Efficacy of Detergent Rinse Agents to Remove *Salmonella* and *Shigella spp.* from the Surface of Fresh Produce

By

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Efficacy of detergent rinse agents to recover pathogenic bacteria from the surface of fresh produce

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ABSTRACT:

Fresh produce has been implicated in several foodborne outbreaks. A primary site of microbial contamination for produce occurs on the surface during production and handling. An approach to reduce contamination is to sample the surface of produce. This study used different detergent agents at 22°C and 40°C to determine their efficacy for recovery of pathogenic bacteria, from surfaces of several produce types and examined survival of organisms in detergents over time. Strawberries, tomatoes and green leaf lettuce were dip inoculated in a 6-6.5 LOG CFU/ml cocktail of nalidixic acid resistant organisms. After drying, produce were rinsed with either 0.1 % sodium lauryl sulfate (SLS), 0.1% Tween 80, or water at different temperatures. Rinse solutions were plated onto Tryptic Soy agar supplemented with 50-ppm nalidixic acid (TSAN). About 4 LOG CFU/ml of *Salmonella*, and 3-LOG CFU/ml *Shigella* were recovered, with slightly lower recovery from tomatoes. Inoculated strawberries rinsed with SLS, displayed minimal recovery at ~1.5-LOG CFU/ml at 22°C, and <1-LOG CFU/ml at 40°C. When whole strawberries treated with SLS were analyzed, few *Salmonella* were recovered. Lack of recovery of *Salmonella* rinsed with SLS, suggests SLS may be inactivating *Salmonella*, especially at elevated temperatures.   Detergent solutions were inoculated with 3-LOG CFU/ml cocktail and incubated for up to 32 hours at 22°C, and 40°C. Aliquots were plated onto TSAN at varying times. All solutions at 40°C allowed *Shigella* to grow. SLS gave initial drops in

Salmonella populations followed by slight recovery. SLS may cause an initial injury of

Salmonella. While organisms were able to survive in detergents, the application of detergents to

produce was no more effective in recovery of organisms from produce than water.

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INTRODUCTION / JUSTIFICATION:

Fresh fruits and vegetables are responsible for a growing number of foodborne disease outbreaks each year. These products are minimally processed and lack a step for pathogen inactivation, making it difficult to ensure that they are safe for consumers. One way to ensure safety of produce is through the development of a rinsing agent that would be effective in removing pathogenic bacteria from surfaces. Steps need to be taken to develop such a product in the attempt to significantly reduce microbial loads from produce surfaces.

Two enteric pathogens of interest were addressed during this project due to their significance in outbreaks associated with produce. *Salmonella* and *Shigella spp.* will be examined to determine the effect of detergent rinse agents in their recovery from the surfaces of strawberries, tomatoes and green leaf lettuce. Detergents have been studied for their use in aiding other anti-microbial surface rinses. However, currently there is little information regarding the use of detergents alone as fruit and vegetable rinses. Detergents alone, may increase the recovery of microorganisms from surfaces making them successful rinses to be implemented both prior to marketing of the product, as well as for consumers to use in their own homes. Sodium lauryl sulfate and Tween 80 are two detergents that will be examined. These both have different actions, one is an anionic detergent, and the other is a non-ionic detergent. These will be studied at room temperature (22^oC) and 40^oC to determine the effects of temperature.

Strawberries, tomatoes and leaf lettuce were chosen due to their differences in surface topography to better understand how this factor plays a role in the removal of pathogens. In addition, when testing a produce rinse agent, it is important that it would be effective on a variety of produce types. For the years between 1990 and 1998, 16.7% of all produce outbreaks lettuce

was implicated, with a generalized salad bar category the highest at 35.4% (16). Tomatoes accounted for 2.1% of produce outbreaks, and fruit accounted for 20.8% (16).

Surveys have shown that consumers are concerned about the safety of their produce. McWatters et al. (47) surveyed a wide range of age groups and social demographics to find that 87% were concerned about the safety of the fruits available in the grocery store. In addition 53% of those surveyed would consider purchasing an antibacterial solution for use in the home (47). An additional 39% were undecided (47).

Literature Review

CONSUMPTION OF FRESH PRODUCE:

Over the past two decades consumption of fresh produce worldwide has been increasing (61). This can be attributed to many different factors. In 1991 the National Cancer Institute (NCI) (49) launched their "five-a-day" campaign to encourage Americans to consume at least five fruits and vegetables per day to prevent cancer and ensure lasting health (49). In launching this campaign, there was a surge of advertisements through the media stressing the importance of fresh fruits and vegetables in the diet (49). An evaluation of this program in 1999 showed that produce consumption increased from 23 percent of the population achieving the recommended 5 a day in 1991, to 26 percent in 1998 (49). It was recommended that more emphasis be placed in this program, and in April 2002 the NCI formed a partnership with the United States Department of Agriculture (USDA) in attempts to strengthen the program even more (49). As a result, the consumption of fresh fruits and vegetable should increase even more in the years to come making it essential to address their safety. Currently 577 – 718 lbs/year are consumed in the United States alone (19).

Additionally, the geographic sources and distribution of fresh fruits and vegetables has been expanding recently, including a growth in global trade of these products (61). Expanding global trade eliminates the seasonality associated with some produce types. There has also been a growing segment of the produce market shifting to fresh cut (62), minimally processed products, including an increased amount of salad bars in grocery stores and restaurants (61). According to Garg et al. (35) the consumption of raw, or minimally processed vegetables has increased over the consumption of processed canned or frozen vegetables. Consumers tend to view fresh produce as being healthier and increasingly convenient. In addition to the benefits to

consumers, food service provider's also find less labor and waste associated with purchasing produce pre-cut and pre-packaged (39).

FOODBORNE OUTBREAKS RELATED TO PRODUCE:

Coupled with the increase of fresh produce available to consumers, is an increase in produce-related foodborne illnesses. According to Tauxe et al., between 1973 and 1987 an estimated 2% of foodborne illnesses were associated with fresh produce. Comparatively, between 1988 and 1991 it was estimated that cases of foodborne illness associated with produce increased to 8% (61). More recently, it was estimated that in comparison of the years 1973 – 1987 and 1988 – 1998, produce associated outbreaks have more than doubled (19). It is thought, however that these occurances are underestimated. While these numbers relate to the number of cases actually reported, there were probably almost twice as many unreported cases. Tauxe et al. (61), also states that this information is underestimated due to the short shelf life and rapid turnover rates of fresh produce products. These foods are rarely still available by the time a related outbreak is confirmed, making it difficult to determine the infective agent as well as the number of persons that may have been affected. In addition, produce is often combined with other ingredients; therefore it is sometimes hard to determine the exact food carrier (61). Currently, there are an estimated 76 million cases of foodborne illness per year in the United States. Of those cases, 325,000 are hospitalized and 5000 result in death. These numbers are projected for both produce and non-produce related (48). As a result of these increases, President Clinton announced a specific produce and imported food safety initiative, separate from the National Food Safety Initiative in 1997 (19).

The Center for Food Safety and Applied Nutrition (CFSAN), a division of the United States Food and Drug Administration (FDA) conducted a survey in 2000, to determine the risk

that imported produce has on the fresh produce market in the United States. The study found that 4% of this produce is contaminated with either *Salmonella* or *Shigella spp.*; 7.3% of this produce was cantaloupe, 2% lettuce, and 1% strawberries (29). A large portion of the United States produce is presently imported; these numbers are only a representative of a small sample. Therefore the possibility for these numbers to be underestimated is great. Currently CFSAN is performing a survey of domestically grown produce. Interim results show that 2.6% of cantaloupe, 1.6% of cilantro, and 1.8% of lettuce are contaminated with *Salmonella spp* (30). Additionally, 0.9% of cantaloupe, 1.6% of cilantro, 4.1% of green onions, and 1.6% of parsley is contaminated with *Shigella spp* (30). Full reports should be completed in the near future.

A few studies have been conducted to determine the microbial flora found on produce in fresh markets from other countries. Garcia Villanova-Ruiz et al. (34) randomly sampled random produce from Spanish markets. Samples included beet leaves, artichokes, celery, cabbage, cardoon, Brussels sprouts, cauliflower, endive, escarole, asparagus, spinach, lettuce, and parsley. Results indicate that 7.5% of the produce is contaminated with *Salmonella spp*, most notably *Salmonella* Typhimirium, and 86.1% is contaminated with *Esherichia coli* (30). Another study performed in Italy found that 68% of lettuce and 72% of fennel sampled are contaminated with *Salmonella spp* (26). In Malaysia, Arumugasawamy et al. (4) sampled leafy vegetables and bean sprouts at local markets. *Salmonella spp*. was found on 4% of the leaf vegetables, and 20% of the bean sprouts.

There are many factors that can explain the rise in foodborne outbreaks related to produce. Madden (1992) reported that produce fits the definition of potentially hazardous food. This definition includes food products that contain the "nutrients necessary to support rapid and progressive growth of infectious or toxigenic microorganisms" (45). Over the past few decades

there have been changes taking place in the food industry.   While the main factor is the increase in global trade, there have been other changes. There has been a shift away from chemical fertilizer for crops to using manure (61). If the manure is not treated correctly before it is applied to crops, any pathogens that are present can infect the produce. There is also an increased amount of meals consumed outside of the home (61). Food prepared outside of the home always has the chance of being prepared by employees that are poorly trained in food safety issues, this could lead to contamination of food through lack of hand washing and cross contamination.

In addition to these factors, the number of persons that fall in to the "at-risk" category for food borne illness has been increasing. This category includes the elderly, the young, and the immuno-compromised who are more susceptible to infection from these pathogens because of their weakened immune systems (61). Finally, the lack of lethality steps in the processing of produce, and production of fresh produce makes it difficult to alleviate the increased risk of contaminated produce reaching the consumer (61).

PATHS TO CONTAMINATION:

Contamination can come from any step along the path from farm to the consumer's table (62).   Most contamination of a product can be traced to the soil, or fertilizer the produce was grown in. Improper storage or treatment of the product can lead to increased growth of microorganisms present on the surface of the fruit or vegetable (62). Cross-contamination can occur from improperly treated irrigation water or wash water in the packaging plants, as well as by improper handling of the product in the presence of animals (61). It is thought that the majority of foodborne outbreaks traced to vegetables are a result of fecal contamination that comes from one of these sources (53).

Pathogenic bacteria, parasites, and viruses are thought to survive on the surfaces of vegetables for months or years (53). Their survival is dependent on several factors. Primarily, the characteristics of the microorganisms present will determine whether they will grow and flourish, or whether the conditions are not favorable for growth. In addition to this, the physiologic state of the plant tissue will determine whether growth can take place. Another factor is the characteristics of the environment surrounding the plant tissue. For example, whether or not the temperature conditions are optimum for growth of the particular organism. Finally, the effects of any processes or agricultural practices that are done to that food before it is marketed to the consumer can have an effect in whether microorganisms have survived, or grown on the surface of the produce (61).

PRODUCE:

The exterior of produce is thought to be a physical barrier, preventing bacteria from penetrating to the inside of the product (61).   The skins, rind, or peel of a piece of produce is thought to be impenetrable to microorganisms. Due to the way that most produce is grown, it already has a high number of normal non-pathogenic microbial flora on the surface that come from the soil, water and air around where the produce is grown (53). The range of the numbers of bacteria present on vegetables vary depending on the type of vegetable. Most concentrations range from 3 $\log_{10}$ count/g to as much as 7.5 $\log_{10}$ count/g (53). The non-pathogenic microorganisms found on vegetables are typically pseudomonads and *Erwinia spp.* These are mostly responsible for spoilage (53). Mcrobial pathogens are typically not found on produce, and would only be found when the produce has been exposed to human or animal waste materials somewhere along the path from farm to table, with the exception of those that are

already naturally present in soil such as *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium botulinum*, and *C. perfringens* (53).

The normal microbial flora of fruits is slightly different from that of vegetables. Bacteria are usually not as prolific on the surfaces of fruits (53). Yeasts and mold are mostly found on fruit. *Aspergillus flavus*, *Rhodotorula spp*, and *Byssochlamys spp*. are the most commonly found yeasts and molds (53), and are typically not harmful to humans.

It is assumed that if the microbial flora can be reduced on the surface of the product, then if any pathogens were present , their numbers would be reduced as well, and the product would ultimately be safe to eat. However, it is not well understood how the microorganisms attach to the surface of produce. Attachment is thought to be a complex interaction that makes it difficult to remove or wash certain microorganisms off the produce. Once the surface of a fruit or vegetable is disrupted, it is thought that growth of microorganisms inside the product will be rapid (61). There are more available nutrients in the interior of produce to aid the microorganisms in growth; they are also protected from practices that may lower their numbers such as rinsing or drying.

Several studies have explored the survivability of pathogenic bacteria on the surface of different types of produce. Del Rosario et al. (21) preformed a study on the survival of *E. coli* O157:H7 on the surfaces of the rinds of cantaloupe and watermelon. They found that the *E. coli* O157:H7 was able to grow when the produce was stored for 14-22 days at 25°C under highly humid conditions (21). In a similar study, it was found that *E. coli* O157:H7 and other non-pathogenic strains were able to survive and in some cases grow on the surface of strawberries that were allowed to remain at 23°C for 24 hours (68). The *E. coli* O157:H7 was reduced in

population, but still able to survive on the exterior of the fruit at refrigeration (5°C) for three days and freezing temperatures (-20°C) for three days (68).

A study completed by Golden et al. (1993) found that *Salmonella spp* readily proliferates on the surfaces of cut melons when incubated at 23°C for 24 hours. This growth was similar to the growth of *Salmonella spp* in TSB (37). However, when the same study was repeated at 5°C, there was no growth found on the melons (37). This suggests that if *Salmonella* was present on the exterior of the melons, and then introduced through the slicing of a knife through the fruit, the growth of the organism to infectious levels would be rapid.

Salmonella has also been shown to grow on the surfaces of papaya and watermelon (27). Inversely, a more recent study showed that while *Salmonella* can survive on the surface of fresh whole and cut strawberries, they are not able to grow, in fact there is some reduction seen over a long period of time (41)

The surface of plants consists of a continuous layer called the cuticle. This is a protective layer around the fruit preventing the plant from weathering, water loss, and leaching. This is thought to be the first line of defense against plant pathogens (5). The cuticle consists of a structural matrix called cutin and wax (5). The composition of these components varies among fruits and vegetables. In a study done on the composition of cuticular waxes, it was found that even among different apple cultivars, there is a significant difference in the mass of the wax around each cultivar (5). These waxes also had a different composition of chemical components (5). The surface chemical components of the cuticle of different produce may play a key role in the attachment and effect of the pathogens to the consumer.

Surface damage of produce including other crevices in the cellular structure provides a great place for microorganisms to evade any types of disinfection steps. Takeuchi and Frank

(60) found that *E.coli* O157:H7 attaches to cracks in the cuticle, trichome, and stomata on leaf lettuce.   It was also found that when rinsing *Salmonella* Chester from the surfaces of sliced apple disks, there was 13 – 19% more bacteria remaining on discs that had no skin, as opposed to disks that contained skin (43). Further scanning electron microscopy showed that the bacteria may be more easily encapsulated by plant tissues from the flesh of the apple than from the skin, also making it harder to rinse off (43). When examining the attachment of bacteria to different locations on the fruit, it was also found that the bacteria attached more firmly and efficiently to the areas around the stem and calyx of the apple than to unbroken skin. This suggests that bacteria may attach more readily and firmly to injured skin or flesh, than to the surfaces of unbroken skin (43). Organisms are sheltered from disinfection in these areas. Rinse agents need to be able to penetrate these areas to prevent the survival of pathogens and more easily aid in their removal.

STRAWBERRIES:

Strawberries consist of four parts. These are the stem, or calyx of the fruit; the exterior of the strawberry called the fleshy receptacle; the interior of the strawberry called the pith, which consists of vascular bundles; and finally the achenes, or seeds that are found scattered over the surface of the fleshy receptacle (63). The vascular bundle is the white 'pitt' of the fruit from which growth occurs (63). These bundles are concentrated in the center of the fruit and extend smaller bundles to the surface, which consist of the seeds, or achenes (63). Strawberries are very acidic and would typically not support bacterial growth, but can support yeast and mold growth. The pH ranges between 3.0 and 3.6 (53).

The most significant spoilage pathogen for strawberries are fungi. Specifically gray mold causing the deterioration of the fruit making it undesirable to the consumer (68). This

deterioration is caused by the fungus *Botrytis cinera* (53). Another common fungal pathogen to strawberries is *Rhizopus stolonifer* causing spoilage rot of the fruit (53). The presence of water on the surface of strawberries greatly increases the chances of fungal growth; therefore the strawberries are not rinsed at all in order to keep them desirable for consumers (68). Due to the lack of a rinse step during processing, transportation and storage it is very possible for bacterial growth to occur if the product has come in contact with any pathogens.

TOMATOES:

Tomatoes come from the family *Solanaceae* (65). Most tomatoes that are consumed are varieties of *Lycopersicon esculentum* Mill (65). These are the most cultivated tomatoes in the United States (65). There are currently 408,740 acres of tomatoes grown in the U.S. accounting for $1,852,148,000 in sales annually (65).

It was estimated in 1990 that the total consumption of tomatoes in the United States was 36.1 pounds per person per year, with fresh market tomatoes making up 44% of that total, and processed tomato products making up 66% (65).

Tomatoes are made up of pericarp, placental tissue, and seeds. The pericarp is a well-defined epidermal layer that is about 3-4 cells thick (65). The pericarp is surrounded by a relatively thick cuticle that is made up of large thin walled cells (65). Tomatoes are made up of 94% water, which allows them to have an extremely high water activity to support microbial growth. However, they are fairly acidic with a pH between 4.2 and 4.39 for red ripened varieties (64). The intact skin would typically protect bacteria on the surface from the lower pH, however broken skin or wounds, would allow the bacteria to become exposed to the lower pH. Some bacteria, however, can be highly acid resistant. *Escherichia coli* O157:H7, and some serotypes of *Salmonella* can withstand a lower pH (64). In their study, Wei et al. (64) found that

Salmonella Montevideo could grow rapidly on the surface of tomato slices where the pH was between 4.31 and 4.52.

Tomatoes have several areas for potential infection or internalization by plant pathogens as well as potential infection or internalization of foodborne pathogens (65). These structures include the blossom end closures, and the stylar scar at the butt end of the fruit (65). Lukasik et al. (44) found that there is preferential attachment of *Salmonella* spp. and *E.coli* O157:H7 around the stem scar, blossom scar, and surface scars as opposed to the intact surface of the produce. In addition to preferential attachment, Wei et al. (64) found that the bacteria also survive better in these areas as opposed to the intact skin.

Tomatoes are classified as fruits, and their normal microflora would be more fungal than bacterial. Some typical plant pathogens for tomatoes include *Alternaria alternata* causing black rot; *Rhizopus stolnifer* causing watery rot, and *Geotrichum candidum* causing sour rot (53). Much like strawberries, it is important to keep tomatoes dry to not aid in additional mold growth on the surface of the fruit.

GREEN LEAF LETTUCE:

Lettuce comes from the family *Asteraceae*, meaning 'Sunflower family' (66). The leaf lettuce in this study is called *Latuca sativa* L. var. crispa (66). Lettuce is the third most important vegetable crop in the United States with an annual value of 1.2 billion dollars (66). In the past 25 years, the consumption of head, iceberg lettuce has remained the same, but the consumption of leaf lettuces (including romaine lettuce) has doubled (66). This can probably be attributed to the growing popularity of ready to eat modified atmospheric packaged salad mixes. These mixes are primarily made up of leaf varieties of lettuce.

In 1999 it was estimated that 31.5 pounds of lettuce per person was consumed (66). Twenty-four percent of this was the loose-leaf varieties of lettuce, with the remaining 76% being head lettuces (66). Much like tomatoes, lettuce is mainly composed of water, giving microorganisms an environment in which to thrive.

A study completed by Li et al. (42) found that when cut lettuce was inoculated with ~3.2 LOG CFU/ml of *Escherichia coli* O157:H7 and stored at refrigeration temperatures for 7 days, growth occurred to reach levels of ~6 LOG CFU/ml. This demonstrates that bacteria can receive required growth nutrients from produce and grow during refrigeration. Leakage of fluids from lettuce tissue as a result of transportation and storage practices could provide nutrients to support growth of bacteria (42).

MINIMAL PRODUCE PROCESSING PRACTICES:

There are several different ways that consumers can purchase fresh produce in the grocery stores. Most produce is purchased in bulk. 'Raw' vegetables are harvested and shipped directly to retail, or sometimes retained in storage for a period of time (53). These types of produce are typically not "processed" or washed at all before being placed in bags, boxes, or crates and shipped to stores (53). On the other hand, raw fruits are typically cleaned and sorted. Some of the cleaning processes can include waxing and dipping in warm water ($40 - 50^{o}C$) (53) which are done to preserve the fruit, not to remove pathogens. These rinses aid in the reduction of fungal loads. Dips sometimes contain fungicides such as benomyl, thiabendazole, or sodium *o*-phenylphenate (SSOP) (53). Following these rinses fruits are typically packaged for shipping in boxes or crates that allow for separation of individual pieces to prevent bruising or further damage to the exterior (53).

The other type of fresh produce that is available to the consumer is 'ready to use' (RTU) produce. These products are fresh cut or minimally processed for added convenience to the consumer. Microorganisms typically proliferate more readily on fresh cut produce because there is greater availability of water and other nutrients (53). It is important to undergo several steps to discourage microbial growth. Ready-to-use produce are typically examined for quality; peeled, cut or shred; washed; dried; and finally packaged before they are presented for sale (2). The most common RTU items include the bagged salads and shredded cabbage that can be found in most grocery stores.

Ready-to-use products and some of the harder fruits are the only type of fresh produce that may receive an anti-microbial, preservative rinse (32). In addition to reducing the microbial load of the product, these rinses also help to reduce enzymatic activity, which spoils the produce (2). Typical rinses are 50-100-ppm chlorine used at a pH between 7.5 and 8.5 for water that will wash produce that contains a large amount of soil or organic matter (24), or 1% citric or ascorbic acid (32). These processes are typically done to reduce the entire microbial load of the product, thus reducing any spoilage organisms to give the product an extended shelf-life (32). These procedures are usually automated where the produce is flumed in flowing water or bubbling systems, however this can present a problem because not all types of produce can be flumed or sprayed with wash waters due to the physical stress that is placed on the food (8). For this reason, it is important to look at other methods of decontamination for all types of produce. According to Garg et al. (35) these rinses have been found to be effective in laboratory research, but not necessarily effective in an industrial setting. Chlorine is used in recycled rinse water primarily to keep microorganisms from growing in the water, than to disinfect product before it is put on the market. As the water is recycled (sometimes over days) it can become

contaminated with bacteria and fungi (24). If the chlorine levels drop, or become ineffective, the bacteria can readily infect the produce through lenticels, stomates, and other injuries producing another step along the path where produce can become contaminated (24).

After rinsing, produce is typically centrifuged prior to packaging to remove any excess water that may be present (32). These products are also usually placed in modified atmospheric packaging, further reducing the risk of pathogen growth (32). Modified atmospheric packages utilize different percentages of gases to create an optimum balance of gas inside the package that would delay spoilage (2).   The optimal gas ratio is 2-5% carbon dioxide, 2-5% oxygen, with the balance nitrogen (2). With all of these processes prior to sale of the product, RTU vegetables are usually not associated with foodborne outbreaks.

RINSE AGENTS:

There are many different rinse agents that are being studied for their efficacy in removing pathogenic bacteria from different food products. It is thought that these would be used as a last minute, pre-packaging or shipping method to increase the safety of the produce that is available for the consumer. In addition, an effective and safe rinse agent could be marketed for consumers to purchase and use for rinsing the produce after they have bought it for another line of safety against pathogens.

Currently, rinsing produce with tap water prior to consumption is currently the recommended treatment for reducing any microbial contamination that may be on the surface of the food (38) Chemical treatments have been found to increase efficacy of the removal, however, it can be difficult to receive adequate results from sanitizers without causing deterioration of the food, thus diminishing sensory qualities (38). In addition, some microorganisms can form biofilms on fruit's surface increasing their adherence and making them more difficult to remove

(18). Differing topographies of fruits and vegetables, also make it difficult to remove

microorganisms (18). Some produce; such as cantaloupes have deep crevices offering greater

areas for microorganisms to attach making them more difficult to remove (18).

When researching a reduction in foodborne pathogens, the goal is to ultimately obtain a

5-log kill (18). This recommendation has been set by the FDA for selected food commodities

(18). The Environmental Protection Agency (EPA) assembled the Scientific Advisory Panel in

1997 (25). Their goal was to standardize methods for evaluating produce rinse agents. They

suggested that perhaps a high-log kill would be an unreachable goal. It would be better to reduce

the number of pathogens than have no treatment at all. The panel recommended that when

researching produce, a 2-log reduction in a cocktail using at least 5 strains of *Escherichia coli*

O157:H7, *Salmonella*, and *Listeria* would indicate a good performance standard (25).

CHLORINE:

In order for chlorine to become an effective sanitizer, it must be present in aqueous

solution as free, unbound chlorine, or elemental chlorine (12). It can be added to solution as

hypochlorite salts, or hypochlorous acid. The dissociation of these compounds depends on the

pH of the solution, and the ability for the two products to remain in equilibrium even when being

consumed through microbiocidal activity (6).

According to the U.S. Code of Federal Regulation, sodium hypochlorite is the only

chemical sanitizer or rinse agent allowed to be used to wash fresh produce (28). A maximum of

2,000 ppm can be used. In addition, the effectiveness of decontamination of produce is

controlled by many different factors.   Type of rinse, contact, temperature and the chemical

properties of produce tissue surface all play a role (60).

A study by Park and Beuchat (51), examined the efficacy of chlorine, acidified sodium chlorite, hydrogen peroxide, and Tsunami on the removal of *E.coli* O157:H7 and *Salmonella spp*. from the surfaces of fresh cantaloupes, honeydew melons, and asparagus. They found that they could achieve a 2.6-3.8 log reduction with chlorine, and this served to be the best of the rinses tested (51).

While chlorine seems to be a good choice for a produce rinse, there are some disadvantages. If chlorine is used, then an additional rinse step must be employed to rinse off the residual chlorine on the food product. Chlorine can also react with some constituents in the food products producing toxigenic substances. Chlorine requires very specific pH, temperature, and contact times in order to be effective (2). Specifically, when using hypochlorous acid to provide the free chlorine in solution, its activity and stability depend on the temperature and pH of the water (24). In addition, chlorine can also react with organic matter present in the wash water, therefore available chlorine concentration must constantly be monitored (24). Chlorine has also been found to be ineffective against *Listeria monocytogenes,* which is an organism ubiquitous in soil (6).

HYDROGEN PEROXIDE:

One of the most promising alternatives to chlorine is hydrogen peroxide (H_2O_2). While this treatment is still experimental, one of the advantages is that unlike chlorine, there are no chemical residues produced (18). In addition, the sensory quality of the produce is not decreased (2). Hydrogen peroxide is also Generally Recognized As Safe (GRAS) for use in food products as an antimicrobial agent (54). Its vapor treatment has been found to reduce microbial populations on mushrooms (54) cucumber, bell peppers and zucchini to name a few (2).

Peters (52) found a 4-log reduction when a 3% hydrogen peroxide solution was used alone to wash *E.coli* O157:H7 from tomatoes, and a 5-log reduction in populations of *Shigella sonnei* from fresh cut green pepper.   In other experiments, Sapers et al. (55) found a 3-log reduction. Each of these studies had reductions of pathogens within the limits set by the EPA.

Hydrogen peroxide has also been shown to extend shelf-life of food products through the reduction of spoilage microorganisms. Immersion of fresh cut cantaloupe cubes in 5% H_2O_2 resulted in the extension of shelf life from 7 to 14 days. This extension was greater than that resulting from 20 ppm of chlorine (54).

ACETIC ACID:

Research has also been conducted on acetic acid as a rinse agent for produce. Peters found that a 5% acetic acid solution showed a 3.5 log reduction in populations of *E.coli* O157:H7 on tomatoes; a 3-log reduction in populations of *Shigella sonnei*. on fresh cut green peppers; and a 2.5-log reduction in populations of *Shigella sonnei* on lettuce, and *E. coli* O157:H7 on broccoli (52). When 3% hydrogen peroxide was added to 5% acetic acid solution, the log reductions increased. She found an additional 0.5-log reduction in populations of *E.coli* O157:H7 on tomatoes; an additional 1.5-log reduction in populations of *S. sonnei* on lettuce, and *E. coli* O157:H7 populations on broccoli, and finally an additional 2-log reduction in populations of *S. sonnei* on green peppers (52).

OZONE:

Ozone has also been tested for its efficacy in reducing microbial load on fresh fruits and vegetables as well. Microgram per milliliter concentrations(ppm) have shown to be lethal to bacteria, viruses, fungi and amoebae (6). Ozone has been found to extend the shelf life of oranges, strawberries, raspberries, grapes, apples and pears (11). The problem with ozone,

however, is that it has been shown to cause physiological injury on certain produce at concentrations as low as 1.5 µg/ml (11).

ADDITIONAL RINSE AGENTS:

Some additional rinse agents being researched include chlorine dioxide ($C1O_2$), peroxyacetic acid and, trisodium phosphate (TSP) (18). Chlorine dioxide has shown promise for sanitizing processing equipment, and whole fresh produce. A 1-log kill was associated with 1–5 ppm when used on produce (18). It has been shown to be an easier chemical to work with than chlorine because it is less effected by pH and organic matter, and it shows a reduced number of chlorinated by-products (18). Peroxyacetic acid has been used in flume water and for fresh cut fruits and vegetables and has shown a 2-log reduction when used at 200ppm concentrations (18). Finally, TSP has been shown to give 4-log kills on green tomatoes and lettuce at 1-10% concentrations, however, it can only be used in solution at a pH of 11-12. Therefore it's use is limited.

Organic acids have the potential to be used as produce rinse agents (6). The use of these as washes and sprays, containing specifically lactic acid, has been shown to successfully decontaminate meat carcasses (6). In addition, medium chain fatty acids also have the potential to be used. Twelve to 18 carbon chain fatty acid chains have exhibited antimicrobial activity against gram-positive bacteria and yeasts (6).

There is also the potential for successful biological control of pathogens. Certain species of yeasts have been shown to have an antagonistic effect against pathogenic bacteria in some fruits (6). Additionally, bacteriocins produced by certain lactic acid bacteria are being researched in their effectiveness against controlling growth of spoilage and pathogenic organisms on the surface of produce (6).

With the addition of any type of chemical to food products, there is the issue of sensory differences in the foods. One study determined whether rinsing apples with 1.5% lactic acid, or 1.5% hydrogen peroxide changed their sensory perception. These treatments showed no adverse sensory perception to the consumer, and they also did not affect the sensory quality of the apples following refrigerated storage for 10 days (47).

DETERGENTS / SURFACTANTS:

Water is a very poor wetting agent. When detergents called surfactants are added to water, the water's surface tension is lowered making it a better wetting agent (59). Surfactants consist of hydrophilic heads and hydrophobic tails. Sodium lauryl sulfate (SLS), for example, is an anionic surfactant. The active group on the molecule is the anion.

When large amounts of surfactants, or surface acting agents, are added to water, they will cluster around the surface. Their hydrophobic tails will be pushed upward in the attempt to not be in contact with the water. Once the surface is full of these molecules, the surfactants will form clusters called micelles. The hydrophilic heads of the molecules form a boundary around the hydrophobic tails. Surfactants are active detergents because they will migrate towards the oil on the product. The micelle will open up because now the hydrophobic tails will be attracted to the grease. The tails will embed in the grease and then the micelle will close, trapping the grease inside the micelle. With the grease inside, the micelle is negatively charged on the surface. These new grease micelles will repel each other due to the negative charge, not allowing the grease to get back into the water (59)

These studies offer strong evidence that detergent rinses may be very effective produce rinses. Depending on the surface chemistry of the produce type, and the chemical interaction

with the microorganism, detergents may offer a way to disrupt those interactions and rinse pathogens off surfaces more readily.

SODIUM LAURYL SULFATE:

Sodium Lauryl Sulfate (SLS), is one of the most common surfactants. It is an anionic surfactant that is sub classified as a sulfuric acid derivative. Anionic surfactants are generally the most cost effective of the detergents. The hydrophobic part of the molecule contains a negative charge that makes the molecule ionized. In the case of SLS, the ionized part of the molecule is an alkyl sulfate (50).

These surfactants are typically food foamers, and have an excellent oil/water emulsifying properties. These are usually used in cosmetics and personal hygiene products. They can also be used in conjunction with other surfactants to improve foaming characteristics. Pure SLS is sometimes used as a foaming agent in dental creams for oral care (50).

Sodium lauryl sulfate is similar to dodecyl sodium sulfate, dodecyl sulfate sodium salt, lauryl sulfate sodium salt, SDS, and sodium dodecyl sulfate (57). There is little research regarding the use of SLS for use in produce rinsing.

TWEEN 80:

Tween 80, a non-ionic surfactant, can also be called polyoxyethylene sorbitan monooleate, polyethylene glycol sorbitan monooleate, or simply polysobate 80 (56). It is typically used in selective protein extraction, and isolation of nuclei from mammalian cells (56). It is a registered trademark of Uniquema (56). Tween 80 is composed of approximately 70% oleic acid as a balance of linoleic, palmitic, and stearic acids (56).

In a study performed by Cheng and Beuchat (17), they found that when treating chicken skin with 5% Tween 80and 1% trisodium phosphate (TSP), the removal of *Salmonella spp.* was

enhanced over treatment with 1% TSP alone. Adams et al. (1) they found that when 1000mg/L

of Tween 80 was added to a chlorine treatment, the efficacy of the chlorine's ability to sanitize

was increased.

Lukasik et al. (44) also found that Tween 80, when mixed in PBS solution, was the most

effective rinse agent in their study for enumerating *Salmonella spp* from inoculated strawberries.

Other effective rinse agents that were tested included 0.25M-Lysine with 0.1% Tween, and

0.25M-Glycine with 0.1% Tween (44).

Beuchat et al. (9) completed a study on inoculated sprout seeds using different rinse

agents. Varying concentrations of chlorine were used, as well as the consumer produce rinse,

FIT. The active ingredient in FIT is oleic acid, which is the major constituent in Tween 80.

They found that there were significant reductions in populations of *Salmonella spp* and

Escherichia coli spp with both 20,000 ppm Cl and separately with FIT (9). They concluded that

FIT would be an excellent alternative to chlorine as an anti-microbial used in produce industry

(9).

Harris et al. (38) found similar results on the efficacy of FIT to enumerate pathogenic

bacteria. When used as a produce wash for the surface of tomatoes, FIT reduced numbers of

Salmonella 2 to 4 logs greater than sterile water, and neutralizing buffer (38). A similar study

compared the efficacy of FIT to 200 ppm chlorine showing a 3 to 6 log reduction of *Salmonella*,

and a 3 to 5 log reduction of *L. monocytogenes* from the surface of fresh tomatoes (10).

PATHOGENIC ORGANISMS:

Foodborne pathogens can cause either acute or chronic illnesses, which lead to serious

problems, such as digestive diseases, kidney failure, septicemia, and even death (62). Virtually

any pathogen could be of concern on minimally processed produce. However, in the past few

years, a few pathogens have emerged as the most commonly identified. *Shigella spp*, *Salmonella spp*, *Clostridium botulinum* and *Listeria monocytogenes* have received the most attention over the past few years, as well as parasites and viruses (12). This study will examine two of these, *Shigella* and *Salmonella spp*.

SALMONELLA SPP.

Just as foodborne outbreaks in general have increased in the last two decades, so have outbreaks related to *Salmonella spp*.   It is estimated that cases of foodborne salmonellosis range from 740,000 to 5,300,000 cases annually (31). The CDC estimates that about 500 deaths occur each year from this illness (40). Mortality rates differ according to age of the individual infected with a 4.1% average rate. The mortality rate is slightly higher for individuals less that one-year of age at 5.8%, and significantly higher for individual over 50, at 15%.   The infectious dose also depends on the age of the individual. For a healthy individual, the infectious dose is between 10^7 and 10^8 organisms (40). This number can be significantly less for immunocompromised individuals, including the very young, and the old.

Salmonella spp also account for the second highest number of laboratory confirmed cases, just behind *Campylobacter spp* according to the state surveillance system known as FoodNet (13). Within 8 states, there were 12,631 laboratory confirmed cases of foodborne illness in 2000, and 4237 of those were identified as being caused by *Salmonella* (13).

Infection comes from ingestion of contaminated food or water, typically poultry, beef and pork. Research shows that about 30% of all raw chicken, 15% of all raw pork and 3% of all raw beef contain *Salmonella* (22). Between 1973 and 1987 it was found that in 1.1% of all *Salmonella* outbreaks were caused by fruits and vegetables (40). It is thought that this number

has increased in the last decade. Other transmission can occur from human to human contact, unsanitary practices and infected pets (22).

Cases of salmonellosis tend to peak in the summer months (11). There is no real explanation for this, but is thought to be because there is more temperature abuse of foods. One explanation is the increased occurrence of picnics and cookouts where undercooked foods are more likely to be eaten and foods are typically set out in the danger temperature zone increasing microbial growth.

CHARACTERISTICS:

Salmonella spp. are Gram negative, rod shaped, non-spore forming aerobic organisms (22). The majority of these are non-motile, with the exception of *S.* Gallinarum and *S.* Pullorum (22). They are ubiquitous in the environment, which makes them very hard to control. There are about 2324 different serovars (40). *Salmonella spp.* are serotyped according to the O antigens of their surface (40). They can also be further classified by their H antigens, or flagellar antigens that are also on their surface (40). Recently the classification of *Salmonella spp.* has changed. These 2324 different serovars, are now broken up into two species groups, *S. enterica*, and *S. Bongori* (41). The majority of the *Salmonella* serovars are classified under *S. enterica* (40). The serovars classified under *S. enterica*, are then further divided into five subspecies or groups (40). As a result of this new classification, there has also been a change in the way that these species are referred. For epidemiological purposes, they can also be broken into two three groups. The serovars of most interest to food microbiologists include the host-adapted serovars; those, which are isolated from animals, and can be pathogenic to humans; or those that are unadapted serovars that show no host preference, but can also be pathogenic to humans (40). The final group

contains those that infect humans only (40). These are typically not associated with foodborne illnesses.

Acute symptoms include nausea, vomiting, abdominal cramps, minimal diarrhea, fever, and headache (22).   These symptoms are sometimes also accompanied with prostration, muscle weakness, faintness, restlessness, and drowsiness (40). Typically onset occurs 5 to 72 hours after ingesting the contaminated food product and will last between 1 and 4 days (22). The mode of action of this organism is penetration into the epithelial cells of the villus in the small intestine. It will stay in the small intestine and multiply causing an inflammatory response in the host. Infected persons can shed *Salmonella* from 2 to 8 weeks prior to onset (22). As many as 5% of all persons infected with *Salmonella* can become carriers (40). The most isolated strains are *S.* Typhimurium*, S.* Enteriditis*, S.* Heidelberg, *S.* Newport*, and S.* Hadar (22).

Salmonella spp have fimbriae, which are proteinaceous material emanating from the cells surface. These are also called F antigens of type 1 pilli (22). These may play a large role in the organism's ability to attach to the surface of foods.

Salmonella can grow at a temperature range between 5 and 45 $^{\circ}$C, with 35°C being ideal; at a pH range between 4 and 9, with 7 being ideal; and in a water activity between 0.945 and 0.999 (22). The effects of pHs below 4.0 or above 9.0 can be bactericidal (40). In addition to these requirements, *Salmonella* is unable to tolerate salt conditions and amount of 9% or higher can also be bactericidal (40).

OUTBREAKS:

Outbreaks are most notably associated among produce in tomatoes, sprouts, watermelon, cantaloupe, orange juice and apple cider (61). However, *Salmonella spp*. have also been found

in mustard cress, artichokes, beet leaves, cabbage, cauliflower, celery, chili, eggplant, endive, fennel, lettuce, parsley and spinach (11).

Other outbreaks have occurred as a result of non-pasteurized orange juice. *Salmonella* Hartford caused one outbreak in Florida in 1995, and *S*. Muenchen caused another outbreak in 1999 (Jay, 2000). The later of the two outbreaks affected 15 states and 2 Canadian provinces resulting in 300 illnesses (40).

Outbreaks as a result of fruits and vegetables are becoming more likely. In 1995 there were 242 confirmed cases of salmonellosis resulting from alfalfa sprouts (40). More recently there have been several outbreaks concerning cantaloupes. In 2001, *Salmonella* Poona contaminated cantaloupes imported from the Mexican border or South America caused 30 individuals to become ill, resulting in 8 deaths (33). In May of 2002, a recall was issued for all Susie Brand cantaloupes (36). These cantaloupes were also imported to the United States and Canada from Mexico. During April 2002, there were 50 confirmed cases of salmonellosis found to be caused by *Salmonella* Poona (36). These cases were found in 4 U.S. states and 2 Canadian provinces (36). Similarly, in 1991, *S*. Poona contaminated cantaloupe cause 400 illnesses in 23 states (33). There was another outbreak in 2000, which affected 39 people in 5 states. All of these outbreaks were traced to imported produce from other countries (33).

In 1998, there was a total of 257 bacterial foodborne outbreaks that resulted in 8410 cases. Of these cases there were a total of 428 confirmed cases of salmonellosis that originated from produce (14). Additionally there were 723 cases where the contaminated food product was never discovered therefore, the number related to produce could be higher than the number that was confirmed (14). Some of the confirmed produce included: salad, tomatoes, alfalfa sprouts, potatoes, tomatillos, and mango (14).

In 1999 there were a total of 222 bacterial foodborne outbreaks that resulted in 6593 cases of salmonellosis. Of these cases there was a total of 1034 cases where the salmonellosis originated from produce (15). Additionally there were 698 cases where the contaminated food product was never discovered (15). Some of the confirmed produce included: alfalfa sprouts, orange juice, fruit, salad, watermelon, mung bean sprouts, clover sprouts, cilantro and mango (15).

Shigella spp.

Shigella spp. are the third most confirmed cause of foodborne illness (13). According to FoodNet, in 2000 among the 12,631 confirmed cases of foodborne illness, there were 2324 laboratory confirmed cases on shigellosis with in those states alone (13). There are about 15,000 to 20,000 cases of shigellosis reported each year in the United States (23). Its main contact with food is from water contaminated with feces (62). Shigellosis is also known as bacillary dysentery (58).

CHARACTERISTICS:

Shigella spp are Gram negative, straight rods that are facultatively anaerobic (23). Some other characteristics are that they are non-motile and oxidase negative (40). They come from the family enterobacteriaceae, which also includes escherichiae and salmonellae (40). They are similar to other enteric bacteria, and will not grow below 10°C or above 48 ^{o}C (40). The optimum temperature for ideal growth is around 35°C. Other growth conditions include a pH range between 6 to 8, and a water activity of 0.94 or greater (40). Unlike other members of the enterobacteriaceae family, humans are the only reservoir for this organism; therefore contamination of food usually comes from fecal-oral contamination by food handlers or

contaminated water (40). Foods that are most at risk for contamination are raw produce and other foods that are handled extensively by workers.

Main symptoms include: abdominal pain, cramps, diarrhea, fever, vomiting,, and blood, pus, or mucus in stools (23). Typical onset occurs 1 to 7 days after ingestion of the organism and can last from a few days, to 2 weeks (23).

The infective dose is relatively low. As few as 10 cells have been found to be infective (23). Shigellosis occurs in the terminal ileum and colon. The organism penetrates epithelial cells of the intestinal mucosa. Inside, they multiply intracellularly and spread to adjacent cells resulting in tissue destruction (23). Shigella also produces a shiga toxin that is slightly enterotoxic, neurotoxic and cytotoxic (23). Shigellosis is typically treated with ampicillin because of its resistance to many other antibiotics (23).

There are only four known species of *Shigella*. These are *S. sonnei*, *S. flexneri*, *S. boydii*, and *S. dysenteriae*. *Shigella dysenteriae* is not typically of concern with foodborne illness. It is mainly implicated in bacillary dysentery, which is most prevalent in underdeveloped countries. Bacillary dysentery results from improper sanitation and cleanliness. It is most likely transferred through contaminated, or under-treated water. The remaining three species, however, are of concern in foodborne illness. These three are all in separate serological groups due to their differing O antigens (40). In 1984, of all the strains of Shigella spp. that were isolated from humans in relation to foodborne outbreaks, 64% were *S. sonnei*, 31% were *S. flexneri*, and 3.2% were *S. boydii* (40).

OUTBREAKS:

Shigella outbreaks among produce are most noted in lettuce and scallions (61). However, *Shigella spp* have been found on onions, green onions, and parsley (11). Between

1973 and 1987, Shigella accounted for 12% of all foodborne illness cases where the etiological agent was identified (40).   The only organisms that were more prevalent were *Staphylococcus aureus* and *Salmonella spp* (40). For the period between 1983 and 1987, there was a reported 44 different outbreaks of shigellosis resulting in 2 deaths (40).

Since *Shigella spp.* are typically spread through the oral-fecal route, there is the potential for many different types of foods to be implicated. Some of the more notable outbreaks involving Shigella spp as the etiological agent will be discussed. One of the largest outbreak occurred in 1987 at a gathering of the Rainbow family. It is thought that over half of the 12,700 people present came down with shigellosis (46). The exact food product implicated was never discovered, but it was thought to be transferred due to the dense crowd of people that was present (46).

Another common environment for shigellosis to occur is on cruise ships. This is thought to be due to the close living quarters aboard the ships and the small areas allocated for food preparation. In 1989 14% of passengers, and 3% of crew members aboard a cruise ship were thought to have become ill from *Shigella spp*. that was isolated from a German potato salad that was served (46).   Another outbreak occurred on the *SS Viking Serenade* in 1994 (46). Five hundred and eighty six of the passengers, and 24 of the crewmembers became sick, and there was 1 reported death resulting from contaminated spring onions (46).

One outbreak occurred in 1990 during Operation Desert Shield. Shigella was isolated from 113 soldiers that were reporting gastrointestinal illness (46). It was thought that the food products that caused the illness were contaminated fresh vegetables (46). This outbreak has particular significance because gastrointestinal illnesses can greatly debilitate troops in the field reducing their effectiveness (46).

In 1998 there were a total of 844 cases of shigellosis that was traced back to produce (14). Some of the produce types that were implicated include: guacamole, parsley, and salsa (14). Additionally, there were 232 cases of shigellosis where the implicated food product was never identified; therefore the number of outbreaks related to produce could have been higher.

In 1999, there was a decrease in the total number of cases of shigellosis. The number that was traced to produce was 42, and 86 cases where the food product was unknown (15). Some of the produce that were implicated include lettuce and basil (15).

Most recently, there was a case of shigellosis infecting at least 140 people in Ottawa, Canada (3). A Greek-style pasta salad that was made in Toronto between May 2, 2002, and May 18, 2002 was found to contain *S. sonnei* bacteria (3). The number of those infected is expected to rise due to the bacteria's long incubation period, and the ability for those that are ill to spread the disease (3).

RESEARCH OBJECTIVES:

The objectives of this project were to evaluate the efficacy of detergent rinses for removing pathogenic bacteria from the surface of fresh produce. Three different types of produce were tested; strawberries, tomatoes, and green leaf lettuce. Topography may play a role in the recovery of organisms from produce surfaces. In addition, seeds, pits, stems, and other scars that may be present on produce may hinder recovery. In order to determine the effectiveness of a rinse, they were tested on several different produce morphologies to compare it's effectiveness.

Salmonella spp and *Shigella spp*. are two important pathogens of interest due to their frequent produce related foodborne outbreaks, and their possible presence on the surfaces of imported produce. Both of these enteric pathogens can contaminate produce when sanitary conditions are not employed during production, harvesting, and marketing.

Sodium lauryl sulfate, and Tween 80 in comparison to water. These will be examined for their effectiveness to remove pathogens at two different temperatures; room temperature (22°C), and 40°C. These detergents could be used in industry as a rinse in plants prior to shipping bulk produce to grocery stores, or it could be marketed to consumers for use in the home prior to consumption.

The objectives of this study were:

1.   To determine the efficacy of a detergent rinse step in the recovery of pathogenic bacteria from fresh produce prior to consumption.

2.   To determine the effect of heat in the use of detergents to remove bacteria

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Running Head: Efficacy of Detergents Rinse Agents to Recover Pathogenic Bacteria from the Surface of Fresh Produce.

Efficacy of Detergent Rinse Agents to Remove *Salmonella* and *Shigella spp*. from the Surface of Fresh Produce

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Abstract:

Fresh produce has been implicated in several foodborne outbreaks. A primary site of contamination can occur on the surface during production and handling. An approach to reduce contamination is to treat fresh produce with rinsing agents. This study used different detergent agents at 22° and 40^oC to determine their efficacy for recovery of *Salmonella* and *Shigella spp.* from the surface of strawberries, tomatoes and leaf lettuce. Produce was dip inoculated at room temperature in a 6.5 LOG CFU/ml cocktail of Nalidixic acid resistant organisms. After air-drying for one hour, samples were rinsed with either a 0.1 % solution of Tween 80, 0.1% sodium lauryl sulfate (SLS), or water as the control at 22° or 40^oC. Rinse solutions were spiral plated onto Tryptic Soy agar supplemented with 50ppm nalidixic acid (TSAN). In each trial 4 LOG CFU/ml *Salmonella* and 3.0 LOG CFU/ml *Shigella* were recovered from each sample, respectively. Recovery of *Salmonella* was 1 LOG CFU/ml lower on tomatoes, and recovery of *Shigella* was 1.5 LOG CFU/ml lower. *Salmonella* inoculated strawberries rinsed with SLS, displayed minimal recovery of 1.5 LOG CFU/ml at 22^oC, and <1 LOG CFU/ml at 40^oC. When whole strawberries were analyzed, minimal organisms were recovered. Reduced recovery of *Salmonella* from strawberries rinsed with SLS, suggests SLS may have a lethal or sub-lethal effect on *Salmonella*, especially when a 40^oC solution is used. Overall, detergents were no more effective in recovery of organisms than water. The detergents examined would not be effective broad-spectrum produce rinse agents.

Fresh fruits and vegetables are responsible for a growing number of foodborne outbreaks each year. These products are minimally processed, lacking a microbial inactivation or removal step. This makes it difficult to ensure fresh produce safety for consumers. For the years 1973-1987 and 1988-1998, produce associate outbreaks have more than doubled (6). These increases are related to both imported and domestic produce (7, 8). It is thought, however that these percentages are underestimated.

In 2000, the FDA conducted a survey of imported produce. It was found that 4% of this produce is contaminated with either *Salmonella* or *Shigella spp.*; 7.3% of this produce was cantaloupe, 2% lettuce, and 1% strawberries (7). The FDA is currently conducting a similar study domestic produce. Interim results show that 2.6% of cantaloupe, 1.6% of cilantro, and 1.8% of lettuce are contaminated with *Salmonella spp* (8). Additionally, 0.9% of cantaloupe, 1.6% of cilantro, 4.1% of green onions, and 1.6% of parsley is contaminated with *Shigella spp* (8).

While the exterior of produce is thought to be a physical barrier, preventing bacteria from penetrating (17), surface damage that may occur during picking or shipping, can provide a great place for microorganisms to bypass the skin, rind or peel. Takeuchi and Frank (17) found that *E.coli* O157:H7 could attach to cracks in the cuticle, trichome, and stomata on leaf lettuce making them harder to remove, and even evade chlorine treatments. It has also been found that many pathogenic organisms are able to survive on produce surfaces. Yu et al (19) found that *Escherichia coli* O157:H7 and Knudsen et al (11) found that *Salmonella spp* were able to survive on strawberry surfaces.

There are many different rinse agents being studied for their efficacy in removing pathogenic bacteria from different food products. These could be used as a last minute, pre-

packaging or shipping rinses to increase the safety of the produce. In addition, a rinse agent could be marketed for consumers to purchase and use for rinsing the produce they have bought for another line of safety against pathogens. One survey has shown that consumers are concerned about safety of their produce. McWatters et al. (12) surveyed a wide range of age groups and social demographics to find that 87% were concerned about the safety of the fruits available in the grocery store, and 52% would consider purchasing an antibacterial solution for use in the home (12).

Rinsing produce with tap water is currently the recommended treatment for reducing any microbial contamination that may be on the surface (9). Chemical treatments have been found to increase efficacy of removal, however, it is difficult to receive adequate results without causing deterioration of the food (9). Some microorganisms can also form biofilms on fruit's surface increasing their adherence, making them more difficult to remove (4). Detergents may provide a safe, effective rinse treatment that would not diminish structural integrity of produce.

Two detergents considered are Sodium Lauryl Sulfate (SLS) and Tween 80. SLS is one of the most common anionic surfactants (13). These are food foamers, with excellent oil/water emulsifying properties (13). Tween 80, a non-ionic surfactant, is typically used in selective protein extraction, and isolation of nuclei from mammalian cells (16).

Many studies have shown detergents effectiveness in aiding a sanitizing agent. Cheng and Beuchat (3) found that when treating chicken skin with 5% Tween 80 and 1% trisodium phosphate (TSP), the removal of *Salmonella spp.* was enhanced over treatment with 1% TSP alone. In addition, Yu et al. (19) found a 1.1 to 1.2 LOG reduction in *E. coli* O157:H7 from the surfaces of strawberries when 100 and 200-ppm Tween 80 was used.

The objectives of this study were to evaluate the efficacy of detergents to recover *Salmonella* or *Shigella* from surfaces of varying types of produce, and to determine whether temperature plays a role.

MATERIALS AND METHODS:

Inoculum preparation: A five strain *Salmonella* cocktail and three strain *Shigella* cocktail was used. All strains of *Salmonella* except *S.* Typhimirium were received from Dr. Larry Beuchat (Research Professor, University of Georgia). *Salmonella* Typhimirium was obtained from American Type Culture Collection (ATCC; Manassas, Va.). *Salmonella* strains include: *S.* Agona (alfalfa sprout outbreak)*, S.* Baildon (lettuce/tomato-associated outbreak)*, S.* Michigan (cantaloupe associated outbreak)*, S.* Montevideo (tomato-associated outbreak)*, and *S.* Typhimirium (ATCC 14028). All *Shigella spp.* were obtained from ATCC. These included *Shigella flexneri* (12023), *S. sonnei* (25931), and *S. boydii* (9207). Cultures were made nalidixic acid resistant by consecutive 24-hour transfers of isolated colonies from Tryptic soy agar (TSA; Difco, Detroit, Mich.) with increasing concentrations of naldixic acid (Sigma, St. Louis, Mo.) until colonies were resistant at a level of 50ppm.

Bacterial cultures were kept frozen in 1:1 glycerol at -80°C. Prior to each experiment, one vial of each culture was thawed, streaked on Tryptic Soy Agar slants containing 50ppm N (TSAN) and incubated for 24 hours at 35°C. One colony was then transferred onto Tryptic Soy Broth tubes containing 50 ppm N (TSBN; Difco, Detroit, Mich.) and incubated for 24 hours. This culture was diluted 1:100 to obtain ~6 LOG CFU/ml inoculum levels.

Produce preparation: All produce was purchase the day of the experiment from a local grocery store. Prior to inoculating, the produce was set out at room temperature (22°C) for 2 hours, after which all produce types were able to reach room temperature. Consistent weights of each

produce type were taken. Samples included: three strawberries weighing 65 ± 15 grams, one vine-ripened tomato equaled one sample weighing 135 ± 20 grams and one leaf of lettuce weighing 3.4 ± 1.5 grams.

Produce inoculation: Produce was placed in a sterile Nalgene© bucket with 4 Liters of 6 LOG CFU/ml of inoculum at 22°C and agitated for 2 min. Samples were then dried in a Laminar flow hood (Nuaire, Plymouth, Mn.) on a sterile stainless steel drying rack for 1-hour.

Rinse Treatment: After air drying, each sample was placed in 500 ml Whirlpak© bags (VWR, Bridgeport, N.J.), and given one of six 250 ml treatments at random. Treatments included: sterile water (22oC), sterile water (40oC), 0.1% sodium lauryl sulfate (22oC) (Sigma, St. Louis, Mo.), 0.1% SLS (40oC), 0.1% Tween 80 (22oC) (Sigma, St. Louis, Mo), 0.1% Tween 80 (40oC). Samples were then agitated on an orbital shaker (Janke & Kunkel, West Germany) at 200 rotations per min. for 2 min.

Microbiological analysis: Undiluted rinsate was plated onto TSAN plates using a spiral plater (Spiral Biotech, Norwood, MA). Plates were incubated for 24 hours at 35oC. Organism confirmation was done at random using *Salmonella-Shigella* agar (Difco, Detroit, Mich.) and API 20E test strips (BioMerieux, France).

Homogenization of whole samples: After drying, whole samples were placed in sterile filtered stomacher bags (Fisher Scientific, Pittsburgh, PA) and 9 ml peptone was added. Samples were homogenized for 1 minute in a Stomacher lab blender 400 (Fisher Scientific, Pittsburgh, PA). Calculations were done to determine amounts of sample and peptone to make a 1:10 dilution.

Statistical analysis: The effects of detergents (no detergent, 0.1% SLS, and 0.1% Tween 80) at (22°C and 40°C) on the removal of *Salmonella* and *Shigella* from the surface strawberries, tomatoes and green leaf lettuce and their interactions on microbial population (log CFU/ml) was

statistically analyzed by a split plot design. All treatments were randomized with 6 replicates of each combination. Each replicate was preformed on different days (1-6). Separate treatment days were nested within the whole-plot factors throughout the model. All three and four-way interactions were disregarded. Tukey's HSD was also preformed to determine significant differences ($\alpha = 0.05$) in treatment means with-in plots. Data was analyzed by JMP statistical software (Statistical Analysis System, Cary, N.C.).

RESULTS:

The population of *Salmonella* cocktail in inoculum was 6.5 LOG CFU/ml, and for *Shigella* was 6.0 LOG CFU/ml. Approximately 5 LOG CFU/ml *Salmonella* were present on strawberries after drying (Figure 1). Therefore there was an initial 1 LOG reduction from inoculum levels due to lethality of the drying step. Lower numbers of *Salmonella* were present on tomato surfaces at 4 LOG CFU/ml, showing about a 2.5 LOG reduction from inoculum levels as a result of drying. Finally, *Salmonella* inoculated on lettuce showed the highest attachment levels of 5.5 LOG CFU/ml, displaying 1 LOG reduction similar to strawberry inoculum levels. *Shigella* levels were similar to those of *Salmonella*, but generally 0.5 LOG CFU/ml lower.

For all negative controls, no nalidixic acid resistant background flora was recovered from any produce type. All API 20E strips were positive for the correct organism, and all SS agar plates displayed typical colony types for *Salmonella* or *Shigella*.

Table 1 shows treatment means for all trials. The statistical model explained 88% of the variation. On average, effects of organism produce type, and detergents were all statistically significant (P<0.0001). Temperature effects were not as significant (P=0.0011). Most two-way interactions were statistically significant. Recovery of *Salmonella* and *Shigella* differed depending on type of produce it was attached to (P<0.0001). Generally, higher populations of

Salmonella were recovered. Higher populations of organisms were recovered from strawberries and lettuce than tomatoes. Organism recovery also differs depending on type of detergent used (P<0.0001). Water had highest recovery rates, with Tween 80 and SLS being less effective.

As shown in Figure 2, for all detergents and temperatures 4 LOG CFU/ml *Salmonella* was recovered with the exception of SLS. Rinses for *Shigella* inoculated strawberries typically recovered 3.5 LOG CFU/ml. This was slightly lower than the general recovery of *Salmonella.* There was minimal recovery of 2 LOG CFU/ml for *Salmonella* inoculated strawberries rinsed with SLS at 22$^{\mathrm{o}}$C, and <1 LOG CFU/ml for those rinsed at 40$^{\mathrm{o}}$C. Further analyses were completed to help explain the phenomena.

Whole strawberries were analyzed to determine whether internalization of bacteria into the core of the strawberry was taking place. Figure 3 shows that as temperatures of SLS increased, fewer bacteria were recovered. There may be an initial internalization, but ultimately cells were unrecoverable. SLS at elevated temperatures showed minimal recovery of <1 LOG CFU/ml from strawberries surface, as well as from stomached strawberries.

Experiments were replicated to determine if SLS was causing injury to cells and not actual lethality. There was no difference between numbers on TSAN and numbers on TSA indicating that there was no sub-lethal injury of bacteria taking place.

Tomatoes yielded the lowest recovery numbers (Figure 4). About 3.5 LOG CFU/ml *Salmonella* was recovered with slightly lower numbers (~0.5 LOG CFU/ml) for rinses at 40$^{\mathrm{o}}$C. About 2 LOG CFU/ml *Shigella* was recovered. Slightly lower numbers (~0.25 LOG CFU/ml) were recovered from rinses at elevated temperatures.

Figure 5 shows data for recovery of organisms from leaf lettuce surfaces. Leaf lettuce yielded the most consistent recovery numbers for *Salmonella spp*. About 4 LOG CFU/ml

Salmonella was recovered for all detergent rinses at all temperatures. Shigella was recovered at concentrations of between 2.5 and 3.5 LOG CFU/ml with the lowest recovery from rinses where SLS was used.

DISCUSSION:

Inoculum concentrations will vary due to the topography of different produce types. Cherry (4) reported that varying surface topographies of fruit can aid or prevent attachment of organisms on surfaces. Produce with more cracks and crevices on the surface will tend to support higher attachment levels (4). Higher attachment levels found with strawberries and lettuce over tomatoes demonstrates this. Beuchat et al. (2) found that tomatoes inoculated with ~7 LOG CFU/ml Salmonella and allowed to dry, showed a 1.5 LOG CFU/ml reduction after just 40 minutes. Strawberries have very porous surfaces covered with achenes (seeds) that can provide shelter for organisms and greater surface areas for attachment. In addition, lettuce is much the same. Pores provide greater surface area, and folds provide areas where drying may not be as rapid as the smooth surfaces of tomatoes, aiding in higher attachment rates. Adams et al. (1) suggests that failure of rinse treatments for lettuce can be a result of folds, or hydrophobic pockets where bacteria can hide. Electron microscopy in their study supported this theory (1). Conversely, tomatoes have a very smooth surface void of large pores or folds. Bacteria would not be as likely to survive the drying time. This accounts for the differences in surface inoculum levels over produce types.

Lower inoculum levels of *Shigella* may be explained because *Shigella* is typically not as fastidious an organism as *Salmonella*. After accounting for 0.5 LOG CFU/ml lower inoculum levels, results in some cases are very similar when comparing *Salmonella* and *Shigella*. In general, recovery of *Shigella* was always lower than recovery of *Salmonella* (P=0.0001), but that

can again be explained by robustness of organisms. In Table 1 it is shown that treatment means for *Shigella spp* tended to show greater variation than *Salmonella spp*.

Reducing the number of *Salmonella* on surfaces would be more significant than reducing the number of *Shigella*. *Salmonella* generally has an infectious dose of $10^7 - 10^8$ cells depending on immunity of the individual (10). If these levels could be reduced, theoretically infectivity would be less likely. This would be much harder to achieve with *Shigella*, where numbers would essentially have to be eliminated to prevent infection.

For *Salmonella* and strawberries, there may be small amounts of internalization into the strawberry pulp, making it difficult to recover organisms from surfaces. Recovery was greatly hindered at elevated temperatures. Rinse pH was examined following rinsing procedures. These values for all solutions were between 4 and 5. Typical pH values for strawberry pulp is between 3.0 and 3.6 (14). Values were taken after solution exposure to the surface of the strawberry, not internal pulp, which explains why they are elevated. *Salmonella* can survive and grow in pH between 4 and 9 with some strains able to survive in more acidic conditions (10); therefore, acidity of solutions would not be responsible for low numbers. More research is needed to determine exact mechanism; however there may be some factor in the strawberry that is causing structural damage to *Salmonella*, which is not occurring with *Shigella*. The presence of flagella on *Salmonella*, but not of *Shigella* (10), may play a role as well.

Lower recovery from tomatoes can be explained by the fact that fewer bacteria may have adhered onto the smooth surface of the tomato. The fewer bacteria attached to produce, the fewer that will be able to be recovered. Beuchat et al. (2) found that water rinsed tomatoes that were inoculated with *Salmonella*, showed 4.5 LOG CFU/ml recovery. These results are similar

because inoculation levels were higher (~7 LOG CFU/ml) in the Beuchat et al. (2) study. The smooth surface of the tomato lacks places where bacteria can hide to evade death by drying.

Higher recovery with lettuce may be explained by the surface topography as discussed before in relation to inoculation levels. In addition, if folds of lettuce were able to shield bacteria in water pools from drying, then these would be much easier to recover due to weaker attachment. Seo and Frank (15) found that *E. coli* O157:H7 attached to leaf surface, trichomes, stomata and cut edges, through confocal scanning laser microscopy. Preferential attachment was seen inside the stomata and cut edges of the lettuce where nutrients would be abundant (15).

Detergents appeared to be slightly more effective at 22°C than at 40°C. This was considered unusual because it was hypothesized that at the higher temperatures, molecules would be more active, enhancing their effectiveness. In industry, raw fruits are sometimes dipped in warm water (40-50°C) in order to enhance cleaning (14). However, this is optimal, because the fewer steps involved in cleaning produce, the more likely that protocol would be adopted.

In accordance with standards proposed by EPA for at least a 2 LOG reduction (6, 9), it is difficult to compare because recovery numbers do not necessarily equate to reductions. In this study, whole samples were not analyzed to determine amounts still attached after rinsing. If it is assumed that recovered bacteria equal bacteria removed from the surfaces, then a 2 to 4 LOG reduction was achieved for each produce type and detergent rinse, with none being more effective than water at 22°C. With the most effective rinse being water at 22°C, it would not be recommended that detergents be used in produce rinsing.

High recovery amounts could be due to the fact that the produce was only allowed to dry for 1 hour. While each produce type appeared dry, perhaps complex attachment of organisms to surfaces may not have had enough time to be established. If organisms were loosely attached,

then they would be much easier to recover through a rinse solution. It would be beneficial to determine the affects of 24-hr dry time. If produce was contaminated, contamination could occur days before consumed.

Acknowledgements:

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TABLE 1. Mean bacterial populations (n = 6) in rinse solutions after a two-minute rinse.

| Rinse Solutions	Bacterial Populations Present in Rinse Solutions (log CFU/ml ± SD)		
	Strawberries	Tomatoes	Leaf Lettuce
Salmonella spp.			
Water (22°C)	4.3 ± 0.21 A	3.8 ± 0.19 A	4.1 ± 0.13 A
Water (40°C)	4.2 ± 0.11 A	3.1 ± 0.51 A	4.1 ± 0.17 A
SLS (22°C)	2.0 ± 1.05 B	3.6 ± 0.38 A	4.1 ± 0.27 A
SLS (40°C)	1.0 ± 0 C	3.1 ± 1.03 A	4.1 ± 0.21 A
Tween 80 (22°C)	4.3 ± 0.15 A	3.7 ± 0.13 A	4.2 ± 0.18 A
Tween 80 (40°C)	4.1 ± 0.13 A	3.4 ± 0.44 A	4.2 ± 0.19 A
Shigella spp.			
Water (22°C)	3.6 ± 0.14 A	1.9 ± 0.35 A	2.9 ± 0.46 AB
Water (40°C)	3.4 ± 0.40 AB	1.8 ± 0.55 A	3.4 ± 0.24 A
SLS (22°C)	3.4 ± 0.27 AB	1.7 ± 0.55 A	2.5 ± 0.80 AB
SLS (40°C)	2.9 ± 0.55 B	1.3 ± 0.36 A	2.3 ± 0.71 B
Tween 80 (22°C)	3.2 ± 0.37 AB	1.8 ± 0.20 A	3.0 ± 0.60 AB
Tween 80 (40°C)	3.2 ± 0.36 AB	1.8 ± 0.64 A	3.1 ± 0.21 AB

*Mean values in the same column within the same microorganism that are followed by different letters are significantly different (P<0.05).
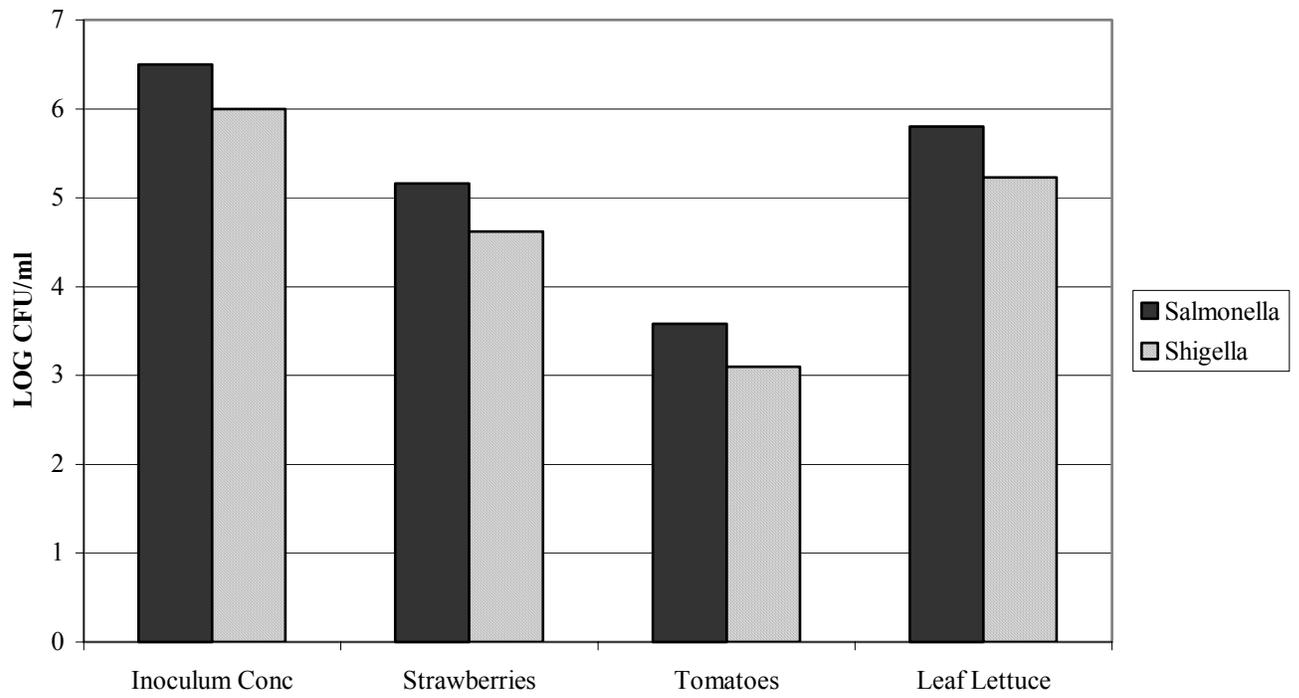
Figure 1. Inoculum solutions in comparison to attachment concentrations (LOG CFU/ml) achieved on surface of different produce types.
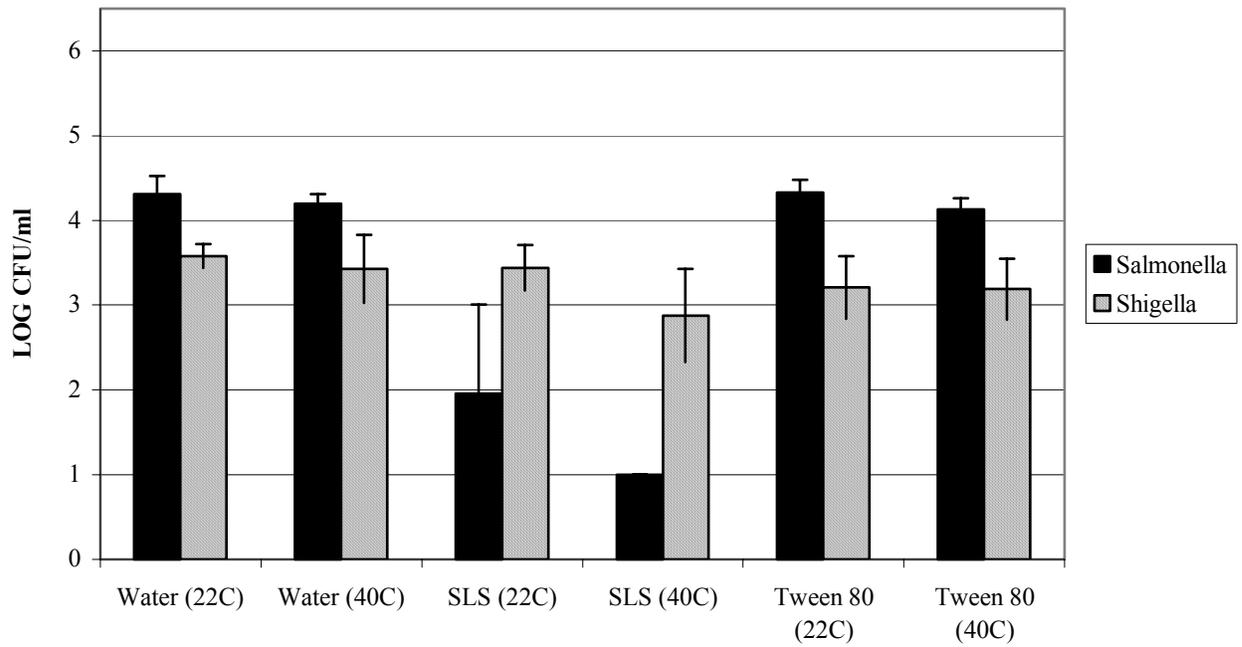
Figure 2. LOG CFU/ml of *Salmonella spp.* and *Shigella spp.* recovered from the surface of fresh strawberries rinsed for two-minutes in sodium lauryl sulfate and Tween 80 at 22 and 40°C.
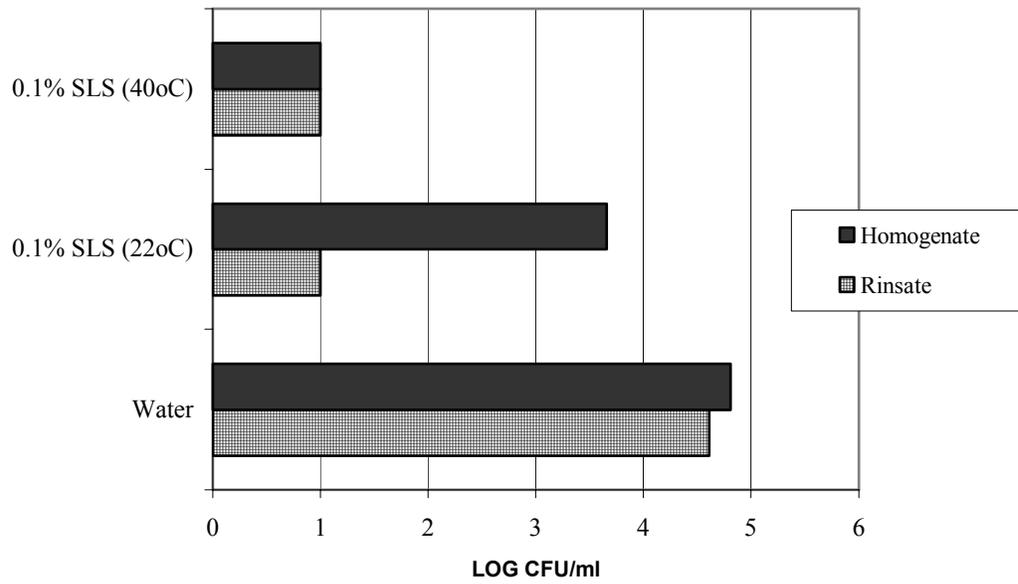
Figure 3. *Salmonella* recovered from two-minute surface rinse solutions and homogenized strawberries when rinsed with 0.1% SLS at 22 and 40°C.
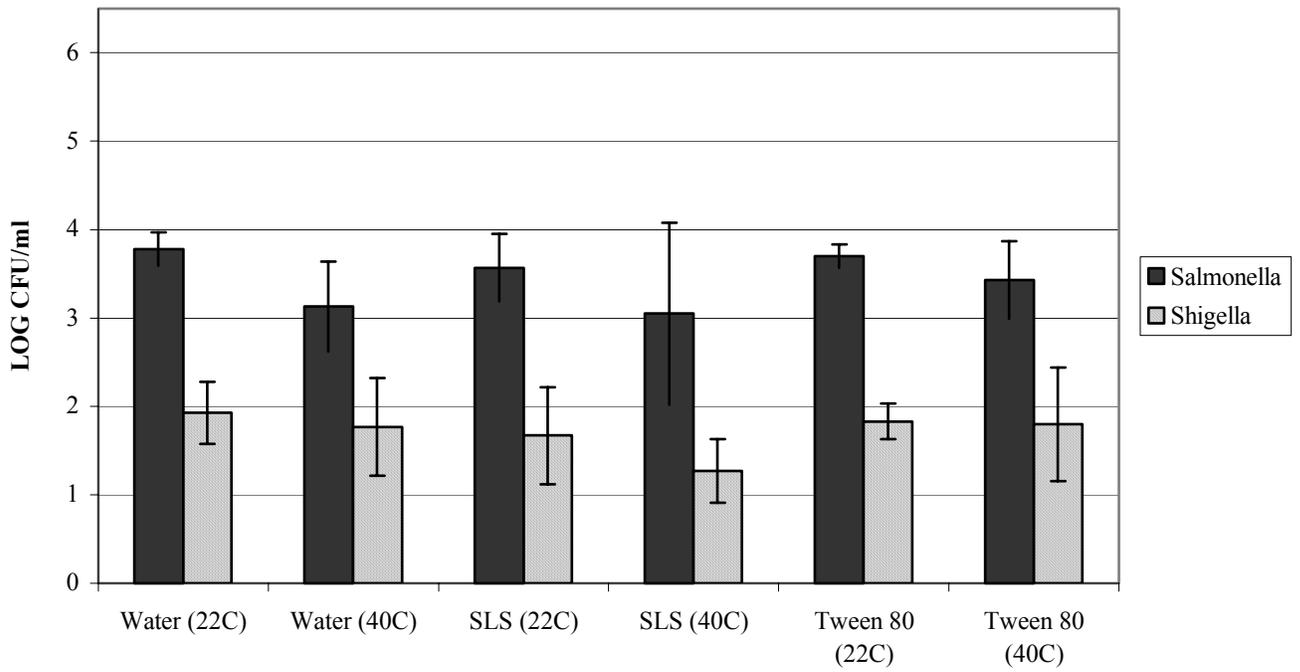
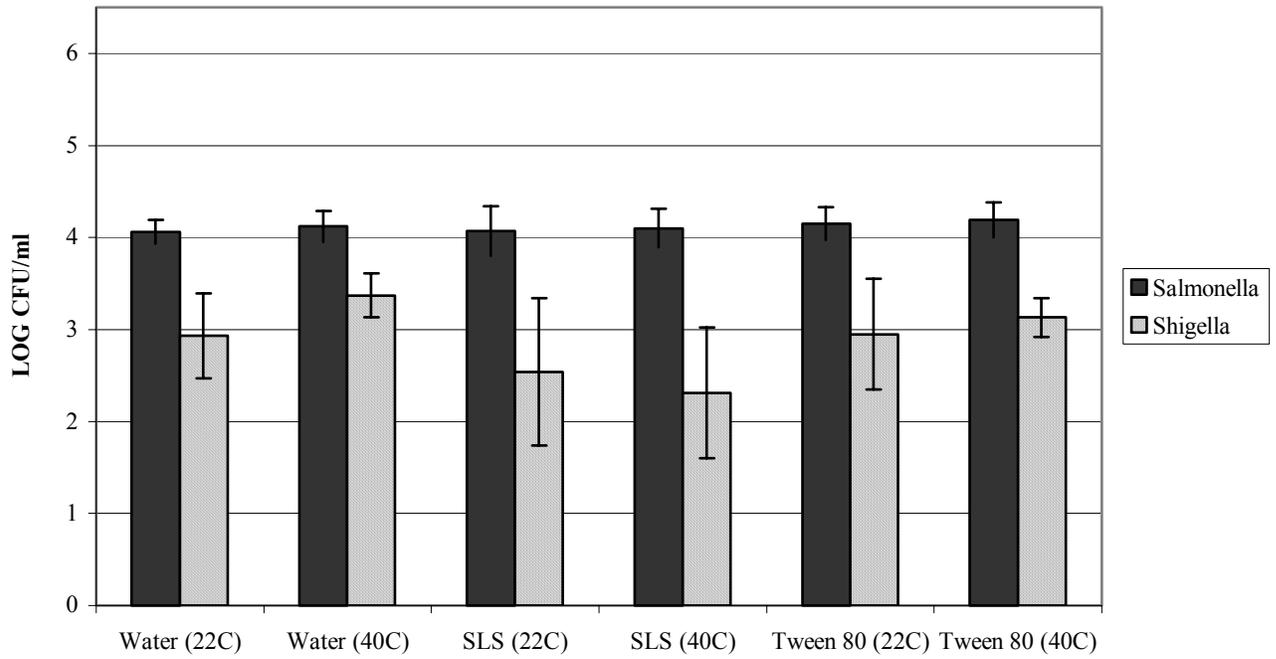**Figure 4.** LOG CFU/ml of *Salmonella spp*. and *Shigella spp*. recovered from the surface of fresh tomatoes rinsed for two-minutes in sodium lauryl sulfate and Tween 80 at 22 and 40°C.

Figure 5. LOG CFU/ml of *Salmonella spp.* and *Shigella spp.* recovered from the surface of fresh green leaf lettuce rinsed for two-minutes in sodium lauryl sulfate and Tween 80 at 22 and 40°C.

Running Head: Survivability of Pathogenic Bacteria in Several Detergents at Different Temperatures.

Research Note

Survivability of *Salmonella* and *Shigella spp*. in Sodium Lauryl Sulfate (SLS) and Tween 80 at 22 and 40°C.

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KEYWORDS: Detergents, *Salmonella*, *Shigella*, Survivability

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ABSTRACT:

Fresh produce has been implicated in several foodborne disease outbreaks. A primary site of contamination occurs on the surface during production and handling. One approach to reducing contamination is to treat fresh produce with rinsing agents. Studies have examined efficacy of detergents and other rinses to recover pathogens from the surface of fresh produce. Determining the effects these solutions have on *Salmonella* and *Shigella spp.*, may aid in understanding the mechanisms behind their removal. This study examines the survivability of *Salmonella* and *Shigella* in two detergent agents at 22°C and 40°C. Detergent solutions of 0.1% sodium lauryl sulfate (SLS), 0.1% Tween 80 and water were inoculated with 3 LOG CFU/ml cocktail of organisms and incubated for 32 hours at 22°C, and 40°C. Samples were taken over time and plated on Tryptic Soy Agar supplemented with 50-ppm nalidixic acid (TSAN). All solutions allowed for survival of *Salmonella* over time. Slight growth was seen in water (4.0 LOG CFU/ml at 22°C and 5.0 LOG CFU/ml at 40°C) and Tween 80 (3.3LOG CFU/ml at 22°C and 4.0 LOG CFU/ml at 40°C). SLS showed the most difference, with an initial drop in populations of 0.5 LOG CFU/ml, then recovery back to inoculation levels. SLS may cause injury to *Salmonella* explaining initial drops in the population, as the cells become more adjusted to SLS concentrations, growth may be enhanced. Survival of *Shigella* was found in all solutions at 22°C, and populations decreased over time at 40°C. Elevated temperatures may cause lethality over time in *Shigella spp.* Overall, organisms showed survival in detergents tested for 32 hours.

Fresh fruits and vegetables are responsible for a growing number of foodborne outbreaks each year. These products are minimally processed and lack a thermal processing step, making it difficult to ensure that they are safe for consumers. One way to ensure safety of produce is through the development of a rinsing agent that would be effective in removing pathogenic bacteria from surfaces.

Many rinse agents are being studied for their efficacy in removing pathogenic bacteria from different food products. It is thought that these would be used as a last minute, pre-packaging or shipping methods to increase safety of the produce before reaching the consumer. In addition, an effective and safe rinse agent could be marketed for consumers to purchase and use in their homes. Some of these rinses include chorine, hydrogen peroxide, acetic acid, and ozone, all of which are known to be lethal to microorganisms at certain doses (2). While these seem ideal for removal of pathogens from produce, some of these methods cause sensory deterioration of some produce types (1). Elevated temperatures of rinse agents can also be used. Some types of fresh fruit are dipped in a hot water bath (40-50°C) in some cases to aid in removal of bacteria (5).

Water is a very poor wetting agent. When detergents called surfactants are added to water, the water's surface tension is lowered making it a better wetting agent (6). This phenomena offers evidence that detergent rinses may be effective produce rinses. Depending on the surface chemistry of the produce type, and the chemical interaction with the microorganism, detergents may offer a way to disrupt those interactions and rinse pathogens off surfaces more readily.

The objective of this study was to evaluate the ability of *Salmonella* and *Shigella spp.* to survive, or grow in several detergent rinse agents that could be used for fresh fruit and vegetable

rinses. Rinse agents include water as the control, sodium lauryl sulfate (SLS) an anionic surfactant, and Tween 80 a non-ionic surfactant. By determining the ability of survival in several solutions, the effect of these chemicals on the organisms can be deduced and mechanisms for mode of action for produce rinsing may be determined.

MATERIALS AND METHODS:

Preparation of inoculum. Five *Salmonella* strains were used. All strains except *S.* Typhimirium were received from Dr. Larry Beuchat (Research Professor, University of Georgia). *Salmonella* Typhimirium was obtained from American Type Culture Collection (ATCC; Manassas, Va). *Salmonella* strains include: *S.* Agona (alfalfa sprout outbreak)*, S.* Baildon (lettuce/tomato-associated outbreak)*, S.* Michigan (cantaloupe associated outbreak)*, S.* Montevideo (tomato-associated outbreak)*,* and *S.* Typhimirium (ATCC 14028). All *Shigella spp.* were obtained from ATCC. These included *Shigella flexneri* (12023), *S. sonnei* (25931), and *S. boydii* (9207). Cultures were made nalidixic acid resistant by consecutive 24-hour transfers of isolated colonies from Tryptic soy agar (TSA; Difco, Detroit, Mich.) with increasing concentrations of naldixic acid (Sigma, St. Louis, Mo.) until colonies were resistant at a level of 50-ppm.

Bacterial cultures were kept frozen in 1:1 glycerol at –80°C. Prior to each experiment, one vial of each culture was thawed out, streaked on Tryptic Soy Agar slants containing 50-ppm nalidixic acid (TSAN) and incubated for 24 hours at 35°C. One colony was then transferred into Tryptic Soy Broth (Difco, Detroit, Mich.) tubes containing 50-ppm nalidixic acid (TSBN) and incubated for 24 hours.

Detergent preparation: Water, 0.1% sodium lauryl sulfate, and 0.1% Tween 80 were prepared in 250ml bottles (Fisher Scientific.). The inoculum was diluted to obtain ~3 LOG CFU/ml in each bottle. Bottles were incubated at 22°C or 40°C for 32 hours.

Microbiological analysis: Samples were taken from incubated bottles at 0, 4, 8, 24, and 32 hours and plated on to TSAN using a spiral plater (Spiral Biotech, Norwood, MA). Plates were incubated for 24 hours at 35°C. Organism confirmation was done at random using Salmonella-Shigella agar (Difco, Detroit, Mich.) and API 20E test strips (BioMerieux, France).

Statistical analysis: Survivability of *Salmonella* and *Shigella spp*. in various detergents (no detergent, 0.1% SLS, and 0.1% Tween 80 at 22°C and 40°C sampled at 0, 4, 8, 24, and 36 hours were statistically analyzed for effect on microbial population (LOG CFU/ml) by a split plot design. All treatments were randomized with 3 replicates of each combination. Each replicate was preformed on different days (1-3). Separate treatment days were nested throughout the whole-plot factors in the model. All three and four-way interactions were disregarded. Tukey's HSD was also preformed to determine significant differences ($\alpha = 0.05$) in treatment means with-in plots. The data was analyzed by JMP statistical software (Statistical Analysis System, Cary, N.C.).

RESULTS AND DISCUSSION:

Initial inoculum populations of *Salmonella* and *Shigella* were 3.0 LOG CFU/ml respectively.   All API 20E confirmations were positive and all SS agar plates displayed typical colonies.

No statistically significant difference was seen between organism types (P=0.1714) indicating that there was no difference in the effects of detergents on both organisms. However, interactions between organism type and temperature were significant (P<0.0001) showing that in

some cases, temperature gave a different effect on the organisms. The temperature and time

sampled was statistically significant (P<0.0001). These factors may play a role in survivability

of organisms in the various detergents. Interactions were seen between the effects of temperature

over time (P=0.0017), as well as effects of temperature throughout different detergent types

(0.0121). It can be concluded that temperature and treatment affected the survivability and

growth of organisms.

Mean values between organism and detergent type were compared using Tukey's HSD

(Table 1). All categories showed no difference over time, except *Salmonella* inoculated water

(22°C and 40°C), and Tween 80 (40°C); and *Shigella* inoculated SLS (22°C) and Tween 80

(40°C).

Salmonella spp. showed survivability in all solutions at both temperatures. In water and

Tween 80, slight growth was seen throughout the course of 32 hours incubation (Fig. 1 and Fig.

2). In water *Salmonella* populations increased by ~1.0 LOG CFU/ml at 22°C, and 2.0 LOG

CFU/ml at 40°C. Similarly, in Tween 80, *Salmonella* populations increased by 0.3 LOG CFU/ml

at 22°C and 1 LOG CFU/ml at 40°C. The exception was when *Salmonella* was incubated in

SLS. Initial population decrease over the first 8 hours was seen at both temperatures (Fig. 1 and

Fig. 2). After 8 hours, slight growth occurred bringing populations back to original inoculation

concentrations.

To explain these results further, separate trials were run in SLS inoculated with

Salmonella at both temperatures to determine the death curve of organism's short time (0-4

hours). Additionally, solutions were plated on both TSA and TSAN to determine whether

nalidixic acid contributes to injury of the cells or plays a role in recovery (Figure 3). The curve

showing the decline of organisms is gradual, not logarithmic, indicating that the decrease in

bacterial populations is not an immediate. Figure 4 gives the comparison between agar types, considering TSA a non-selective and TSAN a selective agar. There is little difference between the two indicating that injury is not occurring, and the cells are dying off, rather than being injured by the solution. If injury is not occurring, the SLS may be acting on some external factor that *Salmonella* carries on its membrane causing death.

Shigella spp showed survivability in all solutions at 22°C. In all cases at 22°C, slight growth occurred over 32 hours (Fig. 5). In water populations increased 2 LOG CFU/ml, in SLS populations increased 1 LOG CFU/ml, and in Tween 80 populations increased 0.2 CFU/ml. Conversely, at 40°C, population decline was apparent (Fig. 6). In water populations decreased 1 LOG CFU/ml, in SLS populations decreased 0.5 LOG CFU/ml, and in Tween 80 populations decreased 1 LOG CFU/ml.

While in most cases survival was apparent, population growth may have occurred due to contents of the inoculum. When inoculum was added to solutions, it was suspended in TSA broth. Though the amounts of broth were very small, over time it may have aided in the small amounts of growth that were seen. Growth of organisms was higher at 40°C, which can be explained because 40°C is closest to the organism's optimal growth temperature of 35°C. *Salmonella* can survive at a temperature range of 5° and 45°C with 35°C being ideal (3) and *Shigella* can survive at a temperature range of 10° and 48°C, with 35°C being ideal (4). It would be expected that *Shigella* would survive slightly better at the higher temperatures than *Salmonella* because *Shigella* has a higher temperature range. However, the opposite was found. This could be due to the fact that *Shigella* is typically less hardy that *Salmonella*, accounting for its susceptibility to detergents at higher temperatures.

One further area for investigation may include extending *Shigella* growth periods at 40°C, to determine how low the populations will decline, and whether re-growth of populations will occur. This may give some explanation as to the mechanism behind the population declines. In addition, suspending organisms in non-nutrient media such as peptone water may produce different results, as well as more limited growth over time.

Overall results indicate that if detergent solutions were used as surface rinses, there aid in recovery of organisms would be a result of the disruption of attachment of the cells to the surfaces rather than an actual lethality to the cells.

ACKNOWLEDGEMENTS:

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Table 1. *Salmonella spp.* and *Shigella spp.* Populations Present in Detergent Solutions at Different Temperatures. (n=3) for 36 hours.

Bacterial Populations Present in Detergent Solutions

| Rinse Solutions	(LOG CFU/ml) Water		SLS		Tween 80	
Salmonella spp.						
22oC						
0 hr	3.0 ± 0.05	A	2.8 ± 0.03	A	3.0 ± 0.09	A
4 hr	3.0 ± 0.02	A	2.3 ± 0.13	A	2.9 ± 0.11	A
8 hr	3.0 ± 0.09	A	2.3 ± 0.33	A	3.0 ± 0.19	A
24 hr	3.4 ± 0.12	B	2.5 ± 1.43	A	3.4 ± 0.97	A
32 hr	3.8 ± 0.26	C	2.6 ± 0.17	A	3.3 ± 1.23	A
40oC						
0 hr	2.9 ± 0.27	A	2.9 ± 0.13	A	3.1 ± 0.08	A
4 hr	3.2 ± 0.08	A	2.4 ± 0.66	A	3.1 ± 0.13	A
8 hr	3.5 ± 0.05	AB	2.5 ± 0.27	A	3.3 ± 0.33	A
24 hr	4.2 ± 0.56	B	3.2 ± 0.8	A	3.2 ± 0.23	B
32 hr	4.8 ± 0.08	B	3.2 ± 0.57	A	4.0 ± 0.17	B
Shigella spp						
22oC						
0 hr	3.3 ± 0.26	A	3.2 ± 0.33	A	3.3 ± 0.24	A
4 hr	3.3 ± 0.17	A	3.1 ± 0.21	A	3.1 ± 0.29	A
8 hr	3.4 ± 0.36	A	3.3 ± 0.15	A	3.1 ± 0.40	A
24 hr	3.8 ± 1.32	A	4.8 ± 0.09	B	3.5 ± 1.14	A
32 hr	4.4 ± 1.37	A	5.2 ± 0.21	B	3.6 ± 1.36	A
40oC						
0 hr	3.2 ± 0.62	A	2.8 ± 0.67	A	3.3 ± 0.28	A
4 hr	2.9 ± 0.46	A	2.4 ± 0.31	A	3.0 ± 0.29	A
8 hr	2.8 ± 0.56	A	2.0 ± 0.65	A	2.5 ± 0.22	AB
24 hr	2.1 ± 0.81	A	1.6 ± 0.57	A	2.1 ± 0.58	AB
32 hr	2.4 ± 1.00	A	1.6 ± 0.57	A	1.9 ± 0.45	B

*Mean values in the same column within the same microorganism followed by a different letter are significantly different (P<0.05).
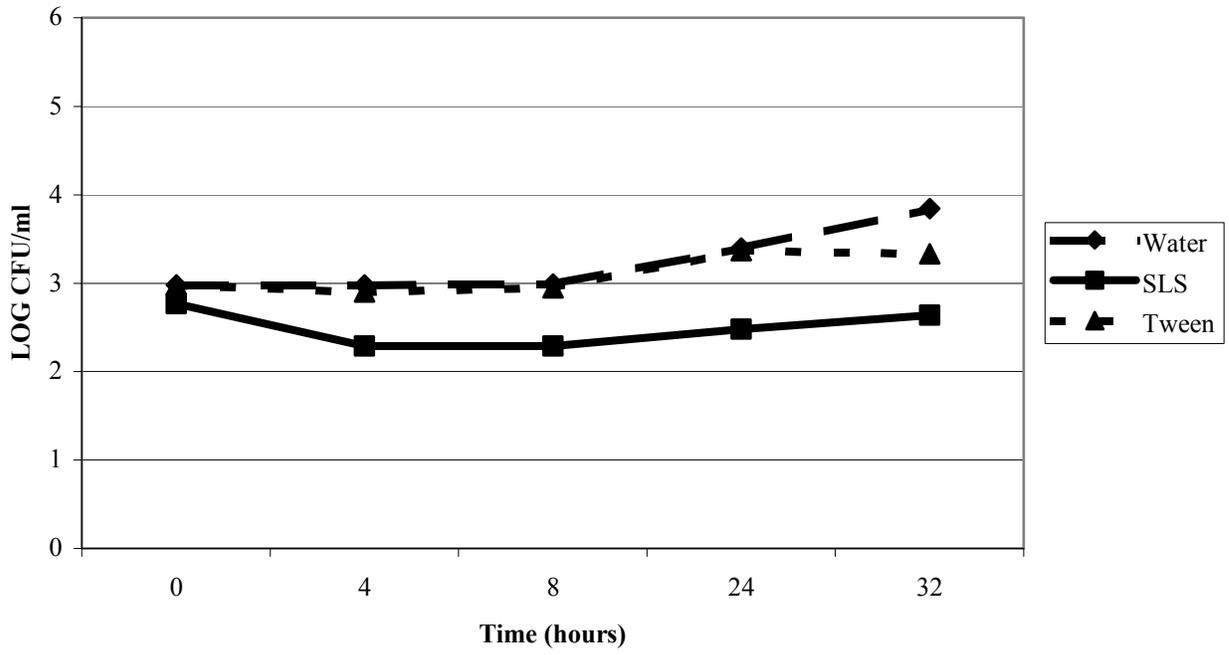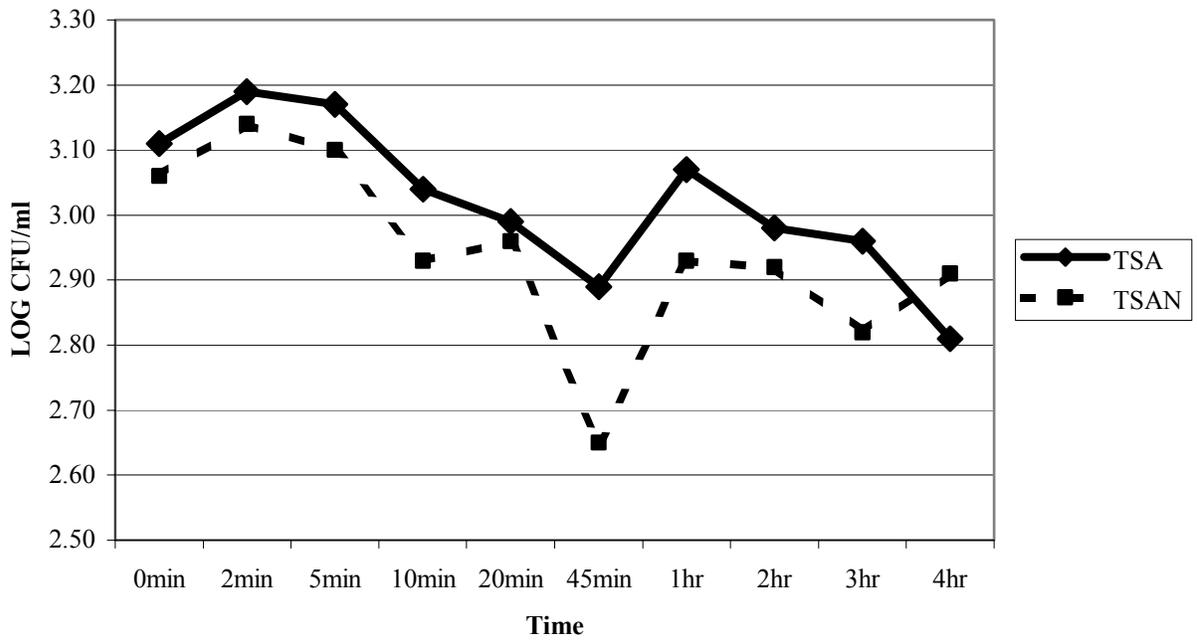
Figure 1. Survivability of *Salmonella spp* at 22°C in different detergent rinse solutions over 32 hours.
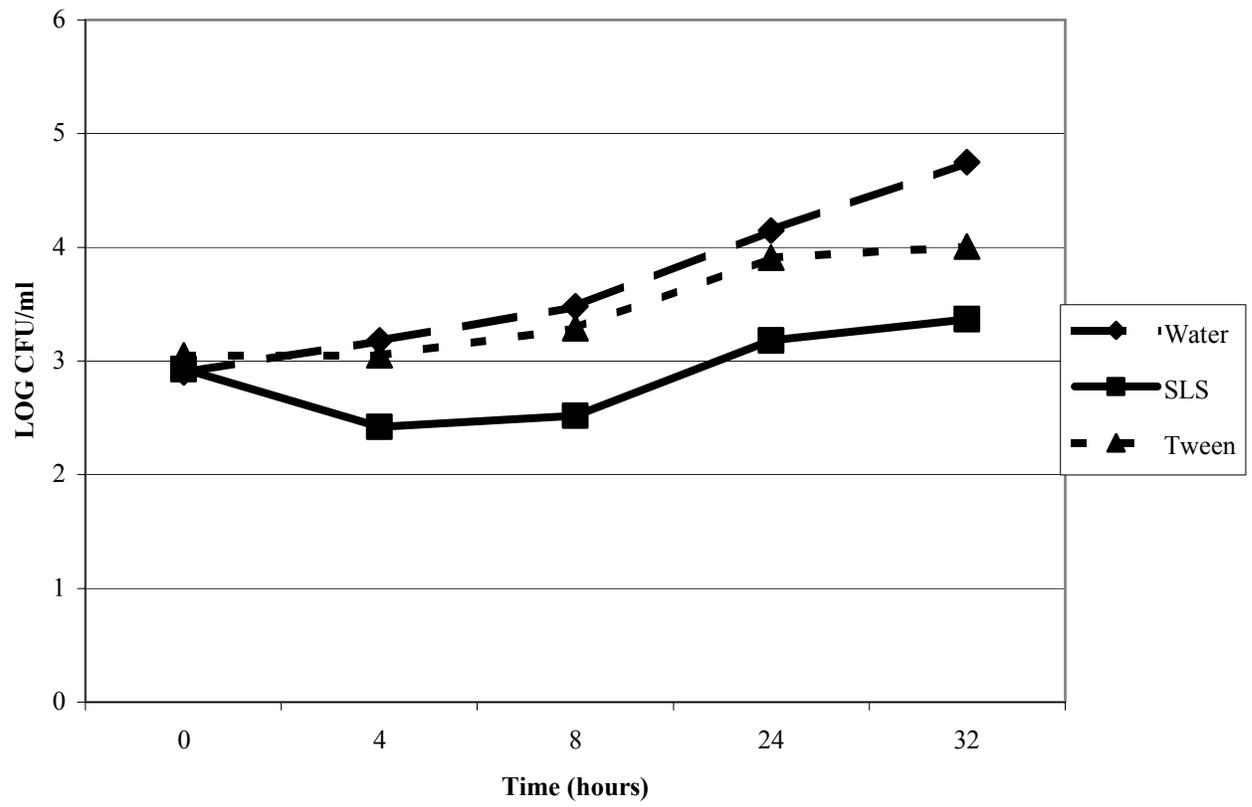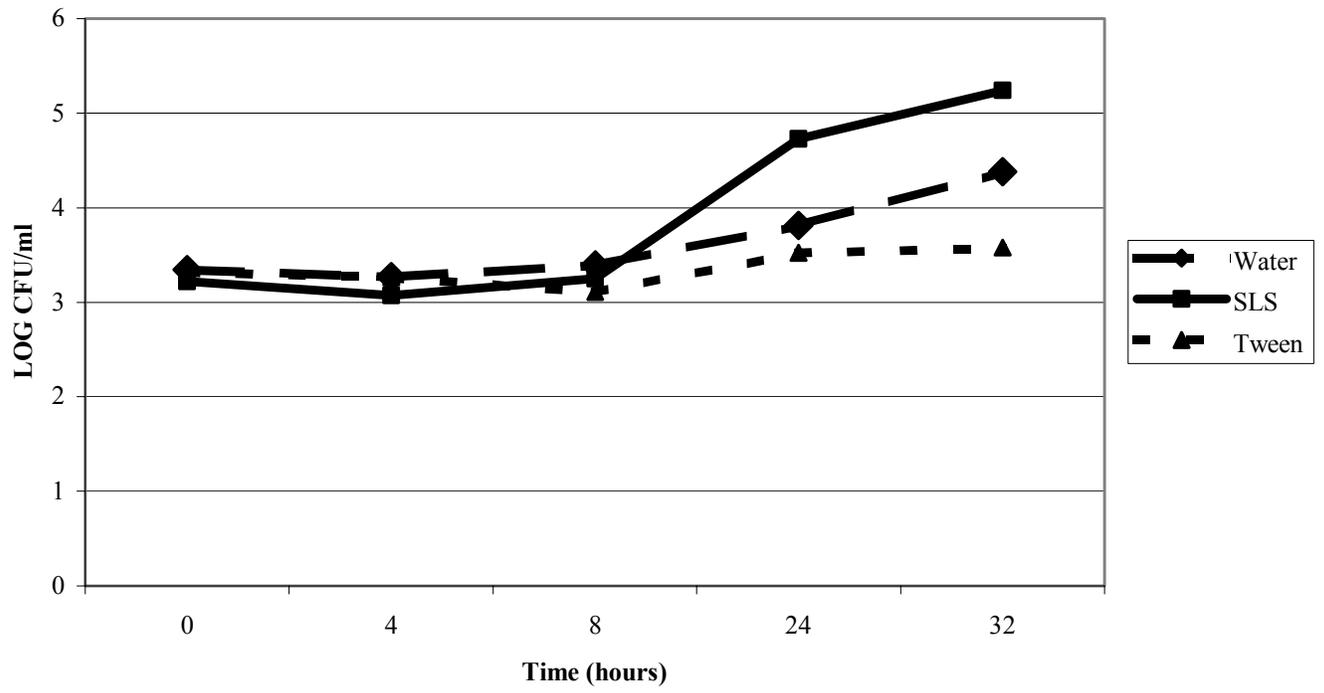
Figure 2. Survivability of *Salmonella spp* at 40°C in different detergent rinse solutions over 32 hours.
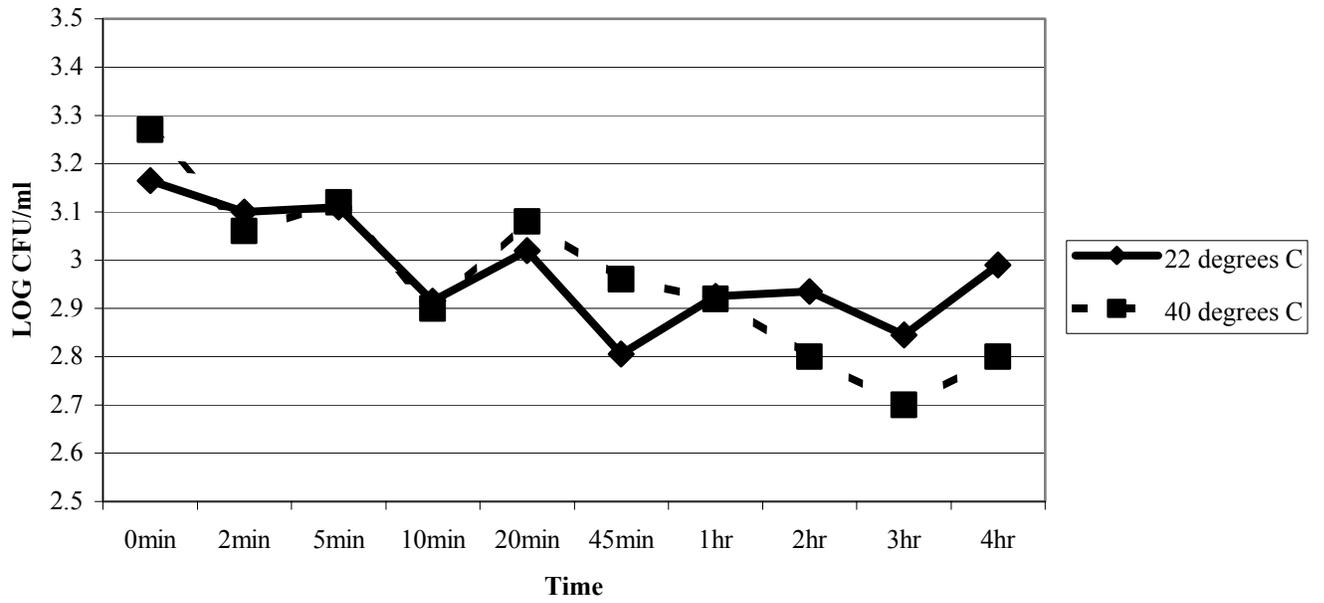
Figure 3. Survivability of *Salmonella spp*. at 22°C and 40°C in SLS over 4 hours.

Figure 4. Survivability of *Salmonella spp* at 22°C in SLS over 4 hours. Recovery when plated on two different agar types.

Figure 5. Survivability of *Shigella spp* at 22°C in different detergent rinse solutions over 32 hours.

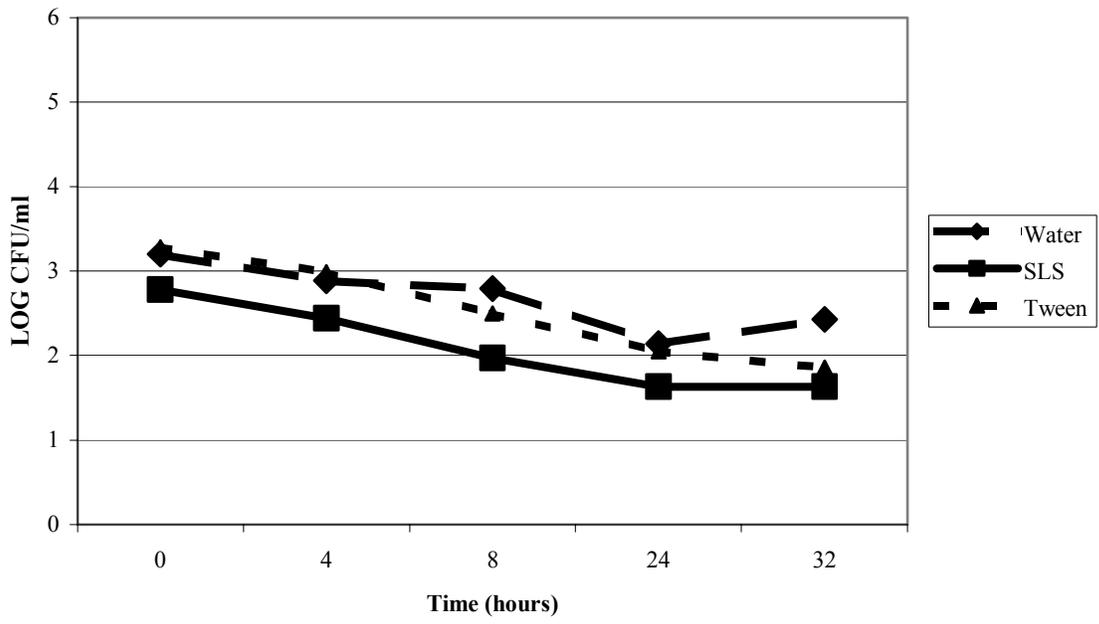**Figure 6.** Survivability of *Shigella spp.* at 40°C in different detergent rinse solutions over 32 hours.

Appendix I: Methods for internalization of *Salmonella* in strawberries:

Inoculum preparation: A five-strain nalidixic acid resistant *Salmonella* cocktail was used. All strains of *Salmonella* except *S.* Typhimirium were received from Dr. Larry Beuchat (University of Georgia) and isolated from produce related foodborne outbreaks. *Salmonella* Typhimirium was obtained from American Type Culture Collection (ATCC; Manassas, Va.). *Salmonella* strains include: *S.* Agona (alfalfa sprout outbreak)*, S.* Baildon (lettuce/tomato-associated outbreak)*, S.* Michigan (cantaloupe associated outbreak)*, S.* Montevideo (tomato-associated outbreak)*,* and *S.* Typhimirium (ATCC 14028).

Bacterial cultures were kept frozen in 1:1 glycerol at -80^oC. Prior to each experiment, one vial of each culture was thawed, streaked on Tryptic Soy Agar slants containing 50ppm nalidixic acid (TSAN) and incubated for 24 hours at 35^oC. One colony was then transferred onto Tryptic Soy Broth tubes containing 50 ppm nalidixic acid (TSBN; Difco, Detroit, Mich.) and incubated for 24 hours. This culture was diluted 1:100 to obtain a 6 LOG CFU/ml culture.

Strawberry preparation: Strawberries were purchased the day of the experiment from a local grocery store. Prior to inoculation they were set out at room temperature for 2 hours. After 2 hours internal temperatures of strawberries were taken to ensure that fruit were at room temperature prior to inoculation. One sample included three strawberries weighing 65 ± 15 grams.

Produce inoculation: Strawberries were placed in a sterile Nalgene® bucket with 4 Liters of inoculum and agitated for 2 min. Samples were then dried in a Laminar flow hood (Nuaire, Plymouth, Mn.) on a sterile stainless steel drying rack for 1-hour $\pm$ 15 minutes.

Rinse Treatment: After drying, each sample was placed in 500 ml Whirlpak® bags (VWR, Bridgeport, N.J.), and given one of three treatments at random. Treatments included: sterile

water (22°C), 0.1% sodium lauryl sulfate (22°C) (Sigma, St. Louis, Mo.), and 0.1% SLS (40°C).

Samples were then agitated on an orbital shaker (Janke & Kunkel, West Germany) at 200

rotations per min. for 2 min.

Microbiological analysis: Rinse water from original rinse was plated onto TSAN using a spiral

plater (Spiral Biotech, Norwood, MA). Remaining rinse water was decanted from bag and 250

ml of buffered peptone water (Difco, Detroit, Mich) was added making a dilution of ~0.5.

Strawberries were homogenized in a Stomacher lab blender 400 (Fisher Scientific, Pittsburgh,

PA) for 1 minute. Homogenate solution was plated onto TSAN using spiral plater. Plates were

incubated for 24 hours at 35°C. Organism confirmation was done at random using Salmonella-

Shigella agar (Difco, Detroit, Mich.) and API 20E test strips (BioMerieux, France).

Appendix II: Methods for whole fruit, Surface Inoculum analysis:

Materials and Methods:

Inoculum preparation: A five strain *Salmonella* cocktail and three strain *Shigella* cocktail was used. All strains of *Salmonella* except *S.* Typhimirium were received from Dr. Larry Beuchat (University of Georgia) and isolated from produce related foodborne outbreaks. *Salmonella* Typhimirium was obtained from American Type Culture Collection (ATCC; Manassas, Va.). *Salmonella* strains include: *S.* Agona (alfalfa sprout outbreak)*, S.* Baildon (lettuce/tomato-associated outbreak)*, S.* Michigan (cantaloupe associated outbreak)*, S.* Montevideo (tomato-associated outbreak)*,* and *S.* Typhimirium (ATCC 14028). All *Shigella spp.* were obtained from ATCC. These included *Shigella flexneri* (12023), *S. sonnei* (25931), and *S. boydii* (9207). Cultures were made nalidixic acid resistant by consecutive 24-hour transfers of isolated colonies from Tryptic soy agar (TSA; Difco, Detroit, Mich.) with increasing concentrations of naldixic acid (Sigma, St. Louis, Mo.) until colonies were resistant at a level of 50ppm.

Produce preparation: All produce was purchase the day of the experiment from a local grocery store. Prior to inoculation produce was set out at room temperature for 2 hours., after which all produce types were able to reach room temperature. Consistent weights of each produce type were taken. Samples included: three strawberries weighing 65 ± 15 grams, one vine-ripened tomato equaled one sample weighing 135 ± 20 grams, and one leaf of lettuce weighing 3.4 ± 1.5 grams.

Produce inoculation: Produce was placed in a sterile Nalgene® bucket with 4 Liters of inoculum and agitated for 2 min. Samples were then dried in a Laminar flow hood (Nuaire, Plymouth, Mn.) on a sterile stainless steel drying rack for 1-hour $\pm$ 15 minutes.

Whole fruit analysis: After drying, each sample was placed in filtered bags and 9ml peptone was added. Fruits were homogenized for 1 minute (Stomacher). Calculations were done to determine amounts of sample and peptone to make a 1:10 dilution. Three trial of each organism with each produce type was performed.

Microbiological analysis: Stomached fluid was plated onto TSAN using a spiral plater (Spiral Biotech, Norwood, MA). Plates were incubated for 24 hours at 35°C. Organism confirmation was done at random using Salmonella-Shigella agar (Difco, Detroit, Mich.) and API 20E test strips (BioMerieux, France).

Appendix III: Methods for Detection of Sub-lethal injury of *Salmonella spp.* on Strawberries with SLS Rinse study:

Inoculum preparation: A five strain *Salmonella* cocktail was used. All strains of *Salmonella* except *S.* Typhimirium were received from Dr. Larry Beuchat (University of Georgia) and isolated from produce related foodborne outbreaks. *Salmonella* Typhimirium was obtained from American Type Culture Collection (ATCC; Manassas, Va.). *Salmonella* strains include: *S.* Agona (alfalfa sprout outbreak)*, S.* Baildon (lettuce/tomato-associated outbreak)*, S.* Michigan (cantaloupe associated outbreak)*, S.* Montevideo (tomato-associated outbreak)*,* and *S.* Typhimirium (ATCC 14028).   Cultures were made nalidixic acid resistant by consecutive 24-hour transfers of isolated colonies from Tryptic soy agar (TSA; Difco, Detroit, Mich.) with increasing concentrations of naldixic acid (Sigma, St. Louis, Mo.) until colonies were resistant at a level of 50ppm.

Strawberry preparation: All strawberries were purchase the day of the experiment from a local grocery store. Prior to inoculation they were set out at room temperature for 2 hours, after which all produce types were able to reach room temperature. Consistent weights of each produce type were taken. Each sample included three strawberries weighing 65 ± 15 grams.

Produce inoculation: Produce was placed in a sterile Nalgene© bucket with 4 Liters of inoculum and agitated for 2 min. Samples were then dried in a Laminar flow hood (Nuaire, Plymouth, Mn.) on a sterile stainless steel drying rack for 1-hour $\pm$ 15 minutes.

Rinse Treatment: After drying, each sample was placed in 500 ml Whirlpak© bags (VWR, Bridgeport, N.J.), and given one of two treatments at random. Treatments included: 0.1% sodium lauryl sulfate (22°C) (Sigma, St. Louis, Mo.), 0.1% SLS (40°C). Samples were then agitated on an orbital shaker (Janke & Kunkel, West Germany) at 200 rotations per min. for 2 min.

Microbiological analysis: Rinse solution was plated onto TSA and TSAN using a spiral plater

(Spiral Biotech, Norwood, MA). Plates were incubated for 24 hours at 35oC. Organism

confirmation was done at random using Salmonella-Shigella agar (Difco, Detroit, Mich.) and

API 20E test strips (BioMerieux, France).

VITAE:

Renee Raiden was born in Springfield, Virginia where she graduated from Robert E. Lee High School. Following high school, she attended Radford University receiving her Bachelor's degree in Biology with a Minor in Chemistry in 1999.

Following graduation, Renee worked for American Research Corporation of Virginia in Radford. There, she worked as a lab tech for biological and chemical related research. After working for 1 year, she decided that she wanted to go back to school to work towards a Master's degree in Food Science and Technology at Virginia Tech. She began the program in the Fall of 2000. While at Virginia Tech, Renee was an active member of the Food Science Club, serving as Secretary for the 2001-2002 term. She was also very active in the graduate school. She served as the graduate representative for the Food Science and Technology departments Graduate committee, as well as the delegate from the department for the Graduate Student Association(GSA). As a member of the GSA, she also served as the College of Agriculture and Life Sciences representative on the budget board. She was also a student member in the Institute of Food Technologists (IFT) and the International Association of Food Protection (IAFP).