)*.

Pathogenic bacteria of concern that have been found on fresh produce include *Shigella* species, *Salmonella* serotypes, *Escherichia coli* serovars, *Campylobacter* species, *Yersinia enterocolitica*, *Aeromonas* species, *Listeria monocytogenes*, *Staphylococcus aureus, Bacillus cereus*, and *Clostridium* species *(14)*. Viruses and parasites are also known to cause illness associated with fresh produce *(14)*. For the current study, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* were focused on due to their prevalence in the produce industry along with the potential ability to survive in fresh juice.

Salmonella serotypes

Salmonella was estimated to cause one million illnesses and 380 deaths annually between 2000 and 2008 according to the Centers for Disease Control and Prevention *(30)*. The burden of this organism is especially profound in poultry, other meats, and eggs, and *Salmonella* has been reported on fresh fruits and vegetables through surveys of market produce in a number of different countries *(14)*. For example, *Salmonella* was detected on 62.5% of celery samples taken from a vendor in India *(142).* A survey completed in the United States found that *Salmonella* was detected on 8% of surveyed

vegetables *(109)*. Contamination of fresh fruits and vegetables by *Salmonella* may be due to contact with animals and birds which are natural reservoirs as *Salmonella* can colonize their gastrointestinal tracts *(59)*.

Salmonella is particularly concerning for fresh juice made from contaminated fruits and vegetables due to documented acid tolerance and adaptation *(48, 75)*. Parish and others documented the ability of different acid-adapted serovars of *Salmonella* to survive in orange juice of varying acidity *(100)*. The study detected viable cells up to 27 days at a pH of 3.5, though the population of 10^6 cells began to decline the same day as inoculation. Higher pH orange juices (pH 4.4) allowed survival times up to 73 days *(100)*. The normal pH range for many fruit and vegetable juices are 5.0-5.5 and above 6.0, respectively, though lemon juices are much more acidic (approximately pH 2.0) *(40)*. Ultimately, the pH of mixed fruit and vegetable juice could be high enough to allow *Salmonella* survival for a sufficient amount of time to be present upon consumption.

***Escherichia coli* O157:H7**

Escherichia coli O157:H7 is estimated to cause 73,000 illnesses and 61 deaths each year in the United States *(107)*. This pathogen is of serious importance due to the risk of acute renal failure during infection especially for young children. Ground beef is commonly associated with *E. coli* O157:H7 outbreaks; however, since it was first noted with produce in 1991, fruits and vegetables have since been determined to cause a statistically significant larger number of outbreaks than ground beef *(107)*. Contamination of produce with *E. coli* O157:H7 can occur in the field due to animals or dirty irrigation water *(14)*. Moreover, *E. coli* can survive in soil for up to 21 days which may allow for harvesting to occur before cell death *(93)*.

Like *Salmonella*, *E. coli* O157:H7 has been demonstrated to become adapted to acidic environments *(88)*. Leyer et al. (1995) found that culturing *E. coli* O157:H7 in pH 5.0 media allowed for better survival when subsequently subjected to lactic acid, meat fermentation, and apple cider (pH 3.4) *(76)*. Un-adapted cells have also been demonstrated to survive at low pH *(55, 88)*. *E. coli* O157:H7 inoculated into strawberry juice (pH 3.6) and acidified media (pH 3.4 and 6.8) survived when kept at 4°C for up to 3 days. *E. coli* O157:H7 was inactivated at 37°C and pH 3.6 but grew at pH 4.7 *(55)*. Similarly, a study concerning *E. coli* O157:H7 acid tolerance in apple cider (pH 3.7) found viable cells for up to 21 days at 4°C *(88)*. That study also explored acidified trypticase soy broth in which *E. coli* O157:H7 survived at pH 2.0 for 24 h and survived better at 4°C than 37°C *(88)*. Refrigeration is key for extending shelf life and assuring minimal bacterial growth for fresh juices. These acidic environment studies demonstrate lower temperatures can allow for *E. coli* O157:H7 to survive longer in high acid solutions which could pose a serious problem for fresh juices *(55, 88)*.

E. coli O157:H7 has been demonstrated to differ in its resistance to different organic acids *(110)*. Both adapted and un-adapted cultures were explored for resistance to malic, citric, lactic, and acetic acid at pH 5.4, 5.1, 4.8, 4.5, 4.2, and 3.9. Acetic acid was inhibitory at higher pH (5.4) than the other three acids. Following acetic acid was lactic acid which was inhibitory at pH 4.5. Both malic and citric acids were found to be inhibitory at pH 4.2 *(110)*. Because fruit and vegetable juices are mostly acidified with organic acids such as malic and citric acid, the results that *E. coli* O157:H7 can survive at lower pH when malic and citric acids are the acidification source are concerning for fresh juice.

Listeria monocytogenes

Listeria monocytogenes does not cause as many cases of foodborne illness as *Salmonella* or *E. coli* O157:H7 though an estimated 1,600 illnesses and 260 deaths occur each year due to listeriosis *(112)*. Older adults, pregnant women, and immunocompromised individuals are most commonly affected; however, though it is uncommon, anyone can become sick due to exposure to *L. monocytogenes* though it is uncommon *(33)*. This organism can be found in healthy animal and bird feces, and it is widely accepted that *L. monocytogenes* is ubiquitous in soil and water *(13)*. There is evidence that *L. monocytogenes* is a saprophyte though many of the strains isolated on decaying plants are nonpathogenic *(146, 147)*. Because of its relationship with soil and water, contamination of produce with *L. monocytogenes* in the field is possible. Outbreaks due to fresh produce such as melons may be cause for concern; surveys of fresh produce in the United States have found *L. monocytogenes* in up to 36.8% of samples *(35, 61)*. To date there have not been any known cases of juice related *L. monocytogenes* outbreaks though its ability to survive and grow at refrigeration temperatures is potentially problematic for fresh juices.

The survival of *L. monocytogenes* in acidic environments has been investigated and is well established *(2, 55, 116)*. Ahamad and Marth studied the effects of acetic, lactic, and citric acids on *L. monocytogenes* and found that citric acid was least harmful followed by lactic acid then acetic acid *(2)*. *L. monocytogenes* was found to be inhibited by strawberry juice at pH 4.7. In acidified media, inhibition did not occur until citric and malic acid acidified media were at pH 3.4 suggesting that a combination of acids in juice is more harmful than a single organic acid to *L. monocytogenes (55)*. Parish and Higgins

found that a more than 4-log reduction of *L. monocytogenes* was achieved at 4°C after 25 days at pH 3.6, 43 days at pH 4.0, and 81 days at pH 4.6 in broth model systems *(99)*. Incubation at 30°C decreased the time needed to achieve the same log reduction *(99)*. Lower pH was shown to reduce populations more rapidly though it took more than 3 weeks for populations to begin to fall below detection limits at pH 3.6 at 4°C. Because fresh juice may be consumed after a few days of storage in refrigeration temperatures, prolonged survival in fresh juice could allow a sufficient number of viable cells to remain upon consumption, and illness may occur. In addition, *L. monocytogenes* has exhibited the ability to become acid-adapted like *Salmonella* and *E. coli* O157:H7 *(69)*. Because complete inactivation of *L. monocytogenes* in acidic environments could take weeks at refrigeration temperatures and the ability of *L. monocytogenes* to become acid-adapted, fresh juice is a possible vehicle for foodborne disease by *L. monocytogenes*.

Produce sanitization

Though preventing contamination is the best way to prevent pathogens on fresh produce, using sanitizers is advised since field environments can be unpredictable. According to the U.S. Food and Drug Administration, for produce, sanitize is defined as "to treat clean produce by a process that is effective in destroying or substantially reducing the numbers of microorganisms of public health concern, as well as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer *(131)*." A perfect sanitizer that can eliminate all pathogens on produce has not been discovered, and there are drawbacks for all currently used *(98)*. The inactivation ability of sanitizers is often affected by factors such as produce surface

characteristics, exposure time, sanitizer concentration, pH, and temperature *(98)*. Sanitizer use should be monitored and adapted to specific processes.

Sanitizers that are currently used in the produce industry include hypochlorite, chlorine dioxide, acidified sodium chlorite, organic acids, PAA, hydrogen peroxide, and commercial sanitizers utilizing a combination of chemicals *(98)*. Some fruits and vegetables, such as strawberries, cannot be wetted with liquid sanitizers because it shortens shelf life. Sanitizer choice is important since type of produce, cost, logistics, efficacy, and worker safety can all be factors. For the current study, hypochlorite, PAA, and chlorine dioxide were tested due to their current and potential ease of use in a small-scale environment such as a juice vendor. Chlorine dioxide is widely used in aqueous solutions, but in the current study, it is applied as a gas.

Chlorine

Chlorinated water has been widely used for numerous years as a produce sanitizer to control microorganisms *(98)*. Chlorination can be achieved by adding elemental gas (Cl_2), sodium hypochlorite, or calcium hypochlorite to water *(36)*. In water, chlorine compounds which include Cl_2, hypochlorous acid (HOCl), and hypochlorite ion (ClO^-) exist in equilibrium. The main bactericidal component of these sanitizers is HOCl which exhibits a broad range of activity for killing microorganisms though some cysts and spores show resistance *(98)*. The exhibited broad range of activity for chlorine has been suggested to be greater than for all other approved sanitizers *(148)*. However, the mode of action is not clear. Many hypotheses have been put forward to elucidate the mode of action for hypochlorous acid though there is no agreement on the principal mechanism. Proposals include amino acid oxidation, amino acid chlorination, protein synthesis

disruption, deoxyribonucleic acid (DNA) breakage, DNA synthesis disruption, cell leakage, and metabolism disruption *(47, 118, 127)*.

For chlorine to be effective, the pH must be monitored to ensure the sanitizer is mostly hypochlorous acid rather than the hypochlorite ion to ensure presence of adequate levels of the active compound (Equation 1). Lower pH values give greater amounts of hypochlorous acid, but pH values less than 6 can be corrosive, and chlorine gas may begin to escape which is a safety hazard *(98)*. An acceptable range of pH for use is between 6.0 and 7.5, and commonly used concentrations for produce washing are between 50 and 200 ppm *(98)*. In addition to pH affecting bactericidal activity, organic matter and light decrease the hypochlorite concentration which in turn decreases the efficacy of the sanitizer *(98)*.

Equation 1. $$Cl_2 + H_2O \underset{\text{Increasing pH} \rightarrow}{\rightleftharpoons} HOCl \rightleftharpoons OCl^-$$

Many studies have explored the effectiveness of hypochlorite on a wide range of fruits and vegetables, and microbial reductions are generally between 1 and 2 log CFU for 1-2 min of contact time *(22)*. A study on the effect of washing on the microflora of lettuce leaves found that 100 ppm chlorine at approximately pH 9 reduced populations by 97.8%, and reducing pH increased the bactericidal activity *(1)*. In contrast, Behrsing and others found that chlorine dips did not greatly outperform water for reducing *E. coli* on broccoli and lettuce *(7)*. Studies have also been performed to determine chlorine effectiveness against pathogens such as *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7. Liao and Sapers demonstrated a log reduction of 0.87 log CFU/disk for *Salmonella* Chester on apple disks when immersed in a 17,600 ppm sodium hypochlorite at pH 6.8 *(79)*. *Salmonella* Montevideo was reduced by approximately 1.5 log CFU/cm^2

when subjected to 320 ppm free chlorine on the surface of mature green tomatoes for 2 min *(154)*. Lettuce and cabbage dipped for 10 min in 200 ppm chlorinated water had levels of *L. monocytogenes* populations reduced by 1.7 and 1.2 log CFU/g, respectively *(153)*. A study performed with *E. coli* O157:H7 inoculated lettuce did not find any significant reduction difference between a 20 ppm chlorine wash and water *(77)*. The physical nature of fruit and vegetables e including properties like shape, roughness, and waxiness can all affect chlorine's antimicrobial effectiveness *(36)*. Generally, 1-2 log CFU reduction through chlorine exposure cannot be relied upon to render contaminated produce safe *(131)*.

Treatment with chlorine sanitizers can cause undesirable sensory changes in some vegetables *(5, 36, 140)*. These changes include altered aroma, color, and flavor properties. Leafy greens and celery have both been shown to exhibit negative sensory quality changes after chorine exposure *(5, 140)*. In contrast, a study on whole and fresh-cut melons found that sensory qualities were higher for fresh-cut melons treated with 50 ppm chlorine as compared to the water control when stored at 5°C for a period of 9-12 days *(137)*. These results were attributed to reduction of spoilage microorganisms that would improve shelf-life. A study on grape tomatoes reported no significant sensory quality change after treatment with 200 ppm chlorine for 2 min *(80)*. Possible undesirable changes may depend on the type of produce being treated with chlorine.

Residues and by-products from chlorine use are major concerns due to associated health hazards. Chlorination of water and organic material can give rise to toxic compounds such as trihalomethanes (THMs) and haloacetic acids *(78)*. Higher pH can increase THM formation though the opposite holds true for haloacetic acid formation as

lower pH increases formation *(78)*. Toxic by-product formation is also influenced by temperature, presence and levels of organic material, chlorine dose, and length of application *(31)*. Conditions need to be closely monitored and controlled when using chlorine-based sanitizers to ensure microbial risk is not replaced with toxicological risk.

Peracetic acid

Peracetic acid (PAA) is a peroxide derived from acetic acid that is antimicrobial in nature due to its oxidation ability *(67)*. PAA is approved by the U.S. Food and Drug Administration for use with certain food products when used at levels not exceeding 80 ppm *(136)*. Commercial PAA sanitizers are available as mixtures of acetic acid, hydrogen peroxide, PAA, and water *(53)*. PAA is formed when acetic acid and hydrogen peroxide react and will eventually decompose into acetic acid, water, and oxygen (Equation 2)*(19)*. Commercial PAA sanitizers may also contain stabilizers due to the unstable nature of PAA which can decompose by 1-2% per month *(67)*. These stabilizers can help extend the shelf-life and ensure the concentration remains as 10-15% PAA. Though hydrogen peroxide will also impart some bactericidal activity from these commercial mixtures, PAA is the better oxidizer and should be protected *(67)*.

Equation 2. $CH_3CO_2H + H_2O_2 \rightarrow CH_3CO_3H + H_2O \rightarrow CH_3CO_2H + H_2O_2 \rightarrow CH_3CO_2H + H_2O + O_2$

The mechanism of action for PAA relies on the oxidizing power of the compound. Hydroxyl and organic radicals formed from PAA have been suggested to be the lethal species responsible for antimicrobial action *(37, 86)*. Sensitive sulfhydryl, sulfur, and double bonds are all possible targets *(67)*. Protein denaturation, enzyme inactivation, cell wall permeability disruption, and DNA modification all likely contribute to the

bactericidal activity of PAA *(50, 67, 127).* The germicidal activity of PAA differs for different organisms. Generally, the order of susceptibility is bacteria > viruses > bacterial spores > protozoan cysts *(4).*

Several studies have explored the efficacy of PAA for killing microorganisms found on fresh produce; efficacy can differ greatly. PAA has been found to reduce *E. coli* O157:H7 by 5 log CFU/apple; however, the concentrations that produced such a decrease exceeded the current recommended and approved level by factors of 2.1 to 14 *(149).* Conversely, *E. coli* O157:H7 was reduced by only 1.83 log on lettuce leaves when treated with 30 ppm PAA *(152).* The effectiveness of 80 ppm PAA against *L. monocytogenes* on lettuce was found not to be significantly different from a 100 ppm chlorine wash *(15).* Neo et al. (2013) found similar results for both chlorine and PAA in a study that explored the reduction of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* inoculated on mung bean sprouts *(91).* Treatment with 70 ppm PAA reduced *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* by 2.3, 1.8, and 2.1 log CFU/g, respectively *(91).* In addition, that study reported increased resistance of acid-adapted cells to the sanitizers *(91).* The potential ability of PAA as a produce sanitizer is evident through studies using high concentrations, but regulations limit PAA to a maximum of 80 ppm in wash water.

Sensory quality of produce would ideally not be affected through the use of sanitizers. PAA has been found to produce mixed results in regards to affecting sensory quality of produce. Potato strips packaged under vacuum after treatment with a PAA wash did exhibit browning *(8).* Similarly, grated carrots treated with 250 mg PAA/L were found to differ greatly in flavor and odor than carrots washed with water; a noticeable sour taste was perceived immediately after treatment *(138).* Conversely, a study

performed on fresh-cut carrots did not produce the same changes in sensory quality as PAA treatment did not affect color, texture, or flavor during storage *(71)*. Any flavor or odor changes due to PAA may be due to acetic acid presence in the formulation. PAA will break down into acetic acid and hydrogen peroxide and will not form toxic organic by-products *(67)*. Overall, PAA can provide a safe means for disinfection for produce though it may result in undesirable flavors or odors.

Chlorine dioxide

Chlorine dioxide (ClO_2) as a disinfectant in water can be traced to the late nineteenth century in Europe, and the FDA allows use of aqueous ClO_2 as a wash for fruits and vegetables when a water rinse follows *(9, 131)*. ClO_2 in water has been demonstrated to have advantages over traditional chlorination methods. Advantages include oxidation capacity being 2.5 times greater for ClO_2 than for chlorine, no chlorophenolic odors or tastes, and lack of carcinogenic chloramine and trihalomethane formation *(125)*. Concentrated ClO_2 is explosive and for safety reasons is usually generated which causes the need of on-site generation *(125)*. Methods of generation of ClO_2 include chlorate ion reduction and sodium chlorite acidification either in generating equipment or dry chemical sachets *(125)*. Furthermore, the increasing ease of generation of gaseous ClO_2 through dry chemical means have created an influx of research on the decontamination ability of this gas.

The mode of bacterial inactivation of ClO_2 is not clear. Evidence exists that cell membranes, protein synthesis, amino acid synthesis, messenger RNA, co-enzyme NADH, and ribosomes are all targets for oxidation *(6, 10, 11, 95, 108)*. The efficacy of gaseous ClO_2 has been shown to be influenced by humidity and dosage *(119, 125)*. A

study on fresh berries found that ClO_2 treatment in 75-90% relative humidity was more effective in reducing *Salmonella* than lower relative humidity *(119)*. Lethality of *E. coli* O157:H7 on green pepper was also increased when relative humidity was increased *(54)*.

Numerous studies have investigated the ability of gaseous ClO_2 to decontaminate different fruits and vegetables (Table 2). Produce types that have been subjected to ClO_2 treatments in these studies include leafy greens, berries, apples, potatoes, and many others (Table 2). Gas concentrations varied greatly among the studies with some as high as 40 mg/l to as low as 0.1 mg/l *(16, 150)*. It is evident that ClO_2 has the potential to be an efficient produce sanitizer as 5 log and greater reductions have been demonstrated in some instances. *L. monocytogenes* and *Salmonella* inoculated on blemish-free tomatoes were reduced by >5 log CFU/cm^2 when treated with 0.5 mg/l ClO_2 for 12 minutes at 95% relative humidity *(16)*. ClO_2 gas treatments can be grouped into two distinct processes: batch and continuous. Batch treatment is a simpler system in that gas is introduced to product in a closed chamber with a maximum concentration reached in a specified amount of time. Conversely, a selected concentration is constantly maintained for a selected amount of time within a continuous gas treatment. Continuous gas treatment has been found to produce greater log reductions than batch treatments of the same concentration on strawberries *(57)*. The degradation or absorption of the gas without replenishment during the batch treatment presumably results in decreased decontamination ability *(57)*.

The effect of ClO_2 on the sensory quality of treated produce has also been explored. Browning and bleaching have been reported after ClO_2 treatment. Lettuce, cabbage, peaches, and apples were found to exhibit browning after ClO_2 exposure *(51,*

73, 82, 111, 120). Bleaching of lettuce, carrots, strawberries, and tomato wounds after ClO_2 treatment has also been found to occur *(82, 85, 115, 119, 120).* In contrast, other studies have reported no deleterious effects from ClO_2 treatment on sensory quality. Blueberries, raspberries, tomatoes, onions, strawberries, cantaloupe, lettuce, and carrots have been found to not be affected significantly by various ClO_2 treatments *(66, 81, 83, 119, 120, 126).* Dosage and treatment time may need to be monitored to ensure no sensory quality changes occur due to ClO_2 gas.

Suitable produce sanitizers should not form excess toxic by-products; use should be monitored to ensure such toxin formation is at a minimum. ClO_2 does not form toxic organochlorine compounds, but ClO_2, chloride, chlorite, and chlorate residues have been detected after gaseous ClO_2 treatment *(126).* Acceptable human daily intakes are set for 0.03 and 0.01 mg/kg of body weight for both chlorite and chlorate in drinking water by the World Health Organization, respectively; thus, these residues should be monitored to ensure safe levels *(64).* ClO_2 is recognized to decompose sufficiently in water before consumers drink treated water; therefore, there is not a recognized guideline value *(65).* A study on tomatoes found ClO_2, chlorate, and chlorite residues disappeared after one day of storage. A brief storage may eliminate residues from treatment *(126).* Gaseous ClO_2 can be a safe and effective sanitizer for produce if use is monitored to ensure minimum toxic by-product formation.

Table 1: List of foodborne illness outbreaks associated with juice from 1922-2015.

Year	Organism	# of Cases/Deaths	Juice type	Comments	Reference
1922	*Salmonella* Typhi	23/0	Apple*	Dirty wash water used on apples	*(97)*
1944	*S.* Typhi	18/1	Reconstituted orange	Asymptomatic handler	*(44)*
1962	Hepatitis A	24/0	Reconstituted orange	Infected handler	*(45)*
1965	Unknown	563/0	Reconstituted orange	Possible water contamination	*(121)*
1974	*S.* Typhimurium	296/0	Apple*	Drop fruit used; manure as fertilizer; no sanitization in plant	*(23)*
1980	Suspected *Escherichia coli* O157:H7	14/1	Apple*	From local market/fair	*(117)*
1989	*S.* Typhi	46/0	Reconstituted orange	Asymptomatic handler	*(18)*
1991	*E. coli* O157:H7	23/0	Apple*	Drop fruit used	*(12)*
1991	Norwalk-like virus	3,053/0	Orange	None	*(74)*
1992	Enterotoxigenic *E. coli*	6/0	Orange*	From roadside vendor	*(113)*
1993	*Crytosporidium* spp.	160/0	Apple*	Cattle grazed in area; drop fruit used	*(87)*
1993	*Salmonella* spp.	18/0	Watermelon*	Homemade juice	*(132)*
1995	*S.* Gaminara, *S.* Harford, *S.* Rubislaw	62/0	Orange*	Plant sanitation problems; distributed at theme park	*(24, 38)*
1995	*Shigella flexneri*	14/0	Orange*	Possible contamination of hands of workers	*(123)*
1996	*Crytosporidium parvum*	20/0	Apple*	Dairy farm nearby; *E. coli* in water samples	*(26)*

Table 1 (continued)

Year	Organism	# of Cases/Deaths	Juice type	Comments	Reference
1996	*E. coli* O157:H7	6/0	Apple*	Non-commercial; for local church	*(133)*
1996	*E. coli* O157:H7	14/0	Apple*	Drop fruit used	*(26)*
1996	*E. coli* O157:H7	70/1	Apple*	Drop fruit used; incorrectly used wash	*(25)*
1997	*E. coli* O157:H7	6/0	Apple*	Field trip to cider pressing farm	*(62)*
1998	*E. coli* O157:H7	14/0	Apple*	Cattle in orchard; drop fruit used; not washed	*(122)*
1999	*E. coli* O157:H7	25/0	Apple*	None	*(34)*
1999	*S.* Anatum	6/0	Orange*	Roadside vendor	*(68)*
1999	*S.* Muenchen	207/1	Orange*	Illegally added ice; transported before bottled; plant positive for *Salmonella*	*(27)*
1999	*S.* Typhimurium	405/0	Orange*	Retail level	*(89)*
2000	*S.* Enteriditis	14/0	Citrus*	Retail level	*(21)*
2002	*Shigella sonnei*	78/0	Mixed fruit	Consumed at a resort	*(34)*
2003	*C. parvum*	144/0	Apple*	Insufficient ozone treatment	*(143)*
2004	*E. coli* O111 and *C. parvum*	213/0	Apple*	Retail level	*(143)*
2004	Hepatitis A	351/0	Orange*	Source of contamination likely worker handling or contact with sewage	*(49)*

Table 1 (continued)

Year	Organism	# of Cases/Deaths	Juice type	Comments	Reference
2005	*S.* Typhimurium and *S.* Saintpaul	152/0	Orange*	Retail level; likely HACCP noncompliance	*(63)*
2005	*Trypanosoma cruzi*	25/0	Sugar cane	Roadside vendor	*(103)*
2005	*T. cruzi*	27/0	Açaí juice	Sales outlet	*(103)*
2006	*Clostridium botulinum*	4/0	Carrot	Retail level	*(28)*
2007	*E. coli* O157:H7	9/0	Apple*	None	*(34)*
2007	Hepatitis A	3/0	Mixed fruit - açaí, banana, strawberry, sugar cane*	Food service level	*(34)*
2007	*T. cruzi*	103/1	Guava*	Left overnight at school; insects may be source of contamination	*(41)*
2008	*E. coli* O157:H7	5/0	Apple*	Mobile service	*(34)*
2008	*S.* Panama	33/0	Orange*	Retail level	*(94)*
2009	Unknown; thought to be viral	38/0	Not specified	Juice served on coach trips	*(141)*
2010	*E. coli* O157:H7	7/0	Apple*	Retail level	*(135)*
2011	Norovirus Genogroup II	207/0	Not specified	Occurred at indoor workplace	*(34)*
2015	*E. coli* O111	13/0	Apple*	Retail level	*(39)*
2015	*E. coli* O103 and *E. coli* O111	104/0	Apple*	Occurred at a fall festival	*(3)*

* denotes reported unpasteurized product

Table 2: Bacterial pathogen inactivation values from gaseous chlorine dioxide treatments on produce from 2000-2016.

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2000	Green pepper (injured)	*E. coli* O157:H7	0.62 mg/l	30	90-95	3.03 log CFU/sample	Injured by blade scrape; used ClO_2 gas generator (CDG)	*(58)*
			1.24 mg/l			6.45 log CFU/sample		
2001	Green pepper (uninjured)	*L. monocytogenes*	0.3 mg/l	10	90-95	3.05 log CFU/5g	Injured by blade scrape; used CDG	*(56)*
			3.0 mg/l			7.39 log CFU/5g		
	Green pepper (injured)	*L. monocytogenes*	0.3 mg/l	10	90-95	1.88 log CFU/5g		
			3.0 mg/l			3.60 log CFU/5g		
2002	Apple surface	*L. monocytogenes*	1.0 mg/l	10	90-95	3.2 log CFU/site	4 apples evenly spaced per treatment; used CDG	*(42)*
			3.0 mg/l			3.3 log CFU/site		
			4.0 mg/l			5.5 log CFU/site		
	Apple calyx	*L. monocytogenes*	1.0 mg/l	10	90-95	2.8 log CFU/site		
			3.0 mg/l			2.9 log CFU/site		
			4.0 mg/l			3.2 log CFU/site		
	Apple stem area	*L. monocytogenes*	1.0 mg/l	10	90-95	2.2 log CFU/site		
			3.0 mg/l			3.1 log CFU/site		
			4.0 mg/l			3.6 log CFU/site		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2002	Lettuce	*E. coli* O157:H7	0.5 ml/l	5	80	0.73 log CFU/g	Used CDG	*(114)*
				10		1.17 log CFU/g		
				15		1.25 log CFU/g		
			0.75 mg/l	5	80	0.81 log CFU/g		
				10		1.67 log CFU/g		
				15		1.90 log CFU/g		
			1.0 mg/l	5	80	1.04 log CFU/g		
				10		1.91 log CFU/g		
				15		2.21 log CFU/g		
	Baby carrot	*E. coli* O157:H7	0.5 mg/l	5	80	0.80 log CFU/g		
				10		1.45 log CFU/g		
				15		1.72 log CFU/g		
			0.75 mg/l	5	80	1.15 log CFU/g		
				10		1.89 log CFU/g		
				15		2.31 log CFU/g		
			1.0 mg/l	5	80	1.39 log CFU/g		
				10		2.54 log CFU/g		
				15		3.00 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2003	Apple surface	*E. coli* O157:H7	1.1 mg/l	10	90-95	2.8 log CFU/site	Used CDG	*(43)*
				20		4.7 log CFU/site		
			3.3 mg/l	10		3.9 log CFU/site		
				20		5.9 log CFU/site		
			4.8 mg/l	10		4.8 log CFU/site		
				20		≥6.3 log CFU/site		
			7.2 mg/l	10		≥5.8 log CFU/site		
				20		≥7.3 log CFU/site		
			12.0 mg/l	10		≥7.0 log CFU/site		
			15.0 mg/l	10		≥7.0 log CFU/site		
			18.0 mg/l	10		≥7.0 log CFU/site		
	Apple calyx	*E. coli* O157:H7	1.1 mg/l	10	90-95	2.1 log CFU/site		
				20		2.5 log CFU/site		
			3.3 mg/l	10		2.1 log CFU/site		
				20		3.6 log CFU/site		
			4.8 mg/l	10		2.6 log CFU/site		
				20		3.8 log CFU/site		
			7.2 mg/l	10		2.9 log CFU/site		
				20		3.8 log CFU/site		
			12.0 mg/l	10		3.7 log CFU/site		
			15.0 mg/l	10		4.0 log CFU/site		
			18.0 mg/l	10		3.8 log CFU/site		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2003 cont.	Apple stem area	*E. coli* O157:H7	1.1 mg/l	10	90-95	1.6 log CFU/site	Used CDG	*(43)*
				20		2.0 log CFU/site		
			3.3 mg/l	10		1.6 log CFU/site		
				20		2.9 log CFU/site		
			4.8 mg/l	10		2.1 log CFU/site		
				20		3.3 log CFU/site		
			7.2 mg/l	10		2.5 log CFU/site		
				20		3.9 log CFU/site		
			12.0 mg/l	10		3.0 log CFU/site		
			15.0 mg/l	10		3.0 log CFU/site		
			18.0 mg/l	10		3.8 log CFU/site		
2004	Strawberry	*E. coli* O157:H7	0.2 mg/l	15	90-95	1.2 log CFU/strawberry	Used CDG; both batch and continuous ClO_2 treatments explored (continuous data shown); batch found to be less effective	*(57)*
				30		2.4 log CFU/strawberry		
			0.6 mg/l	15		1.9 log CFU/strawberry		
				30		3.0 log CFU/strawberry		
		L. monocytogenes	0.2 mg/l	15		1.8 log CFU/strawberry		
				30		2.8 log CFU/strawberry		
			0.6 mg/l	15		2.6 log CFU/strawberry		
				30		3.6 log CFU/strawberry		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2004	Lettuce	*E. coli* O157:H7	0.215 mg/l	30	Not recorded	3.4 log CFU/g	Dry chemical sachet used to generate ClO_2	*(72)*
			0.335 mg/l	60		4.4 log CFU/g		
			0.435 mg/l	180		6.9 log CFU/g		
		L. monocytogenes	0.215 mg/l	30		5.0 log CFU/g		
			0.335 mg/l	60		5.2 log CFU/g		
			0.435 mg/l	180		5.4 log CFU/g		
		Salmonella Typhimurium	0.215 mg/l	30		4.3 log CFU/g		
			0.335 mg/l	60		5.3 log CFU/g		
			0.435 mg/l	180		5.4 log CFU/g		
2005	Blueberry skin	*Salmonella*	4.1 mg/l	30	75-90	2.95 log CFU/g	Dry chemical sachet used to generate ClO_2	*(119)*
			6.2 mg/l	60		3.56 log CFU/g		
			8.0 mg/l	120		3.67 log CFU/g		
	Blueberry calyx	*Salmonella*	4.1 mg/l	30		2.20 log CFU/g		
			6.2 mg/l	60		1.88 log CFU/g		
			8.0 mg/l	120		2.44 log CFU/g		
	Blueberry stem scar	*Salmonella*	4.1 mg/l	30		2.43 log CFU/g		
			6.2 mg/l	60		2.36 log CFU/g		
			8.0 mg/l	120		3.24 log CFU/g		
	Strawberry skin	*Salmonella*	4.1 mg/l	30		2.32 log CFU/g		
			6.2 mg/l	60		3.33 log CFU/g		
			8.0 mg/l	120		3.76 log CFU/g		
	Strawberry stem scar	*Salmonella*	4.1 mg/l	30		2.22 log CFU/g		
			6.2 mg/l	60		2.80 log CFU/g		
			8.0 mg/l	120		4.41 log CFU/g		
	Raspberry	*Salmonella*	4.1 mg/l	30		0.52 log CFU/g		
			6.2 mg/l	60		1.06 log CFU/g		
			8.0 mg/l	120		1.54 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2005	Cabbage	*Salmonella*	1.4 mg/l	10.5	48-85	1.24 log CFU/g	Dry chemical sachet used to generate ClO_2	*(120)*
			2.7 mg/l	20.0		1.89 log CFU/g		
			4.1 mg/l	30.8		4.42 log CFU/g		
		E. coli O157:H7	1.4 mg/l	6.4		1.53 log CFU/g		
			2.7 mg/l	12.3		2.68 log CFU/g		
			4.1 mg/l	20.5		3.13 log CFU/g		
		L. monocytogenes	1.4 mg/l	10.0		1.76 log CFU/g		
			2.7 mg/l	19.3		3.31 log CFU/g		
			4.1 mg/l	29.3		3.60 log CFU/g		
	Carrot	*Salmonella*	1.4 mg/l	10.5	51-88	2.15 log CFU/g		
			2.7 mg/l	20.0		3.11 log CFU/g		
			4.1 mg/l	30.8		5.15 log CFU/g		
		E. coli O157:H7	1.4 mg/l	6.4		2.03 log CFU/g		
			2.7 mg/l	12.3		3.18 log CFU/g		
			4.1 mg/l	20.5		5.62 log CFU/g		
		L. monocytogenes	1.4 mg/l	10.0		3.28 log CFU/g		
			2.7 mg/l	19.3		5.35 log CFU/g		
			4.1 mg/l	29.3		5.88 log CFU/g		
	Iceberg lettuce	*Salmonella*	1.4 mg/l	10.5	36-84	1.14 log CFU/g		
			2.7 mg/l	20.0		1.21 log CFU/g		
			4.1 mg/l	30.8		1.58 log CFU/g		
		E. coli O157:H7	1.4 mg/l	6.4		0.64 log CFU/g		
			2.7 mg/l	12.3		0.72 log CFU/g		
			4.1 mg/l	20.5		1.57 log CFU/g		
		L. monocytogenes	1.4 mg/l	10.0		0.81 log CFU/g		
			2.7 mg/l	19.3		1.23 log CFU/g		
			4.1 mg/l	29.3		1.53 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2005 cont.	Apple	*Salmonella*	1.4 mg/l	6	35-68	3.21 log CFU/g	Dry chemical sachet used to generate ClO_2	*(120)*
			2.7 mg/l	12		4.21 log CFU/g		
			4.1 mg/l	25		4.21 log CFU/g		
	Tomato	*Salmonella*	1.4 mg/l	6	34-62	1.11 log CFU/g		
			2.7 mg/l	12		2.04 log CFU/g		
			4.1 mg/l	25		4.33 log CFU/g		
	Onion	*Salmonella*	1.4 mg/l	5.4	35-64	0.83 log CFU/g		
			2.7 mg/l	10.4		1.89 log CFU/g		
			4.1 mg/l	20		1.94 log CFU/g		
	Peach	*Salmonella*	1.4 mg/l	5.4	55-78	1.00 log CFU/g		
			2.7 mg/l	10.4		1.52 log CFU/g		
			4.1 mg/l	20		3.23 log CFU/g		
2006	Bell pepper surface	*Salmonella*	4.8 mg/l	60	≈ 50	2.12 log CFU/produce	Injured by puncturing with paper clip; dry chemical sachet used to generate ClO_2	*(151)*
	Bell pepper stem scar	*Salmonella*	4.8 mg/l	60		2.28 log CFU/produce		
	Injured bell pepper	*Salmonella*	4.8 mg/l	60		2.31 log CFU/produce		
	Cucumber surface	*Salmonella*	4.8 mg/l	60		>4.97 log CFU/produce		
	Cucumber stem scar	*Salmonella*	4.8 mg/l	60		>5.97 log CFU/produce		
	Injured cucumber	*Salmonella*	4.8 mg/l	60		>4.68 log CFU/produce		
	Strawberry surface	*Salmonella*	4.8 mg/l	60		>4.76 log CFU/produce		
	Strawberry stem scar	*Salmonella*	4.8 mg/l	60		3.99 log CFU/produce		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2006 cont.	Injured strawberry	*Salmonella*	4.8 mg/l	60	≈ 50	1.15 log CFU/produce	Injured by puncturing with paper clip; dry chemical sachet used to generate ClO_2	*(151)*
2007	Strawberry	*Salmonella*	0.5 mg/l	2	99.9	0.6 log CFU/strawberry	Used CDG	*(81)*
				10		2.7 log CFU/strawberry		
			1.5 mg/l	10		3.0 log CFU/strawberry		
			3.0 mg/l	10		4.0 log CFU/strawberry		
			5.0 mg/l	2		1.9 log CFU/strawberry		
				6		3.0 log CFU/strawberry		
				10		4.3 log CFU/strawberry		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2007 cont.	Strawberry	*E. coli* O157:H7	0.5 mg/l	2	99.9	1.2 log CFU/strawberry	Used CDG	*(81)*
				10		2.4 log CFU/strawberry		
			1.0 mg/l	10		3.0 log CFU/strawberry		
			1.5 mg/l	10		3.0 log CFU/strawberry		
			3.0 mg/l	6		3.0 log CFU/strawberry		
				10		4.5 log CFU/strawberry		
			5.0 mg/l	2		1.8 log CFU/strawberry		
				6		3.0 log CFU/strawberry		
				10		4.6 log CFU/strawberry		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2007 cont.	Strawberry	*L. monocytogenes*	0.5 mg/l	2	99.9	0.8 log CFU/strawberry	Used CDG	*(81)*
				10		2.3 log CFU/strawberry		
			3.0 mg/l	6		3.0 log CFU/strawberry		
				10		4.6 log CFU/strawberry		
			5.0 mg/l	2		0.8 log CFU/strawberry		
				4		3.0 log CFU/strawberry		
2007	Blueberries	*Salmonella*	4.0 mg/l	740	99.9	3.62 log CFU/g	Dry chemical sachet used to generate ClO_2	*(105)*
		E. coli O157:H7	4.0 mg/l	740		4.25 log CFU/g		
		L. monocytogenes	4.0 mg/l	740		3.94 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2008	Lettuce	*Salmonella enterica*	0.5 mg/l	2	90-95	0.7 log CFU/5 cm^2	Used CDG	*(82)*
				10		1.9 log CFU/5 cm^2		
			1.0 mg/l	2		0.7 log CFU/5 cm^2		
				10		2.4 log CFU/5 cm^2		
			1.5 mg/l	2		1.0 log CFU/5 cm^2		
				10		2.4 log CFU/5 cm^2		
			3.0 mg/l	2		1.2 log CFU/5 cm^2		
				10		2.5 log CFU/5 cm^2		
			5.0 mg/l	2		1.5 log CFU/5 cm^2		
				10		2.8 log CFU/5 cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2008 cont.	Lettuce	*E. coli* O157:H7	0.5 mg/l	2	90-95	0.5 log CFU/5 cm^2	Used CDG	*(82)*
				10		1.6 log CFU/5 cm^2		
			1.0 mg/l	2		0.9 log CFU/5 cm^2		
				10		2.7 log CFU/5 cm^2		
			1.5 mg/l	2		1.2 log CFU/5 cm^2		
				10		3.1 log CFU/5 cm^2		
			3.0 mg/l	2		1.6 log CFU/5 cm^2		
				10		3.1 log CFU/5 cm^2		
			5.0 mg/l	2		1.6 log CFU/5 cm^2		
				10		3.3 log CFU/5 cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2008	Cantaloupe	*Salmonella*	0.5 mg/l	2	90-95	0.9 log CFU/5 cm^2	Used CDG	*(83)*
				10		3.2 log CFU/5 cm^2		
			1.0 mg/l	2		1.2 log CFU/5 cm^2		
				10		3.5 log CFU/5 cm^2		
			1.5 mg/l	2		1.5 log CFU/5 cm^2		
				10		4.7 log CFU/5 cm^2		
			3.0 mg/l	2		3.2 log CFU/5 cm^2		
				10		≥5 log CFU/5 cm^2		
			5.0 mg/l	2		3.2 log CFU/5 cm^2		
				10		≥5 log CFU/5 cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2008 cont.	Cantaloupe	*E. coli* O157:H7	0.5 mg/l	2	90-95	0.6 log CFU/5 cm^2	Used CDG	*(83)*
				10		2.7 log CFU/5 cm^2		
			1.0 mg/l	2		1.1 log CFU/5 cm^2		
				10		2.7 log CFU/5 cm^2		
			1.5 mg/l	2		1.1 log CFU/5 cm^2		
				10		2.8 log CFU/5 cm^2		
			3.0 mg/l	2		2.2 log CFU/5 cm^2		
				10		3.4 log CFU/5 cm^2		
			5.0 mg/l	2		2.2 log CFU/5 cm^2		
				10		4.6 log CFU/5 cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2008 cont.	Cantaloupe	*L. monocytogenes*	0.5 mg/l	2	90-95	1.2 log CFU/5 cm^2	Used CDG	*(83)*
				10		3.3 log CFU/5 cm^2		
			1.0 mg/l	2		1.8 log CFU/5 cm^2		
				10		3.2 log CFU/5 cm^2		
			1.5 mg/l	2		2.1 log CFU/5 cm^2		
				10		3.7 log CFU/5 cm^2		
			3.0 mg/l	2		2.1 log CFU/5 cm^2		
				10		3.8 log CFU/5 cm^2		
			5.0 mg/l	2		2.2 log CFU/5 cm^2		
				10		4.3 log CFU/5 cm^2		
2009	Injured tomato	*Salmonella* Typhimurium	≥5 mg/3 fruit	120	Not recorded	>5 log CFU/fruit	Injured by removing epidermis layer; aqueous solution of ClO_2 used to emit ClO_2 gas	*(84)*

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2010	Tomato	*S. enterica*	0.5 mg/l	12	85-90%	>5 log CFU/cm^2	Used CDG	*(16)*
		L. monocytogenes	0.5 mg/l	12		>5 log CFU/cm^2		
2010	Potato	*Pseudomonas aeruginosa*	16 mg/l	150	Not recorded	2.2 log CFU/potato	Dry chemical sachet used to generate ClO_2	*(150)*
			20 mg/l	300		2.7 log CFU/potato		
			24 mg/l	150		3.3 log CFU/potato		
			30 mg/l	300		4.3 log CFU/potato		
			32 mg/l	150		3.9 log CFU/potato		
			40 mg/l	300		5.8 log CFU/potato		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2010	Tomato	*Salmonella*	2 mg/l	0.167	90-95	0.53 log CFU/cm^2	Used chlorine gas and sodium chlorite cartridges to generate ClO_2 gas	*(126)*
				0.5		0.50 log CFU/cm^2		
				1		0.94 log CFU/cm^2		
				2		1.25 log CFU/cm^2		
				3		2.41 log CFU/cm^2		
			5 mg/l	0.167		1.05 log CFU/cm^2		
				0.5		1.04 log CFU/cm^2		
				1		1.77 log CFU/cm^2		
				2		1.91 log CFU/cm^2		
				3		2.43 log CFU/cm^2		
			8 mg/l	0.167		1.08 log CFU/cm^2		
				0.5		2.14 log CFU/cm^2		
				1		2.94 log CFU/cm^2		
				2		3.38 log CFU/cm^2		
				3		3.77 log CFU/cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2010 cont.	Tomato	*Salmonella*	10 mg/l	0.167	90-95	2.71 log CFU/cm^2	Used chlorine gas and sodium chlorite cartridges to generate ClO_2 gas	*(126)*
				0.5		2.92 log CFU/cm^2		
				1		3.19 log CFU/cm^2		
				2		3.86 log CFU/cm^2		
				3		4.87 log CFU/cm^2		
2011	Orange	*S. enterica*	0.1 mg/l	14	90-95	>5 log CFU/sample	Used CDG	*(17)*
			0.3 mg/l	10		>5 log CFU/sample		
			0.5 mg/l	10		>5 log CFU/sample		
2012	Spinach	*Salmonella*	1.2 mg/l	30	Not recorded	0.3 log CFU/g	Dry chemical sachet used to generate ClO_2	*(90)*
			2.1 mg/l	60		0.6 log CFU/g		
		E. coli O157:H7	1.2 mg/l	30		0.7 log CFU/g		
			2.1 mg/l	60		0.7 log CFU/g		
2013	Tomato		10 mg/l	3	90-95	4.8 log CFU/cm^2	Used chlorine gas and sodium chlorite cartridges to generate ClO_2 gas	*(124)*
		E. coli O157:H7				3.6 log CFU/cm^2		
		L. monocytogenes				3.0 log CFU/cm^2		
	Cantaloupe	*Salmonella*	10 mg/l	3		4.0 log CFU/cm^2		
		E. coli O157:H7				2.9 log CFU/cm^2		
		L. monocytogenes				3.3 log CFU/cm^2		
	Strawberry	*Salmonella*	10 mg/l	3		4.8 log CFU/cm^2		
		E. coli O157:H7				2.7 log CFU/cm^2		
		L. monocytogenes				3.0 log CFU/cm^2		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2015	Spinach	*Salmonella* Typhimurium	0.14 mg/l	1	50	0.66 log CFU/g	Used CDG; explored other gas lower concentrations of gas with differing humidity	*(102)*
					70	0.60 log CFU/g		
					90	1.09 log CFU/g		
				5	50	0.92 log CFU/g		
					70	0.73 log CFU/g		
					90	2.34 log CFU/g		
				10	50	1.08 log CFU/g		
					70	1.30 log CFU/g		
					90	5.19 log CFU/g		
				15	50	1.21 log CFU/g		
					70	1.49 log CFU/g		
					90	>5.56 log CFU/g		
				20	50	1.25 log CFU/g		
					70	2.54 log CFU/g		
					90	>5.56 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2015 cont.	Spinach	*E. coli* O157:H7	0.14 mg/l	1	50	0.74 log CFU/g	Used CDG; explored other gas lower concentrations of gas with differing humidity	*(102)*
					70	0.50 log CFU/g		
					90	0.77 log CFU/g		
				5	50	1.10 log CFU/g		
					70	0.82 log CFU/g		
					90	2.47 log CFU/g		
				10	50	1.31 log CFU/g		
					70	1.44 log CFU/g		
					90	5.08 log CFU/g		
				15	50	1.50 log CFU/g		
					70	1.80 log CFU/g		
					90	>5.53 log CFU/g		
				20	50	1.58 log CFU/g		
					70	2.45 log CFU/g		
					90	>5.53 log CFU/g		

Table 2 (continued)

Year	Produce type	Pathogen	Gas concentration	Time (min)	Relative Humidity (%)	Log reduction	Other Notes	Reference
2015 cont.	Spinach	*L. monocytogenes*	0.14 mg/l	1	50	0.64 log CFU/g	Used CDG; explored other gas lower concentrations of gas with differing humidity	*(102)*
					70	0.78 log CFU/g		
					90	0.76 log CFU/g		
				5	50	1.26 log CFU/g		
					70	1.28 log CFU/g		
					90	1.98 log CFU/g		
				10	50	1.31 log CFU/g		
					70	1.42 log CFU/g		
					90	4.71 log CFU/g		
				15	50	1.55 log CFU/g		
					70	1.89 log CFU/g		
					90	>4.85 log CFU/g		
				20	50	1.78 log CFU/g		
					70	2.02 log CFU/g		
					90	>4.85 log CFU/g		
2016	Tomato	*Salmonella* Typhimurium	0.15 mg/l	50	92.6	2.94 log CFU/g	ClO_2 batch treatment system from liquid reservoir; other concentrations were explored to determine a model	*(92)*
			0.85 mg/l			7.37 log CFU/g		

CHAPTER 3

MATERIALS AND METHODS

Bacterial strains

Five-serotypes or five-strains of each pathogen (*Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*) were used in the inoculum. Serotypes of *Salmonella enterica* included an isolate from an alfalfa sprout-associated outbreak (serotype Agona), clinical isolates from outbreaks associated with tomato (serotype Baildon and serotype Montevideo), an isolate from orange juice (serotype Gaminara), and an isolate from a cantaloupe-associated outbreak (serotype Poona). The five strains of *E. coli* O157:H7 used were a clinical isolate from an outbreak associated with lettuce (H1730), an isolate from an unpasteurized apple cider-associated outbreak (SEA 13B88), a clinical isolate from an outbreak associated with alfalfa sprouts (F4546), a clinical isolate from an outbreak associated with cantaloupe (CDC 658), and a clinical isolate in a cider-associated outbreak (C7927). The five strains of *L. monocytogenes* included an isolate from celery (serotype 4b, F8027), an isolate from peach and plum (serotype 1/2b, F8255), an isolate from corn (serotype 1/2a, F8369), a clinical isolate from an outbreak associated with coleslaw (serotype 4b, G1091), and an isolate from raw potato (serotype 1/2a, H0222).

Each culture was obtained from the culture stock of the Department of Food Science and Technology at the University of Georgia, Athens, GA. Frozen

Salmonella, *E. coli* O157:H7, and *L. monocytogenes* cultures stored on cryogenic beads (Microbank™ Bacterial and Fungal Preservation System, Pro-Lab Diagnostics, Toronto, Canada) at -80°C were activated by transferring one frozen bead to 10 ml tryptic soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, NJ) and incubating at 37°C for 24 h. At least two successive loop (ca. 10 µl) transfers into 10 ml TSB incubated at 37°C for 24 h were done to activate cultures.

Antibiotic-resistant strain preparation and culture maintenance

To minimize interference from natural produce background microflora, antibiotic-adapted serotypes and strains of *Salmonella* and *E. coli* O157:H7 were prepared and used in the study; *L. monocytogenes* strains were not adapted due to the presence of a number of antibiotics in the selective media used for recovery. *Salmonella* serotypes and *E. coli* O157:H7 strains were adapted to different antibiotics to also decrease the likelihood of interference from each other. *Salmonella* serotypes were adapted to nalidixic acid, and *E. coli* O157:H7 strains were adapted to rifampicin.

Wild-type *Salmonella* serotypes were adapted to 50 µg/ml nalidixic acid (Alfa Aesar, Ward Hill, MA) by a loop transfer from a 24 h TSB culture into 10 ml TSB containing 50 µg/ml nalidixic acid (TSBN) which was incubated at 37°C for 24 h. Each nalidixic acid adapted serotype was confirmed by streaking the 24 h culture onto xylose lysine desoxycholate agar (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 50 µg/ml nalidixic acid (XLDN). After the XLDN plates were incubated at 37°C for 24 h, a typical

colony was transferred onto slants of tryptic soy agar (TSA; Becton, Dickinson and Company, Franklin Lakes, NJ) containing 50 μg/ml nalidixic acid and incubated 37°C for 24 h and stored at 4°C. New TSA slants containing 50 μg/ml nalidixic acid were inoculated with the adapted *Salmonella* strains every 2 – 4 weeks and grown at 37°C for 24 h and stored at 4°C to ensure cells remained viable.

E. coli O157:H7 strains were adapted to 100 μg/ml rifampicin (Fisher Scientific, Hampton, NH) by a loop transfer of the wild-type 24 h TSB culture into 10 ml TSB containing 100 μg/ml rifampicin (TSBR) which was incubated at 37°C for 24 h. Rifampicin resistant strains were confirmed by streaking each 24 h culture onto sorbitol MacConkey agar (Remel, Lenexa, KS) supplemented with 100 μg/ml rifampicin (SMACR). After the SMACR plates were incubated at 37°C for 24 h, a typical colony of each strain was transferred onto slants of TSA containing 100 μg/ml rifampicin and incubated 37°C for 24 h and stored at 4°C. New TSA slants containing 100 μg/ml rifampicin were inoculated with the adapted *E. coli* O157:H7 strains every 2 – 4 weeks and grown at 37°C for 24 h before storing at 4°C.

Growth rates of wild-type and antibiotic resistant serotypes and strains were determined to ensure the adapted serotypes and strains behaved similarly to the wild-type parents.

L. monocytogenes strains were transferred to TSA slants from a 24 h culture in TSB and incubated at 37°C for 24 h. The slants were stored at 4°C.

New TSA slants of the strains were inoculated every 2 – 4 weeks and grown at 37°C for 24 h and stored at 4°C.

Inoculum preparation

A loopful of each serotype or strain was taken from the slant and cultured for 24 h at 37°C in 10 ml TSB containing the appropriate antibiotic if needed. A loopful of each of the 24 h cultures of *E. coli* O157:H7 and *L. monocytogenes* was transferred to 10 ml TSBR and TSB, respectively, and incubated for 24 h at 37°C; 0.1 ml of the 24 h *Salmonella* cultures were transferred to 100 ml TSBN and incubated for 24 h at 37°C. After incubation, 10 ml cultures were centrifuged at 2,500 x g (Model 5810; Eppendorf, Hamburg, Germany) for 10 min to pellet the bacterial cells; 100 ml cultures were centrifuged under the same conditions for 20 min. The supernatant was discarded, and the pellet was suspended in 10 ml of 0.1% peptone water (PW; Becton, Dickinson and Company, Franklin Lakes, NJ). Cellular suspensions was centrifuged again for 5 min at 2,500 x g; the supernatant was discarded and the pellet was suspended in 1 ml of 0.1% PW. One ml of suspension for each serotype and strain was combined to give 15 ml of a mixture containing five-serotypes of *S. enterica*, five-strains of *E. coli* O157:H7, and five-strains of *L. monocytogenes*. The final inoculum cocktail contained approximately 10^9 CFU/ml of each pathogen. The bacterial cocktail was serially diluted in 0.1% PW and surface plated on XLDN, SMACR, and Oxford agar (OXA; Oxoid, Basingstoke, UK) to enumerate and verify the bacterial cocktail populations. XLDN and SMACR plates were incubated at 37°C for 24 h, and OXA plates were incubated at 37°C for 48 h.

Produce tested

Valencia oranges (*Citrus sinensis* L.), celery (*Apium graveolens* L.), cucumbers (*Cucumis sativus* L.), and curly kale (*Brassica oleracea* var. *acephala*) were obtained from a produce distributor in Atlanta, GA. Fresh, unblemished, blemish-free produce was used. Produce was stored at 4°C for a maximum of 7 days before use. Each treatment contained 8 oranges (4 fresh, unblemished; 4 aged), 14 celery stalks (7 fresh, unblemished; 7 damaged), 8 cucumbers (4 fresh, unblemished; 4 damaged), and 14 kale leaves (7 fresh, unblemished; 7 wilted); 2 extra samples per produce type were also included in the experiments for confirming the inoculation level. Mean weights of oranges, celery stalks, cucumbers, and kale leaves were 206 ± 41 g, 73 ± 23 g, 460 ± 80 g, and 40 ± 16 g, respectively.

Produce defined as damaged, aged, or wilted was prepared in the laboratory after equilibrating to room temperature (22 ± 2°C) for 2 h (Figure 1). Aged, less firm oranges were obtained by storing at room temperature (22 ± 2°C) for 10 days before use. Damaged celery and cucumbers were physically blemished by damaging the surface with a food peeler before storing at room temperature (22 ± 2°C) for 1 day. Celery was scraped with the tip of the food peeler by six swift motions down the stalk in a row with a length of 16 cm. Cucumbers were injured by using the tip of the food peeler to puncture the skin at a depth of 0.5 cm and at a 45° angle two times approximately 2 cm apart. The food peeler was flame sterilized between uses. Kale was considered wilted due to wilting and discoloration which was achieved by storing leaves for 2 days at room

temperature (22 ± 2°C) before use. Fresh, unblemished produce was allowed to equilibrate to room temperature (22 ± 2°C) for 2 h before inoculation.

Inoculation and assignment of produce

Six to eight randomly selected samples from each produce type were spot inoculated using a micropipette with 100 µl of the cocktail of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* containing approximately 10^9 CFU/ml to achieve 10^8 CFU of each pathogen on the produce surface away from stems and the ends of each sample. To facilitate drying, the inoculum was deposited using ten to fifteen approximately equal volume spots on each produce surface. Damaged celery and cucumbers were inoculated on the blemished areas and inside the punctures. Kale was inoculated along the midvein on the front of the leaf. A small "1" or "2" was written on each inoculated sample with a permanent marker (Sharpie, Oak Brook, IL) near the stems to ensure no interference with inoculum; the marking allowed for distinguishing the samples that were analyzed after treatments. Samples were allowed to dry in a biosafety hood for 16 h at 22 ± 2°C; uninoculated samples were also stored for 16 h at 22 ± 2°C. Due to the inability to recover all cells, target recovery for samples was 10^6 CFU/sample, and this was confirmed by subjecting two samples from each produce type to microbiological analysis as described in a later section.

Chlorine dioxide gas treatment

All four inoculated produce types in the specified quantities were placed in a heat sterilized 62.8 liter (66.04 cm L x 45.72 cm W x 30.48 cm H) polycarbonate food storage box (Carlisle, Oklahoma City, OK) as shown in

Figure 2. The container was sectioned into 4 areas; damaged, aged, or wilted and fresh, unblemished produce of the same type were randomly placed in one of the sections (Figure 3). Inoculated produce samples were placed in the far corners in the respective assigned section. Samples labeled 1 were placed in the corners and samples labeled 2 were directly adjacent. Damaged, aged, or wilted and fresh, unblemished samples were randomly assigned to either the top or bottom position though inoculated areas were positioned to be facing toward each other (Figure 2, 3). A temperature and humidity recorder (Model RHT10, Extech Instruments, Nashua, NH) was fastened to the container lid (Carlisle, Oklahoma City, OK) to monitor conditions inside the treatment container. Two 12.7 cm portable fans (Model FD05004, O2COOL, Chicago, IL) were also fastened to the lid of the container on opposite sides to provide gas circulation within the container. A chlorine dioxide gas releasing sachet (ICA Trinova, LLC, Newnan, GA) containing reactant chemicals (zeolite carrier impregnated with sodium chlorite and solid impregnated with acid activator, ferric chloride) was taped to the center of the container lid between the two fans. The amount of reactant chemicals used was calculated based on total treatment weight and the desired gaseous dose. Figure 4 shows the release of ClO_2 over time for the reactant chemicals. The formulation was calculated and weighed to release 20 mg ClO_2/kg of produce in 30 min. After the sachet was placed inside the container, the lid was secured, and aluminum foil (Reynolds Wrap, Lincolnshire, IL) then 4-inch Parafilm (Bemis Flexible Packaging, Neenah, WI) was wrapped around the lid and container seam to seal it. The container was placed in a dark ventilated area for the treatment

duration. A positive control was also performed by placing produce in a container in the same manner and conditions but without chlorine dioxide gas sachet.

Aqueous solution treatment

The 4 produce types in the aforementioned quantities were placed in a heat sterilized nickel chrome wire basket (50.8 cm L x 35.56 cm W x 14.61 cm H; Choice, Lititz, PA) that fit inside a heat sterilized 62.8 liter (66.04 cm L x 45.72 cm W x 30.48 cm H) polycarbonate food storage box (Carlisle, Oklahoma City, OK). The produce types were assigned to locations in the basket in the same manner as the chlorine dioxide gas treatments. Hypochlorite solutions were prepared by diluting bleach (8.25%, Clorox, Oakland, CA) to 200 ppm with deionized water. The final pH was adjusted to 6.5 using 10 M sulfuric acid (Aqua Solutions, Deerpark, TX). Total chlorine concentration was confirmed using a Vacu-viles chlorine kit (Catalog K-2513, CHEMetrics, Midland, VA) in a photometer for water quality (Model V-2000, CHEMetrics, Midland, VA). PAA solutions were prepared by diluting a commercially available produce wash (15% peracetic acid; 15932 Victory, Ecolab, St. Paul, MN) to 80 ppm with deionized water. Concentration of PAA was verified using a Vacu-viles peracetic acid kit (Catalog K-7913, CHEMetrics, Midland, VA) in a photometer. Produce filled basket was placed inside the food storage box containing 24 L of the appropriate solution. Produce was allowed to soak for 2 min in either 200 ppm hypochlorite solution or 80 ppm PAA solution before the basket was removed from the treatment container. A deionized water control was also performed in the same manner.

Microbiological analysis

Two inoculated, untreated samples and two inoculated, treated samples from each inoculated produce type (orange, celery, cucumber, or kale; fresh, unblemished or damaged, aged, or wilted) in each treatment (0 mg ClO_2/kg, 20 mg ClO_2/kg, 200 ppm hypochlorite, 200 ppm PAA, or water) were analyzed. The selected celery stalks were cut down to 16 cm lengths (uninoculated material removed), and kale leaf petioles were cut from selected leaves before analysis.

Each selected produce sample was placed into a 400 ml blender bag (Fisher Scientific, Hampton, NH) with 20 ml of Dey-Engley neutralizing (DE) broth (Becton, Dickinson and Company, Franklin Lakes, NJ). The bag was shaken (30 s), manually massaged (30s), and shaken again (30 s). Serial dilutions of rinses were made in 0.1% PW and spiral plated (Autoplate 4000, Spiral Biotech, Norwood, MA) onto XLDN, SMACR, and OXA for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. One negative control for each produce type (uninoculated, untreated) was sampled by rinsing and plating by the same approach to check for possible background microflora. XLDN and SMACR plates were incubated for 24 h at 37°C before *Salmonella* and *E. coli* O157:H7 colonies were enumerated. OXA plates were incubated for 48 h at 37°C before *L. monocytogenes* colonies were enumerated. Five to ten presumptive-positive *Salmonella* colonies from the XLDN plates were randomly selected for confirmation using lysine iron agar (LIA; Becton, Dickinson and Company, Franklin Lakes, NJ), triple sugar iron agar (TSI; Becton, Dickinson and Company, Franklin Lakes, NJ), and *Salmonella* latex test (Microgen Bioproducts,

Camberley, UK). Five to ten presumptive-positive *E. coli* O157:H7 colonies from the SMACR plates were randomly selected for confirmation using a Dryspot *E. coli* O157 kit (Oxoid, Basingstoke, UK). Five to ten presumptive-positive *L. monocytogenes* colonies from the OXA plates were randomly selected for confirmation using the Reveal 2.0 *Listeria* test kit (Neogen Corporation, Lansing, MI). A *Listeria* diagnostic kit (MicroID, Remel, Lenexa, KS) was also used throughout the experiment to randomly test presumptive-positive *L. monocytogenes* colonies for further confirmation.

To ensure the detection of pathogens in the event levels were below the countable range, an enrichment step was performed. Each DE broth rinse was enriched by adding 200 ml of universal pre-enrichment broth (UPB; Becton, Dickinson and Company, Franklin Lakes, NJ) before incubating at 37°C for 24 h. If direct plating did not yield colonies, the enriched samples were streaked by a loop (ca. 10 µl) for isolation onto XLDN, SMACR, and OXA. XLDN and SMACR plates were incubated at 37°C for 24 h, and OXA plates were incubated at 37°C for 48 h before examination. Presumptive-positive colonies were confirmed by the above-mentioned confirmation tests.

Statistical analysis

All experiments were replicated three times with duplicate produce samples of each type sampled within each replication. Log_{10} reductions (CFU/ml of DE broth rinse) were calculated as the response variable with initial counts represented from analysis of the inoculated, untreated samples. Values that were below the detection limit (CFU/ml of rinsate = 400) and positive from

corresponding enrichment were set to 300 CFU/ml of rinsate (log_{10} value of 2.5). Values below the detection limit and negative from corresponding enrichment were set to 100 CFU/ml of rinsate (log_{10} value of 2.0). R software (www.r-project.org) was used to fit a random effect model with interactions with six factors (produce type, microorganism, treatment, condition, type:treatment interaction, type:condition interaction) as fixed effects and replication as the random effect. Wilted kale inoculated with *Salmonella* and in the no gas control was used as the baseline. Analysis of variance and Tukey's honest significant difference test were also performed on the variables individually to determine differences within produce types, injury status, microorganism, and treatments. The level of significance was set at $p = 0.05$.

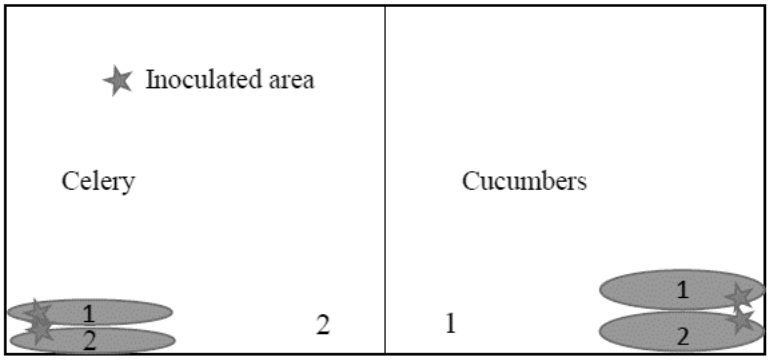
(A) (B)

(C) (D)

Figure 1: (A) Fresh, unblemished celery (top) stalk beside a celery stalk damaged (bottom) by scraping with the tip of the food peeler by six swift motions in a row in stalk grooves. (B) Fresh, unblemished orange (left) beside an aged orange (right) through storage at room temperature (22 ± 2°C) for 10 days. (C) Fresh, unblemished cucumber (right) beside cucumber damaged (left) by using the tip of the food peeler to puncture the skin at 0.5 cm and at a 45° angle two times approximately 2 cm apart. (D) Fresh, unblemished kale leaf (right) beside wilted kale leaf (left) through storage at room temperature (22 ± 2°C) for 2 days.

Top View

Side View 1



Side View 2

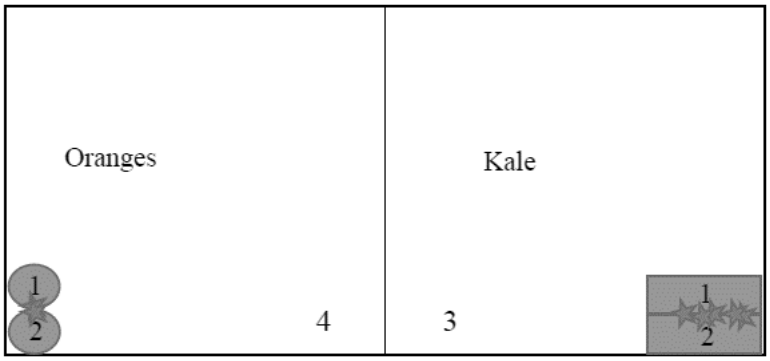


Figure 2: Positions of the produce in the treatment basket or container in an example treatment scheme for both liquid and gas experiments.

(A) (B)

Figure 3: (A) Metal baskets filled with produce used for dipping in liquid treatments using scheme represented in Figure 2. (B) Gas treatment container filled with produce using scheme represented in Figure 2.

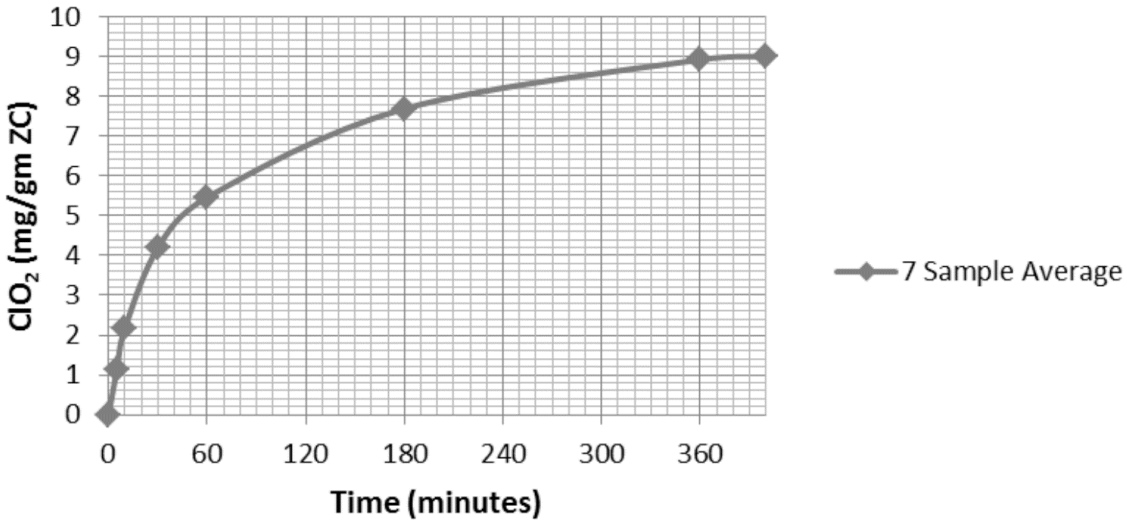


Figure 4: The average release profile of Part A precursor of the ICA Trinova, LLC chlorine dioxide dry chemical system. The average ClO_2 release is reported in total milligrams per gram of Part A, sodium chlorite impregnated precursor. Release curve was obtained from the manufacturer.

CHAPTER 4

RESULTS

The relative humidity in gas and no gas treatments was monitored and increased during the treatment. Relative humidity increased over the treatment time from a mean of 57 to 89% for all replications. For all pathogens, produce types, and conditions grouped together by treatment, ClO_2 gas treatment was found to be the most effective (Table 3). Hypochlorite and PAA treatments were not significantly different from each other, but both were found to be more effective than the water control. The overall treatment effectiveness ordering of ClO_2 gas > (hypochlorite, PAA) > water > no gas > baseline (initial population) was found to be statistically valid. However, there were individual instances in which this ordering is not true. For example, the gas treatment was only marginally better than PAA for celery. Therefore, produce type of each injury status was individually subjected to ANOVA to determine sanitizer effectiveness within each microorganism (Tables 4-6).

The largest average log reduction of 3.70 log CFU/ml of rinsate was obtained from *E. coli* O157:H7 inoculated on wilted kale treated with ClO_2 gas (Table 5). Though in most instances, reduction of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* levels due to ClO_2 gas did not differ significantly from that observed for PAA and hypochlorite treatments. Fresh, unblemished and damaged celery with *Salmonella*, fresh, unblemished and damaged cucumber with

Salmonella, fresh, unblemished and aged orange with *Salmonella*, fresh, unblemished celery with *E. coli* O157:H7, fresh, unblemished cucumber with *E. coli* O157:H7, fresh, unblemished and aged orange with *E. coli* O157:H7, and all instances with *L. monocytogenes* except for wilted kale showed no significant differences between ClO_2 gas, PAA, and hypochlorite (Tables 4-6). ClO_2 gas treatments showed significantly higher reductions than the other sanitizers in all instances with both fresh, unblemished and wilted kale except for *L. monocytogenes* on fresh, unblemished kale. In addition, damaged celery and cucumbers seemingly produced lower average mean log reductions for all three pathogens than the fresh, unblemished counterparts. Kale and oranges did not show this same trend.

Table 3: Mean reductions (log CFU/ml of rinsate) of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* inoculated on kale, oranges, celery, and cucumbers after treatment with chlorine dioxide gas, hypochlorite, peracetic acid, no gas control, and water control as compared to baseline (initial populations).

Treatment	Population Reduction (log_{10} CFU/ml of rinsate)[a]
No Gas Control	[D] 0.57 ± 0.43
ClO_2 Gas	[A] 2.66 ± 0.72
Water Control	[C] 0.99 ± 0.58
Hypochlorite	[B] 1.89 ± 0.88
Peracetic Acid	[B] 1.96 ± 0.94

[a]Mean values not preceded by the same letter are significantly different ($p < 0.05$).

Table 4: Mean reductions (log CFU/ml rinsate) of *Salmonella* inoculated on kale, oranges, celery, and cucumbers after treatment with chlorine dioxide gas, hypochlorite, peracetic acid, no gas control, and water control as compared to baseline (initial populations).

	Population Reduction (log_{10} CFU/ml of rinsate)[a]									
	No Gas		ClO_2 Gas		Water		Hypochlorite		Peracetic Acid	
Kale										
Fresh, unblemished	B	0.57 ± 0.43	A	2.75 ± 1.08	B	0.64 ± 0.67	B	0.99 ± 0.52	B	1.17 ± 0.66
Wilted[b]	B	0.21 ± 0.52	A	2.74 ± 1.30	B	0.43 ± 0.41	B	0.59 ± 0.45	B	0.61 ± 0.51
Celery										
Fresh, unblemished	C	0.24 ± 0.27	A	3.43 ± 0.48	B	1.34 ± 0.35	A	2.63 ± 0.76	A	3.16 ± 0.38
Damaged[c]	B	-0.32 ± 0.20	A	0.96 ± 0.70	AB	0.16 ± 0.36	A	0.73 ± 0.69	A	0.79 ± 0.41
Cucumber										
Fresh, unblemished	B	1.09 ± 0.53	A	3.58 ± 0.21	B	1.40 ± 0.95	A	3.17 ± 0.26	A	2.78 ± 1.12
Damaged[d]	B	0.26 ± 0.42	A	1.35 ± 0.55	AB	0.36 ± 0.61	AB	0.63 ± 0.68	AB	0.60 ± 0.69
Orange										
Fresh, unblemished	BC	1.38 ± 0.27	A	3.08 ± 0.86	C	1.17 ± 0.42	ABC	1.89 ± 1.19	AB	2.74 ± 1.02
Aged[e]	B	1.02 ± 0.41	A	2.96 ± 0.90	B	1.29 ± 0.56	AB	2.10 ± 0.69	A	2.71 ± 1.30

[a]Within the same row, mean values (n = 6) not preceded by the same letter are significantly different ($p < 0.05$).
[b]Wilted kale defined as wilted, discolored leaves.
[c]Damaged celery defined as scraped stalks.
[d]Damaged cucumber defined as surface punctured.
[e]Aged orange define as aged with less firm texture.

Table 5: Mean reductions (log CFU/ml of rinsate) of *E. coli* O157:H7 inoculated on kale, oranges, celery, and cucumbers after treatment with chlorine dioxide gas, hypochlorite, peracetic acid, no gas control, and water control as compared to baseline (initial populations).

	Population Reduction (log_{10} CFU/ml of rinsate)[a]				
	No Gas	ClO_2 Gas	Water	Hypochlorite	Peracetic Acid
Kale					
Fresh, unblemished	B 0.33 ± 0.60	A 3.17 ± 0.61	B 0.56 ± 0.60	B 0.98 ± 0.52	B 1.10 ± 0.49
Wilted[b]	B 0.43 ± 0.51	A 3.70 ± 0.26	B 0.37 ± 0.39	B 0.66 ± 0.39	B 0.68 ± 0.38
Celery					
Fresh, unblemished	C -0.03 ± 0.61	AB 2.27 ± 1.03	B 1.41 ± 0.36	AB 2.56 ± 0.81	A 3.28 ± 0.33
Damaged[c]	C 0.11 ± 0.31	A 1.92 ± 0.74	BC 0.29 ± 0.23	B 0.92 ± 0.59	B 0.92 ± 0.24
Cucumber					
Fresh, unblemished	B 1.19 ± 0.85	A 3.49 ± 0.20	B 1.34 ± 0.54	A 3.05 ± 0.25	A 2.64 ± 0.98
Damaged[d]	B 0.36 ± 0.39	A 1.82 ± 0.64	B 0.56 ± 0.54	B 0.85 ± 0.53	B 0.87 ± 0.48
Orange					
Fresh, unblemished	B 1.23 ± 0.27	A 2.94 ± 0.70	B 1.39 ± 0.33	AB 2.04 ± 0.98	A 3.02 ± 0.68
Aged[e]	C 0.99 ± 0.39	A 3.03 ± 0.74	BC 1.29 ± 0.56	AB 2.27 ± 0.72	A 2.75 ± 1.05

[a]Within the same row, mean values (n = 6) not preceded by the same letter are significantly different ($p < 0.05$).
[b]Wilted kale defined as wilted, discolored leaves.
[c]Damaged celery defined as scraped stalks.
[d]Damaged cucumber defined as surface punctured.
[e]Aged orange define as aged with less firm texture.

Table 6: Mean reductions (log CFU/ml of rinsate) of *L. monocytogenes* inoculated on kale, oranges, celery, and cucumbers after treatment with chlorine dioxide gas, hypochlorite, peracetic acid, no gas control, and water control as compared to baseline (initial populations).

	Population Reduction (log_{10} CFU/ml of rinsate)[a]									
	No Gas		ClO_2 Gas		Water		Hypochlorite		Peracetic Acid	
Kale										
Fresh, unblemished	A	0.67 ± 0.55	A	2.23 ± 1.15	A	0.75 ± 0.90	A	2.08 ± 1.25	A	1.67 ± 0.63
Wilted[b]	B	0.30 ± 0.41	A	3.13 ± 0.25	B	0.38 ± 0.51	B	1.53 ± 1.46	B	1.37 ± 1.36
Celery										
Fresh, unblemished	C	0.72 ± 0.61	AB	2.36 ± 1.21	BC	1.51 ± 0.45	AB	2.39 ± 0.44	A	2.67 ± 0.00
Damaged[c]	C	0.18 ± 0.30	AB	2.11 ± 1.10	BC	0.53 ± 0.58	A	2.69 ± 1.63	ABC	1.83 ± 1.33
Cucumber										
Fresh, unblemished	C	0.79 ± 0.49	A	3.59 ± 0.20	BC	1.64 ± 1.00	A	3.09 ± 0.25	AB	2.46 ± 1.06
Damaged[d]	A	0.47 ± 0.40	A	2.09 ± 0.78	A	0.95 ± 0.82	A	2.14 ± 1.52	A	1.64 ± 1.50
Orange										
Fresh, unblemished	B	0.75 ± 0.28	A	2.50 ± 0.65	A	2.20 ± 0.59	A	2.71 ± 0.81	A	3.01 ± 0.55
Aged[e]	B	0.69 ± 0.43	A	2.57 ± 0.58	A	2.07 ± 0.73	A	2.61 ± 0.45	A	2.62 ± 0.85

[a]Within the same row, mean values (n = 6) not preceded by the same letter are significantly different ($p < 0.05$).
[b]Wilted kale defined as wilted, discolored leaves.
[c]Damaged celery defined as scraped stalks.
[d]Damaged cucumber defined as surface punctured.
[e]Aged orange define as aged with less firm texture.

CHAPTER 5

DISCUSSION

In this study, the ability of ClO_2 gas produced from a self-contained sachet to act as a produce sanitizer was explored by comparing its effectiveness to commonly used hypochlorite and peracetic acid. Self-contained sachets that produce ClO_2 gas were investigated due to the relative ease of use when compared to aqueous sanitizers. The concentration to be released from the sachet is directly related to the measured weight of the dry chemicals; whereas, determining and measuring liquid sanitizer concentration is much more laborious as pH measurements and water samples must be taken. The logistical ease of use is a clear advantage of ClO_2 gas. In terms of efficacy, when all variables (produce type, injury status, and microorganism) were pooled, ClO_2 gas produced significantly greater log reductions than all other treatments and controls with a mean reduction of 2.66 log CFU/ml of rinsate. Produce type, injury, and microorganism all affect the efficacy of sanitizers; thus, individual analysis showed varying degrees of decontamination ability for each treatment.

Juice producers have the option of using less attractive fruit and vegetables that may not sell directly to consumers. In some instances, produce used are damaged, aged or wilted in a manner that may increase the potential for pathogen harboring. It is important to determine the effectiveness of sanitizers on fresh and less attractive produce. An objective of this study was to determine if injury lessened decontamination ability of ClO_2 gas. Results differed by produce type due to the manner of injuring the produce.

Kale and orange injury in this study did not show great differences in average means of population reduction for any treatments; whereas, damaged cucumber and celery resisted decontamination during treatments especially when water dipping alone was tested. Kale and oranges were considered wilted or aged; therefore, the surface was not greatly altered. Kale was wilted and did not have physical abrasions. Aged oranges were less firm than fresh, unblemished counterparts, so the injury can be considered internal. These injury types did not seem to better protect surface inoculated pathogens from decontamination. The abrasion and puncture injuries of celery and cucumber altered the produce surface which appeared to affect the ability of pathogens to better survive treatments.

Salmonella, *E. coli* O157:H7 and *L. monocytogenes* levels were all reduced by similar levels on fresh, unblemished and damaged cucumber (3.0-3.6 and 0.6-1.4 log CFU/ml of rinsate, respectively), for the ClO2 and hypochlorite treatments. Han et al. (2001) found similar results between differing reductions of *L. monocytogenes* inoculated on fresh, unblemished and damaged (punctured) green peppers and treated with 3 mg/l ClO_2 gas *(56)*. In the Han et al. (2001) study, population reductions of 7.39 and 3.60 log CFU/5 g were achieved on fresh, unblemished and injured peppers, respectively, which suggests pathogen protection from ClO_2 gas in injured areas *(56)*. In the current study, a significantly greater reduction of *E. coli* O157:H7 populations was noted on damaged cucumber and celery when treated with ClO_2 gas as compared to all other treatments. Consequently, gas treatment may prove more effective in decontaminating damaged areas than immersion sanitizers though produce injury seemed to offer some protection. Han and others also explored the ability of both gaseous and

aqueous ClO_2 to eliminate *L. monocytogenes* from fresh, unblemished and damaged green pepper surfaces, and gaseous ClO_2 was found to be significantly better at reducing populations on both surfaces than the aqueous counterpart *(56)*.

The ability of gaseous ClO_2 to more effectively decontaminate produce may be due to the capability of gas to reach crevices more readily than liquids. Hydrophobicity of fruit and vegetable surfaces and microbial cell surfaces affect the ability of aqueous sanitizers to efficiently decontaminate *(20)*. The highly hydrophobic surface of kale leaves may explain the ability of ClO_2 gas to better decrease pathogen populations than the aqueous sanitizers in this study. Gaseous ClO_2 treatments yielded significantly greater log CFU/ml of rinsate reductions of all three pathogens in five out of the six instances for both fresh, unblemished and wilted kale; this ability may be attributed to the greater penetration ability of gas than liquids for hydrophobic surfaces. A study on attachment and reduction of *E. coli* O157:H7 from apple, avocado, orange, and cantaloupe surfaces found that surface roughness may be more important in affecting pathogen reductions than hydrophobicity *(144)*. Apple and avocado were found to have similar surface hydrophobicity, but residual *E. coli* O157:H7 populations after water, acidic electrolyzed water, and peracetic acid treatments were higher on the rougher avocado surface *(144)*.

Surface roughness may allow pathogen protection by the associated grooves or cavities *(144)*. Wang and others found that a linear relationship existed between surface roughness and increased pathogen protection *(144)*. The current study demonstrated similar results in that *Salmonella, E. coli* O157:H7, and *L. monocytogenes* were reduced by greater numbers on the smoother fresh, unblemished cucumbers than fresh, unblemished oranges for hypochlorite treatments. Similarly, ClO_2 gas seemingly did not

completely overcome surface roughness as reductions for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* for the smoother fresh, unblemished cucumber were generally higher than those for fresh, unblemished oranges. Overcoming this effect on rougher produce surfaces may be possible through increasing gas concentrations and exposure times since these generally increase efficacy..

ClO_2 gas concentration and exposure time has been found to play a significant role in sanitization ability. The current study used 20 mg of ClO_2 per kg of produce (or 0.32 mg/l) for treatments. This concentration was chosen as it is comparable to previous studies, though it could be considered a low concentration. Several studies have noted that increasing concentration and exposure time decreases surviving pathogen populations *(42, 58, 81, 82, 114)*. On green peppers, 30 min ClO_2 treatments of 0.62 mg/l and 1.24 mg/l decreased *E. coli* O157:H7 by 3.03 and 6.45 log CFU/sample, respectively *(58)*. *Salmonella* has been found to be reduced by 1.9 and 2.8 log CFU/5 cm^2 with 0.5 mg/l and 5 mg/l ClO_2 gas treatments for 10 min, respectively *(82)*. It may be beneficial to further explore greater concentrations of ClO_2 to determine if greater reductions are possible than those achieved in this study. In addition, the ability to increase humidity during treatment may increase the efficacy of gaseous ClO_2 efficacy as 90% relative humidity has been found to produce greater pathogen reductions with ClO_2 gas treatment than 50 and 70% RH *(102)*.

Overall, the current study found pathogen reductions with all treatments that are similar to those found in literature. Hypochlorite reduced pathogen populations by 0.63 to 3.17 log CFU/ml of rinsate with an overall mean of 1.89 log CFU/ml of rinsate. Parish and others report that 1-2 log reductions are commonly associated with hypochlorite

treatment, and the type of produce and pathogen can affect population reduction *(98)*. Mean pathogen reductions in the current study from PAA treatment ranged from 0.60 to 3.28 log CFU/ml of rinsate with an overall average of 1.96 log CFU/ml of rinsate. Similarly, *Salmonella* and *E. coli* O157:H7 have been reported to be reduced by 2.6-3.8 log CFU/g on cantaloupe and honeydew melons by 40-80 ppm PAA *(101)*. Dipping in water alone produced an overall pathogen reduction of 0.99 log CFU/ml of rinsate. A study on sanitizer effectiveness found comparable reductions with 10 min of contact time with deionized water on lettuce and baby carrots as populations were reduced by 0.93 and 1.15 log CFU/g, respectively *(114)*. Gaseous ClO_2 treatment in the current study did not produce the ≥5 log reductions that are found in the literature. This may be due to the practical nature of the present study as produce types were mixed, and the treatment container was filled as it would be in actual vendor use.

CHAPTER 6

CONCLUSION

Gaseous ClO_2 was found to be significantly better at produce decontamination than immersion in hypochlorite and peracetic acid in some instances. The ClO_2 gas treatment did not produce a 5 log CFU reduction that desired in the commercial juice industry. The self-contained sachets are easy to use and reduce water waste associated with aqueous sanitizers. The produce also does not need to be immediately removed from the treatment container as the sachets are self-limiting and will stop producing ClO_2 after a specified length of time. Overnight treatments are possible without constant monitoring, and produce that are water sensitive such as berries can be treated without requiring immediate use. Though contamination prevention is the best method in maintaining safe food, this study shows that a low concentration of ClO_2 gas can reduce pathogens in a mixed produce treatment by up to 3.70 log CFU/ml of rinsate.

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APPENDIX A

BACTERIAL GROWTH CURVES

Growth rates of wild-type and antibiotic resistant serotypes and strains were determined to ensure the adapted serotypes and strains behaved similarly to the wild-type parents. Each wild-type pathogen serotype or strain was individually subjected to two consecutive transfers in 10 ml TSB which were incubated at 37°C for 16 h. *Salmonella* serotypes were adapted to 50 μg/ml nalidixic acid, and *E. coli* O157:H7 strains were adapted to 100 μg/ml rifampicin. *Salmonella* and *E. coli* O157:H7 mutants were individually grown at 37°C for 16 h twice in TSBN and TSBR, respectively. A transfer of 0.25 ml of each of the second growth cultures was done into 25 ml of the appropriate broth. Each of these cultures were sampled at 0 h and 8 h then every 2 hours until the culture entered the stationary phase. To sample, 1 ml of each culture was introduced into a 1.5 ml disposable polystyrene cuvette (Fisher Scientific, Hampton, NH) then placed inside spectrophotometer (Model DU 530, Beckman, Brea, CA) set to read absorbance at 600 nm. A graph for each serotype and strain was prepared by plotting the measured optical density (OD) of the culture versus time. The optical density (OD) value was used to represent the relative bacterial concentration in the sample (Figures 5-14).

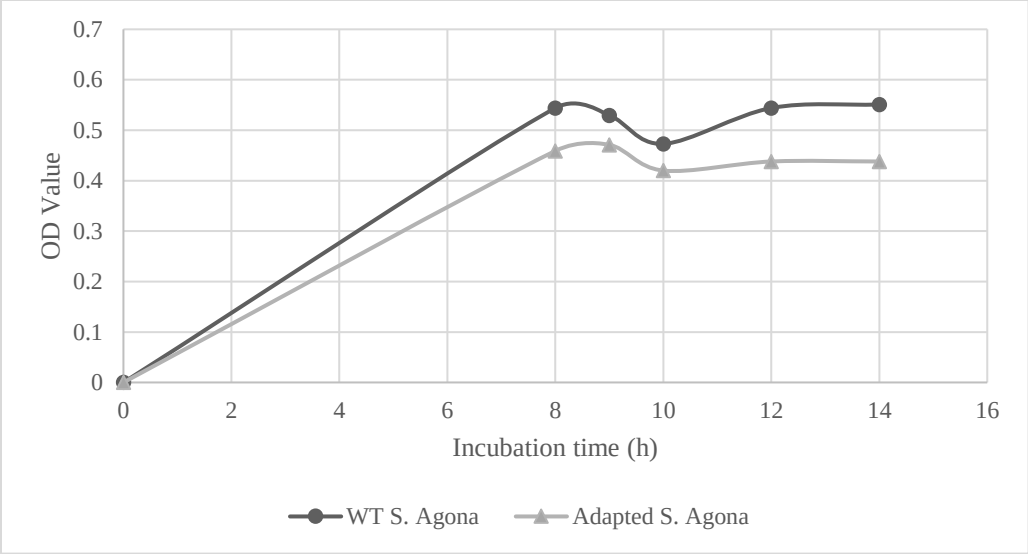

Figure 5: Growth of wild-type (●) and nalidixic acid adapted (▲) *Salmonella* Agona in TSB and TSBN, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

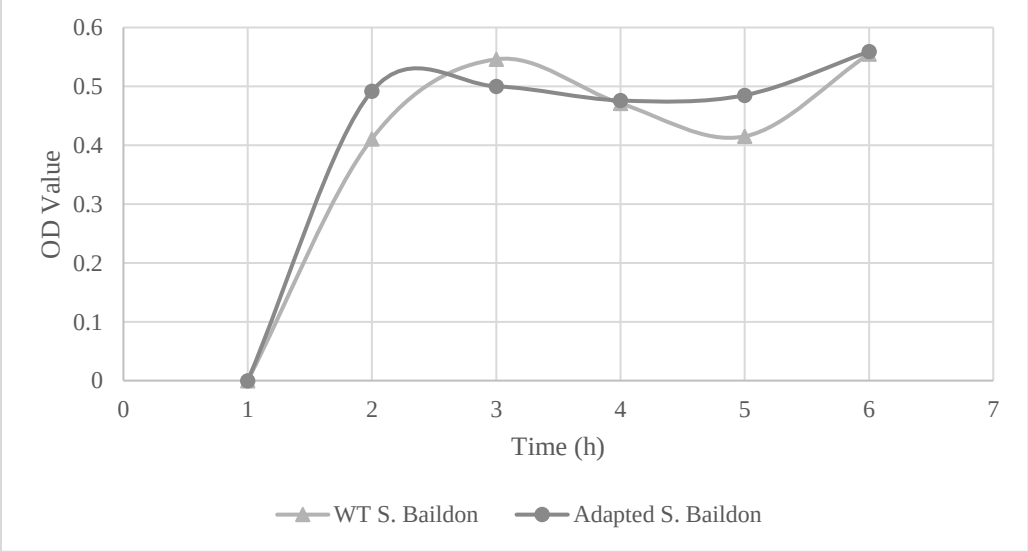

Figure 6: Growth of wild-type (●) and nalidixic acid adapted (▲) *Salmonella* Baildon in TSB and TSBN, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

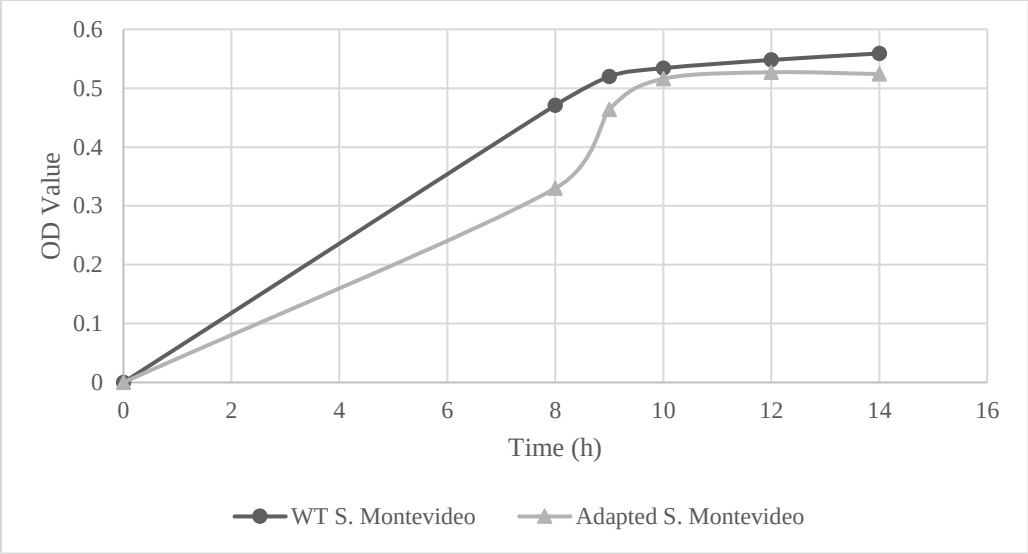

Figure 7: Growth of wild-type (●) and nalidixic acid adapted (▲) *Salmonella* Montevideo in TSB and TSBN, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

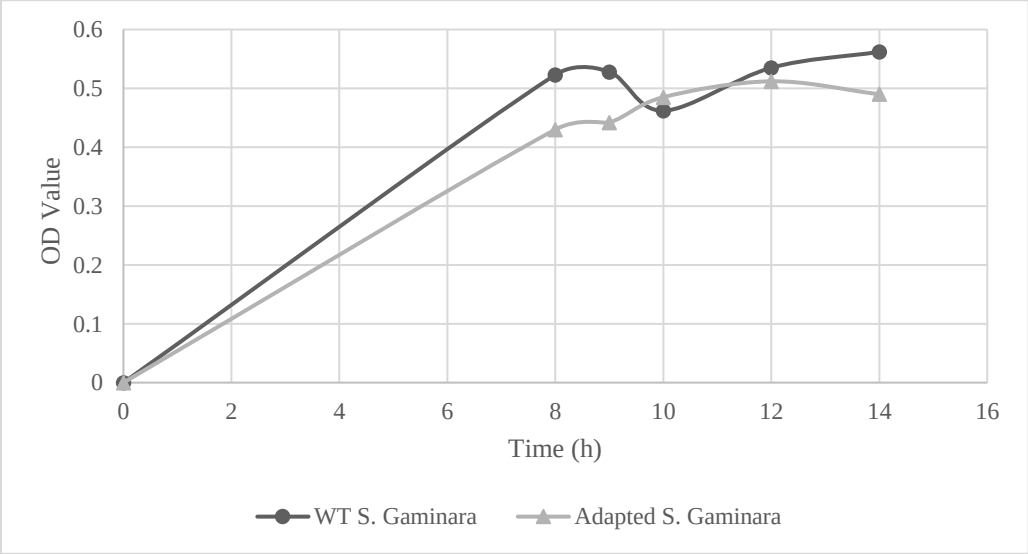

Figure 8: Growth of wild-type (●) and nalidixic acid adapted (▲) *Salmonella* Gaminara in TSB and TSBN, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

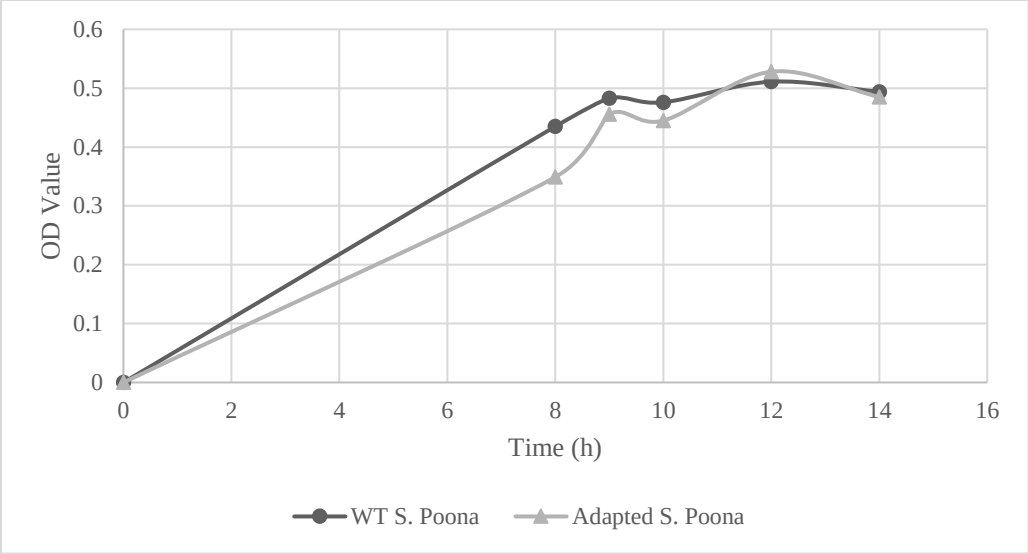

Figure 9: Growth of wild-type (●) and nalidixic acid adapted (▲) *Salmonella* Poona in TSB and TSBN, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

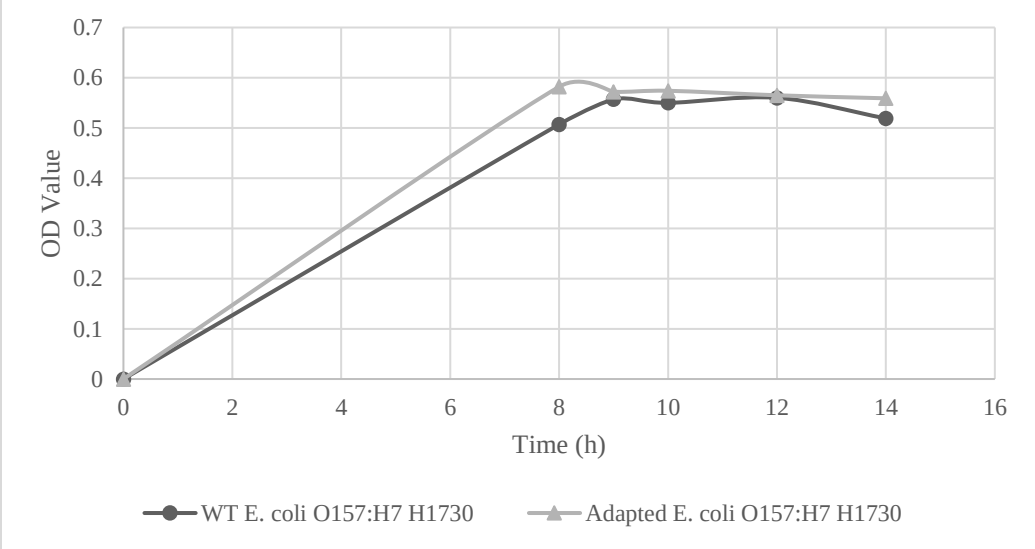

Figure 10: Growth of wild-type (●) and rifampicin adapted (▲) *E. coli* O157:H7 H1730 in TSB and TSBR, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

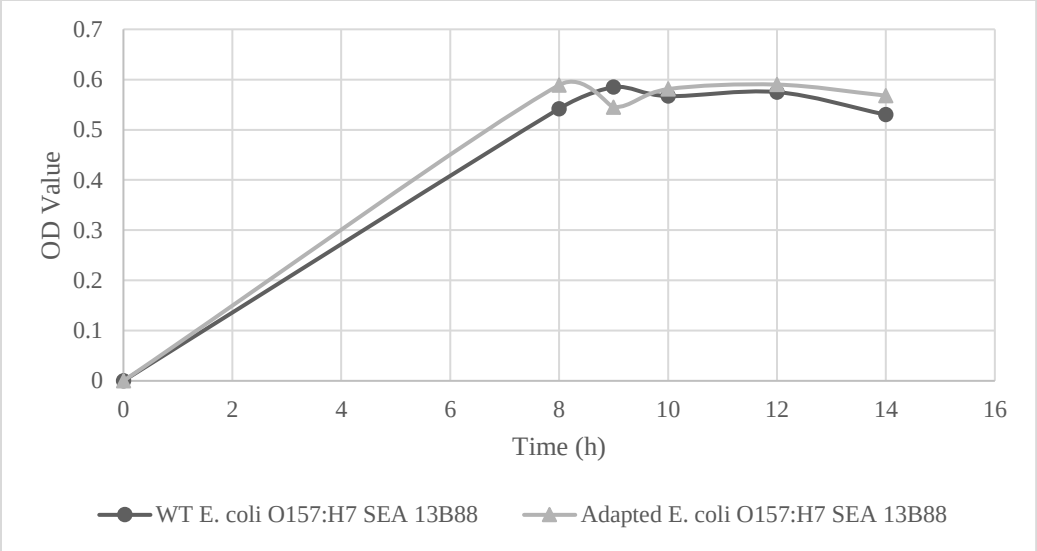

Figure 11: Growth of wild-type (●) and rifampicin adapted (▲) *E. coli* O157:H7 SEA 13B88 in TSB and TSBR, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

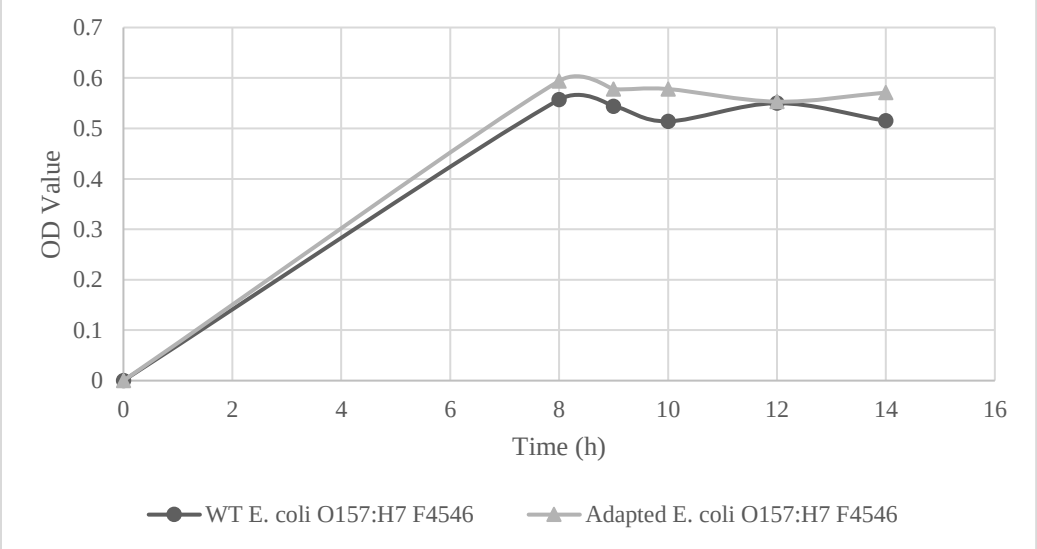

Figure 12: Growth of wild-type (●) and rifampicin adapted (▲) *E. coli* O157:H7 F4546 in TSB and TSBR, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

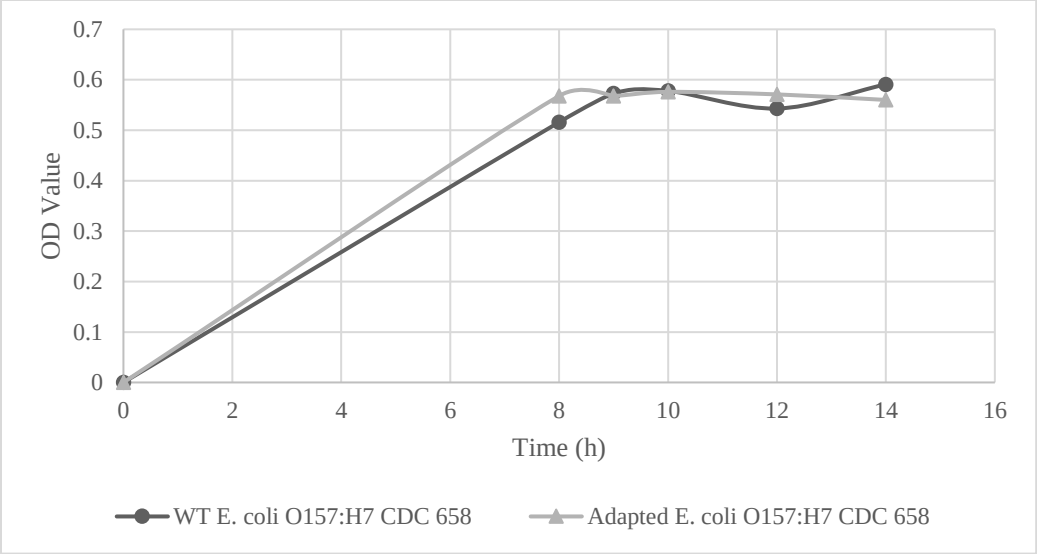

Figure 13: Growth of wild-type (●) and rifampicin adapted (▲) *E. coli* O157:H7 CDC 658 in TSB and TSBR, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

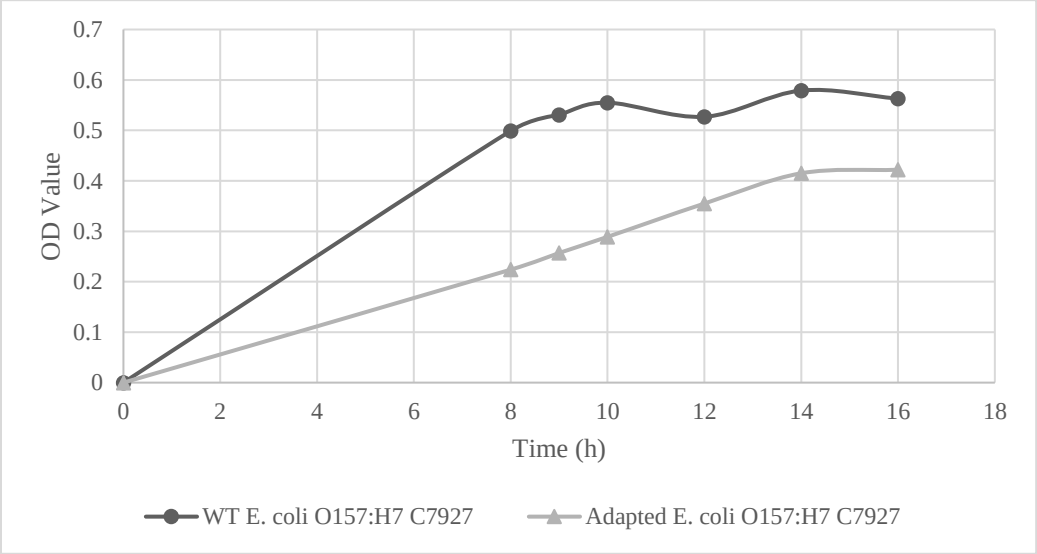

Figure 14: Growth of wild-type (●) and rifampicin adapted (▲) *E. coli* O157:H7 C7927 in TSB and TSBR, respectively, at 37°C as represented by optical density measured from absorbance readings at 600 nm.

APPENDIX B

CROSS-PATHOGEN AND CROSS-STRAIN INHIBITION RESULTS

Due to the mixed pathogen inoculum, each serotype and strain was tested for its ability to inhibit growth of all other serotypes and strains. Each was cultured in 10 ml of the appropriate broth (TSBN, TSBR, or TSB for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively) at 37°C for 24 h. Cross streaks of each serotype and strain were done on TSA then plates were incubated at 37°C for 24 h. Each strain and serotype cross streak junction was examined for inhibition by other strains or serotypes of each pathogen.

Cross-pathogen inhibition was tested by ensuring each pathogen could be recovered from mixed pathogen inoculated test produce. The 15 serotype/strain inoculum was prepared by the method in inoculum preparation section. Two samples of each type of produce (oranges, kale, celery, and cucumbers; fresh, unblemished and aged, wilted, or damaged) were inoculated with 100 µl creating 10-15 spots of approximately equal volume. Recovery of the inoculum was performed according to the microbiological analysis section after the produce was dried for 16 h in a biosafety cabinet. Recovery was performed twice, and numbers were averaged.

Cross-pathogen inhibition during enrichment was also tested. Approximately 10-99 cells of each of the 15 serotypes and strains were inoculated into 200 ml of UPB then incubated at 37°C for 24 h. Streaks of the broth were then performed on XLDN,

SMACR, and OXA. Plates were incubated at 37°C; XLDN and SMACR plates were examined after 24 h while OXA plates were examined after 48 h.

L. monocytogenes strains showed weak, transparent growth on the plates which may mean inhibition from cross-streaks (Figure 15). However, pure cultures of *L. monocytogenes* on TSA grow similarly in that colonies are translucent. Recovery numbers from the produce did not show concern for possible inhibition as *L. monocytogenes* could be recovered in numbers similar to the others (Table 7). In addition, all three pathogens were confirmed from the incubated enrichment broth when inoculated in low numbers.

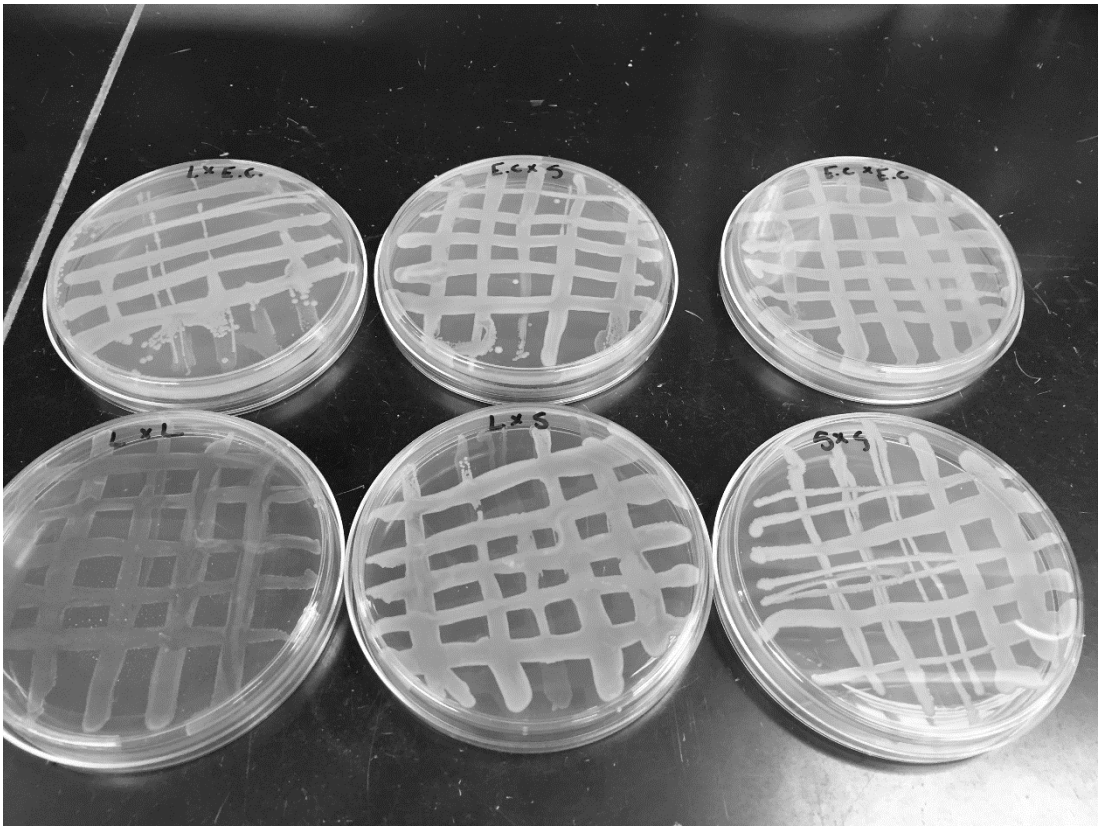

Figure 15: Cross-pathogen and cross-strain streaks of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on tryptic soy agar at 37°C for 24 h. “L X E.C.” represents *L. monocytogenes* crossed with *E. coli* O157:H7. “E.C. X S” represents *E. coli* O157:H7 crossed with *Salmonella*. “E.C. X E.C.” represents *E. coli* O157:H7 crossed with *E. coli* O157:H7. “L X L” represents *L. monocytogenes* crossed with *L. monocytogenes.* “L X S” represents *L. monocytogenes* crossed with *Salmonella.* “S X S” represents *Salmonella* crossed with *Salmonella.*

Table 7: Average recovery of pathogens from cucumber, kale, celery, and orange in CFU/ml of rinsate.

	Salmonella (CFU/ml of rinsate)	*E. coli* O157:H7 (CFU/ml of rinsate)	*L. monocytogenes* (CFU/ml of rinsate)
Cucumber			
Fresh, unblemished	3.90×10^5	1.03×10^6	1.25×10^6
Damaged	1.05×10^6	2.19×10^6	1.49×10^6
Kale			
Fresh, unblemished	9.28×10^5	1.84×10^6	7.08×10^5
Wilted	7.08×10^5	1.46×10^6	5.93×10^5
Celery			
Fresh, unblemished	2.34×10^5	3.83×10^5	3.44×10^5
Damaged	7.03×10^5	2.09×10^6	1.23×10^6
Orange			
Fresh, unblemished	1.86×10^5	3.18×10^5	1.94×10^5
Aged	2.55×10^5	3.65×10^5	1.78×10^5

APPENDIX C

CHLORINE DIOXIDE CONSUMPTION CURVES

Each type of produce and injury status was subjected to 10 mg/kg chlorine dioxide gas treatment, and the consumption curves were obtained through recording the gas concentration over time. The gas concentration in real-time was monitored through a chamber designed and set-up by ICA Trinova, LLC (Newnan, GA). The headspace of the chamber was sampled for ClO_2 gas through a sensor that was connected to a ppmv readout platform. Consumption curves were developed to determine the relative consumption ability of each produce type.

Injury did not seem to greatly affect the consumption speed of produce type though variations were apparent (Figures 16-19). Kale was found to be the fastest ClO_2 consumer (Figure 18) though celery also consumed ClO_2 gas quickly. Oranges were the slowest ClO_2 consumer (Figure 17).

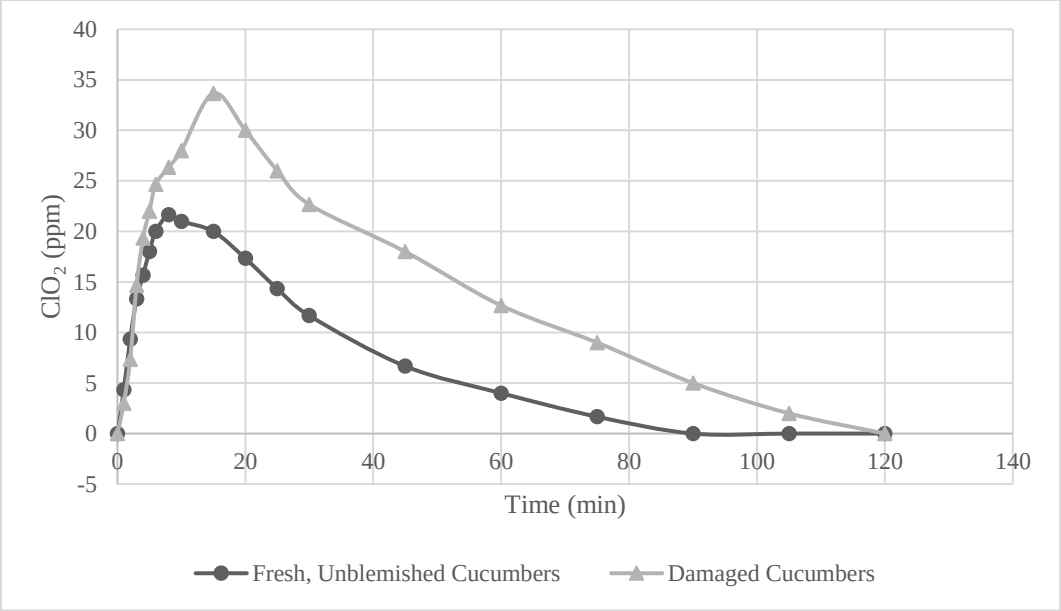

Figure 16: Consumption of ClO_2 (ppm) over time (min) for fresh, unblemished and damaged cucumbers. Data obtained from average of three runs.

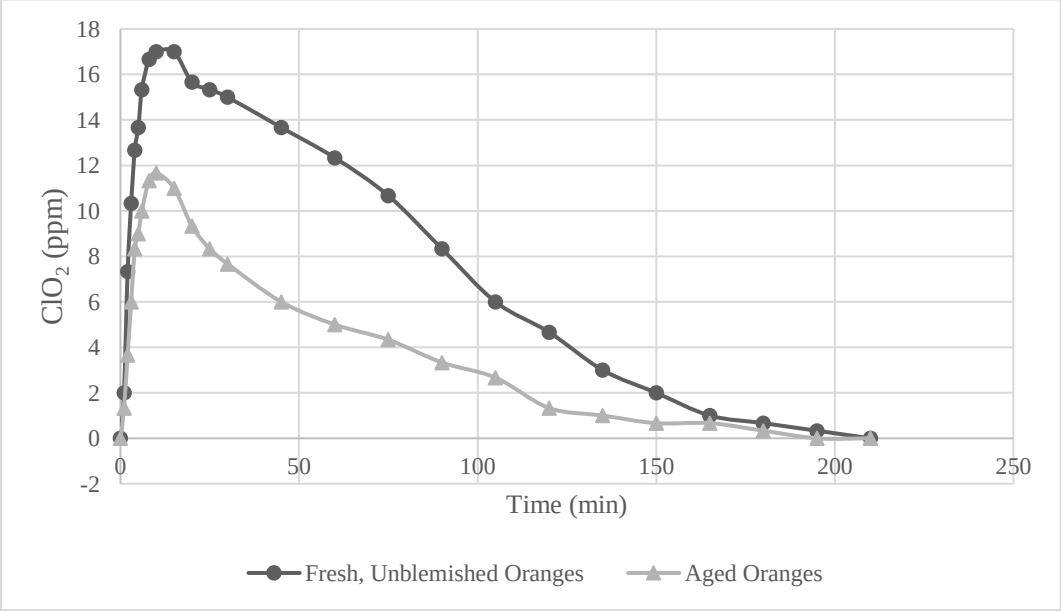

Figure 17: Consumption of ClO_2 (ppm) over time (min) for fresh, unblemished and aged oranges. Data obtained from average of three runs.

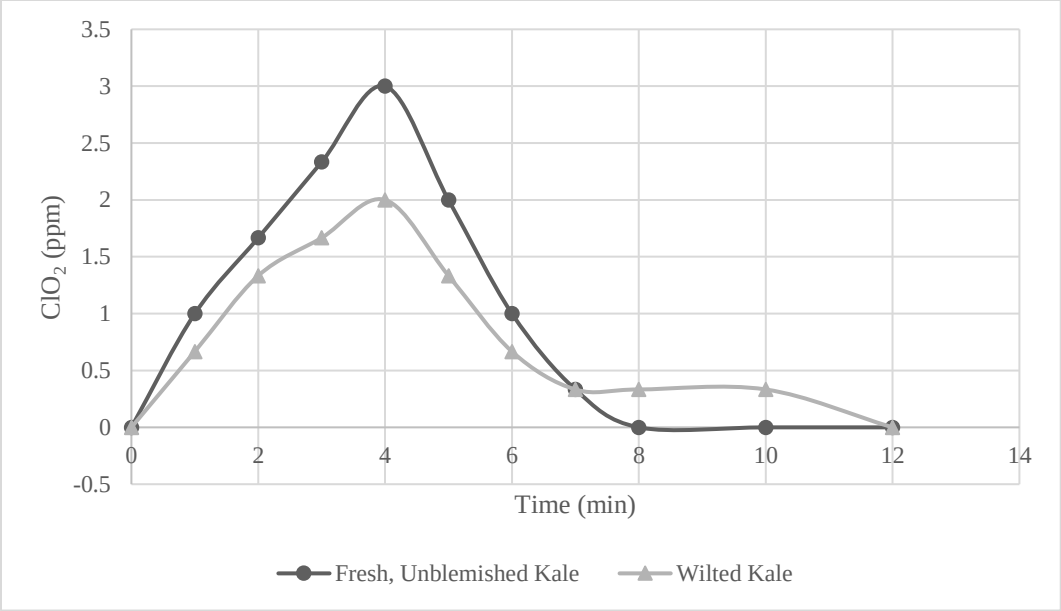

Figure 18: Consumption of ClO_2 (ppm) over time (min) for fresh, unblemished and wilted kale leaves. Data obtained from average of three runs.

Figure 19: Consumption of ClO_2 (ppm) over time (min) for fresh, unblemished and damaged celery. Data obtained from average of three runs.

APPENDIX D

CHLORINE DIOXIDE DISTRIBUTION RESULTS

To ensure that ClO_2 gas reached the corners of the treatment container by the two fans, a simple cucumber bleaching experiment was done. ClO_2 in high concentrations is known to bleach surfaces, and during preliminary testing damaged areas of the cucumbers were found to be highly susceptible to bleaching. A treatment of 100 mg ClO_2/kg cucumbers for 30 min was used on 28 damaged cucumbers. The cucumbers were damaged by using the tip of the food peeler to puncture the skin at 0.5 cm and at a 45° angle two times approximately 2 cm apart on top and bottom of the same end. Damaged areas were placed along the sides of the container (Figure 20).

The cucumbers in the bottom corners of the container were particularly concerning as these were geometrically farthest from the gas generating sachet. Figure 21 shows that punctured areas were subjected to bleaching; therefore, gas was being circulated in the container to reach these areas.

Figure 20: Gas treatment container filled with top and bottom surface damaged cucumbers for treatment with 100 mg ClO_2 gas/kg cucumber.

Figure 21: Cucumber damaged areas bleached after 100 mg ClO_2/kg cucumber treatment.

Vertical cucumber was in direct contact with the bottom cucumber during treatment.