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Optimization of Peracetic Acid as an Antimicrobial Agent in Postharvest Processing Wash Water

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**Optimization of peracetic acid as an antimicrobial agent in
postharvest processing wash water**

A Thesis Presented

By

TIAH GHOSTLAW

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

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Department of Food Science

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postharvest processing wash water**

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ABSTRACT

OPTIMIZATION OF PERACETIC ACID AS AN ANTIMICROBIAL AGENT IN POSTHARVEST PROCESSING WASH WATER

SEPTEMBER 2018

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Directed by: Professor Amanda Kinchla

The Food Safety Modernization Act (FSMA) has a regulation stating that there can be no detectable generic *Escherichia coli* in agricultural water. The objective of this study was to optimize the use of peracetic acid (PAA) as a mitigating strategy in postharvest processing. This project began by investigating the impact of storage temperatures on stability of a commercially available PAA sanitizer. Regression analysis showed a quadratic relationship in the concentration of PAA over 12 weeks when storing the product at 4, 20 and 37°C. Stability of the product was also investigated in the presence of organic load over time at 60ppm PAA. PAA significantly decreased at an organic load level of 750COD. From this, the impact of various ratios of hydrogen peroxide (H₂O₂) and PAA were investigated further to see the efficacy against *E. coli* O157:H7 over three hours with reinoculation of bacteria after 3 hours. A higher level of PAA in the solution had a greater inactivation efficacy against *E. coli* O157:H7. The impact of concentration, organic load, temperature and pH on the efficacy against *E. coli* O157:H7 and stability of PAA over four hours was then investigated. All of the

conditions tested showed a significant decrease in H₂O₂ and PAA concentrations over time, except at concentration of 80ppm, with varying rates of depletion under each condition tested. This study will help to create guidance on optimizing the use of PAA. With this knowledge growers can understand how changes in their post-harvest processing water can affect sanitizer performance.

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LIST OF ABBREVIATIONS

COD- Chemical Oxygen Demand

FDA- United States Food and Drug Administration

FSMA- Food Safety Modernization Act

H₂O₂- Hydrogen peroxide

HUS- Hemolytic-Uremic Syndrome

PAA- Peracetic acid

PSR- Produce Safety Rule

CHAPTER 1

INTRODUCTION

Each year in the United States 48 million people are estimated to become ill due to foodborne diseases, with an occurrence of 3,000 deaths (CDC, 2016). Water is a known vector for pathogens to transfer to produce. The 2011 Jensen Farms cantaloupe outbreak, the 2006 Dole spinach outbreak, and the 2018 romaine lettuce outbreak were all associated to contaminated water that was not disinfected properly (Falkenstein, 2011; Marler, 2011; FDA, 2018). As of 2011, the United States Food and Drug Administration (FDA) has implemented the Food Safety Modernization Act (FSMA), with most compliance beginning between 2018 and 2023, updating the food safety regulations in the United States (FDA, 2018). Within FSMA, there is a rule called the Produce Safety Rule (PSR), which creates laws for food safety on farm. Within the PSR, there is a regulation stating that there can be no detectable generic *E. coli* in any agricultural wash water. This includes water that is used for irrigation, worker hygiene, and postharvest processing. Although the rule went into effect in 2016, there are still many growers that are not yet in compliance with the regulation, much of this is due to a lack of understanding of mitigation strategies.

Wash water is a primary means for cross contamination among produce on farms, as it creates a harborage for debris, dirt and bacteria as produce is passed through it (Joshi, Mahendron, Alagusundaram, Norton, and Tiwari, 2013; Gil, Selma, Lopez-Galvez, and Allende, 2009). One of the common mitigation strategies for treating water on farm is the use of sanitizers (antimicrobial agents) in processing water to combat bacteria that can become present. Although many people commonly use chlorine-based sanitizers on farm, there has been a push for alternative sanitizers, as chlorine has been seen to create harmful disinfection by-products in

the presence of organic matter that have negative effects on environmental and human health (Joshi, Mahendran, Alagusundaram , Norton and Tiwari, 2013). Peracetic acid (PAA) based sanitizers are a common alternative.

PAA based sanitizers are not known to create dangerous byproducts, are less impacted by organic load presence in wash water and are effective over a wide range of pH values (Ölmez and Kretschmar, 2009; Fatica and Schneider, 2009). Compared to chlorine which creates harmful by-products, is more impacted by the presence of organic matter, and has limited pH range (6.0-7.5) with which it is effective (Joshi et al., 2013; Suslow, 2001). PAA is an effective sanitizer for a large range of microorganisms including bacteria, protozoan cysts, spores, and viruses (Kitis, 2004; Lopez-Galvez, 2013). The goal of this project was to study PAA using on farm application in order to better support their use for postharvest processing.

Objectives:

1. Perform a shelf life study on a commercially available PAA-based produce sanitizer under multiple storage temperatures and understand the impact that organic load plays on a commercially available sanitizer over time
2. Investigate the impact that various H₂O₂ and PAA ratios can have on one another in terms of stability and efficacy against *E. coli O157:H7*
3. Understand how various concentrations, organic loads, temperatures, and pH values impact stability and efficacy against *E. coli O157:H7* of a H₂O₂/PAA sanitizer.

CHAPTER 2

PERACETIC ACID ANTIMICROBIAL WASHES AS A CHEMICAL SANITIZER TO REDUCE MICROBIAL LEVELS IN POST HARVEST PROCESSING

2.1 Implementation of FSMA

The Food Safety Modernization Act (FSMA) was passed in 2011 and compliance began in 2016. FSMA was put into place to revolutionize food safety and create an equal playing field of food safety across the market. As of today, many farms are still challenged in becoming compliant with FSMA and are looking for guidance and technical support.

Within FSMA there is the Produce Safety Rule (PSR) which has a regulation specifically stating that there can be “no detectable generic *E. coli*” allowed in any agricultural water with which it is reasonable that if dangerous microbes were present it could lead to the contamination of produce (FDA, 2018). Postharvest processing water, water for hand washing, water on food contact surfaces and irrigation water are examples of agriculture water that fall within this regulation. If water that falls under this classification is contaminated, it is required that the water immediately be discontinued for use, and that a corrective action is taken before the water is used again (FDA, 2018). This review is going to be looking primarily at PAA as a sanitizing option to comply with agriculture wash water regulations. The use of sanitizer in postharvest processing water can help to maintain adequate water quality during processing by reducing the microbial loads in the recycled water systems which thereby help to reduce cross contamination among produce.

2.2 Food safety incidences and cross contamination

Water is a known source of contamination in agriculture, acting as a vector for cross contamination among produce because it allows for a harborage of microbes. As produce is

passed through contaminated water, microbes can adhere to uncontaminated produce, allowing for many pieces of produce to become contaminated quickly, leading to foodborne illness. It is estimated each year in the United States that 48 million people get sick due to foodborne illnesses; leading to 128,000 hospitalizations and 3,000 deaths (CDC, 2016). Fresh produce is the fourth leading carrier of foodborne illness in the United states, of which, leafy greens is one of the most common foods associated with pathogenic *Escherichia coli* specifically (CDC, 2006).

In 2006, one of the most significant outbreaks of pathogenic *E. coli* was due to contaminated spinach. This outbreak spanned 26 states, consisting of 102 hospitalizations, 31 incidences of kidney failure, and three confirmed deaths (CDC, 2006). Investigation into this outbreak found positive results for the same strain of *E. coli* O157:H7 in a water source that was being used on the farm, as on the contaminated spinach (Falkenstein, 2011). In 2011, an outbreak of *Listeria monocytogenes* from contaminated cantaloupes caused 147 people to become infected over 28 states (CDC, 2012). This outbreak lead to 145 hospitalizations, 33 deaths, one miscarriage, and three newborns who contracted the illness (CDC, 2012). This outbreak was linked back to a lack of chlorinating the water that the cantaloupe was being processed in, leading to cross contamination among many produce (Marler, 2011). In 2018, a romaine lettuce outbreak of *E. coli* O157:H7 spanned 36 states with over 200 people becoming ill, including 96 hospitalizations, 27 people developing hemolytic-uremic syndrome HUS, and five deaths (CDC, 2018). This 2018 romaine lettuce outbreak was traced back to contaminated canal water that was being used as an irrigation source on the produce (CDC, 2018).

Produce can become contaminated with a variety of bacteria, including pathogens, at various stages during development and from various sources including, but not limited to,

manure, grower handling, and cross contamination in postharvest processing water (Barrera, Blenkinsop and Warriner, 2011). The level of microbial contamination on produce is affected by multiple factors, including produce type, origin, seasonality of the harvest, and improper postharvest handling (Ailes et al., 2007). If produce becomes contaminated prior to postharvest washing, it can lead to cross contamination, i.e., when one piece of contaminated produce leads to the contamination of many produce, using processing water as a vector. Not maintaining the quality of processing water can lead to cross contamination of every product that passes through the water (Zagory, 1999). Among the many sources that can contribute to contamination of produce, processing water is a known vector for cross contamination among produce. Reuse of water in processing allows for microorganisms, and debris from the crops to build up in the water, creating a harborage for bacteria (Gil, Selma, Lopez-Galvez and Allende, 2009), making it essential that water quality is maintained, and that produce is handled properly throughout processing (IFOAM, 2005).

Washing alone is able to eliminate debris and field heat from the produce, but it is not substantial to eliminate pathogens (Joshi et al., 2013; Gil, Selma, Lopez-Galvez and Allende, 2009). One of the ways to prevent cross contamination in postharvest processing water is the use of chemical sanitizers (Gil, Selma, Lopez-Galvez and Allende, 2009). It is essential that sanitizer levels are high enough to prevent cross contamination before microbes are able to get onto produce or into the cut and damaged surfaces of produce (López-Gálvez, Allende, Selma, and Gil, 2009), but low enough for regulatory compliance (80ppm) (CFR, 2017). Upon entry into these cut spaces microorganisms are no longer susceptible to the sanitizer, which can lead to foodborne illness (López-Gálvez, Allende, Selma, and Gil, 2009; Gil, Selma, Lopez-Galvez and Allende, 2009).

Changes in wash water throughout processing can potentially lead to changes in the efficacy of sanitizers in wash water. Reuse of processing water can result in changes in the levels of organic material in the water, as well as lead to the potential for bacteria to harborage in the processing water. Allende et al. (2008) observed that washing escarole contaminated with *E. coli* with untreated water caused an increase in the microbial count of the processing water from undetectable to 5.5 ± 0.1 log CFU/ml. Although a washing step has the potential to reduce pathogens, it is also able to introduce or spread pathogens to produce (Allende, Selma, Lopez-Galvez, Villaescusa and Gil, 2008). If water is reused, it is important that water quality be monitored throughout use to ensure that sanitizer levels are adequate before produce is washed, to prevent cross contamination among produce.

2.3 Farm operation: postharvest washing

A survey conducted on farms in New England found that although majority of participants indicated implementing food safety practices on farm, there was the lowest knowledge in water safety (68%) and postharvest handling of produce (74%) (Pivarnik et al., 2018). Of the respondents, 77% reported processing leafy greens, and overall 72% reported washing their produce before distribution, with no data collected on the use of sanitizer (Pivarnik et al., 2018). It was seen in this survey that 75% of participants agreed that sanitizer added to the wash water can help to reduce microbial loads (Pivarnik et al., 2018).

In another farm survey conducted in the Northeast region of the United States, 32% of respondents used peracetic acid, compared to 5% using chlorine, and 65% reported not using any sanitizer in their wash water (Schattman, Grubinger, McKeag and Nelson, 2018). Of those that use sanitizer, only 21% reported to monitoring sanitizer concentration, some stated that they monitor at every use, some daily, weekly, monthly or yearly. Of those not using sanitizer during

postharvest washing, 75% reported that they did not feel the need for it (Schattman, Grubinger, McKeag and Nelson, 2018). This raises concern for the region because continued reuse of water can lead to increased levels of pathogens in the water. The extent of water quality changes can go unnoticed to processors, leading to a decreased efficacy of sanitizers throughout operations.

2.4 Processing water mitigating strategies

There are many mitigating strategies to reduce microorganisms in postharvest processing water including ozone, organic acids, and chemical sanitizers such as chlorine and peracetic acid (PAA), yet there are limitations to all of these options. Ozone, at low concentrations (1-5ppm), is an effective disinfectant against bacteria, molds and yeasts (Ölmez and Kretzschmar, 2009). One drawback of ozone is that it can be very unstable, leading to variation in efficacy (Khadre, Yousef & Kim, 2001). Organic acids are also effective against bacteria due its ability to reduce the pH of the solution surrounding the bacteria (Bell, Cutter & Sumner, 1997). A drawback of their use is that they require longer contact times, and they can affect the sensory qualities of the produce (Chang and Fang, 2007; Ölmez and Kretzschmar, 2009). There are many advantages and disadvantages to all sanitizing options, choosing the appropriate sanitizer is essential for each specific operation (Table 1).

Table 1: Summary of advantages and disadvantages of sanitizing options for postharvest wash water

Sanitizer	Pros	Cons
Ozone	<ul style="list-style-type: none"> • GRAS status • No hazardous DBP formation • Strong antimicrobial activity • pH independent • effective at low concentrations 	<ul style="list-style-type: none"> • Toxic when inhaled • Corrosive above 4ppm • Not organic compliant • May interfere with sensory qualities • Unstable
Organic acids	<ul style="list-style-type: none"> • No toxicity 	<ul style="list-style-type: none"> • Long contact time

	<ul style="list-style-type: none"> • Organic compliant 	<ul style="list-style-type: none"> • Interferes with sensory qualities • Lower antimicrobial activity
Chlorine	<ul style="list-style-type: none"> • Low cost • Easily accessible • Short exposure time 	<ul style="list-style-type: none"> • Hazardous DBPs at high concentrations • Lower efficacy with organic matter present • pH dependent
Peracetic acid	<ul style="list-style-type: none"> • Efficacy less impacted by organic matter present • No harmful DBP formation • Efficacy less affected by temperature changes • pH tolerant from 1-8 	<ul style="list-style-type: none"> • Lower antimicrobial efficacy at the levels permitted
Hydrogen peroxide	<ul style="list-style-type: none"> • Easy to use • Cost efficient • No harmful DBP formation 	<ul style="list-style-type: none"> • Low antimicrobial activity • Long contact times • Negative impact on sensory qualities

One of the challenges of chemical sanitizers, is that there are many options on the market with different active ingredients, making it difficult for growers to choose is the best option for their farm. Of the available sanitizers with the same active ingredients, the percentages of active ingredients vary greatly among products (Gombas et al., 2017). It is not known in what capacity these various ratios can play on sanitizer efficacy and stability over time. In PAA based sanitizers there are two active ingredients, hydrogen peroxide (H₂O₂) and PAA. Examples of commercially available PAA sanitizers are Tsunami™ 100 with 15.2% PAA and 11.2% H₂O₂, Sanidate 5.0 with 5.3% PAA and 23% H₂O₂, and Oxidate with 2% PAA and 27.1% H₂O₂. With such large variation on the market further research is needed to understand how this ratio can

factor into efficacy and stability over time to provide guidance to growers choosing the appropriate sanitizer for their operation.

2.5 Benefits of PAA sanitizers

Chlorine is one of the most common sanitizers used in postharvest processing, PAA is another sanitizer that has been approved by the FDA to be used in postharvest processing (Fatica and Schneider, 2009; Gonzalez, Luo, Ruiz-cruz and McEvoy, 2004). Chlorine and PAA are both strong oxidizers that target the cellular membranes (Pfundner, 2011). PAA is a well-known disinfecting alternative that can reduce levels of bacteria, viruses, bacterial spores, and protozoan cysts (Kitis, 2004; Lopez- Galvez et al., 2013), whereas chlorine is unable to reduce spores (Pfundner, 2011). However, there is currently a movement to find alternative sanitizers, and move away from the use of chlorine-based sanitizers due to concerns regarding their environmental and health risks (Gil, Selma, López-Gálvez and Allende, 2009). As the active ingredient, sodium hypochlorite, in chlorine sanitizers breaks down, it can react with halogenated compounds and form harmful disinfection by-products (Fatica and Schneider, 2009).

Compared to chlorine, PAA has several benefits, including its efficacy, it is more stable in the presence of organic load, it does not produce harmful disinfection by products, and it works over a wide range of pH values (Gombas et al., 2017; Ölmez and Kretschmar, 2009; Fatica and Schneider, 2009). A benefit to PAA is that it does not produce harmful by-products as it breaks down over time, because as it decomposes it breaks down into harmless acetic acid and peroxide, which over time breaks down further into water and oxygen (Gombas et al., 2017), making it a safer product than chlorine for human use.

2.6 PAA mode of action and chemical reaction

PAA is a compound generated from a mixture of acetic acid and hydrogen peroxide (Joshi et al., 2013; Yuan, Ni, and Heiningen, 1997). Acetic acid and hydrogen peroxide combine in an equilibrium chemical reaction to form PAA and water (Figure 1). Commercially available PAA sanitizers also list hydrogen peroxide as an “active ingredient” on the label. Although the exact mode of disinfection for PAA is unknown, it is speculated that PAA functions as many other peroxides as an oxidizing agent (Kitis, 2004; Block, 1991). The release of active oxygen drives its disinfectant activity, this disrupts the bonds in proteins, enzymes, and metabolites causing them to oxidize (Liberti and Notarnicola, 1999; Kitis, 2004; Gombas, 2017). This activity disrupts vital biochemical pathways in the cell causing cell death (Kitis, 2004). This oxidizing potential is responsible for PAA’s ability to eliminate bacteria, spores and yeast, by targeting the cell membranes (Joshi et al., 2013).

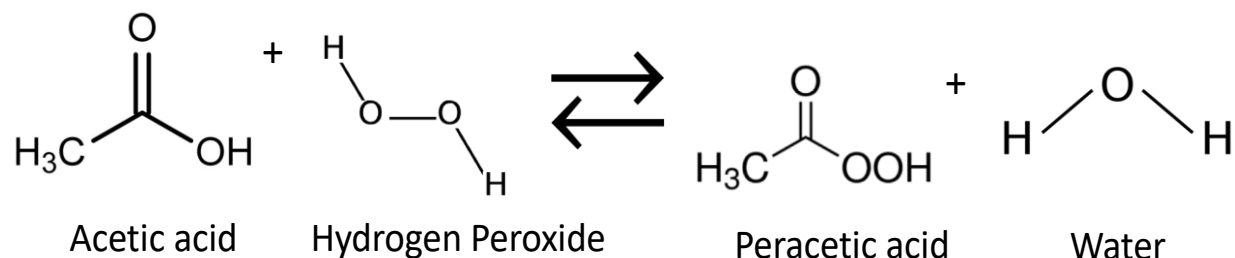


Figure 1: Peracetic acid chemical reaction

Peracetic acid is effective at reducing microbial levels in processing water (Kitis, 2004), it is an effective chemical sanitizer against viruses on produce at 6.4ppm and 12.8ppm PAA (Sánchez, Elizaquível, Anzar, and Selma, 2015). PAA is a suitable sanitizer at reducing microbial levels in processing water, as it has been seen to be effective against bacteria at 0.001%, fungi at 0.003% and spores at 0.3% (Greenspan and MacKellar, 1951). However, the reaction of PAA

with water has the potential to make it difficult to study, as when it is added to aqueous solutions it can cause shifts in the chemical reaction (Figure 1). In production settings, this could lead to changes in PAA concentrations throughout processing, which could have implications on the efficacy of the product during processing. The efficacy of PAA against many microorganisms makes it a great sanitizer for postharvest washing, but more work is needed to establish process controls of their use due to their chemical activity with processing water.

Tsunami™100, a PAA based sanitizer, is a common commercial sanitizer used in research. Tsunami™100 contains 15.2% PAA and 11.2% hydrogen peroxide. Lopez-Galvez et al. (2009) observed that in processing water with an organic load at 500-700COD (chemical oxygen demand), at levels of 500ppm of Tsunami™100 there was a 4-log reduction in *E. coli* levels. Lopez-Galvez et al. (2009) also observed that when investigating cross contamination between uninoculated, and inoculated lettuce there was no cross contamination in the presence of Tsunami™100 (500ppm), and there was no detectable *E. coli* in the residual water that was used for processing. Gonzalez et al. (2004) observed similar results, reporting that in a solution containing 80ppm Tsunami™100 there was no recovery of *E. coli* O157:H7 in the solution after processing carrots. Gonzalez et al. (2004) also observed that the presence of organic load (3,500 COD) did not have an effect on Tsunami™100 efficacy, and that it exhibited the same efficacy as chlorine in tap water. Chriox 5, another commercially available PAA sanitizer, contains 4.6-5% PAA, and 23-25% H₂O₂ (Vandekinderen, Devlieghere, Meulenaer, Ragaert & Camp, 2009). Van Haute et al. (2015) used Chriox 5, a PAA based sanitizer, to assess the efficacy of PAA in solutions with organic load present (500, 800, 1500COD) and reported that in the presence of 500COD, at 1.4ppm of PAA, Chriox 5 reduced *E. coli* O157:H7 levels by approximately 3-log CFU/ml in 30mins. These results demonstrate that PAA based sanitizers have a strong antimicrobial capacity,

and that it is not impacted by high organic load levels in the processing water, making it a strong alternative to chlorine-based sanitizers.

Although Tsunami™100 is a common sanitizer that is used in research, there is little research that has been done looking at other commercially available PAA sanitizers. Commercially available PAA sanitizers can have varying costs and concentrations of PAA and H₂O₂ in the product, which could cause differing efficacies among products. For example, Tsunami™ 100 is 15.2% PAA and 11.2% H₂O₂, Sanidate 5.0 has 5.3% PAA and 23% H₂O₂, Perasan OG has 21.5% PAA and 5% H₂O₂, and Oxidate has 2% PAA and 27.1% H₂O₂. Although having the same PAA sanitizer used in research allows for a greater comparison among research, there is still a gap in understanding the efficacy of other sanitizers in similar conditions. The use of varying sanitizers in different studies also makes it difficult to compare results, as they can have different outcomes based on the sanitizers that are being used. It is important to further investigate how PAA based sanitizers can affect efficacy in postharvest processing because different commercial sanitizers have varying levels of PAA and ratios of PAA and hydrogen peroxide. Another gap in comparing research is that there are many methods that are used in research to create artificial organic load, and there are many levels of organic load that are used in research. Having differences in organic load throughout research in this field create a challenge when comparing results of the same sanitizer, as different levels of organic load can cause different results.

Although PAA has many benefits and is an effective sanitizer, its performance can still be impacted by various factors in the processing water, including organic load, pH, temperature, and concentration. Yet the gap in knowledge of the proper use and handling of peracetic acid, and the impact that various other factors may play on the stability and the efficacy of these sanitizers can

make an impact in ensuring that they have a stable and constant efficacy throughout their use. The remainder of this chapter will analyze research that has been done with PAA based sanitizers and the impact that various processing conditions can have on the efficacy of PAA sanitizers.

2.7 What has been done with PAA sanitizers

Much of the research in postharvest processing focuses on cross contamination between inoculated and uninoculated produce. Tsunami™100 has been observed to reduce levels of *E. coli* O157:H7 on inoculated lettuce by approximately 1.0-1.7 log CF/ml when in the presence of 0-10% organic load (average 2130±1088COD), generated by blending lettuce in tap water and diluting the lettuce juice in a circulation tank (Davidson, Kaminski-Davidson and Ryser, 2017). Levels of *E. coli* O157:H7 were also observed to increase by approximately 1.8, 0.9 and 0.2 log CFU/ml on uninoculated lettuce that was washed in the same PAA solution as inoculated lettuce with varying organic load levels (2.5, 5 and 10% respectively) (Davidson, Kaminski-Davidson and Ryser, 2017). Although understanding the influence that sanitizers can have on reducing cross contamination among produce is important, the PSR specifically mandates that there be no generic *E. coli* levels in the agriculture water itself. This makes it important that research also focuses on understanding how sanitizing agents can reduce microbial levels in processing water itself.

2.7.1 Outside influences on the efficacy and stability of PAA

Chemical sanitizers can be affected by changes in the quality of postharvest processing water, making it essential that postharvest water conditions are monitored. Changes in sanitizer concentrations, increases in organic load, changes in pH and temperature are all factors that have the potential to influence the efficacy of PAA sanitizers.

2.7.1.1 Effect of concentration on PAA

The FDA regulates PAA that can be used in postharvest processing water stating that it needs to be prepared by the reaction of acetic acid and hydrogen peroxide and must not exceed 80ppm in wash water (CFR, 2017). Therefore, it is essential that processors monitor their sanitizer levels to ensure that they are in compliance with this federal regulation. Monitoring sanitizer levels throughout production is important because excess addition of sanitizer without knowing current PAA levels could lead to PAA concentrations exceeding the federal regulation, an unnecessary increase in cost towards sanitizer, and changes in microbial deactivation.

The concentration of PAA influences disinfectant efficacy. Low sanitizer concentrations correlate with low disinfectant efficacy, and extended contact times only have a limited effect on microbial reduction. Vandekinderen et al. (2009) observed that when processing leeks in the presence of the PAA sanitizer Chriox 5, at concentrations ranging from 0 to 250ppm, the microbial inactivation of the sanitizer increased proportionally to the concentrations of PAA. At 20ppm PAA a 0.4 log CFU/g reduction was observed compared to a 1.2 log CFU/g reduction at 120ppm PAA on the leeks (Vandekinderen et al., 2009). PAA was successful at reducing microbial levels when the microorganism was not attached to the produce but was less successful at killing microorganisms that were attached to the surface or in places difficult for the sanitizer to access on the produce (Vandekinderen et al., 2009).

Maintaining the concentrations of peracetic acid at 500ppm in the processing water makes it possible to eliminate microbes before they are internalized into the cells of the produce, once internalized it is almost impossible to eliminate (Beuchat et al., 2001; López-Gálvez et al., 2009), however this is above the federal limit of 80ppm. An accumulation of organic matter in the water can also lead to an increased level of pathogens, as organic material provides a place for pathogens

to attach making it more difficult for sanitizer to eliminate the pathogens (Gawande and Bhagwat, 2002). This allows for the opportunity to expand research investigating the concentration of PAA that could be added to postharvest processing water before it is no longer effective. Peracetic acid is more efficient than chlorine at killing microbes while in aqueous solutions, requiring lower concentrations than the latter to achieve the same effect (Ölmez and Kretzchmar, 2009).

Concentrations of 30ppm peracetic acid are sufficient to prevent cross contamination of *E. coli* when there is no organic matter present in the washing solution (Zhang, Ma, Phelan and Doyle, 2009). In the presence of 10% organic material, peracetic acid's ability to prevent cross contamination is reduced, meaning a higher concentration of PAA is needed to achieve a larger reduction in bacteria levels on produce. As seen by Zhang et al. (2009), PAA at 10ppm showed a recovery of 4.0 log CFU/piece compared to 1.7 log CFU/piece with 30ppm PAA. However, the levels of *E. coli* recovered from uninoculated lettuce are still significantly less than those recovered from washing with water alone (Zhang, Ma, Phelan and Doyle, 2009).

Lopez-Galvez et al. (2009) observed that the presence of Tsunami™100 (15.2%PAA and 11.2%H₂O₂) at 500ppm in processing water, resulted in no *E. coli* detected in processing water after washing inoculated lettuce pieces, where washing with tap water alone had an increase of 3.1 log CFU/ml. The presence of PAA in the processing water can have a large impact on preventing cross contamination among produce.

It can be seen in this research presented, that differences in the concentration of PAA that researchers used, resulted in various reductions of microorganisms, increased concentrations achieved greater reductions. It is also seen that with changes in organic load there is a need for increased concentration of PAA to achieve sufficient reductions in microorganisms in processing water. Changes in the quality of postharvest processing water can lead to a depletion of sanitizer

concentration over time or result in the need for increased PAA levels, impacting efficacy over time as bacteria is continuously introduced to the system throughout processing. Changes in water quality throughout processing impact PAA concentrations, however, the extent that these changes can have on PAA concentration and efficacy over time is yet to be investigated.

2.7.1.2 Effect of organic load on PAA

Organic load is an accumulation of vegetative matter, bacteria and debris in a water solution. It is often measured using chemical oxygen demand (COD), which is a measure of a water samples capacity to consume oxygen during the breakdown of organic matter and the oxidation of inorganic chemicals (COD, 2009), which provides a comprehensive look at the levels of vegetative matter as well as bacteria that are in a sample. Organic load has been known to have an influence on the quality of sanitizers. Compared to chlorine-based sanitizers, the efficacy of PAA is less impacted by the organic load in solution, however, it is still influenced (Zhang, Ma, Phelan, and Doyle 2009). In the presence of 10% organic load, created by blending iceberg lettuce in sterile water, organic load had less of an effect on PAA efficacy than chlorine efficacy at 30ppm (Zhang, Ma, Phelan and Doyle, 2009). Peracetic acid at 30ppm in 10% organic load significantly reduced cross contamination in lettuce leaves inoculated with *E. coli* O157:H7 (Zhang, Ma, Phelan and Doyle, 2009). PAA reduced *E. coli* O157:H7 to undetectable levels on uninoculated produce, compared to chlorine at 30ppm, which showed 1.68 log CFU/ml of *E. coli* O157:H7 on uninoculated lettuce after treatment (Zhang, Ma, Phelan and Doyle, 2009). In organic load levels of 500 and 800COD PAA was observed to reduce microbial loads by 2.5 and 3 log CFU/ml respectively in wash water, with 800COD displaying a faster depletion rate over time (Van Haute et al. 2015).

Organic load levels change with every pass of produce that goes through postharvest processing water, therefore it is important for growers to understand the impact that organic load plays on peracetic acid efficacy and stability, so corrections can be made in processing. The performance of PAA has been seen to be less impacted by the presence of organic load by many researchers, but PAA is sensitive to organic load if it contains high levels of antioxidants, sulfur-rich compounds, and dissolved metals as it can deplete PAA levels (Yuan, Ni and Heiningen, 1997a; Gombas et al., 2017). When choosing a sanitizer, it is important for a grower to understand the changes in water quality that are in their operation, an understanding of their personal operation will lead to a better understanding of what they should be looking for in a sanitizer, this includes understanding the microbes of concern, organic load composition and changes in organic load. Although it has been proven that PAA is more stable and efficient in the presence of organic load compared to other options, limited work has looked specifically at the rate of degradation of PAA, and the efficacy of PAA over extended periods of time in the presence of organic load.

2.7.1.3 Effect of pH on PAA

Decomposition of PAA in solution can be the cause of 3 chemical reactions, spontaneous decomposition, hydrolysis, and metal catalysed decomposition, which have various responses under different pH levels (Yuan, Ni and Heiningen, 1997b). It was observed by Yuan et al. (1997b) that at a pH level of 9.5 the initial decomposition rates of PAA and H₂O₂ were higher than when the solution was at a pH of 8.2. At a pH level of 8.2 (equivalent to the pK_a of PAA) both PAA and H₂O₂ are rapidly consumed by spontaneous decomposition (Yuan, Ni and Heiningen, 1997b). However, as the pH begins to exceed 8.2, spontaneous decomposition of PAA begins to decrease, and the hydrolysis and metal catalysed decomposition reactions begin to increase, causing a more rapid depletion over time (Yuan, Ni and Heiningen, 1997b).

An increase in PAA concentration correlates with a decreased pH within a solution, as PAA is an acidic solution. Microorganisms are more sensitive to the oxidizing potential of PAA at a lower pH, with efficacy of PAA reducing as the pH of the solution gets closer to, and exceeds, the pKa of PAA at 8.2 (Joshi et al., 2013; Sanchez, Elizaquível, Aznar and Selma, 2015; Gonzalez, Luo, Ruiz-Cruz and McEvoy, 2004). *E. coli* O157:H7 cells are tolerant of acidic conditions, so the disinfectant action of the sanitizer cannot rely only on lowering the pH, however it cannot be ignored completely as bacteria is inactivated more quickly at lower pH conditions (Gonzalez, Luo, Ruiz-Cruz and McEvoy, 2004).

Unfortunately, there is limited research looking at the impact of pH on the efficacy and stability of PAA in wash water over time. Work done by Yuan et al. (1997b) allows for an understanding of the relationship between PAA, pH, and time yet it is important to also understand how the influence of pH on the rate of PAA decomposition could potentially influence PAA efficacy in wash water over time. The likelihood of wash water having a high pH value is low, however as pH is a driver in chemical reactions it is an important element to investigate in terms of how it can affect PAA efficacy and stability.

2.7.1.4 Effect of temperature on PAA

Temperature changes in processing water can be common in postharvest conditions. During processing, field heat can transfer from the produce into the water, and in areas where the weather changes often, the postharvest processing water can change in temperature as well. With changes in temperature impacting the efficacy of peracetic acid, it is important for growers to understand how this may impact their postharvest processing throughout the season, so that they can adjust their use of sanitizer accordingly.

PAA is effective over a wide range of temperatures (0-40°C), making it favorable in the food industry as a surface sanitizer (Kunigk et al., 2001; Kitis, 2014). In contrast, increases in temperature can cause peracetic acid decomposition to occur more rapidly in solution (Kunigk et al., 2001). Kunigk et al. (2001) reported that a solution of PAA between 240 and 280ppm had approximately a 50% loss after 72 hours at 45°C, and only a 33% loss after 240 hours at 25°C. The decomposition rate at 45°C was reported to be almost 5 times faster than at 25°C (1.71h⁻¹ and 9.64h⁻¹ respectively) (Kunigk et al., 2001). In a study looking at various processing facilities, the ability of PAA to eliminate *E. coli* in spinach processing was reported to be independent from the temperature of the wash water (approximately 8-18°C) (Barrera, Blenkinsop, and Warriner, 2011). Stampi et al. (2001) reported that there was an increased reduction in enterococci that correlated with an increase in temperature up to 24°C.

Although there is knowledge of the activity of PAA at different temperatures, most research in postharvest wash water has focused on understanding its efficacy specifically, research is lacking in the effect of temperature on PAA rate of degradation and microbial inactivity in processing water. Due to fluctuations in processing water, it is important to understand how extensively temperature can impact stability, as this has the potential to change periodically throughout the processing.

2.8 Conclusion

It has been seen that compared to washing produce in wash water that is untreated, peracetic acid has been able to reduce bacterial levels on produce (Zhang, Ma, Phelan and Doyle, 2009). This shows how the use of peracetic acid sanitizers in post-harvest processing water can improve produce safety. It is common to see postharvest research focusing on cross contamination, although understanding cross contamination and the impact that sanitizers have on preventing

cross contamination is important, the FSMA regulation states that there can be no detectable generic *E. coli* in the processing water, specifically. With this regulation in place, there is more research needed that looks at the processing water, to ensure that there is guidance for growers on how to regulate and combat any *E. coli* that may be present in processing water. It is important to assess the efficacy of peracetic acid in postharvest wash water specifically because looking at the cross contamination between produce does not consider other sources of contamination besides the produce itself. Water can become contaminated through multiple vectors, including, but not limited to, run off water, contaminated well water, and improper employee hygiene. In small on-farm processing, postharvest processing can be very hands on, meaning that there are hands in processing water, which could be a potential source of *E. coli* in the water.

Choosing the right sanitizing option is very farm specific. Ensuring that growers understand that increases in organic load, changes in pH towards an alkaline solution, changes in temperature and variation in peracetic acid concentration can have an impact on the efficacy of a PAA based sanitizer can help to make informed decisions on sanitizer choices. Developing a further understanding of the impact specifically in postharvest processing water, can allow for guidelines to be developed on the optimization of peracetic acid in postharvest processing, that help farmers to ensure a safe food supply.

2.9 Future research

Further research also needs to address how all of these factors play into the stability of PAA over time. Understanding the impact that concentration, organic load, pH and temperature have on preventing cross contamination and eliminating pathogens in agricultural water are important, but it is essential that PAA degradation kinetics are also investigated. Even with all of the research that has been done with PAA based sanitizers, there is a lack of research

investigating if PAA continues to remain effective over time, through a second inoculation of bacteria. If the stability is impacted over time it can impact efficacy as well as have an effect on when a grower needs to adjust the PAA levels in the solution in order to maintain efficacy throughout the entire process. Knowledge in the rates of degradation of PAA in these various parameters will help growers to better understand when it is appropriate to add additional sanitizer and to ensure that it will not exceed FDA regulations on approved concentrations.

CHAPTER 3

IMPACT OF VARIOUS DYNAMIC POSTHARVEST WASH WATER CONDITIONS ON THE EFFICACY AND STABILITY OF PERACETIC ACID SANITIZERS

3.1 Introduction

Peracetic acid (PAA), as a sanitizer, is an acceptable method to reduce cross contamination and inactivate vegetative pathogens, including *Escherichia coli*, found in postharvest wash water to remain in compliance with the Produce Safety Rule's regulation stating that there can be no detectable generic *E. coli* in agriculture water (FDA, 2018). PAA is a strong disinfecting agent because it has a large oxidizing potential that allows it to target cell membranes of bacteria, spores, and yeasts (Kitis, 2004; Joshi, Mahendron, Alagusundaram, Norton, and Tiwari., 2013). PAA has shown to have benefits over the use of chlorine sanitizers in field due to its organic compliance status, reduced environmental concern related to its by-products breaking down to acetic acid and peroxides, and its greater stability in the presence of organic matter (Gombas et al., 2017; Zhang, Phelan, Ma and Doyle, 2009).

PAA is a chemical that is formed from an equilibrium chemical reaction of acetic acid and hydrogen peroxide (H_2O_2) (Figure 1) (Joshi et al., 2013; Yuan, Ni and Heiningen, 1997). Commercially available PAA based products list both PAA and H_2O_2 as active ingredients. However, there are many products in the market with varying ratios of the PAA to H_2O_2 in the product which can make it difficult when choosing a sanitizer for commercial use. For example, Tsunami™ 100 has 15.2% PAA and 11.2% H_2O_2 , Sanidate 5.0 has 5.3% PAA and 23% H_2O_2 , Perasan OG has 21.5% PAA and 5% H_2O_2 , and Oxidate has 2% PAA and 27.1% H_2O_2 . Little

research has been done to better understand the differences in efficacy of various ratios of PAA and H₂O₂ among commercial sanitizers.

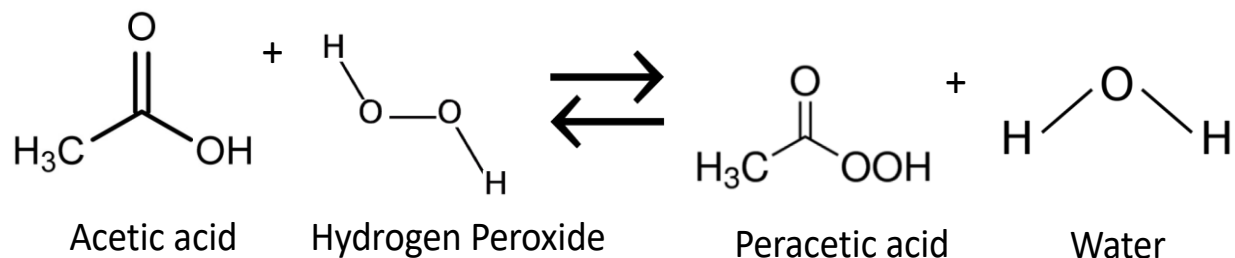


Figure 2: Peracetic acid chemical reaction

While there is strong evidence to support PAA as an antimicrobial agent in postharvest washing and other sanitizer applications, there has been limited research available to establish an optimal ratio of PAA: H₂O₂ and understand the impact of a dynamic agriculture water system, in terms of various concentrations, organic load levels, water temperatures, and pH levels, on PAA efficacy and stability. Organic load can increase with the addition of bacteria and shedding of vegetative matter as produce is passed through the water. Changes in the temperature of processing water can occur as field heat from the produce transfers to the water, introducing temperature as a factor that may influence sanitizer stability and efficacy over time.

Temperature has been reported to be independent from the ability of PAA to eliminate *E. coli* levels in spinach processing (Barrera, Blenkinsop and Warriner, 2011). Changes in wash water quality, as well as variation among commercial sanitizers, make it important to better understand how various factors can have an effect on sanitizer stability and efficacy throughout processing.

This study aims to investigate the parameters that may influence the use, stability, and efficacy of PAA based system. A shelf life study was initially conducted to determine if different storage temperatures influence PAA stability over time. Then the influence organic

load plays on the stability of PAA from a commercial sanitizer over time was investigated to determine at what organic load conditions PAA levels are affected. This study then continued to investigate the relationship between PAA and H₂O₂ and how varying concentrations of the two chemicals in aqueous solutions may impact efficacy against *E. coli* O157:H7, as well as how it may impact the stability of both sanitizing agents in solutions over extended periods of time. Most studies on PAA sanitizers are done with one set of wash water conditions and do not look at multiple conditions side by side. This study investigated the effects of multiple wash water conditions to understand the influence that a range of concentrations, organic load levels, temperatures and pH values have on the efficacy and stability of PAA and H₂O₂ over extended periods of time to mimic a dynamic system.

3.2 Materials and Methods

3.2.1 Storage of PAA at different temperatures. A commercial source of PAA, Tsunami™ 100 (15.2%PAA, 11.2% H₂O₂), was purchased from Ecolab (St. Paul, MN). Samples were transferred into 1-liter ventilated bottles (Bel-Art, Wayne, NJ) and stored at 5, 20, or 37°C for 12 weeks. Samples were collected weekly and H₂O₂ and PAA concentrations were analyzed weekly from each storage condition by diluting the sample in a 500ml DI water sample starting at 60ppm PAA (see section “Measuring hydrogen peroxide and peracetic acid concentrations” for details).

3.2.2 Creating artificial organic load. Baby spinach was purchased from a local grocery store (Amherst, MA) and stored at 4°C and used within 3 days. Baby spinach, 20g, was blended with 100ml of DI water for 30 seconds (Coolife HS-167, Guangdong, China), strained over cheese cloth and diluted using DI water to achieve organic load levels of 100, 500 or 750COD, modeled

after Zhang et al. (2009) and Zhou et al. (2014a), COD values chosen based on statistical differences seen in preliminary experiment. Organic load was measured at 20°C using chemical oxygen demand (COD) via the USEPA reactor digestion method (HACH, Loveland, CO) (HACH, 2002). Spinach solution was then stored at 4°C until use, no longer than 24 hours.

3.2.3 Measuring H₂O₂ and PAA levels. H₂O₂ and PAA levels were measured using a two-part iodometric titration using a Hanna titrator HC901C (Woonsocket, RI), as described by Greenspan and McKellar (1948), with a limit of detection at 2.13ppm H₂O₂ and 4.75ppm PAA.

3.2.4 Preparation of inocula. A slant was created using *Escherichia coli* O157:H7 (ATCC#43894). The slant was then stored at 4°C until use, for no more than 4 weeks. Prior to experiments, a colony was transferred from the prepared streak to a 50ml sample of TSB and incubated at 37°C for 18 hours.

At the time of the experiment, a 2L deionized (DI) water sample was inoculated with 7.0 ± 0.5 log CFU/ml of *E. coli* O157:H7 from 50ml TSB sample. Samples were then treated with H₂O₂ and PAA under different H₂O₂/PAA ratios, concentrations, temperatures, pH levels, and organic load conditions to determine their influence on efficacy and stability after 30 seconds (t=0hrs), 3, 3.5 and 4 hours. Collection times are based on a preliminary experiment (APPENDIX B). The samples were re-inoculated with 7 log CFU/ml at 3.5 hours to determine if the sanitizing capacity was still present in the sample over time. (Growth curve Appendix A).

3.2.5 Change in H₂O₂ and PAA ratios. Following inoculation, a 30ppm sanitizer concentrations was maintained at various H₂O₂ and PAA ratios. Sample variants are presented in Table 1:

Table 2: H₂O₂ and PAA ratios that were added to 2L samples to create 30ppm active ingredients from 100% H₂O₂ to 100% PAA.

H ₂ O ₂		PAA	
Percent	Target ppm	Percent	Target ppm
0	0.0	100	30.0
25	7.5	75	22.5
50	15.0	50	15.0
75	22.5	25	7.5
100	30.0	0	0.0

3.2.6 Change in concentrations using 50/50 H₂O₂/PAA. 2L DI water samples were measured into clean glass bottles (Pyrex™, Fisher Scientific, Hampton, NH). Samples were inoculated with 7.0±0.5 log CFU/ml *E. coli* O157:H7 and treated with H₂O₂ and PAA at a 50/50 ratio (Table 1) at concentrations 0, 30, 60, or 80 ppm active ingredients, to remain below federal limits.

3.2.7 Change in water temperatures. 2 L DI water samples were measured into clean glass bottles (Pyrex™, Fisher Scientific) and maintained at 4, 20, or 37 ±0.5°C and monitored throughout the duration of the experiment using a Hanna titrator HC901C (Woonsocket, RI). All parameters are measured at 20°C except section 3.3.6.

3.2.8 Changes in water pH levels. 2L DI water samples were measured into clean glass bottles (Pyrex™, Fisher Scientific) and inoculated with 7.0 ± 0.5 log CFU/ml of *E. coli* O157:H7.

Solutions were adjusted with sodium hydroxide (Fisher Scientific, Fair Lawn, NJ) to achieve pH levels of 5, 6.5 or 9. pH levels were chosen because a pH 5 is the product itself, pH 6.5 was the pH of DI water used throughout the experiments, and a pH of 9 was above the pKa of PAA (8.2).

3.2.9 Microbial enumeration. To determine sanitizer efficacy, microbial loads were measured before sanitizer treatment to determine an initial *E. coli* O157:H7 value (t=I). Samples were shaken for 30 seconds after the addition of H₂O₂ and PAA. Samples were then pulled at 30 seconds (t=0hrs), 3 (t=3hrs), 3.5 (t=3.5hrs) and 4 (t=4hrs) hours after treatment. After 3.5 hours from initial treatment, *E. coli* O157:H7 was reintroduced to the samples at 7-log CFU/ml to determine if the sanitizer remains effective over time. At each time point samples were enumerated and plated on nalidixic acid treated TSA. Samples were incubated for 18-24 hours at 37°C.

3.2.10 Statistical analysis. All experiments were measured in triplicate for three trials for all analysis. Analysis of variance (ANOVA), regression analysis, and Duncan New Multiple Range Test were used to determine statistical differences at a level of significance of $p=0.05$ using SAS statistical software (SAS Institute, Cary, NC).

3.3 Results and Discussion

3.3.1 Shelf life study of Tsunami™100

The 12-week shelf life study of the selected commercially available PAA based produce sanitizer showed a significant difference over time, and a significant interaction between time

and storage temperature for PAA concentrations. ANOVA also showed a significant difference between storage temperatures for H₂O₂ concentrations but no significant difference over time. With a continuous time variable, regression analysis was performed for PAA concentrations, which showed that there were quadratic relationships over the 12-weeks for all storage temperatures (Figure 3). All three storage temperatures (5, 20 and 37°C) showed similar relationships over time, and had relatively low R² values (0.455, 0.697, and 0.672 respectively), indicating that there is a variation in PAA concentration over time across all three storage temperatures tested (Figure 3). Regression analysis was also performed of H₂O₂ concentrations over time which showed that there was a quadratic relationship with storage at 5°C, a linear relationship with storage at 20°C and a quadratic relationship with storage at 37°C (Figure 3). Storage at 5 and 37°C showed similar relationships between concentration and time. The changes in PAA concentration over the 12-week shelf life study showed approximately a 10ppm range overall within each storage temperature. H₂O₂ concentration changes over 12-weeks showed a variation of approximately 3ppm over the 12-week shelf life study.

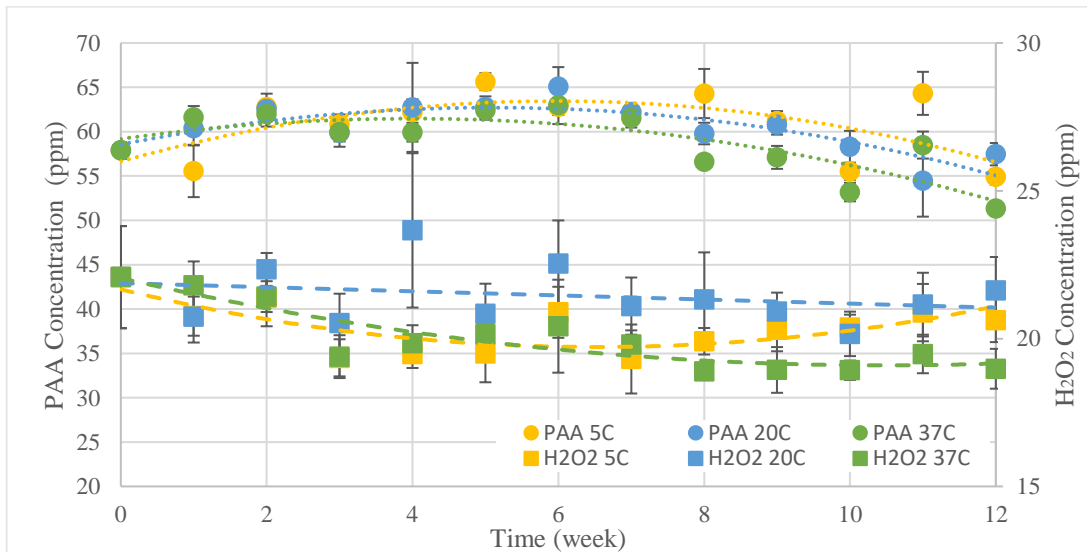


Figure 3: PAA and H₂O₂ concentration as a function of time over a 12-week shelf life study. Standard error bars represent three replicates.

Variability in the PAA and H₂O₂ concentrations is most likely due to the chemical relationship between H₂O₂ and water. PAA reacts with water to form H₂O₂ and acetic acid as products (Joshi et al., 2013). With H₂O₂ and PAA being on opposite sides of the equilibrium reaction it would cause a shift between the two chemicals, therefore influencing concentration. The dynamic relationship between H₂O₂ and PAA in this reaction would explain the pattern of changes in concentration throughout storage (Joshi et al., 2013). In industrial application, such as monitoring postharvest wash water, the dynamic equilibrium changes may impact managing sanitizer levels because variation over time of the PAA concentration makes it challenging to establish validated procedures of needed sanitizer levels. Current literature did not reveal any research that has studied the shelf-life of PAA under different conditions to make a comparison to previous work. The impact that variation during storage can have on efficacy has not yet been explored, this research investigates further the impact that various PAA and H₂O₂ ratios can have on efficacy and stability over time. Changes in postharvest processing water, such as sanitizer concentration, organic load, and temperature, may also impact the efficacy of PAA over time, which may be further influenced by the variability in the commercial sanitizer during storage. Additional experiments were conducted to investigate the influence of PAA concentration, organic load, temperature and pH in postharvest wash water and their influence on PAA stability and efficacy over time.

3.3.2 Influence of organic load on Tsunami™100.

PAA was evaluated under various organic load conditions (0, 100, 500 and 750COD) using Tsunami™100 (15.2% PAA, 11.2% H₂O₂) at approximately 60ppm (Figure 4). Results report that in the presence of organic load levels of 0, 100, 500 and 750COD, PAA had a highly

significant difference between organic loads and a significant difference over time. There was a significant decrease in PAA activity at 500 and 750COD compared to 0 and 100COD, showing that as the organic load increases, the PAA concentrations decrease. At 750COD there is a significant difference between PAA concentrations at t=0hrs and t=3hrs indicating that organic load influences the activity. H₂O₂ had a significant difference between organic load levels, and no significant difference over time (Figure 4) which suggests that H₂O₂ is less impacted by organic load.

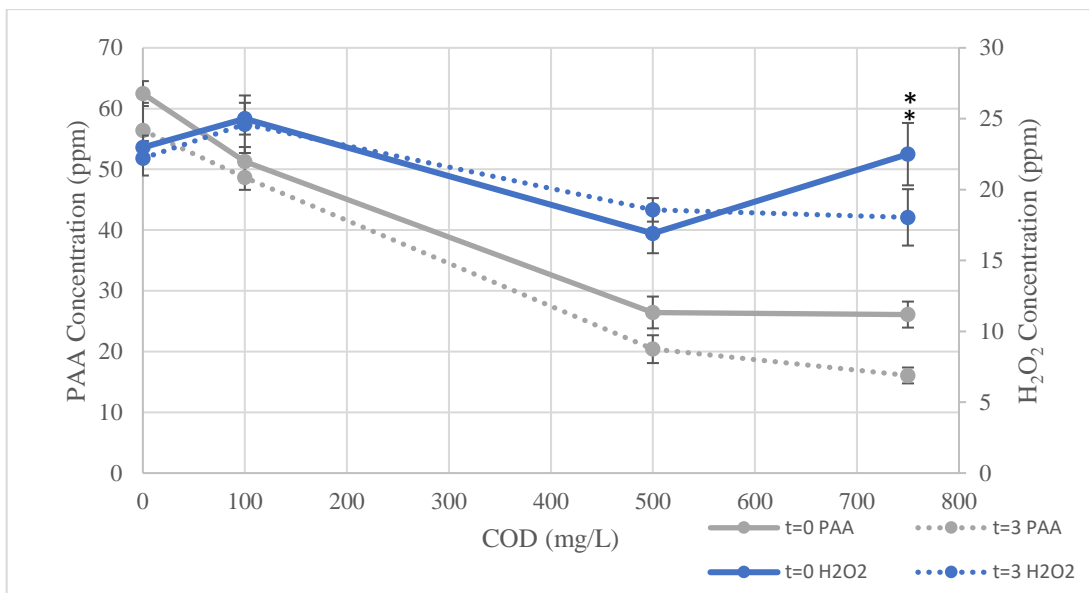


Figure 4: PAA and H₂O₂ concentration changes in various organic loads over time. Error bars represent three trials with three replications.

In processing, organic load increases with every pass of produce through the water, as vegetative material and microbes shed off of the produce. In this study, PAA and H₂O₂ are stable in the presence of 0 and 100COD organic load levels over three hours. However, PAA shows an initial depletion in concentration proportional to an increase in organic load levels. This same initial depletion with organic load was also observed by Van Haute et al. (2015) in a study showing that there was an initial rapid depletion in PAA concentrations within the first

minute that is more rapid than the decay rate over one hour in the presence of organic loads ranging from 30 to 1551 ± 33 COD. Although there was an initial depletion with organic load present, PAA remained present in the sample over three hours with 0, 100 and 500COD indicating that PAA based sanitizers are a good option for leafy green processing because concentrations are less likely to deplete over time. Reuse of processing water for extended periods of time can mean changes in organic load, which can impact PAA levels at both initial addition and over time, and reuse is a common practice in regions such as the Northeast of the United States (Schattman, Grubinger, McKeag, and Nelson, 2018). Therefore, more work is needed to understand how dynamic processing water influence the stability of PAA and H_2O_2 over time.

3.3.3 Influence of various H_2O_2 /PAA ratios on efficacy and stability on efficacy and stability

Five different H_2O_2 /PAA ratios (0/100, 25/75, 50/50, 75/25 and 100/0 H_2O_2 /PAA) were tested against microbial activity over time to determine if the ratio influenced sanitizer performance. Results determined that there was a highly significant interaction between H_2O_2 /PAA ratios and time in microbial activity. While all water samples treated with a H_2O_2 /PAA ratio resulted in a significant decrease in microorganisms, formulated ratios did affect the microbial load reduction. Solutions with higher concentrations of PAA (0/100 and 25/75 H_2O_2 /PAA) showed the greatest reduction in *E. coli* O157:H7 levels after 30 seconds of exposure to the sanitizer (Figure 5). All formulas, except 100/0 H_2O_2 /PAA (all H_2O_2), achieved a 7-log reduction within the first three hours (Figure 5). At $t=3.5$ hrs, *E. coli* O157:H7 was re-inoculated into the sample to determine if PAA had the same disinfectant efficacy over time under various H_2O_2 /PAA ratios, to mimic real world conditions where contamination may be

introduced to the system throughout processing. Ratios 50/50 and 25/75 H₂O₂/PAA had a significant increase in *E. coli* O157:H7 levels after the re-inoculation. After 4 hours, all ratios except 100/0 H₂O₂/PAA had significant decreases in *E. coli* O157:H7 levels, showing undetectable levels of *E. coli* O157:H7. However, for ratio 100/0 H₂O₂/PAA there was a significant increase in *E. coli* O157:H7 levels at t=3.5hrs which did not decrease at t=4hrs.

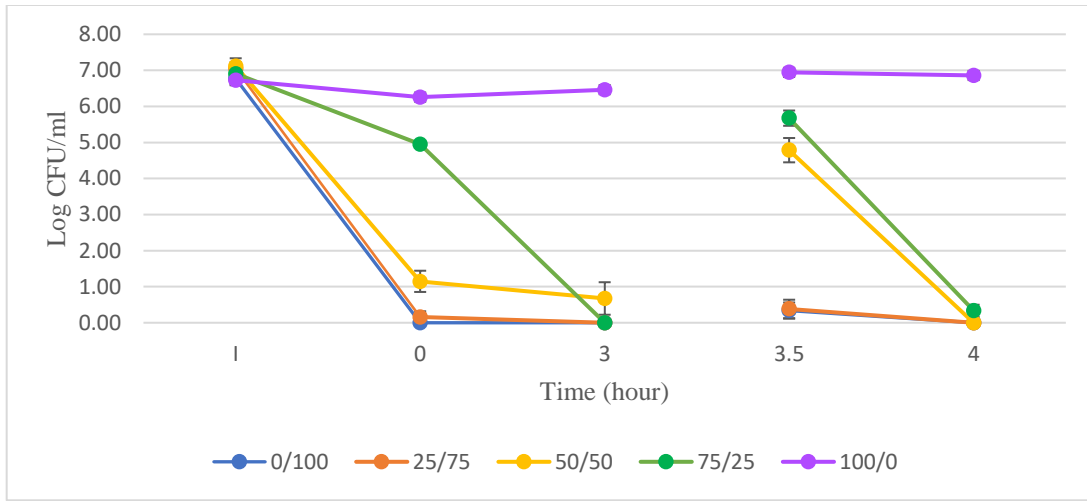


Figure 5: *E. coli* O157:H7 changes over four hours with re-inoculation at t=3.5hrs, under the influence of various H₂O₂/PAA ratios in wash water over time. Ratios are displayed as H₂O₂/PAA respectively. Error bars represent three trials with three replications

With a re-inoculation at t=3.5hrs, it was observed that there was a highly significant interaction between H₂O₂/PAA ratio and time for H₂O₂ levels and a significant interaction for PAA levels. All ratios showed a significant decrease in H₂O₂ and PAA levels across the four hours (Figures 6 and 7). As the ratios began to have higher levels of H₂O₂ in the solution there was a larger depletion in H₂O₂ levels from t=0 to t=3hrs. Variation in the ratio also affects the initial concentration of sanitizer. Sanitizer was added at a calculated 30ppm total active ingredients at t=0hrs for each sanitizer ratio tested, however, it was observed that the total concentrations often were not at the calculated values. This plays back to the chemical reaction

between H₂O₂ and PAA, displaying how aqueous solutions can cause a shift the concentrations that are initially added. Variations in microbial inactivation and sanitizer stability suggest that various H₂O₂/PAA ratios on the market may influence efficacy and stability of PAA systems over time.

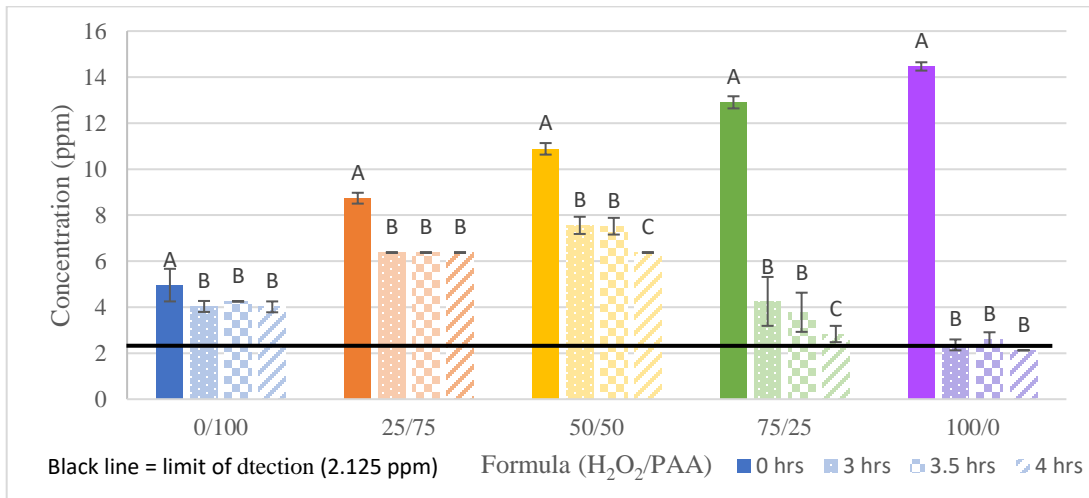


Figure 6: Changes in H₂O₂ concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs of various H₂O₂/PAA ratios. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

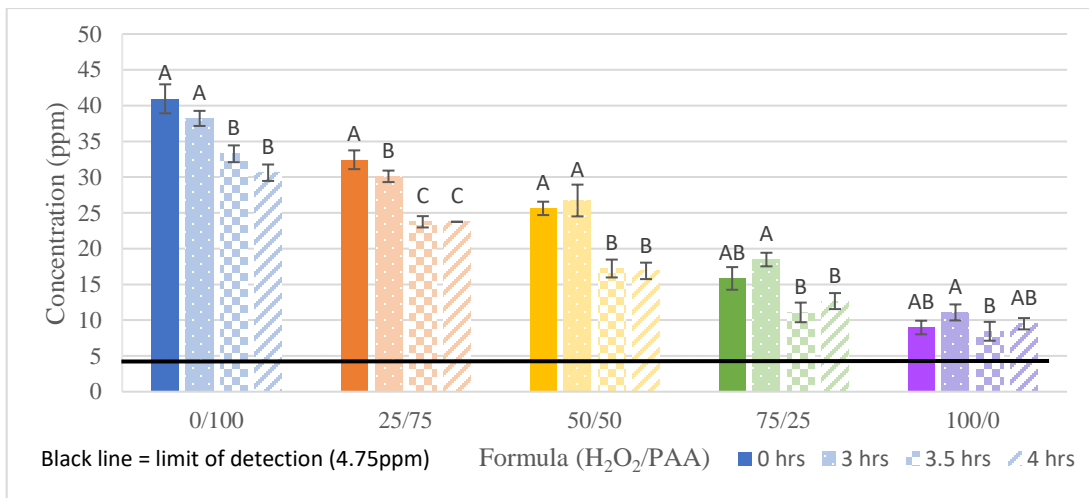


Figure 7: Changes in PAA concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs of various H₂O₂/PAA ratios. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

Reported results demonstrate that the relationship between H₂O₂ and PAA ratios in solution can have an influence on the efficacy and stability of the product. Water treatments with higher PAA ratios resulted in a significantly higher microbial reduction of *E. coli* O157:H7 than those with higher levels of H₂O₂. This matches results seen by Lopez-Galvez et al. (2009) and Van Haute et al. (2015). Lopez-Galvez et al. (2009) observed that in processing water with an organic load at 500-700COD, at levels of 500mg/l of Tsunami™100, there was a 4-log reduction in *E. coli* levels. Van Haute et al. (2015) observed that in the presence of 500COD, at 1.4ppm of PAA, Chriox 5 reduced *E. coli* O157:H7 levels by approximately 3-log CFU/ml in 30mins. Tsunami™100 contains 15.2% PAA and 11.2% H₂O₂ and Chriox 5 contains 5% PAA and 25% H₂O₂. Although the two studies have similar COD levels, the variation in ratios of the two commercial products makes it difficult to directly compare these studies. In order to better assess the relationship of H₂O₂/PAA under other potential influential postharvest wash water conditions, a ratio of 50/50 H₂O₂/PAA was used for additional experiments.

3.3.4 Influence of various concentrations of a 50/50 H₂O₂/PAA ratio on efficacy and stability

A study using a 50/50 H₂O₂/PAA ratio was then conducted to determine the sanitizer efficacy under varied concentrations (0, 30, 60, 80ppm). Results demonstrated a highly significant difference for concentration and time, and a highly significant interaction between concentration and time in microbial activity. As expected, untreated water (0ppm) resulted in no significant difference in *E. coli* O157:H7 levels from t=1 to t=3hrs (Figure 8). However, after re-inoculation of *E. coli* O157:H7 at t=3.5hrs, concentration 0ppm showed a significant increase in *E. coli* O157:H7 levels due to a lack of sanitizer present to inactivate *E. coli* O157:H7. A

concentration of 30ppm was the only concentration with sanitizer present to show a significant increase in *E. coli* O157:H7 following re-inoculation at t=3.5hrs, followed by a significant decrease at t=4hrs. This correlates to a significant decrease in sanitizer PAA levels at 30ppm at t=3.5hrs (Figure 10) . Concentration of 30ppm is likely affected by reinoculation because of the pH of the sample, 30ppm active ingredients had the lowest overall pH of the samples tested which is a large factor in ensuring inactivation of microorganisms in solution (results unpublished). Gonzalez et al. (2004) observed similar results, reporting that in a solution containing 80ppm Tsunami™100 there was no recovery of *E. coli* O157:H7 in the solution after processing carrots for 2 minutes.

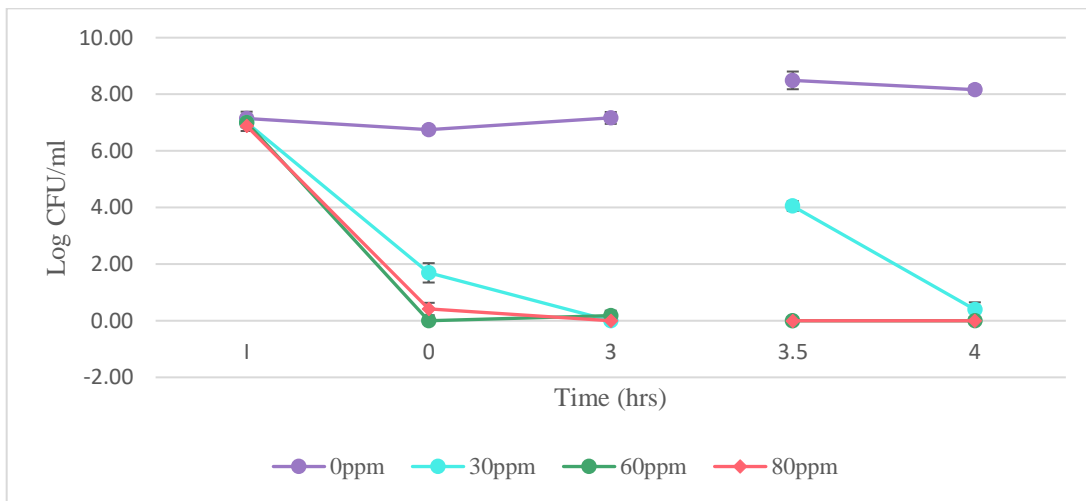


Figure 8: *E. coli* O157:H7 changes over four hours with re-inoculation at t=3.5hrs, with various concentrations of a 50/50 H₂O₂/PAA ratio in wash water over time. Error bars represent three trials with three replications.

There was a highly significant difference between concentration and time for both H₂O₂ and PAA levels. H₂O₂ showed a highly significant interaction between concentration and time, where PAA showed a significant interaction between concentration and time. All of the concentrations tested showed a significant depletion in H₂O₂ levels over 4 hours (Figure 9). PAA at 80ppm total active ingredients (40ppm PAA) had statistically similar PAA levels over

time, while treatments at 30 and 60ppm had a significant decrease after 3.5hrs suggesting that the dose concentration may impact the overall stability of PAA systems over time (Figure 10).

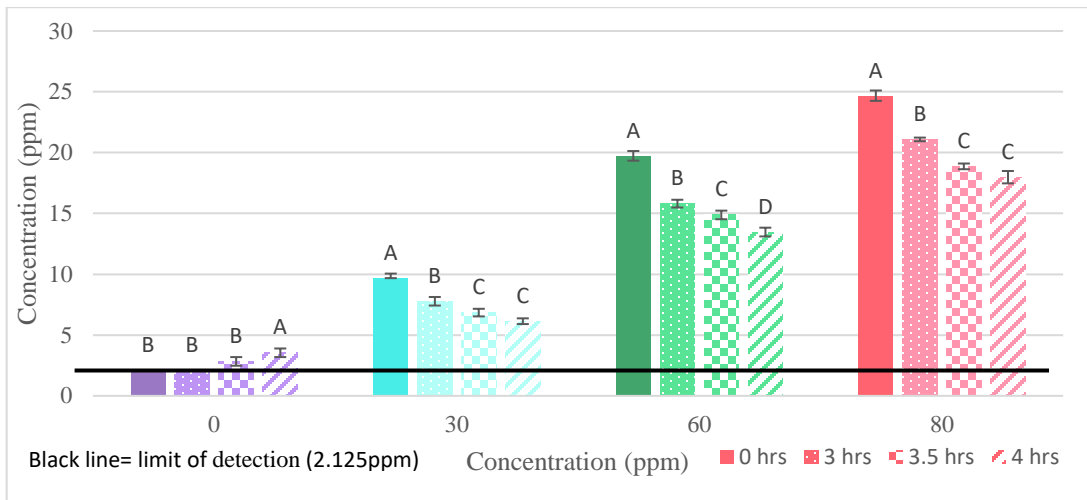


Figure 9: Changes in H₂O₂ concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs with various concentrations of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared overall (p=0.05). Error bars represent three trials with three replications.

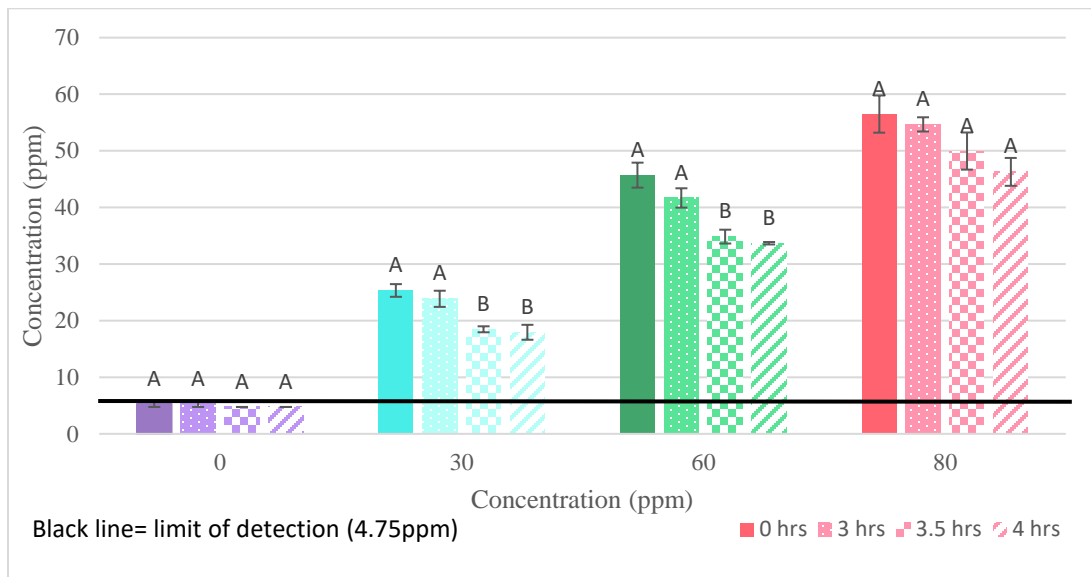


Figure 10: Changes in PAA concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs with various concentrations of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

Concentration impacts the efficacy of a 50/50 H₂O₂/PAA sanitizer solution. The higher the concentration, the more efficient a 50/50 H₂O₂/PAA ratio was at reducing *E. coli* O157:H7 levels. Sánchez et al. (2015) observed that as the concentration of PAA increased there was increasing efficacy against murine norovirus, in the presence of 10ppm PAA there was approximately a 3 log CFU/ml reduction, compared to 80ppm PAA which had approximately a 7 log CFU/ml reduction in the first five minutes. Zhang et al. (2009) measured the changes in *E. coli* O157:H7 levels in processing water after passing inoculated lettuce leaves through the water and observed that as the concentration of PAA increased, less *E. coli* O157:H7 was retrieved from the processing water. With a concentration of 10ppm PAA there was a recovery of 0.88 ±0.84 log CFU/ml of *E. coli* O157:H7 in the processing water, compared to undetectable levels recovered with 30ppm PAA present (Zhang, Ma, Phelan and Doyle, 2009). Both the study presented here, and the study conducted by Zhang et al. (2009), used 30ppm, however the differences were in the contact time, 30 seconds for this study and 1.5 mins for the study by Zhang et al. This study confirmed other research displaying that an increased concentration has an increased efficacy against *E. coli* O157:H7. The variation in efficacy based on concentrations suggest that PAA reduces microbial loads to a certain limit depending on the concentration of sanitizer present. In order to assess the influence of postharvest wash water changes, a 30ppm 50/50 H₂O₂/PAA ratio was used in the additional experiments discussed below to represent a worst-case scenario in sanitizer concentration..

3.3.5 Influence of various organic loads at 30ppm of a 50/50 H₂O₂/PAA ratio on efficacy and stability

Organic load has been known to influence sanitizer efficacy, however PAA has been seen to be less impacted compared to other options, such as chlorine-based sanitizers. Although less

impacted, PAA levels are still influenced by the presence of organic load. Little research has looked at comparing multiple COD levels, making it difficult to compare research that has been done because of differences in methods to generate artificial organic load, differences in sanitizer concentrations, and differences in contact time.

This research looked at the influence of 0, 100 and 750COD on a 50/50 H₂O₂/PAA at 30ppm total active ingredients at reducing *E. coli* O157:H7 levels. Results demonstrated a highly significant difference between organic load levels at 30ppm on microbial activity. Results also demonstrated a highly significant interaction between organic load, time and concentration, comparing with and without sanitizer present for each organic load, on microbial activity. It was seen that for all of the organic loads tested, the presence of sanitizer in the solution had a highly significant difference on *E. coli* O157:H7 levels compared to no sanitizer being present (Figure 11). All three organic load levels had a significant decrease in *E. coli* O157:H7 with a 7-log CFU/ml reduction by t=3hrs. At t=3.5hrs, with the re-inoculation of *E. coli* O157:H7, all three organic load levels showed a significant increase in *E. coli* O157:H7 levels, however 750COD was the only organic load level that did not show a significant decreased in *E. coli* O157:H7 levels from t=3.5 to t=4hrs.

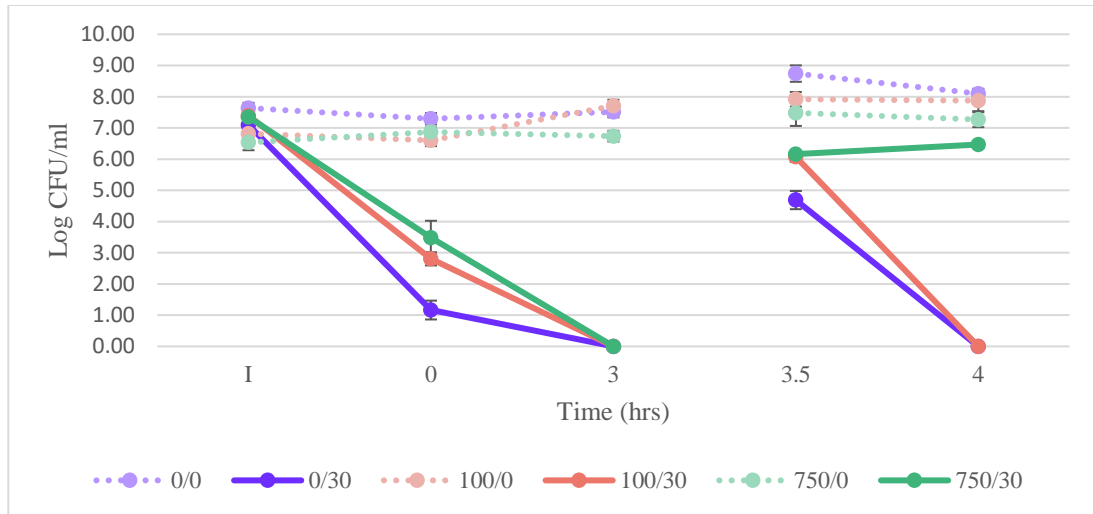


Figure 11: *E. coli* O157:H7 changes over four hours with reinoculation at $t=3.5$ hrs, with in the presence of organic load. Lines represent COD/Concentration. Error bars represent three trial with replications.

Organic load also impacted the stability of H_2O_2 and PAA over time. Results demonstrated a highly significant difference between organic loads for H_2O_2 levels. H_2O_2 levels remained constant at an organic load level of 750COD and decreased for organic load levels of 0 and 100COD (Figure 12). At 750COD with a 30ppm sanitizing solution it is seen that there is no change in H_2O_2 levels over the four hours tested (Figure 12). It is also observed that in the presence of 750COD with no sanitizer present there is a level of H_2O_2 reported, which is not seen elsewhere throughout this experiment. This is most likely due to the release of H_2O_2 from the leaves of spinach during the creation of artificial organic load (Quan, Zhang, Shi and Li, 2008). A higher organic load contains more of the blended spinach than a lower organic load, which could potentially lead to higher levels of H_2O_2 being present in the solution. Although there is H_2O_2 present at a high organic load, this does not mean that there are antimicrobial properties in the solution (refer to Figure 5). PAA levels displayed a highly significant difference among organic load levels and over time, and a highly significant interaction between organic load and concentration. Results also demonstrated a significant difference in

concentration and a significant interaction between organic load and time for PAA concentrations (Figure 13). Further research is needed to investigate a positive PAA level at 750 COD when no sanitizer is present. Monitoring sanitizer levels at 750COD was difficult due to the dark color of the solution interfering with visual changes during the titration. Although 750COD represents a worst-case scenario, changes in appearance of processing water can influence monitoring sanitizer levels, which can become a problem when monitoring levels with test strips. Although test strips and kits, are common in monitoring PAA levels as done by Zhang et al.(2009) and Davidson et al. (2017), these methods are based in color changes and this research suggests that high organic loads can influence efficacy and sanitizer levels, as well as interfere with common methods of monitoring in industry.

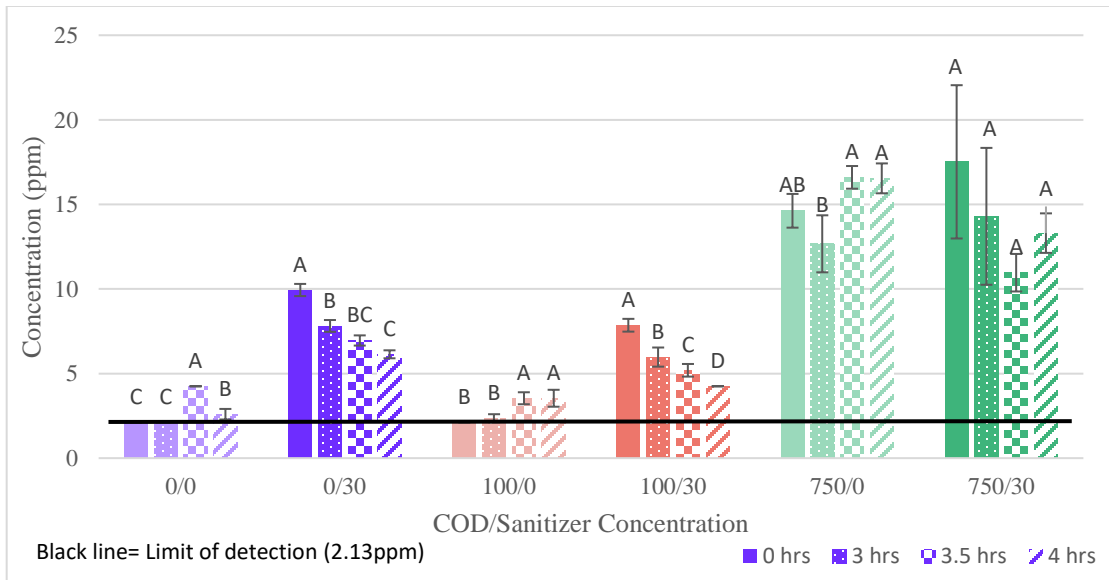


Figure 12: Changes in H₂O₂ concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5 with various organic loads at 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

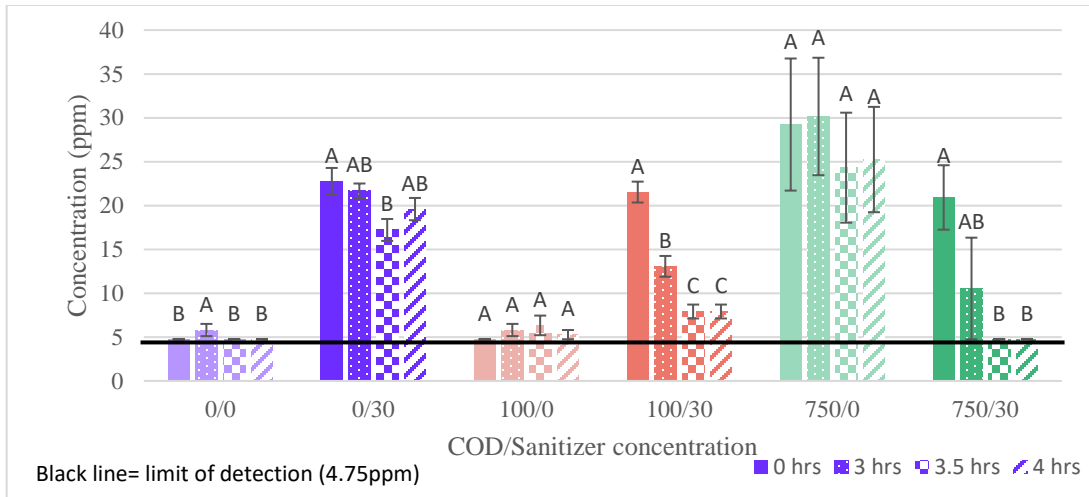


Figure 13: Changes in PAA concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs with various organic loads at 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

Throughout postharvest processing there are changes to the organic load levels in agriculture water. Organic load levels increase with each piece of produce that passes through the water from juices released from the produce, shed particulates, and bacteria that is shed off the produce during post-harvest washing. Increases in organic load can have a large impact on the efficacy and stability of PAA sanitizers over time. It can be seen here that the presence of 750COD decreased PAA concentration to the detectable limit (4.75ppm) by t=3hrs. When compared to the microbial activity in Figure 11 it is seen how this can have negative implications in processing over time as there was an increase in microbial load at t=4hrs. In this experiment, 750COD represents a worst-case scenario for organic load, however tomato processing facilities have been reported to exceed 750COD over 8 hours of processing, achieving over 400COD in the first four hours (Zhou et al., 2014b). In small processing, continued reuse of wash water also has the potential to reach high organic load levels which can impact sanitizer efficacy.

3.3.6 Influence of various temperatures at 30ppm of a 50/50 H₂O₂/PAA ratio on efficacy and stability

The temperature of the processing water at the time of adding sanitizer, and over time, may also impact efficacy. With the addition of 30ppm of 50/50 H₂O₂/PAA to solutions at different temperatures (4, 20 and 37°C) there were varying impacts on the efficacy against *E. coli* O157:H7 upon initial addition. Results showed a highly significant difference in microbial activity between temperatures, and over time, as well as a highly significant interaction between temperature, time and concentration. The greatest reduction in *E. coli* O157:H7 levels at t=0hrs was seen at 37°C, with a reduction of approximately 6-log CFU/ml, followed by 20°C with reduction of approximately 4-log CFU/ml, and 4°C showed approximately a 1-log CFU/ml reduction (Figure 14). However, all three temperatures displayed a significant reduction to undetectable levels of *E. coli* O157:H7 by t=3hrs. At t=3.5hrs, *E. coli* O157:H7 was reinoculated into the sample and all temperatures showed a significant increase in bacterial levels, with 4°C having the highest increase in *E. coli* O157:H7 levels and 37°C having the least increase in *E. coli* O157:H7 levels. However, all temperatures had undetectable levels of *E. coli* O157:H7 at t=4hrs. Little research around agriculture wash water report the temperature that testing is performed at, however, Zhang et al. (2009) reported at 4 log reduction in on lettuce inoculated with *E. coli* O157:H7 at 4C with 30ppm PAA. Compared to this study Zhang et al. (2009) was investigating the reduction in microorganisms on inoculated lettuce and had a contact time of 1.5 minutes compared to the contact time here of 30s, which most likely attributed to the larger reduction in cross contamination.

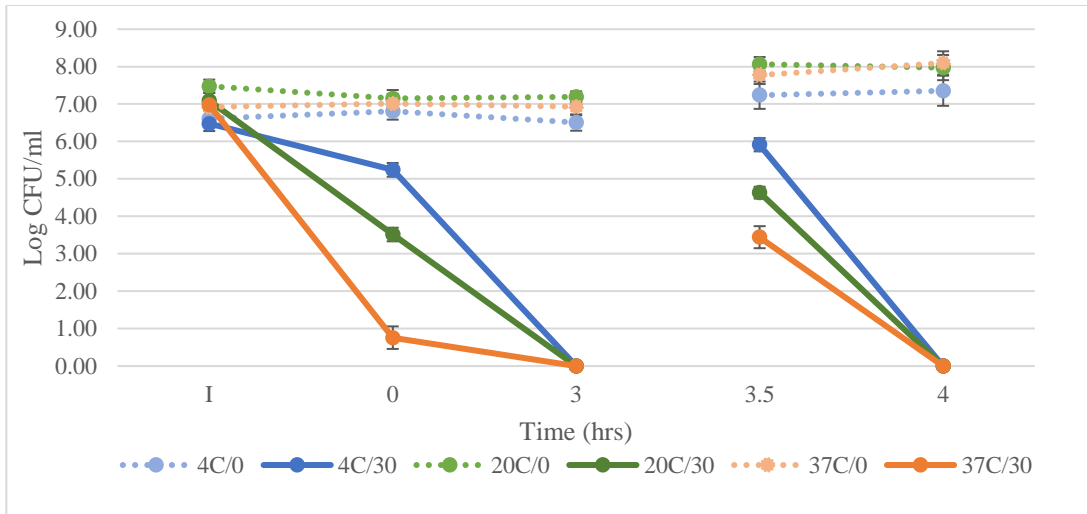


Figure 14: *E. coli* O157:H7 changes over four hours with reinoculation at $t=3.5$ hrs, at various temperatures of processing water. Lines represent Temperature/Concentration. Error bars represent three trials with three replications.

Results showed a highly significant difference in H_2O_2 levels among temperatures, and over time, and showed a highly significant interaction between temperature, time and concentrations. All three temperatures tested (4, 20, and $37^\circ C$) had a significant decrease in H_2O_2 levels over time (Figure 15). At $4^\circ C$ there was a significant decrease from $t=0$ to $t=3$ hrs, with no changes in H_2O_2 levels from $t=3.5$ to $t=4$ hrs. Temperatures 20 and $37^\circ C$ had similar patterns with a decrease in H_2O_2 levels at $t=3.5$ and $t=4$ hrs. This is surprising because $4^\circ C$ was the most impacted by *E. coli* O157:H7 at $t=3.5$ hrs but showed no significant decrease in H_2O_2 following this addition of *E. coli* O157:H7.

As seen in Figure 16, PAA at $37^\circ C$ did not significantly decrease at $t=3$ hrs, where 20 and $4^\circ C$ significantly decreased. It was also observed that $37^\circ C$ showed the most significant decrease from $t=3$ to $t=3.5$ hrs. However, with this significant decrease $37^\circ C$ still had the least amount of changes in *E. coli* O157:H7 levels at $t=3.5$ hrs.

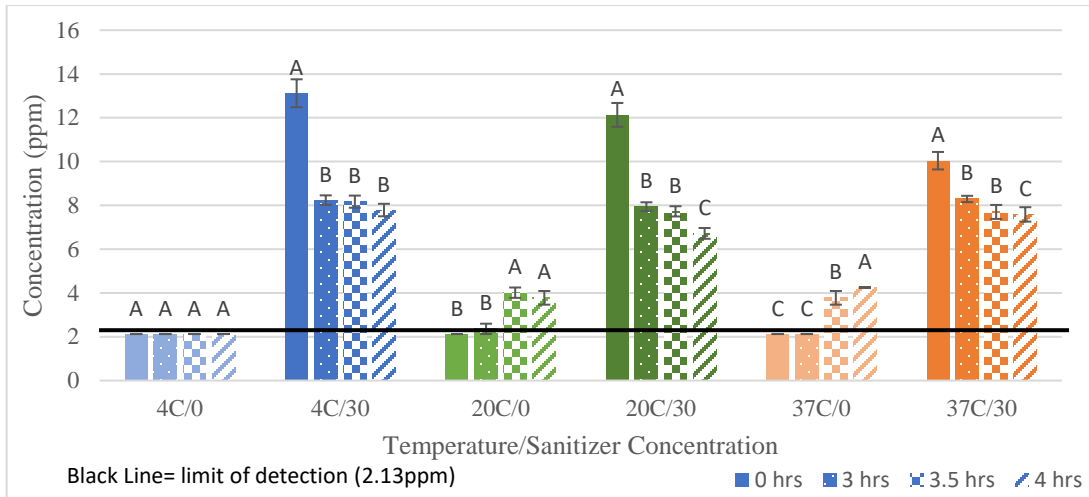


Figure 15: Changes in H₂O₂ concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5 with various water temperatures at 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

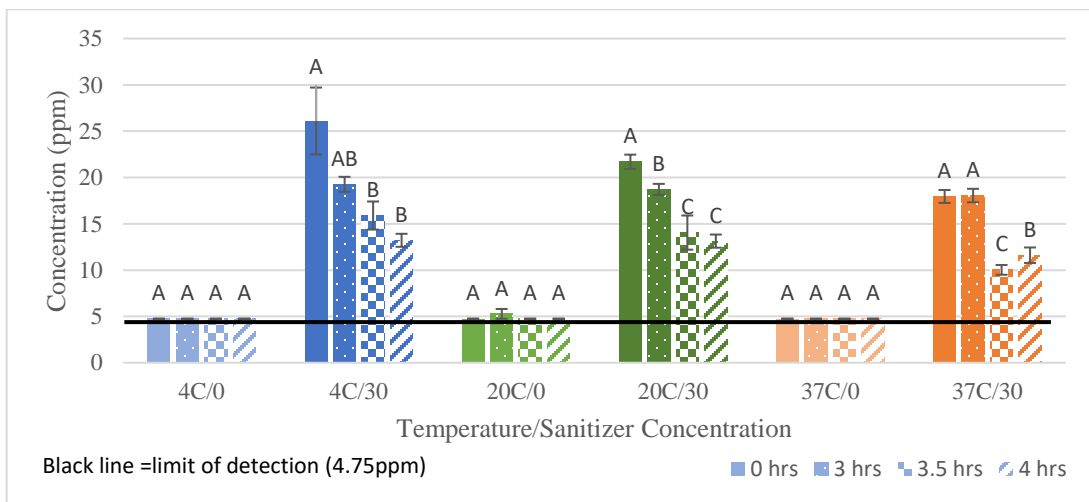


Figure 16: Changes in PAA concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs with various water temperatures at 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

Temperature can play a large role in the postharvest processing of produce. In the United States temperature can vary greatly from day to day, and temperatures of processing water can vary depending on the location at which processing is done and from the transfer of

field heat to wash water. Unexpectedly it was observed in this research that 37°C had the greatest reduction in *E. coli* O157:H7 levels at t=0hrs due to the fact that PAA has been reported to decompose faster at increased temperatures (Kunigk et al., 2001). Based on these findings, at a higher temperature PAA is more effective and more stable over the four hours tested, with the reinoculation of bacteria at t=3.5hrs. PAA is able to remain effective at reducing *E. coli* O157:H7 levels at higher temperatures.

3.3.7 Influence of various pH levels with 30ppm of a 50/50 H₂O₂/PAA ratio on efficacy and stability

Changes in the pH levels of the solution may also play a role on the efficacy of PAA against *E. coli* O157:H7 as it can be a driving factor in chemical reactions causing a shift between H₂O₂ and PAA (Figure 17), as well as cause a change in the composition of PAA if the pH exceeds the pKa of 8.2. Therefore, making it an important factor to study in order to better understand the PAA system. Results displayed a highly significant difference between pH levels, and time, as well as a highly significant interaction between pH and time in terms of microbial capacity. When there was no sanitizer present in the solution there was no significant change in the levels of *E. coli* O157:H7 over time (Figure 17). When sanitizer was present in any capacity, there was a decrease in the *E. coli* O157:H7 levels (Figure 17). pH of 5 is a solution of just the 50/50 H₂O₂/PAA ratio added, with no additional adjustments made to the pH. All three pH values showed undetectable levels of *E. coli* O157:H7 at t=3hrs with pH 5 having the most decrease in *E. coli* O157:H7 levels at t=0hrs. A pH of 9 showed the least amount of *E. coli* O157:H7 reduction from t=1 to t=3hrs with a decrease from approximately 6.54 to 6.13 log CFU/ml, this would be attributed to the pH exceeding the pKa of PAA (8.2). Following the

reinoculation of *E. coli* O157:H7 at t=3.5hrs, all pH values showed a significant increase in *E. coli* O157:H7 levels. Followed by a significant decrease in *E. coli* O157:H7 levels from t=3.5 to t=4hrs.

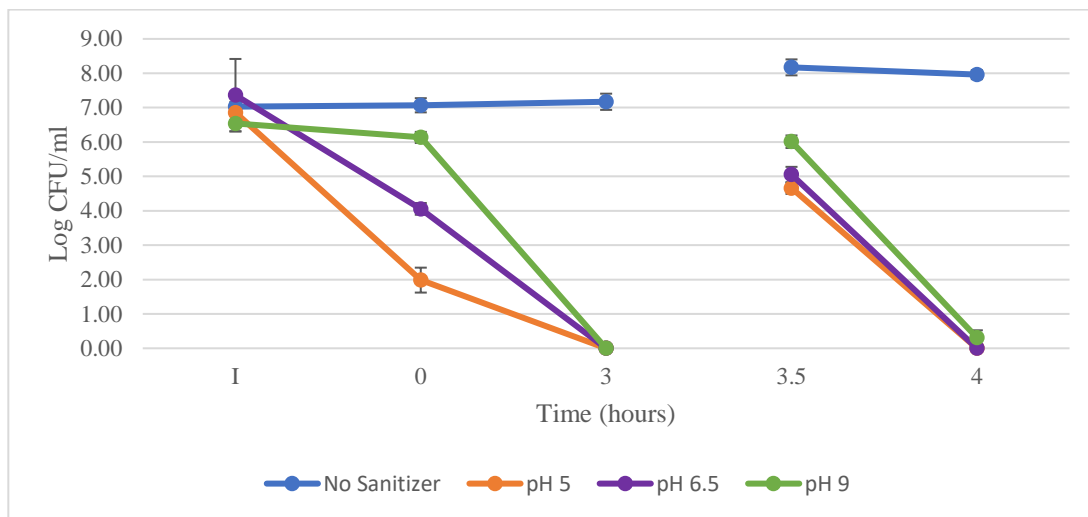


Figure 17: *E. coli* O157:H7 changes over four hours with reinoculation at t=3.5hrs, at various pH values. Error bars represent three trials with three replications.

pH also caused a change in H₂O₂ and PAA levels over the four hours tested. Significant differences were displayed between pH values and time for both H₂O₂ and PAA levels. There was also a highly significant interaction between pH and time for both H₂O₂ and PAA levels. All pH values tested displayed a significant decrease of H₂O₂ levels between t=0 and t=3hrs, pH of 6.5 also had a significant decrease from t=3.5 to t=4hrs (Figure 18). As seen in Figure 19, at pH 5 and 9 there was the most change in PAA levels over time showing a significant decrease at t=3 and t=3.5hrs. pH of 6.5 was the most stable with PAA levels showing a significant decrease from t=3 to t=3.5hrs (Figure 19).

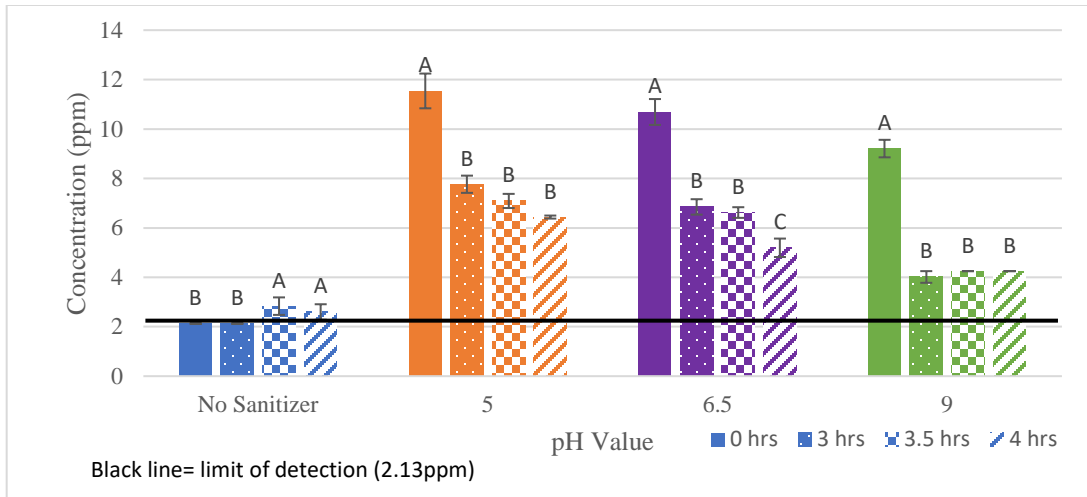


Figure 18: Changes in H₂O₂ concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5 with various pH values with 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

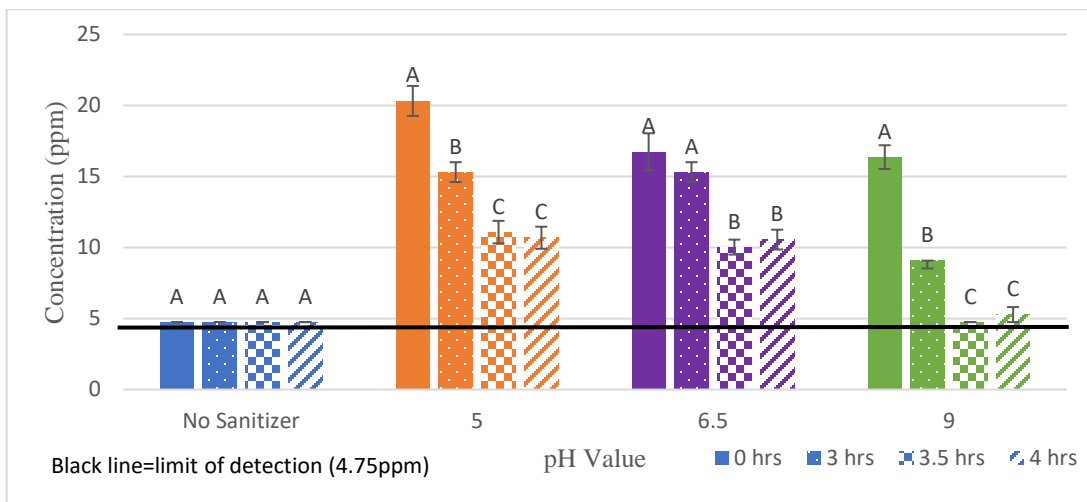


Figure 19: Changes in PAA concentrations over four hours with a re-inoculation of *E. coli* O157:H7 at t=3.5hrs with various pH values with 30ppm of a 50/50 H₂O₂/PAA sanitizing solution. Duncan testing per ratio and not compared over all (p=0.05). Error bars represent three trials with three replications.

These results do not match what has been seen previously. Majority of studies looking at PAA in agriculture water don't adjust or report monitoring the pH level of the water. However, it was seen by Yuan et al. (1997) that at the pKa of PAA (8.2) or higher, levels of H₂O₂ would

increase due to an increase in the hydrolysis of PAA, which is not what was seen here in Figure 18. It was observed here that the H₂O₂ levels and the PAA levels decreased for all pH values tested. The study by Yuan et al. (2009) used 34% PAA and 5% H₂O₂ with the remainder being acetic acid, compared to this study using 50% PAA and 50% H₂O₂. The differences in H₂O₂ and PAA ratios would most likely explain the differences in results compared to the study by Yuan et al. (2009) and would be worth further exploring with various H₂O₂/PAA ratios. Although an increase in pH did affect the stability of both H₂O₂ and PAA in the solution, the lower pH values had less of an impact on the depletion of the chemicals. It is also important to note that although a pH of 5 had more significant decreases of PAA in the solution over time compared to pH 6.5, pH 5 had a stronger antimicrobial capacity against *E. coli* O157:H7 in the solution. In production a change in pH would be most attributed to a decrease or a total loss in sanitizer concentration over time. If sanitizer concentrations are maintained pH will be of little concern but it is important to understand its influence on stability and efficacy as it is a factor that can drive a shift in chemical reactions.

Several times throughout this data it is seen that there is an increase in H₂O₂ levels after the addition of *E. coli* O157:H7 at time t=3.5hrs, when there was no sanitizer added to the sample. It would be beneficial to look into the impact of the addition of TSB both with and without *E. coli* O157:H7 to better understand the impact that the addition is having on those readings, allowing for an explanation as to that phenomenon.

3.4 Conclusion

This experiment showed that the concentrations of PAA and H₂O₂ changes throughout a 12-week storage time at 5, 20 and 37°C, indicating that the relationship between PAA and H₂O₂ is dynamic and constantly working to maintain equilibrium within the chemical equation. This

experiment later showed that efficacy and stability of H₂O₂ and PAA in solution is impacted by the ratio of sanitizers, concentration, organic load present, temperature and pH of the solution. Overall this research showed that adding PAA sanitizer to an aqueous solution can impact sanitizers in various ways, and cause shifts in the chemical reaction between H₂O₂ and PAA. Specifically, it was reported that the ratio of H₂O₂ and PAA can influence sanitizer efficacy, higher concentrations had higher efficacy and stability over time, high organic loads had impacted sanitizer concentrations over time and reporting of results, a higher water temperature was observed to have a faster decrease in *E. coli* O157:H7 levels, and pH 9 showed the least stability over time compared to lower pH values.

These findings show the importance on continuously monitoring water quality and adjusting accordingly. Future research is needed to understand the impact that all of these factors combined can play on the efficacy and stability of PAA. It would also be beneficial to look at these patterns in terms of commercial sanitizers to see if the same patterns are observed.

CHAPTER 4

MATHEMATICAL MODELING TO EVALUATE THE RATES OF DEGRADATION FOR HYDROGEN PEROXIDE AND PERACETIC ACID IN POSTHARVEST WASH WATER UNDER VARIOUS STRESSED CONDITONS

4.1 Introduction

Peracetic acid (PAA) is a strong oxidizer that has been used commercially in agriculture wash water as an alternative to chlorine-based sanitizer (Kitis, 2004). PAA has benefits over chlorine as it has been observed to be more stable in the presence of organic load, does not produce harmful by-products, and it works over a wide range of pH values (Gombas et al., 2017; Ölmez and Kretschmar, 2009; Fatica and Schneider, 2009). Commercially available PAA sanitizers are found in tandem with hydrogen peroxide (H_2O_2), with both listed as active ingredients on the label, which can come in various ratios of PAA and H_2O_2 . For example, Tsunami™ 100 is 15.2% PAA and 11.2% H_2O_2 , Sanidate 5.0 has 5.3% PAA and 23% H_2O_2 , Perasan OG has 21.5% PAA and 5% H_2O_2 , and Oxidate has 2% PAA and 27.1% H_2O_2 . It was reported in Chapter 3 that various H_2O_2 /PAA ratios can impact sanitizer efficacy and stability.

Agriculture wash water is a dynamic system, meaning the quality changes throughout processing. There can be changes in organic load, temperature and pH, which have the potential to impact PAA concentration over time. However, in Chapter 3 it was observed that although PAA is efficient against *Escherichia coli* O157:H7 under various conditions, PAA and H_2O_2 levels deplete over time in dynamic processing water. Previous work has used models to predict available chlorine levels in wash water, however Little research has used models to predict changes in PAA levels over extended periods of time, specifically in agriculture wash water. Based off of the data collected in Chapter 3, this study further explored the changes of H_2O_2 and

PAA levels over time in the presence of various H₂O₂/PAA ratios, concentrations, organic load levels, temperatures and pH levels, by using the Weibullian mathematical model in order to determine rates of depletion and predict H₂O₂ and PAA levels at various times throughout processing.

4.2 Material and Methods

Data collected from Chapter 3 was used in this study to investigate the overall rates of degradation for each of the parameters measured (H₂O₂/PAA ratio, concentration, organic load, temperature and pH).

4.2.1 Modeling of degradation rate using Weibullian Model. H₂O₂ and PAA levels were plotted as function of time and fit to the Weibull model for each parameter measured (refer to Chapter 3). The Weibull model was chosen because it displayed the smallest mean square error of the collected data, indicating the best fit compared to a linear model.

The data was fit with a Weibullian model using the solver tool on Microsoft Excel (Microsoft, Seattle, WA). The Weibullian model used here can be expressed as:

$$C(t) = 1 - bt^n \quad (Eq. 1)$$

where $C(t)$ is the concentration of H₂O₂ or PAA in solution over time, b represents the rate of degradation of H₂O₂ or PAA, t represents time, and n is the shape parameter.

4.2.2 Statistical analysis. All experiments were measured in triplicate for all analysis. Analysis of variance (ANOVA) was used to determine statistical differences at a level of significance of $p=0.05$ using SAS statistical software (SAS Institute, Cary, NC).

4.3 Results

4.3.1 Rate of degradation of various ratios of H₂O₂/PAA ratios

There was a highly significant interaction between the H₂O₂/PAA ratio and time for H₂O₂ levels, and a significant interaction between the H₂O₂/PAA ratio and time for PAA levels. Table 3 shows the degradation rates of the normalized H₂O₂ and PAA levels of the five H₂O₂/PAA ratios that were tested. It is seen that ratio 100/0 (100% H₂O₂), had the fastest rate of degradation at 0.5542hour⁻¹ for H₂O₂ overall. Ratio 25/75 H₂O₂/PAA had the fastest rate of degradation at 0.0053hour⁻¹ for PAA levels. Ratio 50/50 had the slowest rate of degradation for H₂O₂ levels at 0.0819hour⁻¹. As FDA regulates the levels of PAA in agriculture water, with a limit at 80ppm, figure 20 highlights the relationship between 0/100 (100%PAA), 50/50 and 100/0 (100%H₂O₂) H₂O₂/PAA using the Weibullian Model. It can be seen that PAA has faster rates of degradation than H₂O₂ overall. Going forward with the experiment, a 50/50 H₂O₂/PAA ratio was chosen in order to remain unbiased towards either chemical.

Table 3: Degradation rate of H₂O₂ and PAA for five H₂O₂/PAA ratios estimated using the Weibull model (Eq. 1)

Ratio (H ₂ O ₂ /PAA)	Degradation Rate (hour ⁻¹)	
	H ₂ O ₂	PAA
0/100	0.1152	0.0017
25/75	0.1809	0.0053
50/50	0.0819	0.0008
75/25	0.3683	0.0003
100/0	0.5542	0.0000

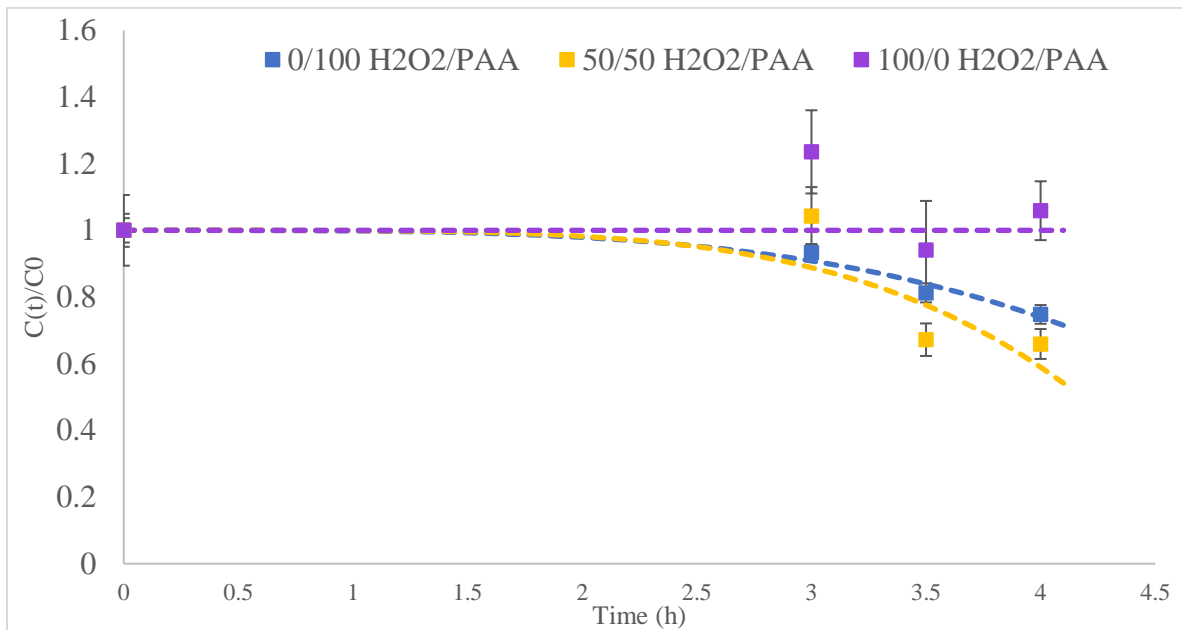


Figure 20: PAA normalized concentrations as a function of time of various H₂O₂/PAA ratios in the presence of *E. coli* O157:H7. Dash lines represent the fit with the Weibullian Model. Filled Symbols represent experimental data. Standard error bars represent three trials with three replications per trial.

Understanding the relationship between H₂O₂ and PAA is important when determining the best sanitizer option for an individual operation because there are many commercially available PAA based sanitizer options with various H₂O₂/PAA ratios. It is demonstrated here that the ratio between H₂O₂ and PAA can affect the stability by influencing the rate of degradation of sanitizer in wash water and that PAA is a more stable compound than H₂O₂ under these conditions. This research suggests that a commercial sanitizer with equal parts PAA and H₂O₂ will result in the slowest rate of degradation over time. No research was found investigating the relationship between H₂O₂ and PAA in agriculture wash water to better understand their influences on one another.

4.3.2 Rate of degradation at various concentrations

There was a highly significant difference between concentration and time for both H₂O₂ and PAA levels. As well as a highly significant interaction between concentration and time for H₂O₂, and a significant interaction between concentration and time for PAA. Table 4 shows the degradations rates of the normalized levels of H₂O₂ and PAA with the four concentrations tested of a 50/50 H₂O₂/PAA sanitizer ratio. It is seen that 80ppm active ingredient had the slowest rate of degradation overall for both H₂O₂ and PAA at 0.0173hour⁻¹ and 0.0002hour⁻¹ respectively, compared to 30 and 60ppm active ingredients. Figures 21 highlights the relationship of concentration and PAA levels over time using the Weibullian Model. It can be seen that 80ppm has the slowest rate of degradation overall and that H₂O₂ levels overall have a faster rate of degradation than PAA concentrations. Going forward with the experiment, 30ppm of a 50/50 H₂O₂/PAA sanitizer ratio will be used because the low levels represent a worst-case scenario within these parameters. The low rate of degradation at 80ppm for PAA aligns with the results observed in Chapter 3, where there were no statistical differences in PAA levels over the four hours.

Table 4: Degradation Rate of H₂O₂ and PAA of various concentrations of a 50/50 H₂O₂/PAA ratio estimated using the Weibull Model (Eq. 1)

Concentration (ppm)	Degradation Rate (hour ⁻¹)	
	H ₂ O ₂	PAA
0	0.0000	0.0003
30	0.0209	0.0024
60	0.0309	0.0062
80	0.0173	0.0002

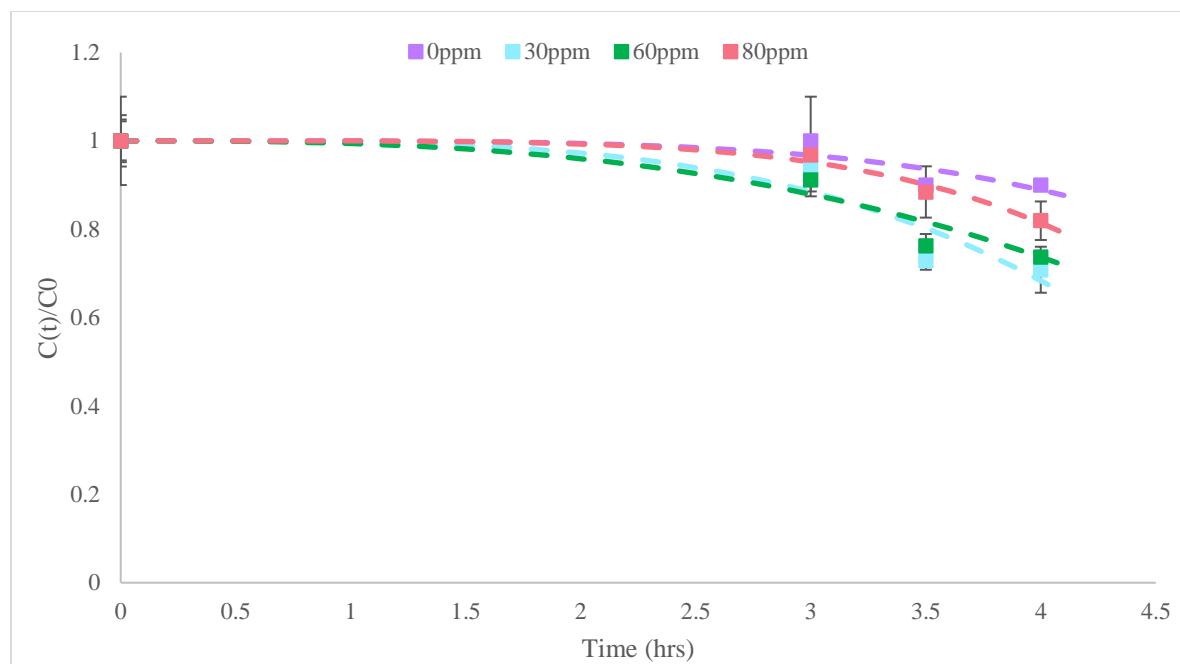


Figure 21: PAA normalized concentrations as a function of time with various concentrations of a 50/50 H₂O₂/PAA sanitizer in the presence of *E. coli* O157:H7. Dash lines represent the fit with the Weibullian Model. Filled symbols represent experimental data. Standard error bars represent three trails with three replications per trial.

It is demonstrated here that when changing the concentration of a 50/50 H₂O₂/PAA sanitizer, PAA is more stable than H₂O₂. Previous work has demonstrated that higher concentrations of PAA in solution have an increased efficacy against pathogens. Vandekinderen et al. (2009) observed that microbial inactivation increased proportionally as the concentration of PAA increased. It was demonstrated that a concentration of 120ppm PAA reduced microbial levels by 1.2 log CFU/ml when processing leeks, compared to a 0.4 log CFU/ml reduction at 20ppm of PAA. Zhang et al. (2009) also reported similar results, observing that in processing water with 10ppm PAA there was a recovery of approximately 0.9 log CFU/ml of *E. coli* O157:H7 in processing water compared to undetectable levels in the presence of 30ppm PAA. Although understanding microbial efficacy is important it is also important to understand

sanitizer stability, no research was found observing the relationship between concentration and time. Increased concentration showed increased efficacy against pathogens in previous work (refer to Chapter 3), and increased stability over time. Comparing the results seen in Chapter 3 to the rates of degradation calculated here for PAA levels, it suggests that changes in concentration will have an influence on when sanitizer levels will need to be adjusted in processing.

4.3.3 Rate of degradation under various organic loads

There was a highly significant difference between organic loads tested for both H₂O₂ and PAA levels. Table 5 shows the degradation rates of the normalized H₂O₂ and PAA levels of the three organic loads tested with 30ppm of a 50/50 H₂O₂/PAA sanitizer ratio. It is seen that 750COD has the fastest rate of degradation for both H₂O₂ and PAA levels at 0.1109hour⁻¹ and 0.1224hour⁻¹ respectively. Figure 22 highlights the relationship between 0, 100 and 750COD. It can be seen that 750COD has the fastest rate of degradation. Under the influence of organic load, PAA demonstrated faster rates of degradation than H₂O₂, which is the opposite of what is seen throughout the rest of the reported results. These results differ from the statistical patterns seen in Chapter 3, where 750COD had no statistical differences in H₂O₂ levels over the four hours, however it is believed that there was interference in the titration method in the presence of a high organic load due to not being able to observe a color change in the titration which is represented more clearly in the previous chapter. Going forward with this experiment there will be no organic load present in the samples to avoid interference with the results.

Table 5: Degradation rate of H₂O₂ and PAA at 30ppm of a 50/50 H₂O₂/PAA ratio under various organic load conditions estimated using the Weibull Model (Eq. 1)

Organic Load (COD)	Degradation Rate (hour ⁻¹)	
	H ₂ O ₂	PAA
0	0.0244	0.0156
100	0.0199	0.0898
750	0.1109	0.1224

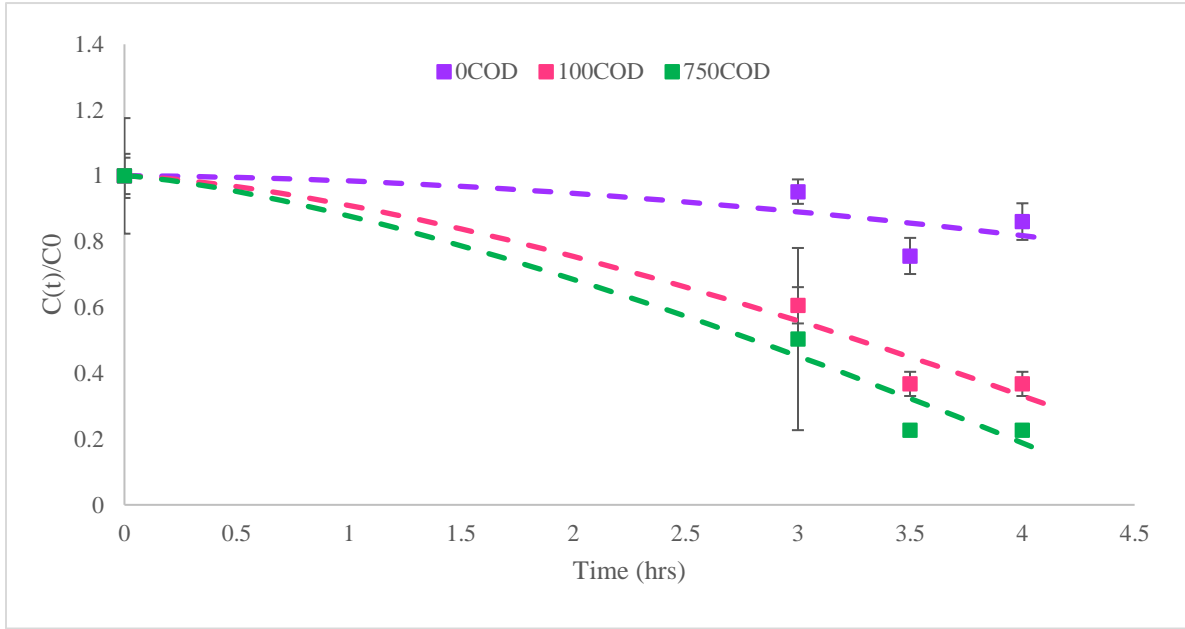


Figure 22: PAA normalized concentrations as a function of time with various organic loads at 30ppm of a 50/50 H₂O₂/PAA sanitizer in the presence of *E. coli* O157:H7. Dash lines represent the fit with the Weibullian Model. Filled symbols represent experimental data. Standard error bars represent three trails with three replications per trial.

There are continuous changes in organic load throughout processing as each piece of produce passes through agriculture water, shedding particulates and bacteria into the water. Organic load plays a large role on the rate of degradation for both H₂O₂ and PAA concentrations. It is seen here that as the organic load level increases, there is an increase in H₂O₂ and PAA rates of degradation. Van Haute et al. (2015) observed that in the presence of 30COD the depletion in PAA concentration was almost negligible compared to when in the presence of 835COD, which

showed a decrease in PAA levels of approximately 46% over sixty minutes. Although these results are similar to those demonstrated here, this study predicted the rate at which H₂O₂ and PAA would degrade in the presence of organic load, rather than just measuring the changes in concentration over time. Monitoring sanitizer levels in food processing often uses monitoring tools that are related to color changes, such as test kits and test strips, however increased organic loads create a darker color in agriculture wash water, which can interfere with interpreting results. Being able to use a mathematical model to establish a rate of degradation and predict PAA levels may allow for a better-established monitoring tool throughout processing.

4.3.4 Rate of degradation with various temperatures

There was a highly significant interaction between temperature and time for H₂O₂ concentrations, and a highly significant difference between temperature and time for PAA concentrations. Table 6 shows the degradation rates of the normalized H₂O₂ and PAA levels of the three water temperatures tested (4, 20 and 37°C) at 30ppm of a 50/50 H₂O₂/PAA sanitizer ratio. It can be seen that 4°C has the fastest rate of degradation overall for both H₂O₂ and PAA levels at 0.2373hour⁻¹ and 0.0264hour⁻¹ respectively. With 37°C having the slowest rate of degradation for H₂O₂ and PAA concentrations at 0.0538hour⁻¹ and 0.0027hour⁻¹ respectively. Figure 23 highlights the relationship between each temperature tested. It can be seen that 37°C has the slowest rate of degradation overall.

Table 6: Degradation rate of H₂O₂ and PAA at 30ppm of a 50/50 H₂O₂/PAA ratio under various temperature conditions estimated using the Weibull Model (Eq. 1)

Temperature (°C)	Degradation Rate (hour ⁻¹)	
	H ₂ O ₂	PAA
4	0.2373	0.0264
20	0.1201	0.0082

37	0.0538	0.0027
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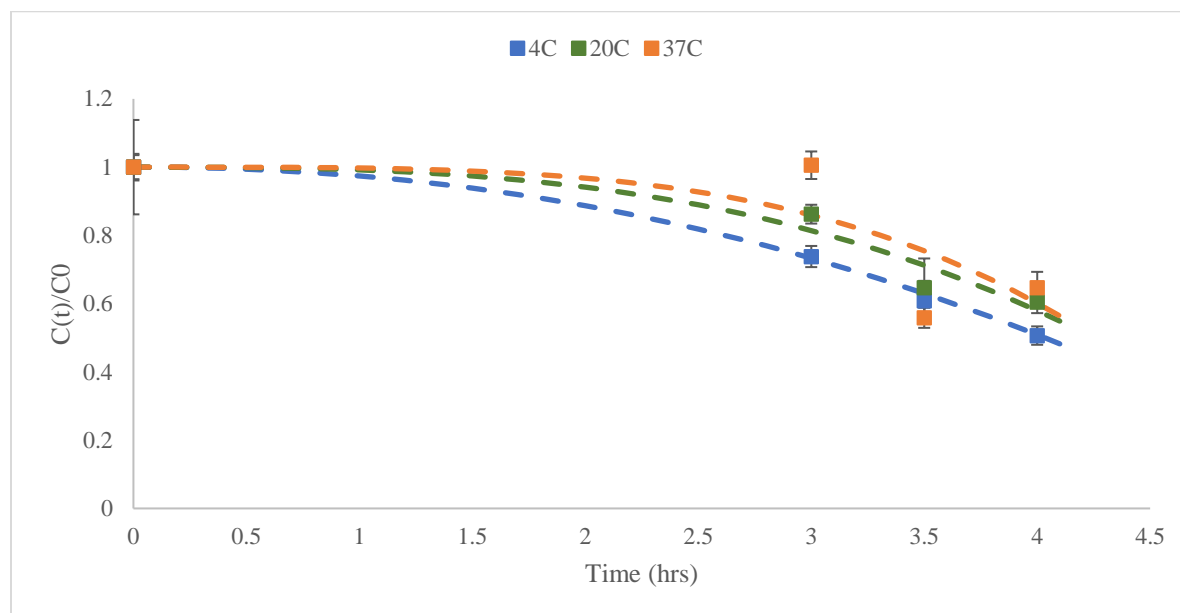


Figure 23: PAA normalized concentration as a function of time with various temperatures at 30ppm of a 50/50 H₂O₂/PAA sanitizer in the presence of *E. coli* O157:H7. Dash lines represent the fit with the Weibullian Model. Filled symbols represent experimental data. Standard error bars represent three trials with three replications per trial.

Temperatures in postharvest processing water can vary throughout processing as field heat is transferred from produce to water during washing. Unexpectedly, 37°C had the slowest rate of degradation compared to 4 and 20°C. This was unexpected because PAA has been reported to decompose faster at increased temperatures (Kunigk et al., 2001). Kunigk et al. (2001) observed that the decomposition rate of PAA at 45°C was almost 5 times faster than at 25°C. This differs from the results reported here, however, Kunigk et al. used a PAA sanitizer containing 5% PAA and 20% H₂O₂, compared to 50% PAA and 50% H₂O₂ used here, as seen in Table 4, variation in the H₂O₂/PAA ratio can impact sanitizer degradation.. In research focused on agriculture wash water, temperature has not been thoroughly explored, and in very little research has reported temperature as a factor that is monitored. These results suggest that

temperature changes during processing influence degradation over time which has the potential to impact efficacy.

4.3.5 Rate of degradation at various pH values

There was a highly significant difference between pH and time, as well as a highly significant interaction between pH and time for both H₂O₂ and PAA levels. Table 7 shows the degradation rates of the normalized H₂O₂ and PAA levels of the pH values that were tested. It is seen that a pH of 9 has the fastest rate of degradation for both H₂O₂ and PAA at 0.3747hour⁻¹ and 0.1243hour⁻¹ respectively. Figure 24 highlights the relationship between each pH level that was tested and time. It can be seen that pH of 9 has the fastest rate of degradation and a pH of 6.5 has the slowest rate of degradation.

Table 7: Degradation rate of H₂O₂ and PAA at 30ppm of a 50/50 H₂O₂/PAA ratio under various pH conditions estimated using the Weibull Model (Eq. 1)

pH	Degradation Rate (hour ⁻¹)	
	H ₂ O ₂	PAA
No sanitizer	0	0
5	0.1038	0.0348
6.5	0.0780	0.0067
9	0.3747	0.1243

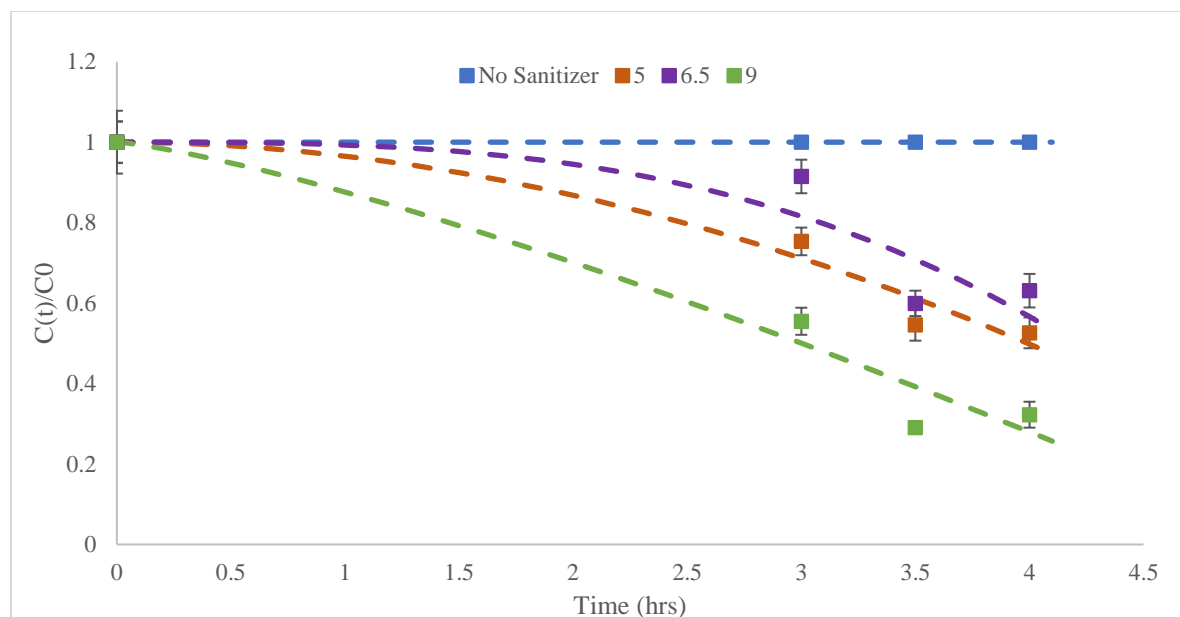


Figure 24: PAA normalized concentrations as a function of time with various pH's at 30ppm of a 50/50 H₂O₂/PAA sanitizer in the presence of *E. coli* O157:H7. Dash lines represent the fit with the Weibullian Model. Filled symbols represent experimental data. Standard error bars represent three trails with three replications per trial.

A pH of 9 having the fastest rate of degradation was expected because this exceeded the pKa of PAA (8.2). It was unexpected that the slowest rate of degradation was at a pH of 6.5, as it was expected that a pH of 5 would have the slowest rate of degradation because the pH was unaltered by sodium hydroxide. Although an increase in pH did affect the rates of degradation for both H₂O₂ and PAA in solution, lower pH values had slower rates of degradation. PAA is reported to be tolerant over a wide range of pH values (1-8) (Fatica and Schneider, 2009), which explains why 5 and 6.5 are most stable over time. As PAA is not pH dependent in agriculture wash water, it is often unmonitored in agriculture water research, however it is worth investigating as it is a factor that can influence chemical reactions and degrade PAA. It would be worth exploring other pH values in processing water to determine what pH is optimal to maintain

a stable PAA concentration over time to gain a better understanding of the chemical reaction between H₂O₂ and PAA.

4.4 Conclusion

The stability of H₂O₂ and PAA in solution is impacted by the ratio of sanitizers, concentration, organic load, temperature and pH of the solution. It was observed that an increased level of PAA in the H₂O₂/PAA ratio, an increased concentration of sanitizer, decreased organic load, increased temperature and acidic pH conditions all decreased the rate of degradation for both H₂O₂ and PAA. Overall H₂O₂ had faster rates of degradation compared to PAA, except in the presence of organic load. Of all of the conditions studied, a concentration of 80ppm had the slowest rate of degradation for PAA concentration overall.

Understanding how all of these factors can cause a depletion in the H₂O₂ and PAA levels over time is important when choosing the right sanitizer for individual operations. Modeling is a tool that can be used to estimate the concentrations of PAA in wash water that is an alternative to using test kits and test strips which may experience interference in their readings based on the water quality. This research shows the importance of monitoring water quality as changes can influence the rates of depletion for H₂O₂ and PAA. As this research demonstrates, modeling can be a useful tool to estimate H₂O₂ and PAA levels within the specified parameters. Developing a model that is able to compile the data presented here into one model, allowing processors to input the qualities of their wash water, in order to estimate sanitizer levels would provide an alternate tool for processors to adjust sanitizer accordingly to remain under federal limits.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The first part of this experiment looked at the influence of temperature on the storage of a commercially available PAA sanitizer. It also investigated the influence of three different organic load levels on the stability of the product over three hours. It was observed that for all of the storage temperatures tested (4, 20, and 37°C) there was a quadratic relationship seen over the 12 weeks. It was also seen that for H₂O₂ concentrations there was an inverse quadratic relationship to the PAA concentrations. This highlights the equilibrium, inverse, reaction between H₂O₂ and PAA in the chemical equation. In the presence of organic load, H₂O₂ showed no changes in concentration over the three hours for any of the organic loads tested (0, 100, 750 COD). PAA showed no concentration changes in the presence of 0 and 100COD, but there was a significant decrease in PAA concentrations from t=0 to t=3hrs in the presence of 750COD.

The experiment then took a closer look at the influence that various H₂O₂ and PAA ratios can have on the efficacy against *E. coli* O157:H7 and stability of H₂O₂ and PAA concentrations over four hours, with a reinoculation of *E. coli* O157:H7 at 3.5 hours. Ratios that had higher levels of PAA in reduced *E. coli* O157:H7 more efficiently. A ratio of 100% H₂O₂ did not decrease *E. coli* O157:H7 levels over the four hours tested. Ratios with higher levels of H₂O₂ had faster rates of depletion over four hours compared to those with higher levels of PAA. Rates of PAA depletion were slower than the rates of depletion of H₂O₂.

The investigation then looked at the influence of concentration, organic load, temperature, and pH on the efficacy against *E. coli* O157:H7 and the stability of H₂O₂ and PAA at 30ppm active ingredients of a 50/50 H₂O₂/PAA solution over four hours, with the addition of more *E. coli* O157:H7 at 3.5 hours. All of the parameters tested were effective against *E. coli*

O157:H7 at various capacities. For all scenarios tested, there was a 7-log CFU/ml reduction in *E. coli* O157:H7 by t=4hrs, except when in the presence of 750COD. With 750COD, following the addition of *E. coli* O157:H7 at t=3.5hrs there was an increase in *E. coli* O157:H7 levels that remained constant through t=4hrs. All scenarios tested also showed a decrease in both H₂O₂ and PAA concentrations over the four hours tested at various rates of depletion. It was seen, however, that 80ppm had the slowest rate of depletion in PAA concentrations at 0.0002hour⁻¹ compared to all of the parameters tested.

Understanding the efficacy and rates of depletion of H₂O₂ and PAA in wash water is important to maintain a level of safety among produce. This research shows the importance of maintaining sanitizer levels, and monitoring wash water quality by illustrating how many variables can influence sanitizer levels over time. Understanding the rates of depletion can help tailor wash water maintenance to specific operations and help to establish validation for operations.

APPENDIX A

ESCHERICHIA COLI O157:H7 GROWTH CURVE

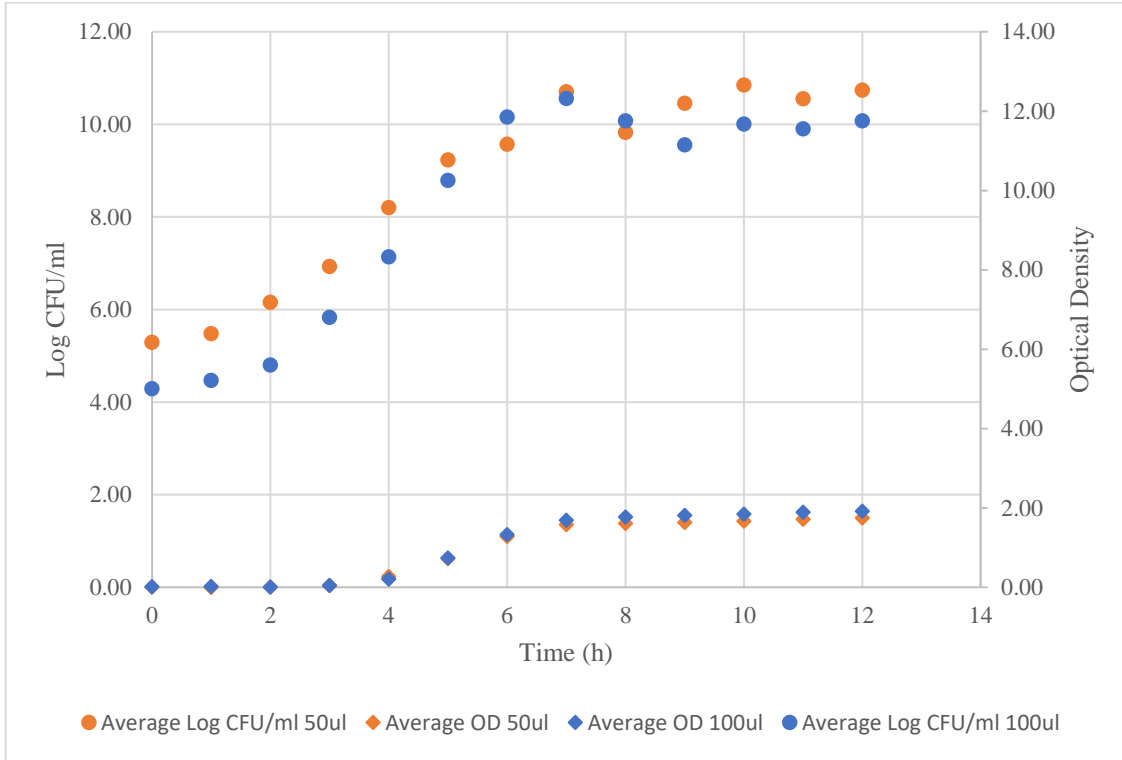


Figure 25: *Escherichia coli* O157:H7 growth curve with 50 and 100 μ g NAL

APPENDIX B

STABILITY OF TSUNAMI 100 OVER 9 HOURS

In a commercial PAA sanitizer, there is a combination of hydrogen peroxide (H₂O₂) and peracetic acid (PAA), along with other ingredients to help maintain the products stability. This experiment was completed to see the depletion of H₂O₂ and PAA over a 9-hour period in the presence of a high organic load (500 COD), starting at a level of 30ppm PAA (vol/vol). At each hour in this time period microbial load, pH, and sanitizer levels were measured, along with measuring COD at time 0 and 9. The results showed that pH had no significant difference over time after the sanitizer was added, and COD did not change from t=0 to t=9, there was only a statistically significant difference seen compared to the initial values for both measurements.

With H₂O₂ and PAA being the active ingredients, it was observed that PAA depletes over time. As seen in figure 26, the H₂O₂ levels did not deplete over the 9-hour period of being

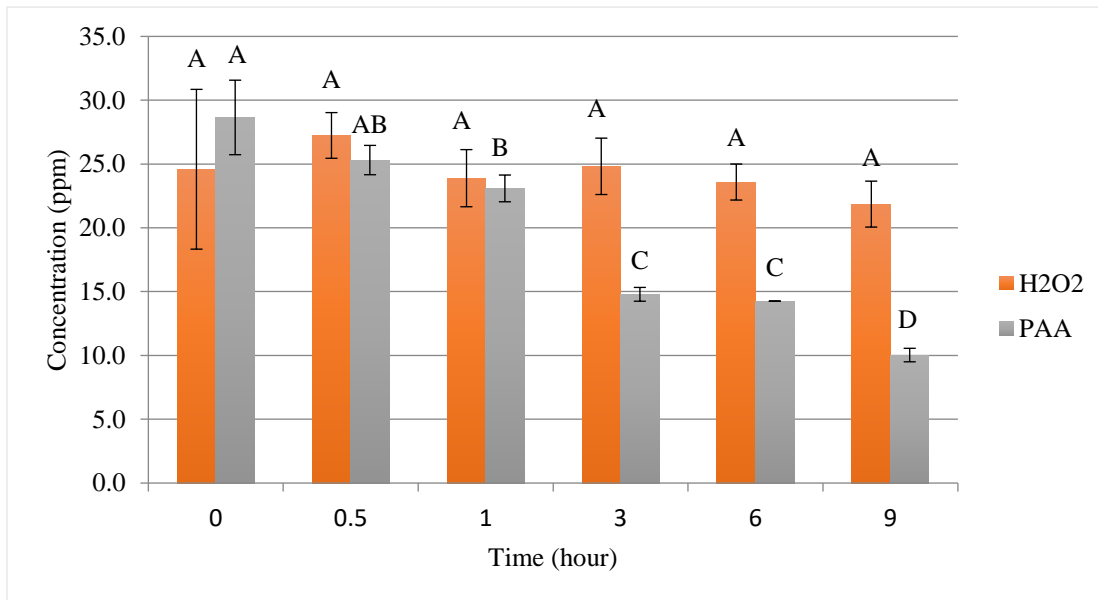


Figure 26: Changes in H₂O₂ and PAA concentrations over 9 hours in the presence of 500COD organic load. Duncan testing performed among individual chemicals and not compared to one another (p=0.05). Error bars represent three trials with three replications per trial

exposed to vegetative material, where the PAA depleted dramatically. Showing more stability of the H₂O₂ in the presence of vegetative material.

Along with measuring the depletion of these two chemicals, depletion in microbial load was also observed. It was seen that there was a 2-log reduction in microbial load, from the initial measurement to 30mins of exposure of the sanitizer. Although this is not sufficient to show that this non-thermal process has a high enough reduction to be effective it is showing that there is some reduction upon exposure. There were a few experimental design flaws that could contribute to this low log reduction: one is that only 0.1ml sample was plated, meaning that a log CFU/ml of 2 was the only value that was obtainable, and the second was the initial inoculum, the initial inoculum was 10⁴, if this value had been higher, then a final value of 2 Log CFU/ml would have been significant enough for a non-thermal process.

From this experiment we were able to move forward with using three hours as the time point in all of the other experiments, due to the significant decrease at three hours. This was a time point that showed a significant decrease in PAA concentrations from t=0, as well as allowing for changes over multiple hours to be observed.

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