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Julianne Elise Drury
Iowa State University

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Inactivation of *Salmonella enterica* on romaine lettuce following spraying with Pro-San™ – a biodegradable foodgrade sanitizer

by

Julianne Elise Drury

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:

Lester Wilson, Co-Major Professor
Aubrey Mendonca, Co-Major Professor
Byron Brehm-Stecher
Chong Wang

Iowa State University

Ames, Iowa

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CHAPTER 1: GENERAL INTRODUCTION

Introduction

The safety of fresh and fresh cut fruits and vegetables in the food supply requires immediate attention. According to Hettiarachchy and others (2010), contaminated leafy greens have been the source of many of the estimated 1.4 million illness and 600 deaths that occur annually in the United States due to *Salmonella* infections. Also, in 2006, there were three multistate outbreaks of *Escherichia* coli (E. coli) O157:H7 on lettuce leaves (CDC: Surveillance of Foodborne Disease Outbreaks, 2009). Together, these two organisms can be attributed to an estimated 1,470,667 illnesses annually, causing an estimated annual cost of over $3 billion (Foodborne Illness Cost Calculator 2010). Furthermore, according to Leverentz and others (2003), "over the past decade, the frequency of reported outbreaks of illnesses due to foodborne pathogens has increased," indicating an increasing urgency for development of a method of treatment to make fresh and fresh-cut produce (which is especially at risk, due to the ability of the bacteria to access leaking nutrients and juices, and also attach in the wound sites) safe for consumption (Bhagwat 2006). Currently, commercial fresh ready-to-eat produce is only rinsed (usually with a 50-200 ppm chlorine solution), and isn't treated with a traditional kill-step. Food safety laws require a reduction of 99.99683% on these products, but unfortunately current conventional washing methods are only capable of 90-99% reduction (Fallik, 2004). And even this reduction is "questionable, particularly when mishandling follows the sanitizing treatment" (Fonseca 2006), and need to take into consideration the deactivation of chlorine solutions due to contact with organic matter in biofilms. Such biofilms could be formed by *E. coli* O157:H7 or *Salmonella* spp. in the field after a contaminate has dried on the lettuce surface. Therefore, much

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research is required to develop a method of sanitation that is capable of producing safe fresh and fresh-cut produce that is not inactivated by organic matter.

Literature Review

PATHOGEN DESCRIPTON

As mentioned above, the two primary bacteria of concern in fresh produce are *Eschericia coli* (*E. coli*) O157:H7and *Salmonella* spp.

Escherichia coli O157:H7 and O104:H4

Escherichia coli is a gram negative rod-shaped bacteria. It is exists in many different strains, most of which are not harmful (or even beneficial to humans), as they are present in the intestines as normal microflora (however, harmful strains also reside in the intestines). There are five classes of *E. coli* that are enterovirulent in humans. Of these five, O157:H7 and O104:H4 are the most dangerous and are enterohemorrhagic in humans, although there is zero tolerance for all five in the United States food system.

E. coli O157:H7 and O104:H4 produce a toxin that attacks the lining of the intestines, causing hemorrhagic colitis, symptoms of which include severe abdominal cramping and bloody diarrhea for an average of 8 days for STEC (Shiga- toxin producing *E. coli*) O157:H7 and 5-7 days for STEC O104:H4 (Bad Bug Book 2009 and Investigation Update: Outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) Infections Associated with Travel to Germany 2011). In young victims more commonly, hemolytic uremic syndrome (HUS) can occur from either of these bacterium, causing renal failure and hemolytic anemia. In elderly victims, HUS as well as thrombotic thrombocytopenic purpura (TPP) (which involves fever and neurological

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symptoms) can occur as a result of *E. coli* O157:H7 infection, causing a mortality rate in the elderly as high as 50% (Bad Bug Book 2009). It is estimated that 73,480 cases of *E. coli* O157:H7 occur annually (Foodborne Illness Cost Calculator 2010), but because *E. coli* O104:H4 has only recently caused a major outbreak, it is not yet known what an annual illness estimate might be.

E. coli O157:H7 has become one of the most influential in the development of food safety systems, because of its lethality and the fact that it is commonly transferred via the fecal-oral route. In particular, it is the reason for the irradiation of ground beef and required pasteurization of apple cider.

Salmonella enterica

Salmonella species are rod-shaped gram negative bacteria. They are present in many animal species, particularly poultry and swine. All species are harmful to humans. They cause disease by infecting the epithelium of the small intestine and causing inflammation (known as "salmonellosis"), which produces symptoms such as fever, nausea, vomiting, abdominal cramps, diarrhea, and can even cause typhoid fever (Bad Bug Book 2009).

There are three serotypes of *Salmonella enteritica,* which include *S. typhi* (which causes typhoid fever), *S. typhimurium* (which isn't as severe as *S. typhi* in humans), and *S. enteritidis* (which infects chicken flocks, and acts similarly to *S. typhimurium*) (A Focus on Salmonella, 2009).

The mortality rate of most forms of salmonellosis is about 1% (S. *typhi* is as high as 10%, and S. *enteritidis* ranges from 1%-3.5% in some populations). The total prevalence of all *Salmonella* species is 2-4 million cases annually, and it appears to be rising. In the past decade, it

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has increased more than 6 fold in the northeast United States, and appears to be spreading south and west (Bad Bug Book 2009).

MAJOR OUTBREAKS

There have recently been numerous outbreaks associated with these three organisms. A few of the largest ones that have occurred recently are described below.

Salmonella outbreak of 2008

In the summer of 2008, there was a large outbreak of *Salmonella* (Saintpaul strain) involving tomatoes and various peppers. In total, 1442 people were infected with this particular strain, which usually accounts for only 1.6% of cases, suggesting a common source of contamination in the cases. After nationwide tomato, serrano pepper, and jalapeño pepper warnings and much investigation, no single direct source was identified, however the contamination was likely to have originated on one of two farms in Mexico, or the packing facility that they shared use of (CDC: Outbreak of Salmonella, 2008).

Salmonella in Sprouts

From February to May of 2009, there was a large outbreak involving *Salmonella* Saintpaul contamination of alfalfa sprouts, which affected 13 states. 228 cases were reported, and were traced back to a single alfalfa seed grower (CDC: Morbidity and Mortality Weekly Report: Surveillance Outbreak of *Salmonella* Serotype Saintpaul Infections Associated with Eating Alfalfa Sprouts 2009).

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E. coli O157:H7 Ground Beef

During the summer of 2008, there were two multistate outbreaks of *E. coli* O157:H7 in ground beef. The first outbreak (which occurred in June), caused 64 confirmed cases and was tied back to a slaughtering facility in Nebraska and resulted in 5.3 million pounds of ground beef being recalled. The second, (which occurred in July), caused 35 cases in 8 states, and initiated recall of ground beef as well as 1.36 million pounds of intact beef cuts. This outbreak was linked to the same slaughtering facility as the first outbreak in June (Two Multistate Outbreaks of Shiga Toxin-Producing *Escherichia coli* Infections Linked to Beef from a Single Slaughter Facility 2008).

Salmonella in Hydrolyzed Vegetable Protein (HVP)

In the spring of 2010, a large recall of many diverse products was initiated after *Salmonella* Tennessee was detected in Hydrolyzed Vegetable Protein (HVP). HVP is a common ingredient in many foods (including soups, sauces, dips, and dressings, to name a few) and typically serves as a flavor enhancer.

Upon detection of *Salmonella* in this ingredient, the company that produced it initiated a recall of all of the products that it went into, and luckily this was done quickly enough to prevent any illnesses from occurring (*Salmonella* Tennessee Identified in a Processed Food Ingredient 2010).

Salmonella in Pistachios

During the Spring of 2009, a recall of pistachios was initiated due to possible *Salmonella* contamination. Setton Pistachio of Terra Bella, Inc., the company that produced the recalled product, sold their pistachios (prior to discovering the potential of contamination) to various

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other companies as ingredients for their products. Therefore, this recall included such varied products as ice cream, snack foods, pies, cakes, and candy bars, as well as general pistachio products. Fortunately, there were no confirmed cases of illness as a result of consuming these products (Update on Pistachio Product Recall 2009).

E. coli O157:H7 in spinach

During the fall of 2006, an outbreak involving *E. coli* O157:H7 on bagged spinach prompted a large recall of this product. Of the 205 people in the United States who became ill as a result of eating the contaminated spinach, roughly 29% developed hemolytic uremic syndrome. Investigation revealed the source to be wild pigs running through ranches in Salinas Valley, California.

Interestingly, during a study of the victims in Utah and New Mexico, it was discovered that washing the spinach before consumption did not have any effect in whether or not they became ill. According to Grant and others (2008), the reasons for this include that *E. coli* could enter the plant structure via the roots, and cut surfaces (such as those on bagged leafy greens) are easier for bacteria to adhere to. Another possible explanation is that the bacteria may have become embedded into the cuticle (the waxy layer).

This suggests the need for an effective method of cleaning contaminated produce that that will make products safe to eat, and therefore prevent future outbreaks.

E. coli O104:H4 in sprouts

Beginning in May of 2011, a major outbreak of the newest pathogenic strain of *E. coli*, O104:H4, occurred in Germany. It causes serious illness, and was responsible for 32 confirmed

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deaths and 852 cases of HUS, besides other unreported illnesses. Sprouts produced on a farm in Lower Saxony, Germany have been identified as the source of the outbreak, which was originally believed to be cucumbers from Spain (Investigation Update: Outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) Infections Associated with Travel to Germany 2011).

Salmonella enteriditis in eggs

Between May 1 and November 30, 2010 a major outbreak of *Samonella enteriditis* occurred in eggs in the United States. The source of the contamination was determined to be Wright County Egg and Hillandale Farms of Iowa. Throughout the outbreak, 1,939 illnesses were reported that were deemed "likely to be associated with this outbreak" (Investigation Update: Multistate Outbreak of Human *Salmonella Enteriditis* Infections Associated with Shell Eggs 2010).

Salmonella in peanut butter

Between November 25, 2008 and January 28, 2009 530 people were reported infected with *Salmonella* Typhimurium, a strain which was confirmed to have been isolated from a sample of King Nut peanut butter. Interestingly, however, illness was more associated with consumption peanut-butter containing products (such as peanut butter crackers) than jarred peanut butter. Also, the same strain that was found in the King Nut peanut butter sample was indistinguishable from the *S*. Typhimurium strain isolated from a previous peanut butter outbreak (2006-2007), which occurred in a factory only 70 miles from the factory that produced King Nut (Multistate Outbreak of *Salmonella* Infections Associated with Peanut Butter and Peanut Butter-Containing Products---United States, 2008-2009 2009).

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CURRENT POSTHARVEST OPERATIONS AND INTERVENTION OPPORTUNITIES

Currently, there are 7 steps from harvesting leafy vegetables to transporting them to retail:

1. Harvest (by hand, or Ramsay Highlander Romaine Harvester)
2. Chlorinated Water Spray (50-200 ppm, sprayed for a few seconds) (Niemira, 2007)
    - The most effective pH for this solution is 6.0, because at that pH there is the highest concentration of hypochlorous acid. However, according to Trevor Suslow, "the best compromise of activity and stability is achieved by maintaining a water pH between 6.5 and 7.5" (1997).
3. Transportation to vacuum cooler (about 2 hours in a refrigerated truck, during which time the spray treatment remains on the lettuce)
4. Vacuum Cooling (about 30 minutes)
    - This reduces the temperature of the leaves from about 28°C to about 0°C, and involves spraying recirculated water onto the leaves to reduce moisture loss (Li, Tajkarimi, and Osburn, 2008)
5. Transport to Packaging Plant (about 96 hours)
6. Cutting, Washing, Packaging (about 30 minutes, typically washed in chlorinated water)
7. Transport to Retail (in a refrigerated truck)

CURRENT METHODS OF TREATMENT OF FRESH PRODUCE

Listed below are several currently used treatment methods for fresh produce, which are applied to kill pathogens that may be present. Typically, these treatments are applied during Step 2 or Step 6 above; however, gaseous sanitizers may be applied during Step 4.

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Hot water

Immersing, rinsing, or brushing fresh produce with hot water has been a classic method of sanitizing. For some products, such as apples, this immersion is actually a very effective method of sanitization; immersion at 80 and 95°C for 15 seconds has been shown to reduce the *E. coli* O157:H7 population by more than 5 logs on fresh apples (Fallik 2004). However, apples are one of many products that experience severe quality degradation when exposed to high temperatures, and therefore are not treated with this method. This treatment also does not access the possible *E. coli* O157:H7 in the apple's core. Furthermore, many other products simply do not experience this level of reduction; fresh cantaloupes only experience a 2 log reduction at 70°C or a 3.4 log reduction at 97°C when held at temperature for 1 minute (Ukuku 2003).

One thing to note is that when using water to wash fresh produce, it is recommended that during prewashing, the wash water be 10°C warmer than the produce to prevent a pressure differential that could cause the uptake and internalization of bacteria from the wash water into the fruit, which could occur if the wash water was colder than the fruit. After this warm prewash, traditional cold wash water (to remove field heat and reduce the respiration rate of the produce) could be used (Al-Zenki and Al-Omariah 2006).

Chlorinated water

Chlorination of the wash water (50-200 ppm) is another frequently used method of sanitization of fresh produce (McWatters 2002). While high levels of chlorine are very deadly to pathogens, chlorine residue in foods can be a problem, as it is capable of producing chlorinated organic compounds, which are potentially carcinogenic, and also reduce the efficiency of chlorine solution as a sanitizing agent when bound (Silveira 2008). Therefore, because of its

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carcinogenicity (and its effects on the aroma of the product), only levels high enough to reduce the microbial load by less than 100 fold are typically used. This level of sanitization isn't particularly helpful in sanitizing the fruit on its own, but is more commonly used to sanitize the wash water itself, so that produce is not re-contaminated (Fallik 2004). This is particularly true if a chlorine stabilizer such as T-128 is used, which slows the depletion of free chlorine by organic material in wash water (Nou 2011). Furthermore, when coupled with hot water, heated chlorinated water treatment of produce before cutting has been shown to be as effective as treatment of the produce after cutting (Fallik 2004).

Hydrogen Peroxide

Hydrogen peroxide is typically more effective at reducing microbial populations than hot or chlorinated water, and is Generally Recognized as Safe (GRAS) (Silveira 2008). As mentioned above, fresh cantaloupes experience a 2 log reduction at 70°C or a 3.4 log reduction at 97°C when held at temperature in wash water for 1 minute. However, when 5% hydrogen peroxide is added to the wash water, a 3.8 log reduction was observed at 70°C when treated for 1 minute (Ukuku 2003). Hydrogen peroxide treatment has also been tested on iceberg lettuce (2% hydrogen peroxide at 50°C for 1 minute) as an antibacterial agent, and according to McWatters and others (2002), "the antibacterial treatment was more effective than the control treatment in maintaining sensory quality over 15 days of storage, provided that the lettuce was initially intensely green," indicating that quality is not necessarily negatively affected by this treatment, and may even be positively affected by it (Silveira 2008). One disadvantage is that it degrades quickly, and therefore solutions must be made fresh before use (Non-chlorine Sanitizer Options for the Wineries). Additionally, if produce is wounded, it cannot penetrate the wound to kill the

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bacteria located there (Fonseca 2006), and is degraded upon contact with catalase or peroxidase, which would be present on the cut surfaces of fruit (Walker and Hui 2007).

Organic Acids: Peracetic Acid (PAA), Acetic Acid, and Lactic Acid

Acetic, peracetic, and lactic acids are all commonly used GRAS organic acids. According to Parish and others (2006):

"The antimicrobial action of organic acids is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid."

Cost effectiveness and effectiveness at killing various pathogens vary among these and other organic acids (Parish and others 2006).

Acetic has been shown to reduce *E.c*oli O157:H7 populations by 5 logs when in a 1.9% solution. However, at this level, the quality of the lettuce that was treated was noted to be unsatisfactory (Fonseca 2006).

Peracetic acid is an equilibrium mixture of acetic acid hydrogen peroxide (Sapers 2001). It is particularly effective against the outer membrane lipoproteins, making it particularly deadly to gram-negative cells. In one study, peracetic acid (in a solution of pH 6) reduced the population of mesophilic bacteria in fruits and vegetables by 2 logs when applied for 1 minute (Silveira 2008). A different study, however, observed as high as a 4.5 log reduction in *Salmonella* Typhimurium after 60 minutes of exposure to aerosolized peroxyacetic acid on lettuce leaves (Oh, Dancer, and Kang 2005). One example of a commercial peroxyacetic acid is Tsunami 200®, which has demonstrated a >5.31 log reduction of *Enterobacter sakazakii* on lettuce (Kim 2007). According to Dr. Randy Worobo, author of "Non-chlorine Sanitizer Options for the Wineries," peracetic acid's disadvantages include "high cost, odor irritancy, corrosive

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nature and inactivation by organic matter." The inactivation by catalase and peroxidase of the hydrogen peroxide component of peroxyacetic acid could prove problematic, especially when it is used to sanitize equipment that has come into direct contact with freshly cut produce.

Lactic acid typically is used in the form of sodium or potassium salts. These salts of lactic acid possess antimicrobial activity because they lower the pH and also disrupt the outer membranes of bacteria, making them particularly effective against gram negative cells. These salts are GRAS certified, and therefore are commonly used in high concentrations in various products. Unfortunately, however, while they do possess antimicrobial abilities when applied to lettuce, the quality of the lettuce was significantly decreased (Bhagwat 2006).

These three organic acids can be used in aqueous, gaseous, or aerosol form. Recently, the aerosol method has been shown to be more effective than aqueous or gaseous, as it combines the advantages of both, which include a wide selection of sanitizers and high penetration activity of punctures, stomata, etc (Oh, Dancer, and Kang 2005). Electrostatic spraying also appears to increase efficacy of organic acids, possibly because it causes slows coalescence and allows droplets to remain small for a longer time, thus coating the leaf more uniformly (Ganesh and others 2010).

Quaternary Ammonium Compounds

Quaternary ammonium compounds are colorless, odorless, have a good penetrating ability, and are more stable than chlorine to organic material. However, they are relatively expensive to use and do not work well with soaps or other anionic detergents (Parish and others 2006).

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Chlorine Dioxide

Chlorine dioxide has more oxidizing power than chlorine, and doesn't produce carcinogenic compounds by reacting with organic molecules as much as chlorine does (Silveira 2008). According to Jorge Fonseca (2006), it is more effective against certain kinds of bacteria than sodium hypochlorite. In one study, *Eschericia coli* O157:H7 and *Salmonella* serovar Typhimurium decreased by 3.4 and 4.3 logs (respectively) on inoculated lettuce leaves after exposure to 4.3 ppm chlorine dioxide gas for 30 minutes. When the time of exposure of the gas was extended, this reduction improved, in particular for *E. coli* O157:H7 (Fonseca 2006). However, it is an unstable compound and can even become explosive (Non-chlorine Sanitizer Options for the Wineries). Furthermore, it must be generated at the same location where it will be used (Bhagwat 2006).

Nicin + EDTA

Nicin is a bacteriocin that primarily targets gram positive cells, and ethylene diamino tetracetic acid (EDTA) is a chelating agent that is often paired with nicin. In one study of this treatment on Galia melons, only a 0.2 log reduction of surface bacteria was observed 7 days after treatment (nisin at 250 mg/L plus EDTA 100 mg/L**)** (Silveira 2008). However, a different study reported 2 log reduction after 7 days on cantaloupe (treated with 50 mg/mL Nisin and 0.02M EDTA), and noted that it might reduce the risk of *Salmonella* spp. on fresh cut cantaloupe melons (Ukuku 2004). Therefore, the efficacy of this treatment is highly dependent upon the matrix and the target bacteria.

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Electrolyzed water

Electrolyzed oxidizing water is a "no added chemical" sanitation alternative (although chemicals are released during the process). It kills bacteria because it possesses a very high oxidation-reduction potential, which is created by using a sodium chloride solution, exposing it to an electric current, and then diluting the solution down to roughly 0.1% sodium chloride. In one study, using a current of 14 A, *E. coli* was reduced by 2.42 logs on lettuce, after exposure of 3 minutes. This was improved further with acidification and chlorination (Park and others 2001).

Surfactants

Surfactants are used in detergents because they are believed to help penetrate the hydrophobic cuticle present on some fruits and vegetables, and help wash water remove dirt and other contaminants more effectively by wetting the produce surface. Commercial detergents for washing fresh produce often contain surfactants such as sodium dioctyl sulfosuccinate, sodium dodeclybenzene sulfonate, sodium 2-ethylhexyl sulfate, or sodium dodecyl sulfate (SDS). In one study, a commercial detergent (DECCO APL KLEEN 246) achieved a >1 log reduction when applied in concentration of 1 and 2% (although other surfactants, including those listed above, did not aid in decontamination when applied in concentrations 0.1% to 0.2%). It was also noted that this detergent was particularly helpful at sanitizing produce when first applied, then rinsed, and then followed with an aqueous sanitizer such as hydrogen peroxide (Sapers and others 1999). However, a different study observed that surfactants did not aid in the detachment of *Salmonella* or *Shigella* from fresh tomatoes when rinsed in water (Sapers and Jones 2006).

It should be noted that the cleaning efficacy of surfactants can be increased by addition of chlorine, as demonstrated in a study by Escudero and others (1999). Furthermore, when some

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surfactants (for example, SDS) are combined with organic acids, their antimicrobial abilities are greatly increased (Zhao, Zhao, and Doyle 2009).

One example of a commercially available sanitizer that uses both an organic acid and an anionic surfactant (which are especially bactericidal when under acidic conditions) is Pro-San™ (Mendonca, Brehm-Stecher, Wilson 2010). The active ingredients in this sanitizer are citric acid and sodium dodecylbenzene sulfonate. The citric acid is an effective antimicrobial compound because of its ability to cross the cell membrane into the cytoplasm, lower the intracellular pH, and as a result inhibit the metabolism of the microorganism. Sodium dodecylbenzene sulfonate is an effective surfactant because it is ability to reduce interfacial tensions, therefore helping to release water-insoluble contaminants that are tightly bound to surfaces. However, it also has bactericidal activity in that it is capable of interacting with microbial proteins and even modifying enzyme conformation, as well as interacting with cell membrane components to promote the release of intracellular organelles out of the cell (Feliciano L 2009). A study done on the ability of 1% (w/v) Pro-San™ to reduce inoculated populations of *Salmonella* and *E. coli* O157:H7 on tomatoes (after 4 minutes of exposure) demonstrated more than 3 log reductions, which was more effective than both sodium hypochlorite (500 ppm) and hydrogen peroxide (5% w/v) solutions. Both Pro-San™ and Pro-San™ Soft were tested in this study, and Pro-San™ was shown to be more effective than Pro-San™ Soft. These sanitizers are biodegradable and GRAS certified, and have been shown to help dislodge debris from fresh produce while also killing microorganisms present (Mendonca, Reitmeier, and Sikinyi 2004).

A similar compound, sodium dodecyl sulfate, has antimicrobial ability as well. It has been shown to denature protein surfaces and damage cell membranes, an effect which is greatly

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increased when the pH is reduced to between 1.5 and 3.0, best obtained by adding organic acids (Zhao, Zhao, and Doyle 2009).

Another type of GRAS certified surfactants are alkaline salts of fatty acids (in particular, oleic, myristic, and lauric acids). One study, which combined lauric acid (LA) with potassium hydroxide (PH), reported that "significantly fewer total plate count (TPC) bacteria, *E. coli* and *Salmonella* Typhimurium were recovered from [poulty] carcasses washed with 2.00% LA-1.00% KOH than from carcasses washed with water" (Hinton and others 2009). However, very limited information is available on the efficacy of these surfactants to reduce microbial populations on fresh produce, therefore additional research on these substances is needed.

One potential problem is the quality effects of surfactants on fresh produce, particularly on lettuce leaves. One study conducted by Guan, Huang, and Fan (2010) demonstrated severe effects on quality (primarily sogginess and tissue softening of lettuce leaves) when combinations of SDS and either levulinic acid or sodium acid sulfate were used on iceberg lettuce leaves, and the leaves were then stored in modified atmosphere packaging for 7 and 14 days. Therefore, future studies will need to be conducted to determine if these effects are also present when SDS is combined with other organic acids, and if they are present in other storage methods.

Irradiation

Irradiation with γ rays is used in many different commodities as a method of sterilization, and has recently been approved by the FDA as a method of killing pathogens on iceberg lettuce and spinach. A 5 log reduction of *Salmonella* on some commodities can be achieved by 1.15-1.55 kGy. However, irradiation of fresh produce causes radiolytic degradation of pectin causing softening, although different products have different tolerances (Niemira 2003). Therefore, a

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maximum treatment of 1.0 kGy is recommended for fresh produce (Fonseca 2006), and is also the maximum level permitted for leafy greens by the FDA (Gomes, Moreira, Castell-Perez 2010). However, recent research has demonstrated that its efficacy can be greatly increased by increasing the radiation sensitivity of the organisms by exposing them to increased concentrations of oxygen during irradiation. In fact, a 5 log reduction in *Salmonella* spp. on baby spinach was observed in 100% oxygen conditions with a treatment of only 0.7 kGy (Gomes, Moreira, Castell-Perez 2010). Another issue to consider, however, is that consumers who are unfamiliar with the process of irradiation may be hesitant to purchase irradiated produce, for fear that they are hazardous to human health. One recent survey released that 66% of those surveyed are "very concerned" about irradiation (Blaine, Kamaldeen, and Powell 2006).

Ultraviolet Radiation

Ultraviolet radiation has recently been suggested as a method of sanitization of fresh produce that is inexpensive and, like γ irradiation, does not leave chemical residue (Yaun and others 2003). Furthermore, it does not require complicated safety equipment, and can also be useful in reducing postharvest decay (Fonseca 2006). A study conducted by Yaun and others demonstrated a 3.3 log reduction of E. coli O157:H7 on red delicious apples with a treatment of 24 mW/cm^2, but much lower reductions on tomatoes and green leaf lettuce, with the reduction on green leaf lettuce not being statistically significant. Therefore efficacy of UV radiation, like many other treatments, is highly dependent upon the produce being treated. Two possible factors in the efficacy of UV radiation as a sanitizer include the presence or absence of wax on the surface of the produce, and the topography of the sample, both of which may provide some shielding of the UV rays (Yaun and others 2003).

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Although many food products have surfaces that may not be very well sanitized by UV light, UV light could still be a very helpful tool in sanitizing other surfaces, such as conveyor belts that food products travel on. One study demonstrated that *L. monocytogenes* populations of ~ 10^7 were reduced to below detection on a variety of types of conveyor belt surfaces when exposed to 5.95 mW/cm^2 UV light intensity for 3 seconds, indicating that this is a promising method of equipment sanitization (Morey and others 2010).

Ozone (liquid and gaseous)

Ozone is a treatment that is effective at low concentrations, and produces an effect after a very short contact time (Parish and others 2006). It is available for sanitization of fresh produce in both liquid and gaseous forms. Ozone gas, however, is much more effective than liquid ozone. Fonseca (2006) reported that ozone gas at 3 ppm reduced bacterial populations on fresh produce (apples, lettuce, strawberries, and cantaloupes) by 5.6 logs, while ozonated water only reduced the populations by a maximum of 3 log CFU/g. According to Singh and others, "The biocidal effect of ozone is caused by a combination of its high oxidation potential, reacting with organic material up to 3,000 times faster than chlorine, and its ability to diffuse through biological cell membranes" (2002). One disadvantage to ozone treatment is that it may affect the sensory quality of the produce; for example, decolorization of lettuce has been observed (Singh and others 2002). However, it should be noted that significant sensory changes were not observed in shredded lettuce samples in a different study, which were exposed to 3 ppm aqueous ozone for 15 second intervals for 5 minutes (Rodgers and others 2004).

Other disadvantages include its unstable, highly reactive nature that is highly corrosive to equipment, and the potential for it to produce toxic effects in processing facilities (Parish and others 2006).

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Hurdle Technology

According to Arvind Bhagwat (2006), "Hurdle technology is the deliberate use of multiple preservation techniques in order to establish a series of microbiological controls that any microorganisms present should not be able to overcome." This combination of treatments has been shown to have a synergistic effect, as almost none of the bacteria are able to jump over every "hurdle." Previously, combinations of chlorine, hydrogen peroxide, electrolyzed water, and other GRAS substances have been used while creating a "hurdle" plan. For example, when fresh-cut cabbage and lettuce is first washed with acidic electrolyzed water, then placed in modified atmosphere packaging (100% Nitrogen), and finally stored at 1°C, bacterial growth was inhibited for 5 days (Bhagwat 2006).

CURRENT RESEARCH

Ultrasound Technology

Ultrasound technology is a new technique that can be used to improve bacterial reduction of some existing methods. The biocidal effect of ultrasound waves has been described by Fonseca (2006) as the following:

"Ultrasonic fields consist of waves at high amplitude that form cavitation bubbles. Cavitation enhances the mechanical removal of attached or entrapped bacteria on the surfaces of fresh produce by displacing or loosening particles through shearing or scrubbing action" (Fonseca 2006).

One study has shown that when applied to a chlorine rinse solution, it increases the microbial reduction by 1 log (Fonseca 2006). However, this is not consistent with the results of a study done by Sanglay and others (2004), which observed no increased bacterial reduction on

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various produce surfaces exposed to 40 kHz of ultrasonic waves. Therefore, more research should be done to determine the efficacy of this method (at various treatment levels) on different kinds of fresh produce.

Trisodium Phosphate (TSP)

Trisodium phosphate is an alkaline disinfectant (Fonseca 2006) that is also less corrosive than other commonly used compounds (Parish and others 2006). Its primary use is in detaching bacteria from the surfaces of fresh produce (Liao 2001). One study showed it to completely eliminate the *Salmonella* population on the surfaces of tomatoes when applied in a concentration of 15%. However, the cores of the tomatoes only experienced a 2 log reduction. Furthermore, when applied to lettuce, concentrations that did not damage the sensory attributes of the lettuce also did not reduce the *L. monocytogenes* populations present on those samples (Fonseca 2006).

A different study reported that when applied to green pepper slices (in concentrations ranging from 3% w/v to 12% w/v for 5 minutes at pH 12.3), the *Salmonella* population present on the disks was reduced 10-100 times. When the pH of this treatment was reduced to 4.5, the ability of the TSP at detaching the target bacteria was reduced by 26%, indicating that it is most effective in basic conditions (Liao 2001). Treatment with TSP is particularly effective when applied first, then rinsed, and finally followed by another sanitizer (Sapers and others 1999).

Calcinated Calcium

Calcinated calcium is obtained from the pearl layer of oyster shells, which is ground and treated electrically with ohmic heating before being ground into a fine powder and dissolved in water. The solution is filtered immediately before use.

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Calcinated calcium has been shown to be a very effective sanitizer, resulting in a 7.59 log reduction in E. coli O157:H7 and a 7.36 log reduction in *Salmonella* on tomatoes (Bari 2002). However, some changes in color and flavor of foods treated with calcinated calcium have been reported (in particular, yellowing and bittering) (Isshiki and Azuma 1995).

Essential Oils

Essential oils extracted from various herbs and spices have recently been shown to have antimicrobial activity, in most cases due to their phenolic content. The main compounds in these oils that have biocidal activity are carvacrol, eugenol, linalool, and thymol, with thymol and carvacrol having the widest ranges of activity (against the most strains of bacteria). Some of these compounds can be very effective sanitizers of produce, as demonstrated by a 0.1% solution of thymol and carvacrol decreasing the *Shigella* spp. population on lettuce leaves (after a 2 minute immersion) to below the detectable limit (Fonseca 2006). However, there is a possibility that the flavor or other sensory characteristics of some fresh produce may be negatively affected by treatment with some of these compounds.

METHODS OF QUALITY MEASUREMENT

During these studies, which involve applying novel sanitizers to fresh produce, the efficacy of the sanitizer to reduce microbial load is not the only attribute that is measured. The effects on the quality of the fresh produce must also be measured and taken into consideration, because even if a treatment can sterilize a food product, if it produces a product with unacceptable quality, no one will consume it and therefore it will not be useful. Thus, the effects of treatments on fresh produce quality must be measured in a variety of ways, to provide an accurate portrait of how much a treatment affects product quality. Below are several ways that

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the quality attributes of fresh spinach and lettuce can be measured, both before and after treatment.

Color

Color is one of the most important attributes in determining the freshness of a product. In lettuce, it can be affected by chlorophyll degradation, edge browning (enzymatic), and russet spotting (Rico and others 2007). Overall color is typically measured using a Hunter L*a*b* colorimeter, which evaluates color based on lightness/darkness (L*), red/green (+/-a*), and yellow/blue (+/-b*) attributes of a specific color. Also, hue angle, chroma, and ΔE (which represents total color difference) can be calculated (McWatters and others 2002). Spot area and shape can be measured and described.

Sensory Evaluation

Sensory evaluation is typically done to evaluate the acceptability of the samples, but can also be used to quantify certain attributes of the samples. Simple yes/no responses of whether or not the sample is acceptable, or a 9 point hedonic scale (1=dislike extremely, 9=like extremely) of overall acceptability can be used. Appearance, color, aroma, flavor, and texture can also be evaluated using a 9 point intensity or preference scale (McWatters 2002). Additionally, an overall difference test such as a triangle test comparing treated and untreated samples can be completed to determine if differences among groups are significant (Rodgers and others 2003).

Panelists can be untrained or trained. Typically untrained panelists are used for acceptability tests, and trained panelists are used for scale tests so that they have a better understanding of the scale.

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Expert Evaluators

Expert evaluators are another option involving human subjects, but instead of using several untrained or moderately trained panelists, a few highly trained personnel are used.

One study used three expert evaluators to evaluate the quality of lettuce leaves after treatment and storage. Weight of leaves was recorded, as well as "turgor, visual quality, decay, stem discoloration, wilting, and other defects (like spotted or torn not counted in other quality evaluations) using a 9-point hedonic scale for each quality attribute," which were evaluated visually or by touching the sample (Park and others 2001).

SPECIAL CONSIDERATIONS WHEN WORKING WITH FRESH PRODUCE

Broken Tissue

When working with fresh produce, investigators must keep in mind the differences in viability of bacteria in intact vs. broken tissue. There can be major differences among the two, as bacteria tend to enter through broken tissue (which is also a better substrate for their growth) and can be harbored there, and therefore are more difficult for sanitizing agents to access them. For example, *Salmonella* Typimurium populations that were reduced on intact vegetables by a chlorine solution by 1 to 1.5 logs were only reduced by 0.3 to 0.6 logs when the vegetables were cut (Fonseca 2006).

Biofilms

Another consideration is that bacteria on a given surface do not always have the same resistance against given sanitizers. Their resistance can be change with time due to the formation of biofilms. According to Speranza, Corbo, and Sinigaglia (2011), a biofilm is defined as "an assemblage of surface-associated molecules that are enclosed in hydrated extracellular polymeric

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substances (EPS)". These substances include polysaccharides, proteins, phospholipids, techoic, and even nucleic acids, and can form single layers or complex 3-dimensional structures. These substances are secreted by "biofilm communities" which may be comprised of one or several bacterial species (Speranza, Corbo, and Sinigaglia, 2011). Once formed they serve the purpose of providing a protective barrier of organic material for the bacteria inside, which makes them much more resistant to washing and sanitizing treatments. In particular, chlorinated solutions are made much less effective when attempting to kill bacteria beneath a biofilm, because they are inactivated by organic matter (they react with it, to form chloro-organic compounds, which have no antimicrobial activity) (Fonseca 2006).

However, this is not the only reason that bacteria inside biofilms are so difficult to kill. There are three primary hypotheses for why bacteria present in biofilms are so resistant to most antimicrobial treatments.

The first hypothesis is quite simply that the penetration of the antimicrobial agent into the biofilm is severely inhibited, causing this to either slow dramatically or be incomplete. While this effect varies greatly among antimicrobials (and is dependent upon such factors as molecule size, hydrophobicity, capacity to react with organic material, and other factors) it can cause an otherwise effective antimicrobial to be completely ineffective at reaching the cells embedded underneath the biofilm (Steward and Costerton, 2001).

The second hypothesis is that within the biofilm there is an altered chemical environment. Anaerobic conditions, buildup of waste products, and pH differences (all of which are results of metabolism of cells) can occur inside the biofilm, which may interfere with the antimicrobial's ability to work, once it has arrived at the location of the cell cluster (Steward and Costerton, 2001).

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The third hypothesis is that once cells create a biofilm, they enter a phenotypic state similar to that of a spore, in that they are highly self-protective. This could further reduce an antimicrobial's ability to kill cells, especially if the antimicrobial was tested and chosen because of its ability to kill free-floating vegetative cells, which are in no such protective state (Steward and Costerton, 2001).

Another problem associated with biofilms is their abundance and the fact that they can exist nearly anywhere. Water systems, heat exchangers, cooling towers, floor drains, conveyor belts, storage tanks, any other food processing surfaces that come into direct contact with food, have all been shown to potentially harbor biofilms. And while biofilm formation is greatly affected by factors such as temperature, medium, and pH, many food processing conditions exist which either do not prevent biofilm formation or possibly even promote it (Speranza, Corbo, and Sinigaglia 2011).

Therefore, given that biofilms pose such a problem in the fresh produce industry, more research is required to develop a method of cleaning that is capable of producing safe fresh and fresh-cut produce. Ideally this newly developed treatment would be effective not only at killing free-floating vegetative cells, but also the cells under biofilms which traditionally are the ones that survive current treatments and are therefore speculated to be the true cause of outbreaks.

Infiltration of Pathogens into Produce

According to Solomon and others (2006), bacteria have the ability to infiltrate plant tissue by entering through stem scars, stomata, lenticels, broken trichomes, and sites of cuticle damage. Because of this, sanitizers such as chlorine, hydrogen peroxide, ozone, trisodium phosphate, and peroxyacetic acid have never been shown to eliminate all bacteria present on produce (Solomon

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and others 2006). Further complicating this issue is the current industry practice of vacuum cooling lettuce soon after harvest, which according to Li and others (2008) has been shown to actually increase the rate of infiltration of *E. coli* O157:H7 into lettuce leaves.

CONCLUSION

As this literature review has demonstrated, there are many sanitizer options for fresh and fresh-cut produce available. However, many have major disadvantages (including cost, instability, detrimental quality effects etc), or are simply not effective enough to fully protect consumers from foodborne illnesses when they have consumed produce significantly contaminated with pathogenic microorganisms (in particular, because they are not able to penetrate wounds on produce, or because they have been internalized into the tissue itself). Others require more research to determine their antimicrobial capability and effect on the quality of the produce that they are applied to. Therefore, the objective of this study is to evaluate the efficacy of various sanitizers (including bleach solutions, which are currently used in the romaine lettuce industry, and organic acid-surfactant combinations, which may be a more effective alternative) on inoculated populations of *Escherichia coli* O157:H7 and *Salmonella enterica* on romaine lettuce, and evaluate the effects on the quality of these products as a result of various treatments. The results of this study will then be used to develop a cost-effective plan to sanitize leafy vegetables on a large scale by incorporating new sanitization practices and sensors into pre-existing handling, processing, packaging, and distributing steps. The data collected will then be shared with other members of this project at Ohio State University, who will work with the mass transfer model of selected treatment methods to improve them further.

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Literature Review References

A Focus on *Salmonella* [Internet]. United States Department of Agriculture; c2009 [Accessed 18 May 2010]. Available from: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/ Foodborne Illness FoodbornePathogensNaturalToxins/BadBugBook/ucm069966.htm

Al-Zenki, Al-Omariah. 2006. Fruits: Sanitation and Safety. In: Hui YH, Barta J. Handbook of Fruits and Fruit Processing. Ames, IA: Blackwell Publishing. p 245-261.

Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook [Internet]. U.S. Food and Drug Administration; c2009 [Accessed 18 May 2010]. Available from: http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm

Bari ML, Inatsu Y, Kawasaki S, Nazuka E, Isshiki K. Calcinated Calcium Killing of *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* on the Surface of Tomatoes. 2002. Journal of Food Protection; 65: 1706-1711.

Bhagwat AA. 2006. Microbiological Safety of Fresh-Cut Produce: Where Are We Now?. In: Matthews KR. Microbiology of Fresh Produce. Washington, DC: ASM Press. p 121-165.

Blaine K, Kamaldeen S, Powell D. 2006. Public Perceptions of Biotechnology. Journal of Food Science; 67: 3200-3208.

CDC : Morbidity and Mortality Weekly Report: Outbreak of *Salmonella* Serotype Saintpaul Infections Associated with Multiple Raw Produce Items [Internet]. 57(34); 929-934. Atlanta, GA: Department of Health and Human Services; c2008 [Accessed 18 May 2010]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5734a1.htm

CDC : Morbidity and Mortality Weekly Report: Surveillance of Foodborne Disease Outbreaks

www.manaraa.com

[Internet]. 58(22); 609-615. Atlanta, GA: Department of Health and Human Services; c2009 [Accessed 18 May 2010]. Available from:http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5822a1.htm

CDC : Morbidity and Mortality Weekly Report: Surveillance Outbreak of Salmonella Serotype Saintpaul Infections Associated with Eating Alfalfa Sprouts --- United States, 2009 [Internet]. 58(18); 500-503. Atlanta, GA: Department of Health and Human Services; c2009 [Accessed 27 May 2010]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5822a 1.htm

CDC : Morbidity and Mortality Weekly Report: Two Multistate Outbreaks of Shiga Toxin—Producing *Escherichia coli* Infections Linked to Beef from a Single Slaughter Facility [Internet]. 59(18); 557-560. Atlanta, GA: Department of Health and Human Services; c2008 [Accessed 27 May 2010]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5918a3.htm

CDC: Morbidity and Mortality Weekly Report: Multistate Outbreak of Salmonella Infections Associated with Peanut Butter and Peanut Butter-Containing Products---United States, 2008-2009 [Internet]. 58(Early Release); 1-6. Atlanta, GA: Department of Health and Human Services; c2009 [Accessed 16 June 2011]. Available from http://www.cdc.gov/mmwr/pre view/mmwrhtml/ mm58e0129a1.htm

Escudero ME, Velázques L, Di Genaro MS, De Guzmán AS. 1999. Effectiveness of Various Disinfectants in the Elimination of *Yersinia entercolitica* on Fresh Lettuce. Journal of Food Protection; 62: 665-669.

Fallik, Elazar. Prestorage hot water treatments (immersion, rinsing, brushing). 2004. Postharvest Biology and Technology ; 32: 125-134.

Feliciano L. 2009. Shelf Extension of Seafood Using Sanitized Ice [MSc thesis]. Columbus,

www.manaraa.com

OH: The Ohio State University. 162 p. Available from: http://etd.ohiolink.edu/etd/send pdf.cgi/Felicia no%20Lizanel.pdf?acc_num=osu1252965039.

Fonseca JM. 2006. Postharvest Handling and Processing: Sources of Microorganisms and Impact of Sanitizing Procedures. In: Matthews KR. Microbiology of Fresh Produce. Washington DC: ASM Press. p 85-120.

Foodborne Illness Cost Calculator [Internet]. USDA Economic Research Service; c 2010 [Accessed 2010 May 27]. Available from: http://www.ers.usda.gov/data/foodborne illness/.

Gomes C, Moreira RG, Castell-Perez E. 2010. Radiosensitization of *Salmonella* spp. and *Listeria* spp. in Ready-to-Eat Baby Spinach Leaves. Journal of Food Science; 76: E141-E148.

Grant J, Wendelboe AM, Wendel A, Jepson B, Torres P, Smelser C, Rolfs RT. 2008. Spinach-associated *Escherichia coli* O157:H7 Outbreak, Utah and New Mexico, 2006. Emerging Infectious Diseases[serial online]. 14. Available from: http://www.cdc.gov/eid/content/14/10/1633.htm. Posted Sept 25, 2008.

Guan W, Huang L, Fan X. 2010. Acids in Combination with Sodium Dodecyl Sulfate Caused Quality Deterioration of Fresh-Cut Iceberg Lettuce during Storage in Modified Atmosphere Package. Journal of Food Science; 75: S435-S440.

Hettiarachchy NS, Ravichandran M, Johnson MG, Griffis CL, Martin EM, Meullenet JF, Rick SC. 2010. Electrostatic Sprays of Food-Grade Acids and Plant Extracts are More Effective than Conventional Sprays in Decontaminating *Salmonella* Typhimurium on Spinach. Journal of Food Science; 75: M574-M575.

Hinton A, Cason JA, Buhr RJ, Liljebjelke K. 2009. Bacteria Recovered from Whole-

www.manaraa.com

Carcass Rinsates Of Broiler Carcasses Washed in a Spray Cabinet with Lauric Acid-Potassium Hydroxide. International Journal of Poultry Science; 8: 1022-1027.

Isshiki K, Azuma K. Microbial Growth Suppression in Food Using Calcinated Calcium. 1995. Japan Agricultural Research Quarterly; 29: 269-274.

Investigation Update: Multistate Outbreak of Human *Salmonella* Enteriditis Infections Associated with Shell Eggs [Internet]. Centers for Disease Control and Prevention; c2010 [Accessed 16 June 2010]. Available from: http://www.cdc.gov/salmonella/enteritidis/

Investigation Update: Outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) Infections Associated with Travel to Germany [Internet]. Centers for Disease Control and Prevention; c2011 [Accessed 16 June 2011]. Available from: http://www.cdc.gov/ecoli/2011/ecoliO104/

Kim H. 2006. Survival and Growth of Enterobacter Sakazakii on Produce, Conditions Affecting Biofilm Formation, and its Sensitivity to Sanitizers. Athens, GA: University of Georgia. 216 p. Available from: http://athenaeum.libs.uga.edu/bitstream/handle/10724/9253/kim_hoikyung_200608_phd.pdf?sequence=1.

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A. 2003. Biocontrol of *Listeria monocytogenes* on Fresh-Cut Produce by Treatment with Lytic Bacteriophages and a Bacteriocin. Applied and Environmental Microbiology 69: 4519-4526.

Li H, Tajkarimi M, Osburn BI. 2008. Impact of Vacuum Cooling on *Eschericia coli* O157:H7 Infiltration into Lettuce Tisse. Applied and Environmental Microbiology; 74:3138-3142.

Liao CH, Cooke PH. Response to trisodium phosphate treatment of *Salmonella* Chester attached to fresh-cut green pepper slices. 2001. Canadian Journal of Microbiology; 47: 25-32.

McWatters KH, Chinnan MS, Walker SL, Doyle MP, Lin CM. 2002. Consumer Acceptance of

www.manaraa.com

Fresh- Cut Iceberg Lettuce Treated with 2% Hydrogen Peroxide and Mild Heat. Journal of Food Protection; 65: 1221-1226.

Mendonca A, Brehm-Stecher B, Wilson L. Inactivation of *Salmonella enterica* and *Escherichia coli* O157:H7 on Romaine lettuce immersed in PRO-SAN, a Biodegradable Foodgrade Sanitizer. Presentation given at USDA NIFSI meeting at Ohio State University, April 13, 2010.

Mendonca A, Reitmeier C, Sikinyi T. Evaluation of a Gras Sanitizer for Enhanced Microbial Safety and Shelf-Life of Whole Tomatoes for Iss and Planetary Outpost. 2004. SAE International; Document number 2004-01-2560.

Morey A, McKee SR, Dickson JS, Singh M. Efficacy of Ultraviolet Light Exposure Against Survival of *Listeria monocytogenes* on Conveyor Belts. 2010. Foodborne Pathogens and Disease; 7: 737-740.

Niemira BA. 2003. Radiation Sensitivity and Recoverability of *Listeria monocytogenes* and *Salmonella* on 4 Lettuce types. Journal of Food Science; 68: 2784-2787.

Niemira BA. 2007. Relative efficacy of sodium hypochlorite wash vs. irradiation to inactivate *Escherichia coli* O157:H7 internalized in leaves of romaine lettuce and baby spinach. Journal of Food Protection; 70:2526–32.

Non-chlorine Sanitizer Options for the Wineries [Internet]. Geneva, NY: Department of Food Science and Technology, Cornell University; [Accessed 19 May 2010]. Available from: http://74.125.155.132/scholar?q=cache:3gVi8jrGscYJ:scholar.google.com/+h2o2+disadvantages+sanitizing+fresh+produce&hl=en&as_sdt=1000000.

Nou X, Luo Y, Hollar L, Yang T, Feng H, Millner P, Shelton D. 2011. Chlorine Stabilizer T-

www.manaraa.com

128 Enhances Efficacy of Chlorine against Cross-Contamination by *E. coli* O157:H7 and *Salmonella* in Fresh-Cut Lettuce Processing. Journal of Food Science; 76: M218-M224.

Oh SW, Dancer GI, Kang DH. Efficacy of Aerosolized Peroxyacetic Acid as a Sanitizer of Lettuce Leaves. 2005. Journal of Food Protection; 68: 1743-1747.

Parish ME, Beuchat LR, Suslow TV, Harris LJ, Garrett EH, Farber JN, Busta FF. 2006. Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce. Comprehensive Reviews in Food Science and Food Safety; 2: 161-173.

Park, C. M., Y. C. Hung, M. P. Doyle, G. O. I. Ezeike, and C. Kim. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. J. Food Sci. 66: 1368–1372.

Rico D, Martín-Diana AB, Barat JM, Barry-Ryan C. Extending and measuring the quality of fresh-cut Fruits and vegetables: a review. 2007. Trends in Food Science and Technology; 18: 373-386.

Rodgers SK, Cash JN, Siddiq M, Ryser ET. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution on apples, lettuce and cantaloupe. Journal of Food Protection; 67: 721-731.

Salmonella Tennessee Identified in a Processed Food Ingredient [Internet]. U.S. Food and Drug Administration. c2010 [Accessed 28 May 2010]. Available from: http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm203067.htm

Sanglay GC, Eifert JD, Sumner SS. 2004. Recovery of *Salmonella* spp. from Raw Produce Surfaces Using Ultrasonication. Foodborne Pathogens and Disease; 1: 295-299.

Sapers GM. 2001. Efficacy of Washing and Sanitizing Methods, Food Technology and Biotechnology; 39: 305–311.

www.manaraa.com

Sapers GM, Jones DM. 2006. Improved Sanitizing Treatments for Fresh Tomatoes. Journal of Food Science M; 71: 252-256.

Sapers GM, Miller RL, Pilizota V, Mattrazzo AM. 1999. Antimicrobial Treatments for Minimally Processed Cantaloupe Melon. Journal of Food Science; 66: 345-349.

Silveira AC, Conesa A, Aguayo E, Artes F. 2008. Alternative Sanitizers to Chlorine for Use on Fresh-Cut "Galia" (*Cucumis melo* var. *catalupensis*) Melon. Journal of Food Science; 73: 405-411.

Singh N, Signh RK, Bhunia AK, Stroshine RL. 2002. Efficacy of Chlorine Dioxide, Ozone, and Thyme Essential Oil or a Sequential Washing in Killing *Escherichia coli* O157:H7 on Lettuce and Baby Carrots. Lebensmittel-Wissenschaft und Technologie; 35: 720-729.

Solomon EB, Brandl MT, Mandrell RE. 2006. Biology of Foodborne Pathogens on Produce. In: Matthews KR. Microbiology of Fresh Produce. Washington, DC: ASM Press. p55-83.

Speranza B, Corbo M, and Sinigaglia M. 2011. Effects of Nutritional and Environmental Conditions on Salmonella sp. Biofilm Formation. Journal of Food Science; 76: M12-M16.

Steward S, Costerton J. 2001. Antibiotic resistance of bacteria in biofilms. The Lancet; 358: 135-138.

Suslow, Trevor. Postharvest Chlorination: Basic Properties and Key Points for Effective Disinfection [Internet]. Publication 8003. Davis, CA: University of California Division of Agriculture and Natural Resources; c1997 [Accessed 20 Sept 2010]. Available from: http://ucanr.org/freepubs/docs/8003.pdf**.**

Update on Pistachio Product Recall [Internet]. U.S. Food and Drug Administration. c2009 [Accessed 28 May 2010]. Available from: http://www.fda.gov/Safety/Recalls/

www.manaraa.com

MajorProductRecalls/Pistachio/Update/default.htm

Ukuku DO, Fett WF. 2004. Effect of Nisin in Combination with EDTA, Sodium Lactate, and Potassium Sorbate for Reducing Salmonella on Whole and Fresh-Cut Cantaloupe. Journal of Food Protection; 67: 2143-2150.

Ukuku DO, Pilizota V, Sapers GM. 2003. Effect of Hot Water and Hydrogen Peroxide Treatments on Survival of *Salmonella* and Microbial Quality of Whole and Fresh-Cut Cantaloupe. Journal of Food Protection; 67:432-437.

Walker LT, Hui YH. 2007. Enzymes. In: Hui YH. Food Chemistry: Principles and Applications Second Edition: 2007. West Sacramento, CA: Science Technology System. p 13-1 – 13-16.

Yaun BR, Sumner SS, Eifert JD, Marcy JE. 2004. Inhibitions of pathogens on fresh produce by ultraviolet energy. International Journal of Food Microbiology; 90: 1-8.

Zhao T, Zhao P, Doyle MP. Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on Lettuce and Poultry Skin by Combinations of Levulinic Acid and Sodium Dodecyl Sulfate. 2009. Journal of Food Protection; 72: 928-936.

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CHAPTER 2. INACTIVATION OF *SALMONELLA ENTERICA* ON ROMAINE LETTUCE FOLLOWING SPRAYING WITH PRO-SAN™- A BIODEGRADABLE FOODGRADE SANITIZER

ABSTRACT

The efficacy of various solutions of Pro-San™ were tested on romaine lettuce leaves against *Salmonella enterica*.

Romaine lettuce samples were inoculated with a 5-strain cocktail of Nalidixic acid resistant *Salmonella enterica* and held at room temperature for 16-18 hours to allow a biofilm to form. Lettuce samples were then sprayed separately with one of six treatments and remained exposed to the treatment for various times (including 30, 45, 60, 75, 90, 105, 120, 280, and 240 minutes) which was at either refrigerated temperature or room temperature. Samples were then plated onto nalidixic acid supplemented Xylose Lysine Desoxycholate (XLD) agar and bacterial colonies were enumerated 48 hours later after being held at 35° C.

The 6 spray treatments included: a distilled water control, a 150 ppm chlorine bleach solution (with pH maintained between 6.4 and 7.5), a 0.78% Pro-San™ LC solution, a 0.19% Pro-San™ LC solution, a 0.78% Pro-San™ LC Soft solution, and a 0.19% Pro-San™ LC Soft solution. Samples of lettuce leaves that were not sprayed served as dry controls. Pro-San™ LC and Pro-San™ LC Soft are both bio-degradable GRAS sanitizers composed of an organic acid and a surfactant, with a pH around 2.2.

After 3 replicates at each application time, data was analyzed using SAS software. Three variables were analyzed: time, treatment, and treatment*time (to determine if there is any interaction between the two variables). The time and treatment variables had significant

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differences ($a<0.05$), but because the time*treatment variable was not significant, the ratio among the treatments was not different among the times. Therefore, *Salmonella* counts for each time were logged and averaged. A Tukey pairwise t-test was obtained from this data, which indicated that the dry control, distilled water control, and 150 ppm bleach solution were not significantly different from each other, but were less effective than the Pro-San™ solutions when the treatments were tested at room temperature. At refrigerated temperature, the same trend among treatments existed but only the 0.78% solutions were statistically more effective than the dry control and distilled water control. Additionally, only the 0.78% Pro-San™ was more effective than the bleach solution.

The ability of the increased concentrations of Pro-San™ LC to kill bacteria present on lettuce leaves is theorized to be due to the ability of the surfactant to aid the delivery of the primary antimicrobial agent (the organic acid) to the cells by penetrating the biofilm (although the surfactant itself also has some antimicrobial activity) (Feliciano 2009). Furthermore, it is hypothesized that when a biofilm is present, the organic matter inactivates the majority of the hypochlorite ion in the bleach solution, making it ineffective in this condition.

The effects of each spray treatment on the color of the lettuce leaves was also analyzed with a Hunter L*a*b* system, and data showed that the color was not significantly different among any treatment groups. Overall quality was also not effected either treatment, based on results of a triangle tests completed with a total of 100 panelists.

INTRODUCTION

The safety of fresh and fresh cut fruits and vegetables in the food supply requires immediate attention. According to Hettiarachchy and others (2010), contaminated leafy greens

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have been the source of many of the estimated 1.4 million illness and 600 deaths that occur annually in the United States due to *Salmonella* infections. Furthermore, according to Leverentz and others (2003), "over the past decade, the frequency of reported outbreaks of illnesses due to foodborne pathogens has increased," indicating an increasing urgency for development of a method of treatment to make fresh and fresh-cut produce safe for consumption. Fresh-cut produce is especially at risk, due to the ability of the bacteria to access leaking nutrients and juices, and also attach in the wounded sites (Bhagwat 2006). Currently, fresh ready-to-eat produce is only rinsed (usually with a 50-200 ppm chlorine solution), and is not treated with a traditional kill-step. Food safety laws require a reduction of 99.99683% on these products, but unfortunately these current conventional washing methods are only capable of 90-99% reduction (Fallik, 2004). And even this reduction is referred to as "questionable, particularly when mishandling follows the sanitizing treatment" by Jorge Fonseca, and need to take into consideration the deactivation of chlorine solutions due to contact with organic matter in biofilms, which could be formed many bacteria in the field after the contaminated material had dried on the lettuce surface (2006).

Various alternative sanitizer options to chlorine exist for fresh and fresh-cut produce, however many have major disadvantages (including cost, detrimental quality effects, instability, etc), or are simply not effective enough to fully protect consumers from foodborne illnesses. The latter is especially true when consumers have consumed produce significantly contaminated with heavy loads of pathogenic microorganisms, because many treatments are not able to penetrate wounds on produce and the target bacteria may have become internalized into the tissue itself. Therefore, much research is required to develop a method of sanitizing that is capable of producing safe fresh and fresh-cut produce.

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The focus of this experiment was on the potential use of Pro-San™, a GRAS sanitizer composed of a surfactant and organic acids in solution that was sprayed onto lettuce leaves inoculated with a 5 strain cocktail of *Salmonella enterica*. Potential advantages to using this sanitizer (vs. traditional chlorine bleach) include penetration of the hydrophobic cuticle of the leaf (the waxy coating), no inactivation due to reaction with the organic material present in a biofilm (which was induced on each leaf prior to treatment to observe the sanitizing abilities of all treatments on the worst case scenario), and disruption of the cell membrane by sodium dodecylbenzene sulfonate, resulting in cell death (Feliciano L 2009). One difference between Pro-San™ LC and Pro-San™ LC Soft is that Pro-San™ LC Soft uses sodium lauryl sulfate as the surfactant instead of sodium dodecylbenzene sulfonate. Also, the levels of the surfactant are different between these two types of Pro-San™, and the Pro-San™ Soft lacks the phosphoric acid and chelating agent present in Pro-San™ LC (Mendonca, Reitmeier, and Sikinyi 2004). Both sanitizers contain lactic acid and citric acid as major acidifying agents.

Therefore, it is hypothesized that when tested against *Salmonella enterica* present in a biofilm, the various dilutions and variations of Pro-San™ will be more effective sanitizers than the 150 ppm chlorine bleach or control treatments.

MATERIALS AND METHODS

Experimental Design

In this experiment, treatments were tested against inoculated cells of *Salmonella enterica* on romaine lettuce leaves. There were 5 treatments tested, as well as 2 controls. The two controls were a dry control and a distilled water control. The treatments included 150 ppm sodium hypochlorite (bleach) solution made in a potassium phosphate buffer of pH 6.8 (final pH adjusted to pH 6.4), 0.78% and 0.19% Pro-San™ LC, and 0.78% and 0.19% Pro-San™ LC Soft.

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The 150 ppm bleach solution was used because it is the currently used treatment for fresh romaine lettuce in industry. These treatments were applied by spraying onto the inoculated leaves and applied for 7 different exposure times (30, 45, 60, 75, 90, 105, 120, 180, and 240 minutes) and two temperature (room temperature and refrigerated temperature) before leaves were removed from the trays and placed into Dey Engley neutralizing broth (which renders the antimicrobial compounds ineffective) in sterile stomacher bags. Stomaching of samples and plating onto appropriate agar followed.

Color measurements of treated lettuce leaves (treatment for 1, 2, 3, and 4 hours) were also taken using a Hunter L*a*b* colorimeter to observe any effects of the treatments on leaf color.

Calibration of Spray Bottle Heads

Each of the treatments was applied using its own spray bottle head throughout the experiment. Before the experiment was begun, these spray bottle heads were numbered and calibrated. First, the knob was adjusted to make sure spray diameter is approximately the same for all bottles, and the spray angle and distance of average spray was recorded. Also, a line was made with a Sharpie® marker on the nozzle to indicate the proper setting, so that if it were adjusted, this would be clear before spraying began.

To calibrate the bottles, each spray head was used on an uninoculated lettuce leaf sample (similar to those used in the actual experiment) to determine how many sprays each bottle head required to cover an entire leaf very thoroughly. After 3 replicates, the median was recorded for each bottle head and used later to determine the amount of liquid sprayed onto the leaves during each treatment. This was done in 3 replicates by spraying each bottle head 10 times into a small

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beaker and measuring the change in weight to obtain grams/spray. The average was calculated and converted to grams per treatment (assuming that each bottle head would be sprayed the same number of times every time that it was used).

Preparation of inoculum

The inoculum was prepared by first preparing a 5-strain cocktail of a nalidixic-acid resistant culture. After this culture had been developed, 0.1 ml of each working nalidixic-acid-resistant culture was added to 10 ml of tryptic soy broth supplemented with 25 ug/ml nalidixic acid (TSBN) and incubated at 35° C for 24 hours. A second 24 hour transfer of the same amount (also into a 10 ml tube of TSBN) was completed on the following day, with the sample coming from the first transfer's tube. After the second transfer had incubated for 24 hours, 6 mL of each from each of the 5 freshest tubes was placed into a centrifuge tube, which was centrifuged for 10 minutes at 4°C and 10,000 x g in a Sorvall® Super T21 Centrifuge (Kendro Laboratory Products). The supernatant was discarded and the cells were resuspended in 30 mL of sterile saline (0.85%). A tenfold dilution was then done immediately into a 9 mL tube of 0.1% bacto peptone water.

Inoculation of lettuce

To prepare the lettuce leaves for inoculation, the outer 3 or 4 leaves were discarded. A sterile scissors was then used to cut the inner leaves into 2 pieces, of which only the outer half was used. Leaves were laid down with the inner portion facing up and inoculated by placing 200 µL (delivered in 16-18 drops) of the inocula in a circle of where the spray hits to ensure that all drops were wetted by treatments. Leaves were then allowed to remain in a laminar flow hood (at room temperature) for 30 minutes to air dry the inocula. The fan was then turned off to allow the

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inoculated leaves to remain at room temperature for 16 to 18 hours before spraying them with either distilled water or antimicrobial solutions.

Preparation of antimicrobial solutions

Distilled water was used for the water-spray control and for preparing all antimicrobial solutions.

To prepare 150 ppm chlorine bleach solution, 0.5 mL of 6% sodium hypochlorite bleach was added to 199.5 mL buffer solution. The buffer solution was prepared by combining ~150 mL 0.05 M dibasic potassium phosphate with ~75 mL 0.05 M monobasic potassium phosphate. Then, 0.5 mL of bleach was added to 199.5 mL of buffer to make a 150 ppm solution. Finally, the pH was adjusted to 6.4 with citric acid as needed. Once the solution was completed, the level of free chlorine was recorded using a Hach Company (Ames, IA) chlorine test kit. Upon testing, each batch resulted in a free chlorine level of 0.

For the Pro-San™ LC and Pro-San™ LC Soft solutions, 1 L of each was prepared using liquid concentrate and distilled water.

Procedure for treating lettuce

Inoculated lettuce leaves were sprayed with distilled water (control) or antimicrobial solutions and allowed to set undisturbed at both room temperature and refrigerated temperature for 15, 30, 45, 60, 75, 90, 105, 120, 180, and 240 minutes, covering the entire leaf (using the amount of sprays determined in spray calibration). A digital clock was used to monitor exposure times. Then, at appropriate time intervals sanitized thongs were used to transfer each control or

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treated lettuce leaf to a separate sterile stomacher bag containing 200 ml sterile Dey-Engley (DE) neutralization broth.

Microbiological analysis and confirmation

The bagged samples were pummeled for 1.0 minute in a Seward Stomacher® 400 Circulator (West Sussex, United Kingdom) operating at medium speed. Appropriate 10-fold serial dilutions of the sample homogenate in 0.1% (w/v) Bacto peptone were prepared. For highly concentrated sanitizers, 1 ml of homogenate (lettuce in DE broth) was plated onto 5 plates in 0.20 ml increments, and for all treatments duplicate 0.1-ml samples of homogenate and dilutions were surface plated on xylose lysine desoxycholate agar with added nalidixic acid (25 ug/ml; XLDN). Black colonies were enumerated after 48 hours in the 35˚C incubator.

The inoculum was also plated on XLDN agar and enumerated after 48 hours, and both yellow and black colonies from the plates were randomly selected to be tested with a latex agglutination assay, to confirm that they were or were not *Salmonella* species.

Measurement of pH of treatment solutions

Measurement of the pH of solutions was taken periodically to ensure that the solutions were stable. This was done by transferring 10 mL of each treatment solution into a 25 mL beaker and measuring the pH with a calibrated Orion pH meter fitted with a glass electrode.

Color Measurements

The outer leaves were removed from each head of fresh (no more than 3 days or storage in the refrigerator after purchase from grocery store) romaine lettuce, then the bottom half of each leaf was discarded. Leaf tops were sprayed with treatments (from calibrated spray bottles)

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and held at room temperature for 1, 2, 3, and 4 hours. 2"x2" square samples were cut from each leaf (in the vertical center of the leaf, but approximately 0.5" from the center rib) after treatment had been completed. Samples were blotted with paper towels to ensure that the surface was reasonably dry and then placed in a clear plastic dish for measurement in the Hunter L*a*b* colorimeter.

The Hunter L*a*b* colorimeter was set to use D65 light at a 10° standard observer with a port size of 1.2" and an area view of 1".

Samples were read in triplicate, with a 120° rotation after each measurement, and all data was recorded. Three replicates for each treatment/treatment application time combination were completed, and ΔE values for each treatment vs. the distilled water treatment for that group were calculated as follows for the two colors (L_1^*, a_1^*, b_1^*) and (L_2^*, a_2^*, b_2^*):

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

Sensory Panel

A triangle test completed by an untrained sensory panel was used to determine if there was any overall detectable difference between lettuce treated with the 0.78% Pro-San™ and 0.78% Pro-San™ Soft solutions vs. lettuce treated with water as well as 0.19% Pro-San™ and 0.19% Pro-San™ Soft solutions vs. lettuce treated with water (including taste, aroma, wilting, color, etc.). In this test, three samples are presented on a plate and randomly coded, 1 or 2 of which are the control (tap water treated) and 1 or 2 of which are the test sample (one of the Pro-San™ solutions). The panelist then selected which sample is the odd sample. There is a 33% chance of the panelist getting the correct answer by random choice. Fifty panelists were used for each test, with a total of 100 panelists overall. The treatments were each applied to the lettuce for

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2 hours, and subsequently washed off with tap water. The lettuce was then torn into approximately 2x2" pieces, placed onto plates, and served with a glass of water.

Statistical Analysis

Both the microbial and the color measurements were analyzed using two-way analysis of variance (ANOVA) models, with treatment and treatment application time as explanatory variables. Tukey's pairwise t-tests were performed to assess differences among groups. P-value ≤ 0.05 was regarded significant.

For the sensory panel results, a triangle test table called "Critical Number of Correct Response in a Triangle Test" was used (Meilgaard, Civille, Carr 2007) to determine if there was any detectable difference among the samples.

RESULTS AND DISCUSSION

Microbial Testing Results

All black colonies tested had positive agglutination results, and all yellow and clear colonies had negative agglutination results, which was expected. The nalidixic acid which supplemented the XLD plates was used to help reduce background flora, and the nalidixic acid resistant culture of *Salmonella* was used to ensure that all *Salmonella* included in enumerations was from the inoculum only.

ANOVA analysis of the microbial test data indicated that there was no interaction between the variables "treatment" and "time," (amount of time of application of treatments) for neither the room temperature study (P=0.977) nor the refrigerated study (P=0.742). Therefore, even though in the room temperature study both variables "treatment" and "time" were

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statistically significant (P<0.0001), results presented in Table 1 (below) show an average log mean count across all times. The table also shows the accompanying Tukey pairwise t-test results because although application time did effect the average number of surviving cells at each time point, the differences among treatments were not affected by time.

Table 1 shows that the dry control (which was inoculated but not treated), the distilled water control, and the 150 ppm bleach treatment were not statistically different from another, but that they were all less effective treatments than the Pro-San™ treatments, which were not statistically different from one another.

Table 1: Tukey Distribution: Average Effects of Treatments on Log CFU/mL of *Salmonella* at Room Temperature

Treatment	Mean Log CFU/mL and Grouping*
Dry Control	4.12[a]
Distilled Water Control	3.99[a]
150 ppm Bleach	3.61[a]
0.19% Pro-San™	2.53[b]
0.19% Pro-San™ Soft	2.43[b]
0.78% Pro-San™ Soft	2.27[b]
0.78% Pro-San™	1.87[b]

*Means with different letters are significantly different, and α=0.05.

Because the amount of time of application was a significant variable but again there was no interaction among variables, Table 2 shows the mean log count (averaged for all treatments)

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for the room temperature study at each time. It shows that the only statistical differences were between the 180 minute and 240 minute application times with some of the other shorter application times. Figure 1 (appendix) shows that at 180 minutes of application, the median count for the treatments of 0.78% Pro-San™ and 0.78% Pro-San™ Soft was actually <1 CFU/mL (shown as 1 CFU/mL on the graph) which was quite low, and all treatments at 240 minutes of application (including the dry control) were lower than the most other application times. Therefore it is likely that in most cases application time is not an important variable to control when using these treatments, especially because there was no interaction among the two variables.

Table 2: Effects of Time on Average Log Count of *Salmonella* (For All Treatments) for Room Temperature Treatments

Treatment Application Time (Minutes)	Log Count Mean and Grouping
15	3.51[a]
60	3.49[a]
45	3.35[a]
75	3.21[ab]
90	3.10[ab]
30	2.93[abc]
105	2.85[abc]
120	2.84[abc]
240	2.37[bc]
180	2.08[c]

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Table 3, below, displays the results of the refrigerated study, which was similar to the room temperature study except that the application time was completed in a refrigerator, and that only 15, 30, 45, and 60 minutes were tested.

The order of treatment efficacies was exactly the same as that in the room temperature study, although the significant differences observed differed slightly. Again, the dry control, distilled water control, and 150 ppm bleach treatments were not different from one another, but only the 0.78% Pro-San™ (and 0.78% Pro-San™ Soft treatments) were statistically more effective than the dry control, distilled water control, and 150 ppm bleach (see Figure 2, appendix).

For the refrigerated study, application time of treatments was not a significant variable, indicating that there were no differences in log counts among the time points.

Table 3: Tukey Distribution: Average Effects of Treatments on Log CFU/mL of *Salmonella* at Refrigerated Temperature

Treatment	Mean Log CFU/mL and Grouping*
Dry Control	3.85[a]
Distilled Water Control	3.67[ab]
150 ppm Bleach	3.21[abc]
0.19% Pro-San™	2.76[abcd]
0.19% Pro-San™ Soft	2.37[bcd]
0.78% Pro-San™ Soft	1.90[cd]
0.78% Pro-San™	1.53[d]

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These results suggest that similar trends exist between the room temperature study and the refrigerated study.

Furthermore, because an average of 4.90 logs of inoculum per mL was observed before drying, it could be assumed that a log reduction of between 0.8 and 1.1 occurs as a result of drying. The purpose of the 16-18 hours of drying in this experiment was to induce formation of a biofilm, which could easily be present in nature and would likely be present after this amount of time (as demonstrated in Annous et al. 2005, which showed fibrillar material 2 hours after inoculation, and EPS 24 hours after inoculation) . This represents the "worst case scenario" and because it is the state in which the bacteria are most protected, it challenges the treatment solutions. By testing the solutions against this level of resistance to sanitizers, it can be determined which treatments are truly the most effective and which are ineffective when this worst-case situation is present.

The results from this portion of the study suggest that the dry control (which experienced no treatment), the distilled water control, and the 150 ppm bleach solution (the currently used industry standard) are no different from one another. Therefore, when a biofilm is present, it is hypothesized that the organic matter present inactivates the majority of the hypochlorite ion, making it ineffective in this condition.

The Pro-San™ and Pro-San™ Soft solutions were also no different from one another, however in the room temperature study they were significantly more effective sanitizers than the dry control, distilled water control, and 150 ppm bleach. The 150 ppm bleach solution provided only a 0.51 log reduction from the dry control. However, there was a 1.85 log reduction from the

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dry control to the 0.78% Pro-San™ Soft, and a 2.25 log reduction from the dry control to the 0.78% Pro-San™ solution. Additionally, the refrigerated study demonstrated that the 0.78% solutions of Pro-San™ and Pro-San™ Soft were statistically more effective than the 2 controls, and that the 0.78% solution of Pro-San™ was more effective than the bleach solution. The order of observed effectiveness (which wasn't necessarily statistically significant) was the same for both temperatures, suggesting that the treatments react similarly to changes in temperature, or possibly that temperature did not have an effect (although this remains to be formally tested).

Overall, the Pro-San™ and Pro-San™ Soft solutions have all proven to be more effective than the currently used bleach solution (and controls) at room temperature, and the 0.78% Pro-San™ solution proved more effective then the bleach (and controls) at refrigerated temperature. Therefore, Pro-San™ and Pro-San™ Soft solutions may be a possible replacement for 150 ppm bleach, because they are more effective when the worst-case scenario (a biofilm) is present.

Color Measurement Results

Two-way ANOVA analysis was done on the L*, a*, and b* values at each time point to compare treatments, and no significant differences were observed between treatments at any time point for any of the three values tested (all P values were greater than 0.05) (see Figures 3, 4, and 5).

Two-way ANOVA was also completed for the ΔE values (the overall color differences between each treatment and the distilled water sample for that set) and again no significant difference was observed among treatments or times (the P value for treatment effect was 0.359), and also there was no interaction among the variables (P=0.783).

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Sensory Panel Results

Of the 50 panelists who completed the triangle tests by evaluating both the lettuce treated with 0.78% Pro-San™ and lettuce treated with 0.78% Pro-San™ Soft vs. water treated lettuce, 13 were able to determine the odd sample among the 0.78% Pro-San™ samples and the water samples, and 20 were able to determine the odd sample among the 0.78% Pro-San™ Soft samples and the water samples. Both of these values were less than the required amount of 23 correct responses at α=0.05 for 50 panelists, and therefore the treatment samples are not statistically different from the control water samples when the treatment is applied for 2 hours and subsequently rinsed off (Meilgaard, Civille, Carr 2007).

Of the 21 panelists who completed the triangle tests for the 0.19% Pro-San™ and Pro-San™ Soft treatments vs. water on the first day of testing, 7/21 selected the correct odd sample for the 0.19% Pro-San™ test, and 8/21 selected the correct odd sample for the 0.19% Pro-San™ Soft test. 12 correct responses was required for this replicate to demonstrate that the samples were different from the water treatment, therefore no difference was demonstrated. For the second day of testing (a replicate of the previous 0.19% Pro-San™ and Pro-San™ Soft tests) an additional 29 panelists completed the tests. However, because 2 panelists did not complete the test correctly, only the data from 27 of them was used. Of these 27 panelists, 8 identified the correct odd sample between the 0.19% Pro-San™ and the water treatments, and 10 identified the correct odd sample between the 0.19% Pro-San™ Soft and water treatments. Because 14 correct responses was required to suggest that these samples were different from the water treatments, again no significant difference was shown.

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CONCLUSION

The Pro-San™ and Pro-San™ Soft solutions tested appeared to be promising sanitizers in the food industry. The 0.78% Pro-San™ and Pro-San™ Soft solutions seem to be particularly effective against *Salmonella* in a biofilm, and neither of the tested concentrations of either type of Pro-San™ caused a sensory quality change of the romaine lettuce when rinsed off before consumption, according to the instrumental measurements of color and the overall difference test completed by the sensory panel. When used at room temperature they are both a better choice than the conventionally used 150 ppm chlorine bleach, and when used at refrigerated temperature 0.78% Pro-San™ is a better choice than bleach, which is quickly inactivated by the organic matter in the biofilm and was shown to be no more effective at killing *Salmonella* in the tested conditions than distilled water.

REFERENCES

Annous BA, Solomon EB, Cooke PH, Burke A. 2005. Biofilm Formation by *Salmonella* spp. on Cantaloupe Melons. Journal of Food Safety; 25: 276-287.

Bhagwat AA. 2006. Microbiological Safety of Fresh-Cut Produce: Where Are We Now?. In: Matthews KR. Microbiology of Fresh Produce. Washington, DC: ASM Press. p 121-165. Meilgaard MC, Civille GV, Carr BT. 2007. Sensory Evaluation Techniques. 4TH ed. Boca Raton, FL: CRC Press. 448p.

Fallik, Elazar. Prestorage hot water treatments (immersion, rinsing, brushing). 2004. Postharvest Biology and Technology; 32: 125-134.

Feliciano L. 2009. Shelf Extension of Seafood Using Sanitized Ice [MSc thesis]. Columbus,

www.manaraa.com

OH: The Ohio State University. 162 p. Available from: http://etd.ohiolink. edu/etd/send pdf.cgi/Feliciano%20Lizan el.pdf?acc_num=osu1252965039.

Fonseca JM. 2006. Postharvest Handling and Processing: Sources of Microorganisms and Impact of Sanitizing Procedures. In: Matthews KR. Microbiology of Fresh Produce. Washington, DC: ASM Press. p 85-120.

Hettiarachchy NS, Ravichandran M, Johnson MG, Griffis CL, Martin EM, Meullenet JF, Rick SC. 2010. Electrostatic Sprays of Food-Grade Acids and Plant Extracts are More Effective than Conventional Sprays in Decontaminating *Salmonella* Typhimurium on Spinach. Journal of Food Science; 75: M574-M575.

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A. 2003. Biocontrol of *Listeria monocytogenes* on Fresh-Cut Produce by Treatment with Lytic Bacteriophages and a Bacteriocin. Applied and Environmental Microbiology 69: 4519-4526.

Mendonca A, Reitmeier C, Sikinyi T. Evaluation of a Gras Sanitizer for Enhanced Microbial Safety and Shelf-Life of Whole Tomatoes for ISS and Planetary Outpost. 2004. SAE International; Document number 2004-01-2560.

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APPENDIX

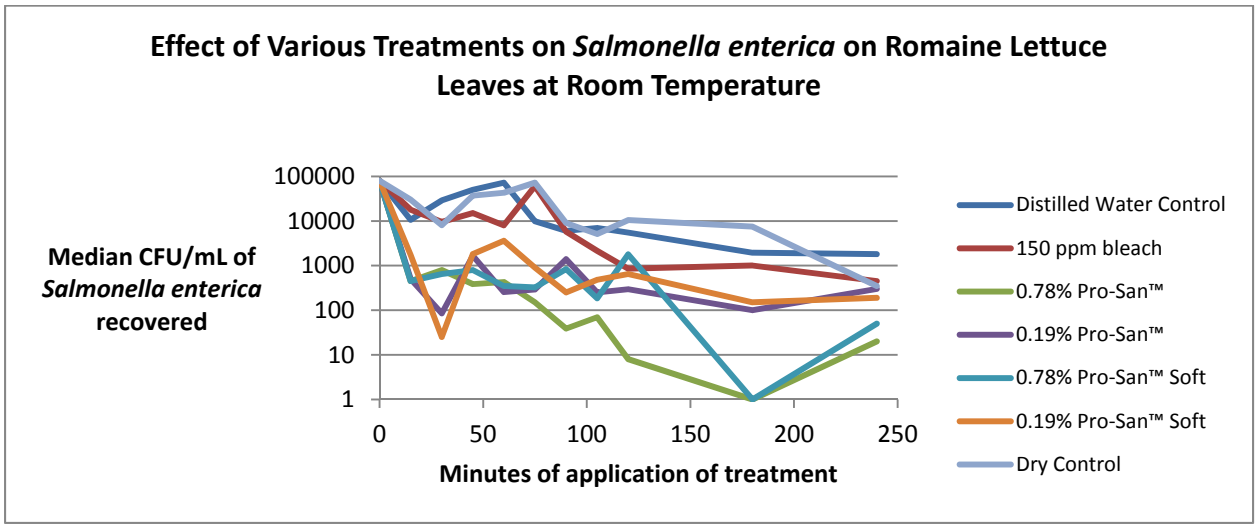

Figure 1: Effect of Various Treatments on *Salmonella enterica* on Romaine Lettuce Leaves at Room Temperature

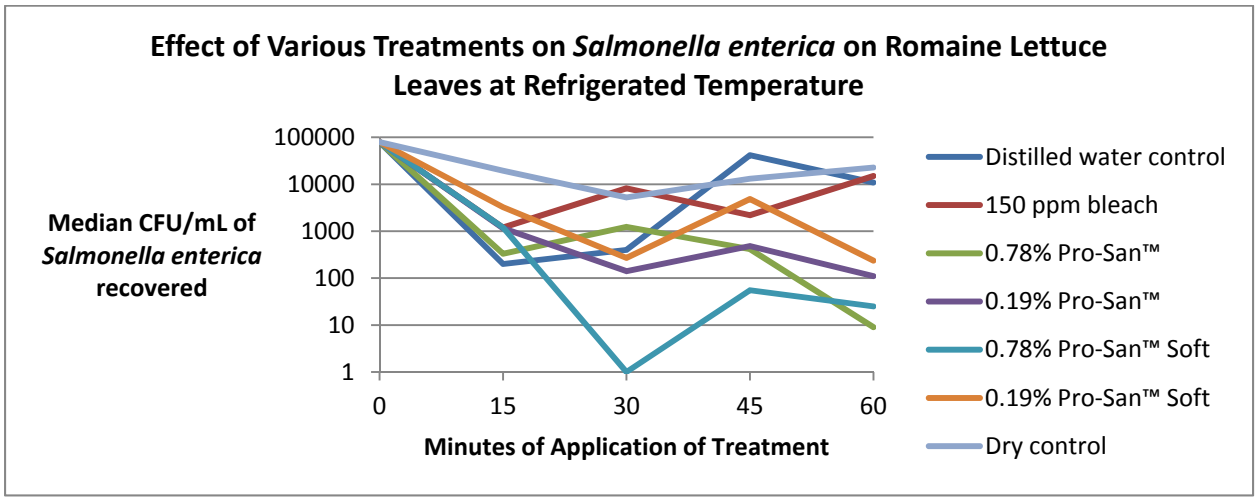

Figure 2: Effect of Various Treatments on *Salmonella enterica* on Romaine Lettuce Leaves at Refrigerated Temperature

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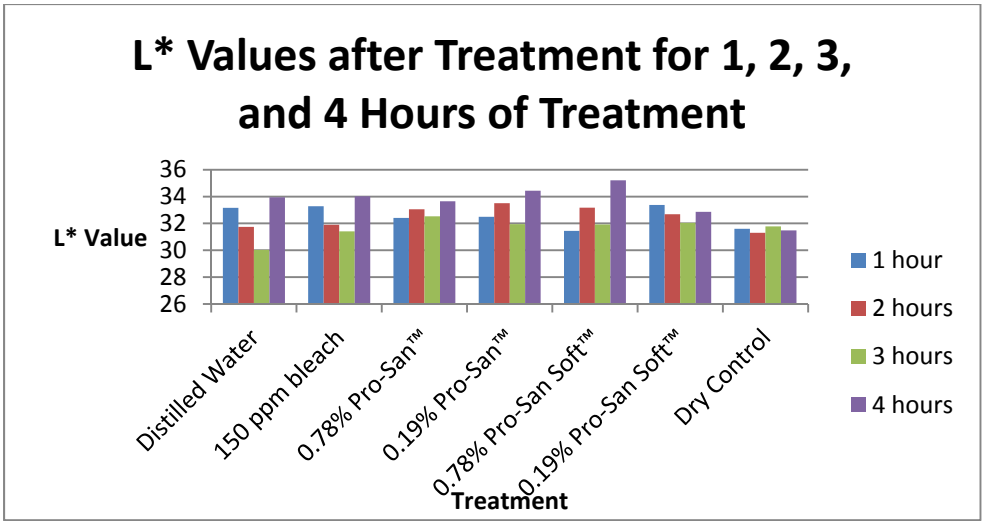

Figure 3: L* Values after Treatment for 1, 2, 3, and 4 Hours of Treatment

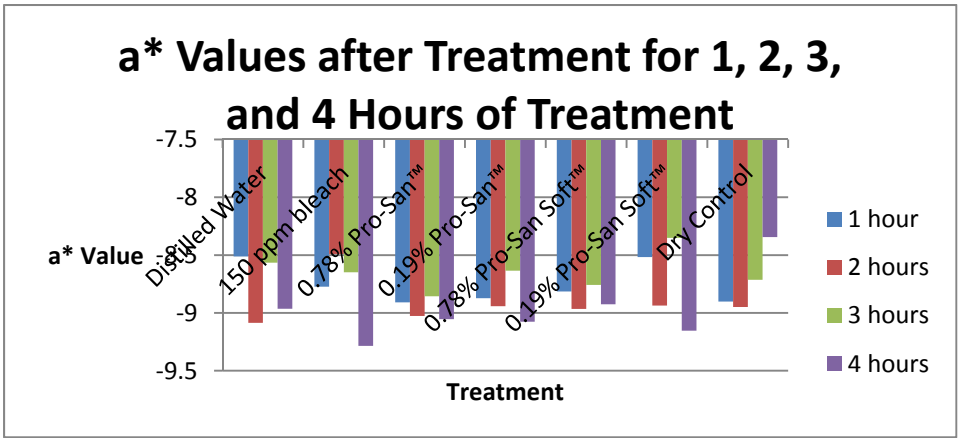

Figure 4: a* Values after Treatment for 1, 2, 3, and 4 Hours of Treatment

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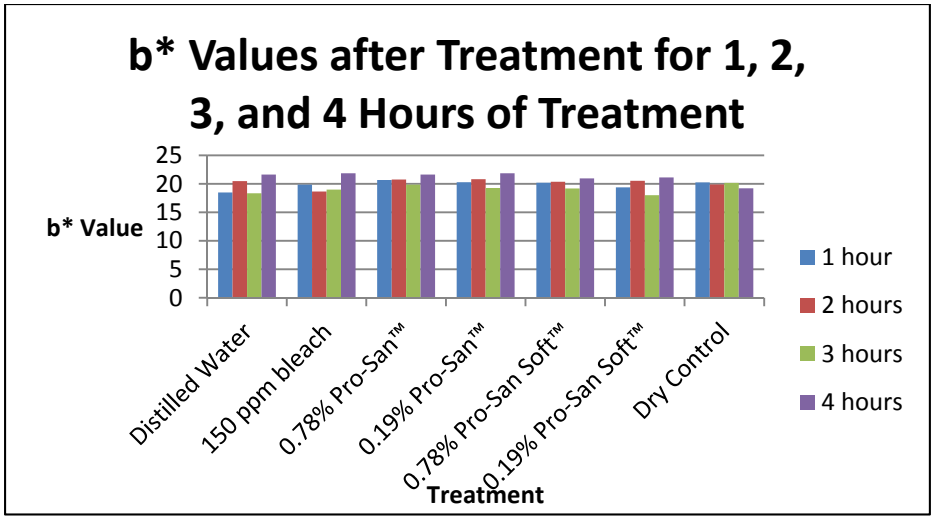

Figure 5: b* Values after Treatment for 1, 2, 3, and 4 Hours of Treatment

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CHAPTER 4: GENERAL CONCLUSIONS

General Discussion

The most commonly used sanitizer in the food industry, chlorine bleach, clearly is not the best choice when sanitizing fresh produce. Organic matter in the wash water, on the surface of the produce, or from a biofilm produced by bacteria quickly bind to free hypochlorite ions, making them not only ineffective at killing microorganisms but also carcinogenic (Silveira 2008). There are many possible alternatives to bleach, but most have other disadvantages, such as cost, negative quality effects, instability, and inability to penetrate hydrophobic surfaces of produce, among other things.

Pro-San™ is a promising sanitizer because it is more effective than bleach when tested against a biofilm, causes no known quality effects (when rinsed off before consumption), is stable in solution, and can penetrate the hydrophobic cuticle of lettuce leaves to access bacteria that may be embedded inside. It is also made entirely from Generally Recognized as Safe (GRAS) ingredients, is biodegradable, and doesn't negatively affect lettuce quality. However, cost may be an issue, because it would be a relatively expensive alternative to chlorine bleach.

Recommendations

Certainly, more research needs to be done in this area in order to make the best decision for both consumers and producers of fresh produce. In particular, other sanitizers should be tested against bacteria in a biofilm to determine their effectiveness when a worst case scenario is present, and Pro-San™ and Pro-San™ Soft need to be tested against *E. coli* O157:H7 on romaine lettuce, and against both *Salmonella* spp. and *E. coli* O157:H7 on various other types of fresh produce.

However, unless new data suggests otherwise, Pro-San™ could potentially replace chlorine bleach as the in-field sanitizer of romaine lettuce, although it should be rinsed off prior to vacuum cooling. The 0.78% concentration should be recommended because although the effects of this

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concentration were no different than those of the lower concentration tested (0.19%) in either experiment, it was the only treatment shown to be more effective than chlorine bleach when tested at refrigerated temperature, and lettuce is quickly refrigerated after harvest. Furthermore, Pro-San™ should be recommended over Pro-San Soft™ because Pro-San™ has been shown to be more effective in previous research (Mendonca, Reitmeir and Sikinyi 2004). Hopefully this concentration of Pro-San™ will prove to be effective against *E. coli* O157:H7 as well, and aid in the effort to reduce future outbreaks of not only romaine lettuce but other types of fresh produce as well.

References

Mendonca A, Reitmeier C, Sikinyi T. Evaluation of a Gras Sanitizer for Enhanced Microbial Safety and Shelf-Life of Whole Tomatoes for Iss and Planetary Outpost. 2004. SAE International; Document number 2004-01-2560.

Silveira AC, Conesa A, Aguayo E, Artes F. 2008. Alternative Sanitizers to Chlorine for Use on Fresh-Cut "Galia" (*Cucumis melo* var. *catalupensis*) Melon. Journal of Food Science; 73: 405-411.

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APPENDIX: DIFFICULTY WITH TESTING OF PRO-SAN™ SOLUTIONS ON *ESCHERICIA COLI* 0157:H7 ON ROMAINE LETTUCE

The experiment with *Salmonella enterica* on fresh romaine lettuce was also done with *Eschericia coli* 0157:H7 on romaine lettuce with just one difference besides the inoculum: the selective agar used was Sorbital MacConkey Agar supplemented with nalidixic acid (SMAN). On this type of agar (especially when supplemented with nalidixic acid), only two types of colonies would be expected to be seen: clear colonies (0157:H7) and pink colonies (other *E. coli*). However, every time that this experiment was completed, there were pink colonies and several different types of clear colonies. Some were more glossy than others, and some were more translucent or opaque, but several different types of clear colonies were observed. When these clear colonies and the pink colonies were tested with an agglutination assay that would agglutinate when it came into contact with *E. coli* O157:H7, a few pink colonies were positive and most were negative, and some clear colonies were positive and some were negative. It became clear that it was impossible to tell which colonies were actually O157:H7 without testing every colony, so the experiment was discontinued. Therefore it is suggested that when completing a similar experiment, a different agar should be used, because SMAN was not successful in distinguishing O157:H7 *E. coli* from other *E. coli* in this experiment.

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