COMPARSION OF EFFICACY OF APPLIANCES TO BE USED AT HOME AND OTHER PLACES FOR FRESH PRODUCE DECONTAMINATION AND CLEANING

by

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ABSTRACT

Consumers need assistance in avoiding food-borne outbreaks associated with bacteria such as *Salmonella*. For fresh produce, heat treatments are not typically an option. In this study, three appliances with non-thermal technology were tested to determine efficiency and efficacy of washing and sanitizing fresh produce. Two of the appliances are currently available to the public, while one is still in development and testing in our laboratory. Grape tomatoes and spring salad mixes were used as samples to provide different surface textures: smooth and rough. They were inoculated using two methods, dip or spot inoculation, with a four-strain *Salmonella* cocktail to achieve an initial population of approximately 8-log CFU/g. The apparatuses involved used either ozone, ultrasonic, or ultrasound to treat the samples, and the samples are treated within 4 gallons of water at 3, 6 and 9 minutes, respectively. The results for inactivation of the *Salmonella* included about a 3-log reduction for tomatoes, and about less than a 1-log reduction for salad when sanitized with ozone, and ozone with ultrasound, and about a 3-log reduction for tomatoes and about a 2-log reduction for salad when sanitized with shortwave ultraviolet light radiation. The effect of duration of treatment time on *Salmonella* inactivation can be noted here, as the longer the samples underwent each treatment, the number of bacterial colonies reduced. At 3 minutes, the UV apparatus had the best inactivation amount compared to the other two apparatuses. In terms of texture and color change, the UV with water agitation did not significantly affect texture and color, the use of ozone significantly affected texture, and the combination of ozone with ultrasound significantly affected texture and color.

In the future, UV with water agitation could be a viable alternative – instead of washing under running water- for consumers who want to keep the quality of their fresh produce.

Chapter 1

INTRODUCTION

People eat food because they need vitamins, minerals, and energy food provides. A big part of that, people have been increasingly consuming fresh produce as part of their diet for decades now to lead healthier lives (Marx-Pienaar and Alet, 2014). With the demand for fresh produce having gone up, the need to increase production came with it. For example, according to Chatziprodromidou (2018), production increased by over 94% between 1980 and 2004. The increased consumption of fresh produce continued even in 2008, when the recession hit the United States, with vegetables being consumed at an increase of 20.6% while fruit was consumed at an increase of 16.2% ("Fresh Produce Consumption Growing Rapidly" 2017). There are programs that have been developed in schools for children to consume more fruits and vegetables, especially in less wealthy areas of the country that may not always have access to fresh produce (Staudigel, 2018).

Most, if not all, people expect the food they buy to be safe to consume, especially organic fresh produce (Henneberry 1999). Yet, without fail, every single year, the FDA reports there are foodborne outbreaks due to pathogenic bacteria (Nutrition, 2021). The kind of bacteria vary between outbreaks; although, the common ones include *Salmonella*, *Listeria monocytogenes*, and pathogenic *Escherichia coli* species (Botondi et al., 2021).

Of those mentioned before, *Salmonella* has been involved in many outbreaks over the last several decades, but definitely within the last four years it has been involved in a multitude of outbreaks (Brown et al, 2016). Varying serovars have been responsible for infections involving leafy greens, including *Salmonella Newport* and *Salmonella Typhimurium* (Chittick et al. 2006). There have also been outbreaks of *Salmonella* that relate back to tomatoes, including a case in Virginia where Salmonella Newport was found in a field of tomatoes in a range of years, spanning from 2002 to 2010 (Angelo, 2014).

Thermal technology is a way of reducing or eliminating bacteria, such as *Salmonella*. According to several studies (Biswas et al. 2019, Dash et al. 2022, Gerdts et al 2021), heat is an effective tool to inactivate the bacteria and prevent further replication. Some people enjoy grilling their fruits and vegetables and the nutritional value is more likely to kept this way ("Benefits of Grilled Vegetables", 2019). But not all fresh produce is consumed in this manner and so while thermal technology is important to keep in mind, it will not be the focus of this review.

In the food industry, steps are taken to ensure that infections of bacteria are minimized. In recent years, there has been a push across the food processing industry for 'cleaner' methods of decontaminating and sanitizing food (Yi, 2001). Traditionally, in the case of fresh produce, chlorine washes are approved by the government, so long as the chlorine residue amounts don't exceed 3ppm (Center for Food Safety and Applied Nutrition, 2019). These chlorine washes- along with other washes- can be limited by low efficacy, potential high cost, and the possibility of

residue being left behind on the food products (Kenney, 2002, Park, 2008, Rahman, 2016). Another way the industry tries to reduce outbreaks is through a series of procedures known as the Hazard Analysis and Critical Control Point (HACCP) (Kharub, 2018). It's known as an effective tool to help manufacturers implement standards important to maintaining a safe environment for food production. It may be effective, but it does not guarantee a company will be able to achieve its highest level of safety (Trafialek, Wojciech 2017).

Where the industry can sometimes fall short on its safety standards, decontaminating and cleaning fresh produce at home could help significantly reduce outbreaks. Yet the chances of an infection occurring at home are still significantly probable (CDC, 2019). It's been recommended that after buying fruits and vegetables, they should be cleaned by placing them under running water (Zander, Bunning, 2010). In more recent years, people have been buying produce washes or they have been using acids such as distilled white vinegar to help reduce bacterial contamination (Zander, Bunning, 2010). Other than this, people have been using ozone as a way to decontaminate their fresh produce, as it's being marketed as a way to get rid of bacteria, viruses, and fungi (Absolute Ozone, 2023).

Besides making sure the produce is safe to eat, people also need to make sure they are following the proper safety guidelines for cooking in the kitchen. Cross contamination is one of the more common ways people can become ill from cooking in their own kitchen: from using the same knife and cutting board to cut lettuce and raw chicken, to not washing or drying their hands separately (Sapatkin, 2015).

Currently, there are few options for consumers to wash their fruits and vegetables, some of which are mentioned above. Compared to simply putting fresh produce under running water or using a produce wash that may leave residues, using shortwave ultraviolet light (UV) with water agitation will decrease the amount of pathogenic and non-pathogenic bacteria attached to fresh produce bought from the store (Guo et al, 2017)

According to Yao (2021), UV has been used to decontaminate drinking water for many years now and according to many studies done (Guo et al., 2017; Guo et al., 2019; Huang et al., 2018; Yao et al. 2021) it can also be used to decontaminate fresh produce. By itself, UV cannot do this alone; it needs water to help scatter the UV rays.

In this study, there are a few objectives that are prioritized to look at and understand how varying non-thermal technologies can affect fresh produce. The first objective is to compare the different technologies at different treatment times between various appliances to determine the efficacy of reducing bacteria, in this case, *Salmonella*. The second objective is to determine food acceptability after each decontamination cycle. This will be done by comparing color values, weight values, and texture values from before and after every treatment cycle. The third objective is to evaluate the effectiveness of every treatment and treatment time for each appliance in order to determine which one is more likely to reduce or eliminate bacteria the most and still maintain the fresh produce's quality and structural integrity. There will not be a tasting panel during this study; perhaps in a future study, this may be evaluated further.

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Chapter 2

LITERATURE REVIEW

2.1 *Salmonella* Characteristics

Salmonella can be identified by their rod shape, are known to be a facultative anaerobic, and are a gram-negative bacterium. There are currently only two known species, *Salmonella bongori* and *Salmonella enterica*. These can be further categorized as either typhoidal or non-typhoidal (Gal-Mor, Boyle, Grassl, 2014). *Salmonella* enterica has thousands of serovars, although a small percentage of them can cause issues within people (Ibarra & Mortimer, 2009). These include Typhimurium, Enteritidis, Infantis, Newport, and Poona (Alvarez-Fernandez et al. 2011)

Salmonella is difficult to inactivate, as it can't be frozen to death (Sorrells et al. 1969, Beuchat & Heaton 1975), but it can be inactivated at relatively high temperatures such as 55°C for as long as an hour and a half, or the time can shorten to just 12 minutes at 60°C (Goodfellow & Brown 1978). At temperatures lower than mentioned, the *Salmonella* can persist (Mandal & Kwon, 2017). For food that is to be cooked, such as chicken or beef, the goal of inactivating the bacteria can be accomplished, but it's a different challenge for fresh produce.

2.1.1 Infection & Outbreaks

According to Heymans et al (2018), salmonellosis is a massive problem for the human population, with an estimation of 93.8 million cases of *Salmonella*

gastroenteritis happening every year and about 155 thousand people die. *Salmonella* can enter many kinds of cells, including epithelial cells, microfold cells, and macrophages (LaRock, Chaudhary & Miller, 2016). Because of its facultative anerobic nature, it can use oxygen to create ATP or it can create ATP by fermentation of less efficient electron acceptors (Garai et al. 2012). This lets the bacteria survive in many places for longer periods of time.

The kind of people that are more likely to become sick from the bacteria include those with lowered immune systems: children, elderly, pregnant people, and immunocompromised people (Center for Veterinary Medicine, 2019). These symptoms can include diarrhea, fever, and headaches, with more serious reactions including meningitis, depending on the *Salmonella* strain (Popa & Papa, 2021). Also, according to Popa & Papa (2021), about 80% of cases are not linked to any known outbreak and are considered sporadic cases.

An outbreak occurs when a specific disease has an unexpected increase in cases in a specific area. *Salmonella* Typhimurium is among one of the top Salmonella serovars to be reported and documented (Kuhn & Ethelberg 2020). Just last year, in 2022, there was an outbreak involving this strain with alfalfa sprouts (CDC 2023). Going a bit further back, in 2021, there were outbreaks involving the serovars Typhimurium and Oranienburg with prepackaged salad and onions, respectively (CDC, 2023). Also, in 2020, *Salmonella* Newport contaminated onions, and in 2018, *Salmonella* Montevideo infected raw sprouts (CDC, 2023). Even going further back to 2013, there was a *Salmonella* Saintpaul outbreak of cucumbers (CDC, 2023). When looking back on the outbreaks, every year for the last fifteen years, there's been an

outbreak with fresh produce involving *Salmonella* (CDC, 2023). The FDA also reports on these outbreaks and detail ongoing and closed investigations (FDA, 2023).

There are a couple of ways for fresh produce to become contaminated with bacteria. In some cases, *Salmonella* can be present within the soil and when the conditions are more wet in the fields, there's a chance for the bacteria to infect the produce (Schierstaedt et al., 2020, Lee et al., 2019). In other cases, the bacteria can be infected during post-harvest handling and transportation due to lack of gloves by workers, increased relative humidity, an increase in light, or the re-use of wash water for the fresh produce (Zhou et al., 2013).

2.2 Technology & Hurdle Effect

Any method for decontamination and cleaning of produce needs to be used in such a way that the consumer will still enjoy the fresh produce in the way it's meant to be enjoyed (Barrett et al., 2010). Using any form of thermal decontamination isn't ideal in the slightest for fresh produce, as this changes the physical properties of the fresh produce negatively (Yao, 2021). So, non-thermal technology will be the focus of this literature review.

The main theoretical foundations used in this study include the use of ozone, ultrasound, and shortwave UV light technologies combined with water and water agitation to achieve appropriate bacterial reduction and maintain the structural integrity of fresh produce. Each technology has its own strengths and limitations.

When it comes to combining technologies together, this is known as the 'hurdle effect' (Ockerman et al, 2014). The efficacy of two or more technologies

combined in one in order to further inactivate bacteria has been studied extensively (Piyasena et al. 2003, Ahmed et al., 2022, Francisco et al., 2017).

2.2.1 Ozone

Ozone can be classified as an inorganic molecule. It is the allotropic form of oxygen and is considered highly reactive (Streng, 1961). It's typically generated by oxygen splitting apart and attaching to a third single atom of oxygen making its known chemical formula O_3 (Premjit et al. 2022). This is usually done via electrical discharge (Batakliev et al. 2014).

Many apply the ozone in its gaseous phase without a liquid medium (Singla, 2021). It's been shown that ozone in its gaseous form is more stable than its aqueous counterpart. (Botondi et. al, 2021). When ozone is in water, it decays much faster and is considered highly soluble (Brodowska et. al, 2017). There are two different ways to apply ozone to either water or food: venturi injection, and a fine bubble diffusion. The venturi injection is applied via pressurized inlets via a vacuum. The fine bubble diffusion is applied by generation of the ozone by an ozone generator through porous aeration material and it expands through the water like bubbles (Aslam et al 2020).

When it comes to the safety of using ozone in the food industry, the FDA gave the potential additive a 'GRAS' -generally recognized as safe- designation. It's been allowed for use in water sanitation since 1982 and in food sanitation in 2001. It's also allowed to be used in organic food production (McHugh, 2015). It's worth noting that

this is under the assumption that the concentrations are less than 0.1 ppm, as any more is considered toxic to humans (Batakliev et al. 2014).

The effectiveness of ozone has been studied in the food industry for many years. For example, at varying ozone concentrations (0.07 ppm, 0.15 ppm, and 0.25 ppm) at different times (2 and 5 minutes), ozone was found to inhibit mold growth and maintain quality in the strawberry sample used (with the exception of the highest concentration of ozone, as this did contribute to negative quality changes) (Aday et al. 2013). In a different study, ozone was used in its gaseous phase for surface decontamination of tomatoes, at about 10 mg/L. According to Das et al. (2006), the ozone treatments were found to be effective at 5 and 10 minutes, respectively, at the reduction of *Salmonella* Enteritidis.

The way ozone interacts with bacteria to kill it is simple: when ozone breaks down into its two molecules (O_2 and O_1), the reactivity of this reaction is enough to destroy the integrity of the cell walls within a microorganism and it oxidizes essential components (such as enzymes or proteins), causing the microorganism to be unable to replicate (Rangel et al., 2021). This reaction is considered incredibly potent and can destroy a range of bacteria, viruses, and fungi (Guzel-Seydim et al. 2004).

There are some limitations to the use of the technology. According to the EPA (1999), too low of a dosage can be inefficient at inactivating some microorganisms. The equipment required, on a large industrial scale, is complex and needs constant maintenance for proper use. The cost of the treatments can become super expensive due to the amount of power required to generate the ozone and break it and any by-

products down. On a smaller scale for the consumers, the same logic can be applied. The appliances currently on the market that consumers can buy are expensive: they can range in price from $69.99 to over $200 (Amazon, 2023). According to the EPA (2014), none of the ozone generators for home use have been approved by any federal agency and the currently available scientific evidence shows it can be difficult to control ozone exposure, especially if the ozone comes into contact with chemicals, which can create unhealthy by-products such as aldehydes and formic acid (EPA 2014).

An appliance on the market currently made by WSTA (Shenzhen Wangcheng New Energy Technology Co. Ltd.), Ozone-Purifier air and water, applies ozone through the tubing and an aeration stone. It can be used by itself or within a bowl of water. Compared to the literature right now, this appliance's purpose, strengths, and limitations should fall into what is expected: the ozone will significantly reduce the number of bacteria present in the food samples (Xu, 1999; Horvitz et al., 2013). This appliance is relatively simple to use: assemble the tubing and aeration stone, place them within a bowl of water (or empty bowl, depending on desired use), and press the buttons once or multiple times for the desired setting. It exudes about 500 mg/h of ozone, or about 237 ppm.

2.2.2 Ultrasound

Ultrasound can be classified as energy made from sound waves at varying frequencies, starting at 20 kHz (Afari et al. 2016). According to Bhargava et al.

(2021), it is typically generated by a transducer, which converts electrical pulses into intense energy. Two types of transducers can create these frequencies: magnetostriction and piezoelectric. The former works by using magnetization and magnetostrictive material (such as cobalt or iron), while the latter works by a piezoelectric material (such as a quartz crystal) undergoing force, it creates electrical charges on the surface (Bhargava et al 2021). Regardless of which transducer is used, when these electrical pulses go through a liquid medium, it creates vibrations (Beitia et al. 2023). Equipment made for generating the ultrasound can have a frequency range of 20 kHz to 10 MHz (Piyasena et al. 2003). This technology is considered to be non-thermal.

When the frequencies are low, but the power level is high, it's known as "power ultrasound" and is capable of something called cavitation (Bilek & Turatas, 2013), which will be discussed in further detail below. It is uncommon for this technology to be used by itself, as various studies (Afari et al 2016, Piyasena et al 2003, Bonah et al. 2021), have shown it is more effective when combined with sanitizers and other technologies. This allows it to be used more broadly (Francisco et al. 2017).

Ultrasound can affect the protein structures of bacteria. Oscillations produced by ultrasound- previously mentioned as cavitation- can start off as stable and uniform gas bubbles, before eventually erupting and collapsing these gas bubbles rapidly (Gallo et al. 2018; Silventoinen, 2020). It's interesting to note, according to Silventoinen (2020), that cavitation can cause other things to happen such as mixing,

microstreaming currents, shear stresses, and turbulence. This is how, when interacting with bacteria, the bacterial cell walls are disrupted and break down (He et al. 2021). When it comes to food, the power and frequency of the ultrasound matter: the application of low frequency and high power will not cause differences in food products, while still affecting bacteria (Kadam et al., 2015).

In the food industry, ultrasound is considered an environmentally friendly technology (Bernardo et al. 2021). It's being used more as it doesn't affect quality or texture of the fresh produce (Seymour et al. 2002). As of now, there isn't a large-scale industrial plant dedicated to only using ultrasound for food production but there is a patented water system that has proven to be efficient in inactivating bacteria (Bilek & Turantas, 2013).

There was a study done with *Salmonella* Typhimurium and using ultrasound as a way to reduce it. In iceberg lettuce, there was a 1.5 log reduction when the ultrasound was set to 40 kHz, and in cherry tomatoes there was a 0.8 log reduction when the ultrasound was set to 45 kHz (Bilek & Turantas, 2013).

An appliance currently on the market by Bestlife (Huiliu), Ultrasonic and Ozone Vegetable & Fruit Sterilizer, applies both ozone and ultrasound in bursts. This apparatus specifically always requires water. According to the literature reviewed already (Ockerman et al. 2014), this appliance should outperform the other two, at least somewhat significantly. It combines the use of ozone and ultrasound, for the hurdle effect (Dietrich et al. 2017). It's easy to use: fill the container to the max fill line, place your food sample inside, top it with the lid, and press the button for the

desired setting. It exudes about or more than 200 mg/hr of ozone, or about 94.6 ppm and has a frequency of 40 kHz.

2.2.3 Shortwave UV

Ultraviolet light is a form of electromagnetic radiation with wavelengths between 100 and 400 nm. These wavelengths can be broken down further into three separate bands depending on the treatment to be done: band one is 320 to 400 (UV-A), band two is 280 to 320 nm (UV-B), and band three is 200-280 nm (UV-C) (Riganakos et al. 2017). For food applications, a wavelength of about 254 nm is used to result in the inactivation of microorganisms (Bintsis et al. 2000). Also, according to Bintsis et al. (2000), the way these wavelengths are generated by mercury lamp tubes without a phosphor coating, as glass can absorb UV-C. The lamps are considered to be low-pressure, so they're meant to be more efficient in power use. Newer technology has allowed for the development of LED lamps. The LED lamps' wavelengths can be tuned to between 255 and 280 nm (Messina et al. 2015).

UV technology has been used on an industrial scale to decontaminate and sanitize water to make it drinkable (Guerrero-Beltran et al., 2004). In the past several years, research has been conducted to determine the full effects it may have on food. In the study from Guo et al. (2017), they found treatments with shortwave ultraviolet light on tomatoes, lettuce, blueberries, strawberries, and lettuce were more effective than a simple tap water wash, ranging on average a 1-3 log reduction higher in reducing *Salmonella*. In the study from Huang et al. (2018), they found that combining

the UV treatments with chlorine washes helped reach higher inactivation of *Salmonella*, to almost undetectable limits.

When bacteria are underneath UV, pyrimidine bases within the DNA form dimers (Guo et al, 2018). These dimers can interfere with and interrupt cell replication, which can cause the bacteria to die since they aren't able to replicate (Guo et al, 2018).

The benefits of UV include not leaving behind any kind of residue and leaving the structural integrity of the fresh produce intact (Yousef et al, 1988). UV-C has benefits in post-harvest production, where it can be used to maintain the quality of the fresh produce to attain a longer shelf-life (Erkan et al. 2008). It also has influence in preventing loss in nutritional value in fresh produce when applied in post-harvest (Gogo et al. 2017). In part due to research and development, the use of UV is inexpensive and can be considered safer because there's no additional chemicals required for the UV to work (Wang et al, 2019).

There are a couple of limitations to the use of UV. For instance, if a sample is more turbid, the treatment using UV is less likely to be effective because of the absorption of the rays (Guerrero-Beltran et al., 2004). There is also concern for people's health, mainly for peoples' eyes and skin, due to the exposure to UV radiation (European Commission, 2010).

The third apparatus, which is currently being assessed for efficacy among different foods and will be compared to the other two, applied UV. With a rotating motor and a detachable paddle, the food placed within the bowl filled with water can

be decontaminated and sanitized. It works similarly to a microwave oven: adjust the motor to the desired speed and press the numbers for desired treatment time. With the literature reviewed, the results to come from this machine should be within the acceptable range of bacterial reduction while minimizing the damage done to the fresh produce.

2.3 Consumer Practices at Home

It's been noted in nearly every study involved with the literature review that the consumer's kitchen is most associated with foodborne illnesses, around nearly 87% of foodborne illness occurs in the common kitchen (Soares et al. 2012). *Salmonella* and *Campylobacter* are considered the bacteria most likely to be linked to infections at home (Barker et al 2003). People tend to be more careless in their own kitchen, which leads to unhygienic practices. One example of this includes the use of cutting boards that aren't washed correctly and then preparing raw meat and fresh produce on the same cutting board (Gkana et al. 2016). About 15% of tested sponges and dish washing cloths were found to contain *Salmonella* (Chaidez et al. 2014). Another contributing factor to infections is how infrequently people wash their hands, leading to potential cross contamination (Cogan et al .2002). If people were better informed and implemented better hygienic practices, this could lead to a decrease in cross contamination and infections.

In terms of what people do now to decontaminate and clean their fresh produce, there's a few different methods people employ. One is to place fresh produce underneath running water and gently scrub it, like the FDA recommends (FDA, 2021). Another method includes using an acidic solution such as diluted vinegar or lemon juice. One study conducted looked at several home-cleaning procedures to determine

efficacy, using a 5% vinegar solution, 13% lemon solution, a vegetable wash, and running water. They found that there wasn't a significant difference between the acidic cleaning solutions and the tap water while applying agitation (Kilonzo-Nthenge et al. 2006).

An ineffective method that some consumers do is using dish detergent, which can leave residues unsafe for consumption (Li-Cohen & Bruhn, 2002).

2.4 Contribution of Study

The contribution this study will provide is determining if the UV with water agitation machine is better than what's currently on the market for consumers right now for cleaning and washing fresh produce. Ideally, reducing bacterial contamination of fresh produce in any capacity is the goal. Additionally, maintaining the structural integrity and color of the produce is a secondary objective.

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Chapter 3

METHODS AND MATERIALS

3.1 Overview

The methodology used in this study is similar to another study (Yao, 2021). It's designed to simulate point contamination and immersion contamination of a sample, regardless of which stage the fruit or vegetable may be in (field to fridge). The consistency of the method is important to maintain when testing three different machines against each other at varying times. The strengths of the outputs of the machines do not change, only the time required to perform the treatment. At first, it was discussed that the timings would be 1, 2, 3, 6, and 9 minutes. Due to results within the initial trials, 1 and 2 minutes were deemed unnecessary and later dropped from the experimental design.

3.2 Methods

3.2.1 Culture of *Salmonella* serovars

The four *Salmonella* serovars, Montevideo, Newport, Heidelberg, and Typhimurium, used for this study were previously grown to be resistant to nalidixic acid (NA) (Fisher Scientific, NH, USA) and streptomycin (ST) (Fisher Scientific, NH, USA). To make individual stock plates of each strain, one loop of each culture from a

vial stored at -18°C was placed individually in test tubes containing 10 mL tryptic soy broth (TSB) (Becton, Dickinson and Company, MD, USA), supplemented with 6% yeast extract (YE) (HiMedia Laboratories Pvt. Ltd., India), NA and ST each at a concentration of 100 ug/mL (TSBYE-NA+ST). The test tubes were placed on an orbital shaker and incubated for 24 hours at 35°C. One loop of each strain was quadrant-streaked onto tryptic soy agar plates (TSA) (Becton, Dickinson, and Company, MD, USA) supplemented with 6% yeast extract, NA and ST each at a concentration of 100 ug/mL (TSAYE-NA+ST). Each stock plate was incubated for 48 hours at 35° C. Then, the plates were properly stored in the fridge at 4°C for four weeks.

3.2.2 *Salmonella* Cocktail Preparation

One colony from each cultivated strain- Heidelberg, Montevideo, Newport, and Typhimurium- was placed into their own respective test tube containing 10mL of TSBYE-NA+ST. The test tubes were placed on an orbital shaker and inoculated for 24 hours at 35°C. 1000 uL of each test tube was added to a glass bottle containing 200 mL of TSBYE-NA+ST. All 4 glass bottles were placed on an orbital shaker and inoculated for 48 hours at 35°C. The bacteria were poured into centrifuge bottles and placed into the centrifuge machine at 4000 x g for 10 minutes at 20°C. After the supernatant was poured off, the bacterial pellets were resuspended and combined in 0.1% peptone water (PW) (Becton, Dickinson and Company, MD, USA): 400 mL for dip inoculations and 10 mL for spot inoculations.

3.3 Produce Inoculation

There were two different types of inoculation performed in the experiments: dip and spot inoculation. These were to simulate two different contamination points for a food product anywhere from the field to the fridge of the consumer. The grape tomatoes and the spring salad mix were bought the day before inoculation.

For dip inoculation, the grape tomato samples were weighed out to 200 g and spring salad mix samples were weighed out to 100 g. Each sample was placed individually into a double-bagged Ziploc bag and the *Salmonella* cocktail was poured in. The samples were immersed in the *Salmonella* cocktail for five minutes. The cocktail was poured out and disposed of properly and the samples were taken out of the Ziploc bags. The samples were placed on a tray to air dry to visible dryness inside a laminar air flow hood for about two to three hours. Then, the samples were transferred to new Ziploc bags and stored at 4°C for 24 hours for further attachment of bacteria. The initial concentration, on average, was 10^8 CFU g/mL.

For spot inoculation, the grape tomato samples were weighed out to 200 g and spring salad mix samples were weighed out to 100 g. They were inoculated using 1 mL of the prepared *Salmonella* cocktail, by pipetting drops of the cocktail on the top surfaces of samples. After inoculation, the samples were placed in laminar air flow hoods to dry to visible dryness for about two to three hours. Then, the samples were transferred to Ziploc bags and stored at 4°C for 24 hours. The initial concentration, on average, was 10^8 CFU g/mL.

3.4 Antimicrobial Treatments

There were three apparatuses used for the treatment of the samples.

Figure 1: The Ozone-Purifier air and water machine (Brand: WSTA, manufacturer: Shenzhen Wangcheng New Energy Technoglogy Co., Ltd.) , pictured unplugged, used for the experiments.

The ozone apparatus (Figure 1) was used by inserting one end of the tubing into the aeration stone- where the ozone was dispersed from- and the other end of the tubing into the machine. The apparatus was placed into a laminar air flow hood as a safety precaution. The samples were placed into a stainless-steel bowl filled with 4 gallons of water, along with the aeration stone. The water is meant to be agitated by the ozone and not an external paddle. The apparatus ran for 3,6, and 9 minutes in triplicate. The water was poured into a large container for decontamination purposes, while the sample was taken out.

Figure 2: The Ultrasonic and Ozone Vegetable & Fruit Sterilizer (Brand: Bestlife manufacturer: Huiliu (Family Dentist) used for the experiments.

The ozone with ultrasound apparatus (Figure 2) was used by placing the metal basket inside the machine. The apparatus was placed into a laminar air flow hood as a safety precaution. The samples were added into the metal basket and 4 gallons of water were added to the machine, adding its lid on top of the apparatus when complete. The water is meant to be agitated by the ozone and penetrated by the ultrasound, and not by any external paddle. The ozone and ultrasound came from micro-holes in the inside corners of the apparatus as it ran. The apparatus ran for 3, 6, and 9 minutes in triplicate. The water was drained into a large container for decontamination purposes, while the sample was taken out.

Figure 3: The shortwave ultraviolet light radiation with water agitation apparatus used for the experiments.

The shortwave UV apparatus (Figure 3) ran for five minutes prior to use to allow the UV lamps to heat up. It was used by placing the fresh produce inside a stainless-steel bowl filled with 4 gallons of water. The bowl was placed inside the apparatus and the paddle was attached to the motor on the inside. The rotation speed was determined and set prior to the bowl being placed inside using a tachometer. For the grape tomatoes, the rotation speed was set to 190 RPM while for the spring salad mix, it was set to 110 RPM. The reason why the salad needed a lower rotation speed is because the faster the paddle went, it would shred the leaves and made it difficult to conduct the quality assessment. The apparatus ran for 3, 6, and 9 minutes in triplicate.

The water was poured into a large container for decontamination purposes, while the sample was taken out.

3.5 Microbial Analysis

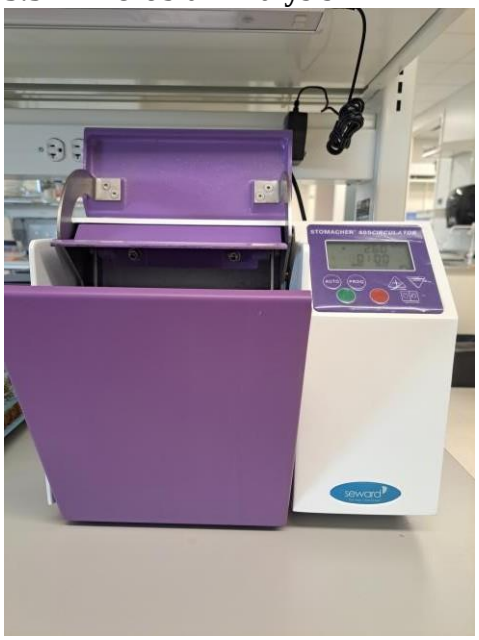

Figure 4: Stomacher 400*Circulator* (Fischer Scientific, NH, USA), set at 260 RPM for 2 minutes, for homogenizing samples within methods sections.

The initial inoculation levels were determined by splitting each sample in half, placing one half in a stomacher bag (while the other half went through treatment) and adding 200 mL of Dey-Exley (DE) broth to a stomacher bag. The bag was pummeled in a stomacher for 2 minutes at 260 RPM (Figure 4). The stomached solutions were serially diluted in 0.1% PW test tubes. The dilutions were spread onto TSAYE-NA+ST plates and the plates were incubated for 48 hours at 35°C.

After every treatment, the samples were placed into stomacher bags. Each bag was pummeled in a Stomacher for 2 minutes at 260 RPM. The stomached solutions were serially diluted in 0.1% PW test tubes. The dilutions were spread onto TSAYE-NA+ST plates and the plates were incubated for 48 hours at 35°C.

The *Salmonella* colonies on all the plates were counted after the incubation period to determine log reduction. After determining the CFU g/mL, which is found by multiplying the dilution factor by three and multiplying that by the number of colonies found, the after CFU count is divided by the initial CFU count and using a log reduction formula, the log reduction can be found.

3.6 Quality Assessment

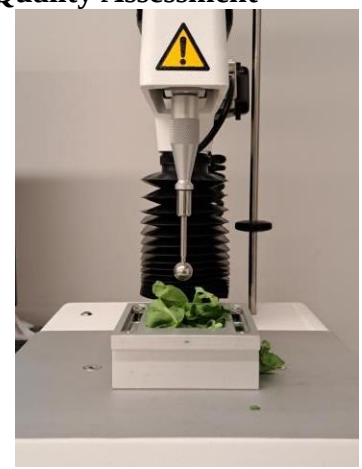

Figure 5: The TA.XT.Plus C (Stable Micro Systems, UK) texture analyzer used to determine the textures of before and after treatment of the samples. Pictured is an example of a mixed salad sample.

The uninoculated samples went through the same treatments as inoculated samples. Prior to treatment and after treatment, the uninoculated samples were weighed using a calibrated scale. Prior to treatment and after treatment, the texture values were taken using the TA.XT.Plus C texture analyzer (Stable Micro Systems, UK) (Figure 5). There was 1 cm distance between the probe and the sample prior to use, and there was total sample height of 3 cm, for the spring salad mix and a sample height of 2 cm for the grape tomatoes. The probe used was a TA-181/2" for the compression tests. The data was recorded using the software Exponent Connect. Prior to treatment and after treatment, the L.A.B. color values taken using a calibrated colorimeter (CR-10, Kocica Minoltda, Ramsey, New Jersery) once the samples were dried. The readings were taken from the non-stem end of the tomato and the top of each kind of leaf found in the spring salad mix. The figure below (Figure 3) shows the equation used to determine the color difference from before and after treatment. There were three readings taken per sample, and this was done in triplicate.

$$\Delta E = \sqrt{(L^*{}_t - L^*{}_u)^2 + (a^*{}_t - a^*{}_u)^2 + (b^*{}_t - b^*{}_u)^2}$$

Figure 6: Equation used for calculating the difference in color change between treated samples compared to prior to treatment

3.7 Statistical Analysis

Microsoft Excel (Microsoft, WA, USA) was used to analyze the raw data collected from the experiments, which again, were performed in triplicate. It calculated averages and standard deviations of the treatments and calculated the log

reductions, and forces from the texture analyzer. It calculated ANOVA variances and determined the significant differences ($P<0.05$) and the likelihood of error. It also calculated the L-A-B color values collected from every sample and determined the difference in color prior to and after treatment.

Chapter 4

RESULTS

4.1 Microbial Counts

Table 1: Log reductions of *Salmonella* on dip and spot-inoculated grape tomatoes (200 g) and spring salad mix (100 g) by ozone generated by ozone apparatus.

	Tomato		Salad	
Time (min.)	Dip	Spot	Dip	Spot
3	3.09 ± 0.28 Aa	2.96 ± 0.18 Aa	0.80 ± 0.17 Ba	0.79 ± 0.13 Ba
6	3.15 ± 0.12 Aa	3.04 ± 0.17 Aa	1.14 ± 0.16 Ba	1.0 ± 0.08 Ba
9	3.11 ± 0.14 Aa	3.06 ± 0.14 Aa	1.44 ± 0.13 Ba	1.46 ± 0.17 Ba

Every number represents the average log reduction of 3 trials along with calculated standard deviations

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

"Dip" and "Spot" indicates two types of inoculation: immersion and point-to-surface, respectively

Table 1 shows the effectiveness of using ozone as the treatment for sanitizing and cleaning. These values were found by finding the initial CFU g/L and the after CFU g/L. Those CFU g/L numbers were placed into a logarithmic formula to find the log reduction. For grape tomatoes for both inoculation methods at all treatment times,

there was around an average of a 3-log reduction of *Salmonella*. For the salad mix for both inoculation methods at all treatment times, using ozone was able to achieve about a 1-log reduction of *Salmonella*. The values between the tomato and salad mix samples are significantly different, although within the groups themselves they are not.

Table 2: Log reductions of *Salmonella* on dip and spot-inoculated grape tomatoes (200 g) and spring salad mix (100 g) by ozone and ultrasound generated with ozone and ultrasound apparatus.

	Tomato		Salad	
Time (min.)	Dip	Spot	Dip	Spot
3	1.42 ± 0.11 Aa	1.44 ± 0.28 Aa	0.58 ± 0.04 Aa	0.52 ± 0.04 Aa
6	3.31 ± 0.31 Aa	3.48 ± 0.65 Aa	1.02 ± 0.03 Ba	1.06 ± 0.07 Ba
9	3.46 ± 0.36 Aa	3.05 ± 0.52 Aa	1.43 ± 0.16 Ba	1.36 ± 0.20 Ba

Every number represents the average log reduction of 3 trials along with calculated standard deviations

Not significantly different ($P > 0.05$): same uppercase letter in same row

Not significantly different ($P > 0.05$): same lowercase in same column

"Dip" and "Spot" indicates two types of inoculation: immersion and point-to-surface, respectively

Table 2 shows the effectiveness of using ozone and ultrasound together as the treatment for sanitizing and cleaning. These values were found by finding the initial CFU g/L and the after CFU g/L. Those CFU g/L numbers were placed into a logarithmic formula to find the log reduction. For tomatoes for both inoculation methods at 3 minutes, there was just over a 1-log reduction of *Salmonella*, whereas

there were over 3-log reductions of *Salmonella* for 6 and 9 minutes. For the salad mix, at 3 minutes, there was less than 1-log reduction achieved of *Salmonella*. At 6 and 9 minutes, there was little more than a 1-log reduction of *Salmonella*. Most of the values are not significantly different from each other.

Table 3: Log reductions of *Salmonella* on dip and spot-inoculated tomatoes (200 g) and spring salad mix (100 g) by shortwave ultraviolet light generated with UV apparatus

	Tomato		Salad	
Time (min.)	Dip	Spot	Dip	Spot
3	3.41 ± 0.20 Aa	3.30 ± 0.26 Aa	2.68 ± 0.11 Ba	2.52 ± 0.04 Ba
6	3.53 ± 0.19 Aa	3.34 ± 0.37 Aa	2.68 ± 0.07 Ba	2.51 ± 0.18 Ba
9	3.59 ± 0.28 Aa	3.37 ± 0.32 Aa	2.81 ± 0.05 Ba	2.77 ± 0.03 Ba

Every number represents the average log reduction of 3 trials along with calculated standard deviations

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

"Dip" and "Spot" indicates two types of inoculation: immersion and point-to-surface, respectively

Table 3 shows the effectiveness of using shortwave ultraviolet light radiation with water agitation. These values were found by finding the initial CFU g/L and the after CFU g/L. Those CFU g/L numbers were placed into a logarithmic formula to find the log reduction. For tomatoes, for both inoculation methods at all treatment times, there was at least a 3-log reduction of *Salmonella*. For salad, for both

inoculation methods at all treatment times, there was at least a 2-log reduction of *Salmonella*. The values between the tomato and salad mix samples are significantly different; however, in between the groups themselves, they are insignificant.

4.2 Effect of treatments on produce properties

Table 4: Determination of color change due to ozone between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad(L)	Salad(S)	Salad(R)
3	2.24 ± 1.3 Aa	0.86 ± 1.08 Bb	0.41 ± 0.63 Bc	0.40 ± 0.54 Dd
6	1.74 ± 1.5 Aa	1.81 ± 0.56 Bb	3.43 ± 0.63 Cc	0.22 ± 0.34 Dd
9	2.37 ± 1.3 Aa	0.41 ± 0.39 Bb	0.46 ± 0.29 Bc	0.80 ± 0.47 Dd

"L" represents green lettuce, "S" represents spinach, and "R" represents red leaf lettuce

The salad sample was a mixed spring mix of varying quality. While the most similar ones were used for comparison, a wider sample size should be used for more accurate results

Every number represents the average and standard deviations of 3 trials of total color change (ΔE)

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

Table 5: Determination of color change due to ozone and ultrasound between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad(L)	Salad(S)	Salad(R)
3	2.41 ± 1.7 Aa	1.96 ± 0.39 Bb	1.92 ± 0.54 Bc	1.79 ± 0.44 Dd

		0.72 ± 0.73	0.47 ± 0.43	
6	3.66 ± 1.2 Aa	Bb	Cc	2.15 ± 0.5 Dd
		0.31 ± 0.75	2.13 ± 0.41	0.85 ± 0.43
9	2.49 ± 1.4 Aa	Bb	Cc	Dd

"L" represents green lettuce, "S" represents spinach, and "R" represents red leaf lettuce

The salad sample was a mixed spring mix of varying quality. While the most similar ones were used for comparison, a wider sample size should be used for more accurate results

Every number represents the average and standard deviations of 3 trials of total color change (ΔE)

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

Table 6: Determination of color change due to shortwave UV between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad(L)	Salad(S)	Salad(R)
	3.80 ± 1.7	2.93 ± 0.42	0.76 ± 0.46	10.06 ± 0.48
3	Aa	Bb	Cc	Dd
	2.78 ± 2.0	0.71 ± 0.39	2.49 ± 0.38	4.19 ± 0.39
6	Aa	Bb	Cc	Dd
	2.31 ± 1.4	0.71 ± 0.30	5.27 ± 0.37	1.03 ± 0.45
9	Aa	Bb	Cc	Dd

"L" represents green lettuce, "S" represents spinach, and "R" represents red leaf lettuce

The salad sample was a mixed spring mix of varying quality. While the most similar ones were used for comparison, a wider sample size should be used for more accurate results

Every number represents the average and standard deviations of 3 trials of total color change (ΔE)

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

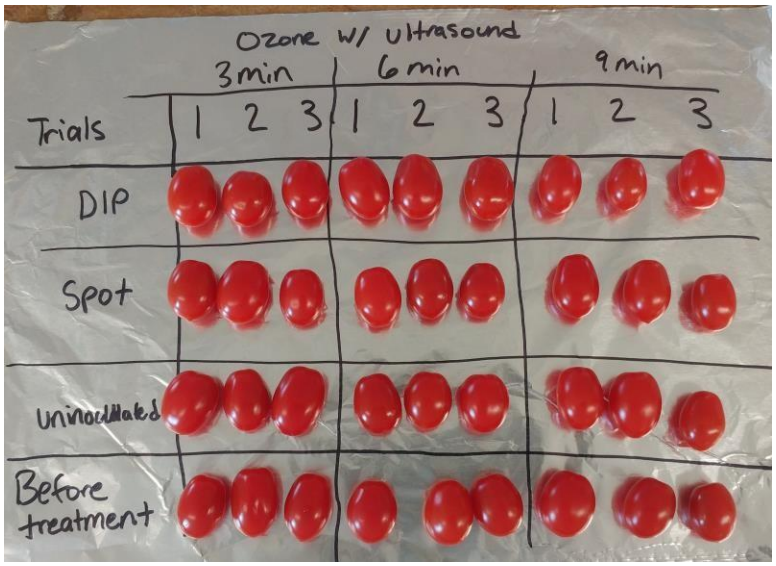

Figure 7: Picture showing how ozone and ultrasound apparatus (shown in Figure 2) impacted the tomato samples. Dip-and-spot-inoculated tomatoes, uninoculated tomatoes, and untreated tomatoes (200 g) were placed within the bin full of water to be washed and sanitized by ozone and ultrasound in alternating bursts. They appear intact; although, their texture was altered.

As the treatment time became longer, the tomatoes did see a color difference, more noticeable if held under water, and not noticeable to the naked eye, as seen in Figure 7. Tables 4, 5 and 6 show the color changes between all treatment times. These values were taken as L*, a*, and b*. L* values represent the light or darkness of a color, the a* values represent how much green or red is in a color, and the b* value

represents how much blue or yellow is in a color. Combined with the equation found in Figure 3, the difference in color can be found.

Table 7: Determination of texture change due to ozone between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	3.33 ± 10.00 Aa	-5.00 ± 12.50 Cc
6	230.00 ± 20.82 Bb	-140.00 ± 37.86 Dd
9	230.00 ± 25.17 Bb	-46.67 ± 26.46 Dd

Every number represents the average and standard deviations of 3 trials

The large standard deviations stem from large numbers from the sampling

Each number represents the difference between the average force required before treatment and average force after treatment (g/sec)

Not significantly different ($P > 0.05$): same uppercase letter in same row

Not significantly different ($P > 0.05$): same lowercase in same column

Table 8: Determination of texture change due to ozone and ultrasound between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	103.33 ± 50.0 Aa	43.33 ± 10.00 Ba
6	160.00 ±20.82 Aa	-141.67 ± 25.00 Bb
9	170.00 ± 15.28 Aa	-146.67 ± 26.46 Bb

Every number represents the average and standard deviations of 3 trials

The large standard deviations stem from large numbers from the sampling

Each number represents the difference between the average force required before treatment and average force after treatment (g/sec)

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

Table 9: Determination of texture change due to shortwave ultraviolet light between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	66.67 ± 32.15 Aa	3.33 ± 10.00 Bb
6	53.33 ± 45.83 Aa	-23.33 ± 25.17 Bb
9	-26.67 ± 26.46 Ab	-46.67 ± 26.46 Bb

Every number represents the average and standard deviations of 3 trials

The large standard deviations stem from large numbers from the sampling

Each number represents the difference between the average force required before treatment and average force after treatment (g/sec)

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

The values found in Tables 7, 8, and 9 represent the difference in force from before treatment and after treatment and these values are in g/sec. The texture differences for both the tomatoes and the salad are insignificant to each other, as

shown in Tables 7, 8, and 9. By physical touch, there is a difference. The tomatoes became squishier the longer they were subjected to treatment times and the structural integrity of the tomato was damaged. The salad became firmer the longer they were subjected to treatment times. Underneath the texture analyzer, the samples did show differences; however, they are insignificant.

Table 10: Determination of weight change due to ozone between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	2.66 ± 0.66 Aa	-6.08 ± 0.77 Bb
6	1.61 ± 0.98 Aa	-10.18 ± 0.69 Bb
9	3.88 ± 0.87 Aa	-16.13 ± 0.86 Bb

Every number represents the average and standard deviations of 3 trials in grams (g)

Every positive number indicates a loss in weight, whereas every negative number indicates a growth in weight

Not significantly different ($P > 0.05$): same uppercase letter in same row

Not significantly different ($P > 0.05$): same lowercase in same column

Table 11: Determination of weight change due to ozone and ultrasound between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	0.36 ± 1.52 Aa	-4.86 ± 1.58 Bb
6	1.19 ± 1.07 Aa	-9.07 ± 0.69 Bb
9	2.65 ± 0.83 Aa	-9.59 ± 0.29 Bb

Every number represents the average and standard deviations of 3 trials in grams (g)

Every positive number indicates a loss in weight, whereas every negative number indicates a growth in weight

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

Table 12: Determination of weight change due to shortwave ultraviolet light between untreated and treated tomato (200 g) and spring salad mix samples (100 g)

Time (min.)	Tomato	Salad
3	0.86 ± 0.98 Aa	-4.14 ± 1.04 Bb
6	0.32 ± 1.42 Aa	-5.09 ± 0.61 Bb
9	1.90 ± 0.19 Aa	-5.73 ± 0.74 Bb

Every number represents the average and standard deviations of 3 trials in grams (g)

Every positive number indicates a loss in weight, whereas every negative number indicates a growth in weight

Not significantly different (P > 0.05): same uppercase letter in same row

Not significantly different (P > 0.05): same lowercase in same column

Figure 8: Pictures showing how Figure 2's ozone and ultrasound impacted a spring salad mix. Dip-and-spot-inoculated, uninoculated, and intreated samples (100g) were placed within the bin full of water to be washed and sanitized by ozone and ultrasound in alternating bursts. The longer they were in the bin, the brighter and fuller the salad became.

The values found in Tables 10, 11, and 12 were calculated by subtracting the weight found after treatment by the weight found before treatment and these values are in grams. The weight gains for the salad mix samples are insignificant. The tomatoes either lost weight or stayed the same, depending on the method and time of treatment, as is shown in Tables 10, 11, and 12. Unexpectedly, due to the weight gain of the salad, the lettuce appeared brighter and crispier, as shown in Figure 8.

The data relates significantly to the hypothesis provided earlier in the paper. Ozone reduced bacterial population by a 3-log reduction. Compared to UV and water agitation, ozone does about the same. Unlike the UV and water agitation, the ozone changes the structural integrity of the tomatoes negatively, whereas for the salad, it

impacts it positively. Compared to the ozone, the ozone and ultrasound combination does about the same in terms of changing the color and texture of the samples. It's important to note it is less effective than ozone, for a difference in color change, texture change, and *Salmonella* reduction, and only more effective for a difference in color change and texture when it comes to the UV and water agitation.

Chapter 5

DISCUSSION

Determining the *Salmonella* reduction of the tomatoes and the salad was one of the main priorities of the project. UV with water agitation, among the three methods, was the better approach when it came to decreasing the amount of *Salmonella* found on the surfaces of the samples. It reached nearly a 4-log reduction when it came to the tomato samples and was between a 2 and 3-log reduction for the salad mix samples. It also reached these amounts faster than the other two approaches in three minutes. Even the ozone machine, which did reach a 3-log reduction of *Salmonella* in three minutes, didn't reach the highest amount of *Salmonella* destroyed. For the UV machine, these results are like those reported by Guo et al. (2017): who showed similar log reductions for tomatoes of a smaller sample size. Although they and Huang et al. (2018) used spinach instead of a salad mix, the two foods can be considered comparable, and they achieved similar results with lower inactivation rates of *Salmonella*. The lower inactivation rates for the spring salad mix, and in the other study, the spinach, may be due to the bacteria's ability to penetrate the porous leaves of the spring salad mix, and because two of the three technologies were most likely only hitting the surface level, it wouldn't have reached the bacteria that internalized within the samples.

The machine that did worse than the other two was the one that used ozone and ultrasound together. This was an unexpected finding. The literature (Aparecida et al. 2021, Dietrich et al. 2017, Kidak et al. 2018) points out the opposite should have happened, i.e., the hurdle effect would cause a higher inactivation rate. As pointed out earlier, the machine does the ozone and ultrasound in bursts. Sometimes, these bursts happen separately, other times both technologies are running at the same time. Because the treatment times were done in intervals of three minutes, it's possible that if done for longer, the machine could have potentially reached a maximum log reduction of *Salmonella*, such as the other two. Another thing to note is the pH. According to Tomoyoshi et al. (2019), when ultrasound is used alongside an acidic solution, it works well. Using alkaline (or basic) solutions causes ultrasound to be less effective. Tap water is typically around a neutral pH, which would also explain why the machine didn't perform as well as it could. Further research using acidic solutions instead of tap water should be investigated.

The color changes of the tomatoes were uniform and easier to calculate. To the naked eye, there were no visible color differences. Underneath the colorimeter, there were some differences, but none of them were significant. According to Sarron et al. (2021), depending on the concentration of ozone, this can cause the color of the tomatoes to change. While the machine used doesn't specify how much ozone is generated, it is enough to indeed change the color, if slightly. The color changes of the salad mix, however, were much harder to calculate. There was too much variety within each salad mix sample and not every piece of green (or red) was exposed to the

ozone, ultrasound, or UV in the same way. Using only three samples per type of leaf, the results should be considered inconclusive. A larger sample size would be preferrable to truly understand how the technologies affect each kind of leaf within the salad mix.

The texture changes of the tomatoes were noticeable straightaway. Even without putting the tomatoes underneath the texture analyzer, the tomatoes were no longer firm after each treatment type and time, the exception being the treatment with the UV apparatus at all times. As noted previously in the results section, they became squishy. It took less force for the texture analyzer to compress the tomatoes after treatment than before the treatment. This is noted in Sarron et al. (2021) as well. The skin of the tomato had been affected. The texture changes of the salad had the opposite effect, especially with the longer treatment times. The leaves became crisper and firmer, and the texture analyzer had to use more force to compress the pile of salad leaves placed beneath it.

The weight changes, while insignificant across the board, may correlate to the texture change. The tomatoes typically lost a bit of weight after each treatment. This resulted in a less firm tomato and in less force being required to compress them. For the salad, the opposite reaction happened. The salad typically gained weight after each treatment, which resulted in firmer textures, requiring more force to be used by the texture analyzer. This happened with nearly all the samples, especially the longer the samples were being treated. Every treatment required the use of water. It’s most likely that the agitation of the water by the ozone, ultrasound, and UV allowed for more

water to enter the salad leaves, whereas it broke the structural integrity of the tomato because it was already firm to begin with and it's less porous.

It was hypothesized that the UV with water agitation would work best versus what is currently on the market for consumers. Based on the findings of this study, this holds true. Ozone works well in inactivating *Salmonella,* but it alters the texture of the food too much, especially at longer time intervals. Ozone and ultrasound together are less effective in inactivating *Salmonella* when just using water. UV with water agitation is effective at inactivating *Salmonella*; it also doesn't influence the color or texture change significantly when compared to ozone and ozone with ultrasound.

Chapter 6

SUMMARY

Three different machines using three different technologies were used to compare efficiency and efficacy compared to each other. Two of the machines are currently on the market for consumers; one of them is not. The machine currently not on the market- the UV with water agitation- was found to be comparable, if even slightly better, than what is currently on the market today. There are limitations to the UV machine: the light cannot penetrate the surfaces of fresh produce as it can only be used on the surface. The initial cost of the apparatus could be high, but possibly come down to being comparable to the machines on the market today. For quality machines, one might expect to spend over one or two hundred dollars. In the future, more studies on different kinds of produce could be used to determine just how effective the UV machine is compared to the ozone and ozone with ultrasound machines. More research into the color and texture changes of these machines should be done as well.

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