EFFECTIVENESS OF UV LIGHT AS A MEANS TO REDUCE *SALMONELLA* CONTAMINATION ON TOMATOES AND FOOD CONTACT SURFACES

by

WINNIE LIM

(Under the Direction of Mark A. Harrison)

ABSTRACT

The effectiveness of ultraviolet light at a wavelength of 254 nm to reduce *Salmonella* contamination on tomatoes and food contact surfaces was evaluated. Inoculated tomatoes were exposed to UV-C light at various doses. All UV treatments significantly reduced *Salmonella* populations ($p<0.05$). The effectiveness of UV-C light in reducing *Salmonella* contamination on different locations on tomato surfaces under various UV doses was also explored. Regardless of the locations, UV treatment was effective in decreasing *Salmonella* populations. Subsequent studies evaluated possible photoreactivation or dark repair of injured *Salmonella* post-UV treatment. Photoreactivation was not detected, nor was dark repair. UV light was also evaluated for its effectiveness to reduce *Salmonella* contamination on food contact surfaces (stainless steel, HDPE, waxed cardboard and PVC). UV-treated coupons were significantly different from the controls ($p<0.05$). *Salmonella* populations decreased the least on waxed cardboard. Application of UV-C light in tomato handling facilities is feasible.

INDEX WORDS: UV-C light, *Salmonella*, tomatoes, photoreactivation, food contact surfaces

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DEDICATION

To my parents and family for their love, support, and encouragement.

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CHAPTER 1

INTRODUCTION

Outbreaks linked to fresh produce have been increasingly reported. The proportion of produce-associated outbreaks has increased from 0.7% in the 1970s *(10)* to 15% by 2007 *(3)*. From 1998 to 2007, there were 684 produce-associated outbreaks involving 26,735 cases *(3)*. *Salmonella* accounted for 17% of the major cause of produce outbreaks *(3)*. Tomatoes have been implicated as a source of salmonellosis. The first multistate outbreaks of salmonellosis linked to tomatoes were reported back in 1990 *(8)*. The latest one occurred in 2008, in which tomato was considered as a possible source in the early outbreak *(4)*.

In order to minimize the risk of foodborne illness, contamination of tomatoes has to be prevented or eliminated. Once tomatoes are contaminated, there are no effective ways to completely eliminate the pathogens, except by cooking and irradiation *(5)*. In 1998, the U.S. Food and Drug Administration (FDA) issued "Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables". This guidance is voluntary and can be used to improve the safety of fresh produce *(12)*. Recently, FDA announced Food Safety Modernization Act (FSMA), in which one of the five proposed rules includes rules for produce safety standards. Prevention is the key point in FSMA. Rather than solving the problems after they occur, FSMA will focus more on prevention. This prevention-based food safety program will enable FDA to better protect public health *(13)*.

Interventions are needed to minimize pathogen contamination of raw produce and to eliminate them if present on produce. Normally, fresh produce receives little microbial intervention other than washing. The most commonly used sanitizer in the produce industry is chlorine, which primary use is to limit cross-contamination during washing *(6)*. However, failure to maintain free chlorine in wash water may increase the microbial loads on fresh produce. Part of the produce tissue may also neutralize chlorine, making it less effective *(1)*. In addition, chlorine compounds can react with organic matter on fresh produce to form carcinogenic organochlorine by-products *(9)*. For these reasons, alternative strategies to decrease pathogenic bacterial levels on fresh produce are needed. One of the alternatives is to use ultraviolet-C light at 254 nm.

UV light has been used for air, water/liquid, and surface disinfection *(2)*. Microorganisms are inactivated through the creation of pyrimidine dimers in DNA. These dimers prevent microbial replication *(11)*. However, many microorganisms have developed mechanisms to repair the UV-induced DNA damage. One of the repair mechanisms is photoreactivation, which is a light-dependent process that involves photolyases to reverse UV-induced DNA damage *(7)*. Photoreactivation increases the possibility that microorganisms might regain viability after UV light treatment and thus raise food safety concerns.

The objectives of this study were: (1) to determine the effectiveness of UV-C light in reducing *Salmonella* populations on tomatoes; (2) to evaluate the effectiveness of the treatment to reduce *Salmonella* contamination regardless of its location on the tomato surface; (3) to determine whether photoreactivation by the visible light or the dark repair mechanisms can result in the recovery of damaged *Salmonella* cells post-UV treatment; and (4) to study the effectiveness of UV light to decrease *Salmonella* contamination on food contact surfaces that could be used in tomato handling facilities.

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CHAPTER 2

LITERATURE REVIEW

Consumption of Produce

The increasing knowledge about how diet impacts health, the importance of maintaining a healthy weight, the demand for foods that are convenient yet diverse, the demand for fresh foods, the increasing research of the health benefits of fruits and vegetables, and the involvement of U.S. government on the health issues associated with fruits and vegetables are some of the trends that have affected the food consumption patterns in the United States *(22)*. Other factors such as income, education, age, eating out, and race/ethnicity also contribute to U.S. food consumption patterns *(62)*. Fruit and vegetable consumption is recognized as part of a healthy diet. Increase intake of vegetables and fruits is recommended in the Dietary Guidelines for Americans 2010 *(90)*. Three of the main reasons are: they provide a number of nutrients, including folate, magnesium, potassium, dietary fiber, and vitamins A, C and K; they may reduce the risk of many chronic diseases (i.e., heart attack, stroke); and they are moderately low in calories *(90)*. The U.S. Department of Agriculture's (USDA) MyPlate also suggests that half of our plate should consists of fruits and vegetables *(91)*.

Based on the Behavioral Risk Factor Surveillance System (BRFSS) conducted by state health departments in collaboration with Centers for Disease Control and Prevention (CDC), an estimated of 32.5% adults consumed fruits 2 or more times daily and 26.3% consumed vegetables 3 or more times daily in 2009. The prevalence of consumption has in fact declined slightly from 34.4% in 2000 to 32.5% in 2009 for fruits and has remained relatively stable for vegetables as it was 26.7% in 2000 *(18)*. However, according to per capita disappearance data from the USDA Economic Research Service (ERS) compiled by Cook *(22)*, both consumption of fresh and processed fruits and vegetables have trended higher. By 2009, it reaches 675 lbs, an increase of 8.4% from 1976. Looking at the total consumption of fruits and vegetables in fresh form alone, the percentage increased from 40% in 1976 to 46% in 2009 *(22)*. Meanwhile, comparing both fresh and processed of fruits and vegetables consumption, vegetables per capita consumption (increased 13% to 384 lbs) outweighed fruits (increased only 3% to 291 lbs) in 2009 *(22)*.

The increase in per capita consumption may be the result of the national effort in actively promoting intake of fruits and vegetables as an essential part of a healthy diet. Increasing the intake of fruits and vegetables remains the objective of Healthy People 2020 *(92)*. Between 2000 and 2020, it is expected that fruit consumption will increase by 24-27% and vegetable consumption will increase by 19-24% *(62)*.

The demand for fresh and processed tomatoes, particularly for fresh tomatoes, has increased in the United States over the past few decades. Tomatoes rank second in both U.S. farm value and vegetable consumption, preceded by potatoes. During the 1960s and 1970s, the per capita use of fresh tomatoes remained stable at 12.2 lbs. However, it increased by 19% to 14.6 lbs during the 1980s. A decade later, it increased again by 14% to 16.7 lbs *(67)*. The USDA ERS estimated that by 2020, per capita tomato consumption will increase slightly by 1.3% from 2000 *(62)*. The reason why consumption of fresh-market tomatoes increases is mainly due to the popularity of salads, salad bars, and sandwiches like the BLT (bacon-lettuce-tomato). The abundance of tomato varieties as well as the health benefits of the diet may contribute to the higher tomatoes consumption as well *(67)*.

Foodborne Pathogens and Outbreaks Linked to the Consumption of Fresh Produce

As the consumption of fresh produce increases in the United States and other countries, challenges keep arising, especially from the standpoint of foodborne illness associated with contaminated fresh produce. Furthermore, with produce production in a variety of countries in which the standards for handling and processing produce are still developing, there is a risk of human illness by foodborne pathogenic microorganisms *(9)*. Scallan et al. *(78)* estimated that 31 pathogens caused 9.4 million foodborne illnesses yearly in the United States, 59% of which was caused by viruses, while 39% caused by bacteria and the rest caused by parasites.

The CDC defines a foodborne disease outbreak as an incidence where the same illness in two or more people results from the same contaminated food or drink *(20)*. Therefore, a produce-associated outbreak is "2 or more cases of the same illness in which epidemiologic investigation implicated the same uncooked produce item, such as fruit, vegetable (including fresh herbs), salad or juice" *(81)*.

The proportion of produce-associated outbreaks in regards to all foodborne outbreaks with an identified food item in the U.S. has increased eightfold from 0.7% in the 1970s to 6% in the 1990s *(81)*. During period of 1973 to 1997, 190 produce-associated outbreaks were reported in 32 states, with *Salmonella* being the most common bacterial pathogen, hepatitis A being the most common virus, and *Cyclospora cayetanensis* being the most common parasite *(81)*. By 2007, produce outbreaks accounted for 15% of outbreaks with a known food source. The main cause of the outbreaks was norovirus, responsible for 51% of all outbreaks, followed by *Salmonell*a (17%), and *E. coli* (7%) *(14)*. Produce items like greens-based salads, lettuce, potatoes, unspecified fruits, and sprouts are the most common vehicles in produce-associated outbreaks *(26)*. Harris et al. *(44)* has extensively reviewed produce-associated outbreaks. The most common bacteria, viruses, and parasites which are responsible for outbreaks of produce-associated illness are: *Clostridium botulinum*, *E. coli* O157:H7, *Salmonella* spp., *Shigella* spp., *L. monocytogenes*, *Cryptosporidium* spp., *Cyclospora* spp., Hepatitis A, and Norwalk/Norwalk-like virus *(44)*.

Salmonella

Salmonella are facultative anaerobic gram-negative rod-shaped bacteria *(25)*. The genus *Salmonella* consists of two species, *S. enterica* and *S. bongori (25)*, which make up more than 2,700 serotypes of *Salmonella (9)*. The primary habitat of *Salmonella* is in the intestinal tract of animals and humans *(51)*. *Salmonella* growth depends on temperature, pH, salinity, water activity, and nutrient availability. The optimum growth of *Salmonella* is at 35-37ºC, but growth temperature between 5ºC and 47ºC has been reported *(12)*. *Salmonella* grows best at pH 6.5-7.5, with possible growth between pH 4.5 and 9.0 *(12)*. Water activity below 0.94 in media with neutral pH *(51)* and the presence of 3-4% NaCl inhibit the growth of *Salmonella (24)*.

Salmonella food poisoning occurs due to ingestion of foods that contain significant numbers of *Salmonella* of a specific strain *(51)*. The infection caused by *Salmonella* is called salmonellosis. High numbers of cells (10^7-10^9/g) are generally necessary to cause illness, although ingestion of low numbers of cells has been encountered in outbreaks *(51)*. Symptoms usually develop 12-72 hours after ingestion *(19)*. Symptoms include nausea, vomiting, abdominal pain, headache, chills, and diarrhea *(51)*. The illness usually lasts 4 to 7 days. Although most people recover without treatment, *Salmonella* infection can be life-threatening, especially for the elderly, infants, pregnant women, and immunocompromised persons. Among *Salmonella* species causing illness, *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Enteritidis are the most common ones in the United States *(19)*.

Salmonella has been associated with fresh fruits and vegetables. Outbreaks of salmonellosis related to tomatoes, lettuce, mixed salads, bean and alfalfa sprouts, raw almonds, cantaloupes, and orange juice have been repeatedly reported *(25)*. Contamination of fruits and vegetables with *Salmonella* could occur as a result of the infiltration of the pathogen through scar tissues, the internalization through root systems, surface contamination of flowering plants and subsequent entrapment of the pathogen during embryogenesis of the fruit or vegetable, and the transfer of the organism from contaminated surfaces onto edible plant tissues during processing *(8, 38, 63, 98)*.

***Salmonella* Outbreaks Associated with Tomatoes**

It is estimated that 1 million cases of foodborne illness caused by *Salmonella*, with estimated 378 deaths, occurs annually in the United States *(78)*. The first large multistate *Salmonella* outbreaks related to tomatoes consumption were reported back in 1990, involving *S.* Javiana which caused 176 illnesses. In 1993, another outbreak with 100 illness cases caused by *S.* Montevideo occurred *(46)*. Both tomato outbreaks were traced to the same South Carolina tomato packing facility. Contamination most likely occurred at the packing house in which tomatoes were washed in the same wash tank where chlorinated water was not monitored *(46)*.

In 2000, a third multistate outbreak associated with raw tomatoes resulted in 86 cases of salmonellosis. *S.* Baildon was recovered from outbreak patients. Traceback of the tomatoes lead to two Florida tomato growers/packers *(23)*. In the summer of 2004, three *Salmonella* outbreaks linked to Roma tomatoes occurred in 18 states of the United States and 1 province in Canada, causing 561 illnesses *(15)*. From 2005 to 2006, four large multistate outbreaks occurred in the United States due to consumption of contaminated raw tomatoes at restaurants. The investigators determined that the implicated tomatoes were supplied from tomato fields in Florida, Ohio and

Virginia. These outbreaks caused 459 cases of salmonellosis in 21 states *(16)*. In 2008, tomatoes were suggested as a source of an outbreak of *S.* Saintpaul which resulted in 1,442 cases. In the end, it was found out that jalapeño peppers were the major source of contamination; however, tomatoes were possibly a source of contamination early in the outbreak *(17)*.

The *Salmonella* source in most of the outbreaks originated from the farm or packer. This indicates that Good Management Practices are still not fully implemented. According to Hedberg et al. *(46)*, controlling contamination of commodities which are ready-to-eat, especially fruits and vegetables, remains a major challenge to industry, regulatory and public health agencies.

Potential Sources of Contamination

Contamination of produce can occur at any point during production, harvest, processing, transport, and distribution, as well as in the home kitchen. Improper food handling and storage prior to consumption will enhance contamination. Contamination can occur directly or indirectly through animals or insects, soil, water, non-hygienic equipment, and human handling *(44)*. During preharvest, irrigation water and manure fertilizer are the two most likely sources of contamination in the field *(71)*. Studies have been done to determine the survival of *E. coli* O157:H7 and *Salmonella* in manure. *E. coli* O157:H7 was found to survive for 21 months in manure collected from inoculated sheep and held outside under fluctuating conditions *(59)*. In another study, *S.* Typhimurium and *E. coli* O157:H7 were found to be able to survive 6 days to 3 weeks in cow manure and 2 days to 5 weeks in cow manure slurry *(47)*. Using irrigation water contaminated with fecal matter potentially allows contamination to occur. *Salmonella* was detected on parsley regularly irrigated with raw wastewater, and it survived for up to 3 days after irrigation. Meanwhile, survival of *Salmonella* on lettuce was more than 3 days *(73)*. Besides irrigation and manure fertilizer, migratory birds and wild and domestic animals could also be the source of contamination during preharvest *(71)*.

During harvest, produce can be contaminated through workers and harvesting equipment. Harvesting equipment, such as knives, clippers, boxes, bins, and truck beds, if not cleaned and sanitized routinely, would potentially transfer pathogen from one produce to the entire batch of produce. Maintaining worker hygiene is also important in order to prevent contamination. Most Norovirus and Hepatitis A outbreaks occurred due to contamination of food from infected food handlers *(40, 66)*.

Source contamination of produce during processing could come from poorly sanitized food contact equipment used for sorting, cutting, packing, and transporting or from contaminated water used for washing or cooling. Sanitizers are usually added to the wash water in order to control the microbial load *(71)*.

Improper food preparation prior to consumption may also introduce pathogens into a product. Poor employee hygiene may result in the spread of viruses and bacterial pathogens. Improper food storage, improper food handling, and cross-contamination during food preparation can increase the likelihood of contamination. Cutting board for meats and seafood should be separated from the one for ready-to-eat food. Knives used to cut meats and seafood should be thoroughly cleaned before use to cut vegetables to be eaten raw *(71)*.

Survival and Multiplication of Pathogens on Produce

Pathogen survival and growth on fresh produce depends not only on the organisms themselves, but also on the ability of the pathogen to attach to fresh produce, and environmental and storage conditions. Generally, pathogens will not grow on the uninjured outer surface of fresh produce, but they are able to survive. The plant’s natural barriers such as cell walls and wax layers may prevent the pathogen growth due to nutrient deficiency. Pathogen survival and proliferation of plant material are enhanced when there is physical damage (punctures or bruises) or degradation by plant pathogens (bacteria or fungi) that would provide nutrients and water to the pathogens *(44)*. In able to survive, bacteria must overcome environmental conditions during preharvest, such as temperature fluctuation, ultraviolet radiation from sunlight, and osmotic stress *(36)*. At various postharvest stages, microbes have to deal with various transportation conditions, wash and rinse water at various temperature, sanitizers, pH fluctuations, oxidative stress, and storage and packaging conditions *(36)*.

Fresh cut produce is known to be vulnerable to microbial contamination. During processing, the produce is injured through peeling, cutting, slicing or shredding, which gives the opportunity for microbes to grow as a result of the release of nutrients *(44)*. Various pathogens are able to grow on cut surfaces or in punctures or cracks. Studies have shown the survival of pathogens on the surface of cut melons, shredded lettuce, and chopped tomatoes *(1, 35, 98)*.

Attachment of pathogens to produce involves a number of mechanisms. Different pathogens use different adhesion mechanisms. Some of the factors influencing the attachment include: extracellular polymeric substances, types of fimbriae, cell surface hydrophobicity, divalent cationic bridges, and bacterial surface charge *(27, 32, 45, 52, 95)*.

Internalization of pathogens into produce can occur in several ways. Bacteria can internalize produce through air entering open stomata on leaves, wounds, and water channels, which are free water in surface opening like stomata. Internalization of microbes can also occur through the stem scar of tomato fruit. Infiltration of water or aqueous cell suspensions of bacteria can happen during harvest and handling as well *(6)*. Wash water contaminated with bacteria can infiltrate when there is temperature and pressure difference between the produce surface and the surrounding water. Usually, internal gas pressure and surface hydrophobicity of produce prevent infiltration of water. However, if the wash water is colder, the pressure difference will draw contaminated water into intercellular spaces through pores, causing infiltration *(6)*. FDA recommends that the wash water temperature is at least 5°C (10°F) higher than the internal tomato temperature *(94)*. Furthermore, adding detergents (surfactants) to the water enhances infiltration, as a result of reduced surface tension *(5)*. Besides, hydrostatic pressure also plays role in promoting infiltration. Produce submerged at the bottom of containers would experience hydrostatic pressure. When the pressure is removed, the differential internal pressure would force water into pores *(6)*. In addition, plant roots are also likely to internalize soil microbes due to wounds form during root growth *(6)*.

Interventions to Inactivate Pathogens on Produce

With the increasing number of outbreaks, interventions on pathogens reduction on produce are needed to minimize the risk of contamination. Washing raw fruits and vegetables in hot water or water containing detergents can partially remove pathogenic and spoilage bacteria. Washing fruits and vegetables in potable water could also facilitate removal of microorganisms, but treatment with disinfectants would still be better as they provide additional 2 to 3 log reductions *(10)*.

Scientists and researchers have developed both chemical and physical interventions and explored their efficacy in reducing pathogens on produce. Whichever treatments are applied, they must be safe for humans. Generally, they should decrease the enteric pathogens by at least 3 logs and preferably 5 logs *(28)*. Interventions applied should also maintain the produce quality. Furthermore, pathogens should not survive in any antimicrobial treatment solutions in order to avoid cross-contamination *(28)*.

Each disinfectant has different efficacy in eliminating pathogens. Under certain circumstances, some sanitizers may be more effective than others. The effectiveness of the sanitizers depend mostly on the type and pH of the disinfectant itself, contact time, temperature, and the chemical and physical characteristics of the fresh produce *(10)*. Limitations of the chemical sanitizing agents do exist and are due in part to the attachment of the pathogens to the tissue and inaccessible sites (pores, cut surfaces, indentations, and other irregularities), biofilm formation, and the effect of the chemical agent on the background microflora *(28)*.

Chlorine is the most common sanitizing agent used in the produce industry to prevent potential cross-contamination during washing. Its effectiveness is influenced by the amount of free available chlorine in the solution, pH, temperature, type of produce, diversity of microorganisms, amount of organic matter, and exposure to air, light and metals. Chlorine is usually used at concentrations of 100-150 ppm free chlorine at pH 6.5-7.5 on produce *(6)*. Other disinfectants such as peroxyacetic acid, acidified sodium chlorite, hydrogen peroxide, alkaline solution, chlorine dioxide, hypochlorous acid, lactic acid, benzalkonium chloride, essential oils, electrolyzed water, and ozone are also effective in reducing bacterial pathogens on fresh produce *(74)*.

Physical interventions on pathogen reduction on produce include thermal treatments, high pressure processing, irradiation, and UV light *(28)*. Surface pasteurization of fresh produce using steam, hot water, or air has been shown to decrease microbial counts on produce with hard surfaces *(87)*. Arroyo et al. *(2)* reported that high pressure treatment of 350 MPa at 10ºC for 20 min was necessary to reduce most Gram-negative bacteria and molds. Irradiation has been shown to be effective at decreasing pathogens on intact and fresh-cut produce. A maximum dose of 1.0 kGy has been recommended for use on fresh produce *(30)*. Yaun et al. *(103)* found that UV light at a dose 24 mJ/cm^2 reduced *Salmonella* or *E. coli* O157:H7 on lettuce and tomatoes by 2 logs and those on apples by 3 logs.

UV Light

Ultraviolet (UV) light is non-ionizing radiation that has a wavelength between X-rays and visible light in the electromagnetic spectrum *(11)*. The UV spectrum can be subdivided into UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm), and the vacuum UV range (100-200 nm) (Fig 2.1). While UV-A is responsible for changes in human skin called tanning, UV-B can cause skin burning and eventually lead to skin cancer. UV-C is the germicidal range as it effectively inactivates bacteria and viruses. Vacuum UV can be absorbed by almost all substances and can only be transmitted in a vacuum *(58)*.

UV-C light has been approved by U.S. Food and Drug Administration (FDA) as an intervention technology to decontaminate liquid foods and water, food contact surfaces, and food surfaces *(93)*. The first application of UV was in the disinfection of water, and it remains so today. Other than water treatment, UV is used as a surface treatment since it is absorbed by most materials and is unable to penetrate beyond the surface of solid objects *(80)*. UV radiation between 250 and 260 nm is lethal to most microorganisms, such as bacteria, viruses, protozoa, mycelial fungi, yeasts, and algae *(11)*. UV generated using low-pressure mercury lamps emits UV primarily at 254 nm. This wavelength is the most efficient as it is absorbed most by nucleic acids *(58)*. The germicidal effects of UV at 254 nm is therefore used for disinfection of surfaces, water, and some food products *(37)*.

UV radiation offers some other advantages over existing sanitation methods: it does not leave any residue; it does not have legal restrictions; it does not require installation of extensive safety equipment *(100, 104)*; it is easy to use; and it is economical *(11)*. The only disadvantage is the limited penetration *(74)*. The efficacy of UV light depends on the surface structure and topography *(34)*, the doses applied, and the distance between the UV light source and the treated sample *(29)*. Temperatures between 5 and 37°C have little, if any, impact on the effectiveness of UV-C light *(56)*.

UV dose is the product of UV intensity or fluence rate I (e.g., in $\mathrm{mW/cm^2}$) and exposure time t (s). Thus, the microbial reduction rate is related to the applied UV dose (in $\mathrm{mWs/cm^2}$ or $\mathrm{mJ/cm^2}$). The germicidal effect can be obtained by applying either low intensity for long exposure times or high intensity for short times *(3)*. UV sensitivity of microorganisms is characterized by the UV doses required to reduce microbial populations by 1 log. The sensitivity of a specific microorganism to different UV doses is presented in survival curves, also known as dose-response curves *(57)*. A summary of UV dose-responses of a wide range of microorganisms which include pathogens, indicators, or organisms encountered in the application, testing of performance, and validation of UV disinfection technologies has been provided by Cairns *(13)*.

UV light sensitivity varies for different microorganisms, which may be due to: cell wall structure, thickness and composition; the presence of UV-absorbing proteins; or differences in the structure of the nucleic acid *(58)*. For instance, spores, yeasts, fungi *(3)* and viruses *(58)* are more resistant than other microorganisms; gram negative bacteria are more susceptible than gram positive bacteria *(58)*; and bacteria suspended in air are more sensitive than those suspended in liquids *(11)*.

Applications of UV Light

The most common and practical application of the UV-C light is for disinfection of surfaces, liquids, and air *(11)*. It is also used for sanitation in commercial businesses (resorts, hotels, restaurants), institutions (hospitals, schools, nursing homes, fish hatcheries, laboratories) and industries (food packagers, brewers, bottling, cosmetics) *(37)*. In regards to disinfection of surfaces, sanitization of conveyer surfaces and packaging materials, such as boxes, bottle caps, cartons, and wrappers, is one of the examples *(56)*. In hotels, UV has been used to sanitize drinking glasses, plates, and cutlery and sanitize walls and fixture in hotel bathrooms *(56)*. In order for these applications to be effective, the surfaces should be clean and free of dirt. Otherwise, the dirt would absorb the radiation and provide protection to the microorganisms *(56)*. In addition, significant microbial inactivation is only achieved when the surfaces are smooth, as crevices may shield microorganisms from UV radiation *(79)*.

Kim et al. *(55)* achieved more than 4 log reductions of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 populations on stainless steel chips radiated with UV at intensity of 500 µW/cm^2 for 3 min (dose of 0.09 J/cm^2). Similarly, Sommers et al. *(84)* reported that *Salmonella* spp., *S. aureus*, and *L. monocytogenes* populations inoculated on both electroplated and bead blasted stainless steel coupons were reduced by more than 5 logs when irradiated with UV-C dose of 0.4 J/cm^2.

Studies about the effectiveness of UV light to disinfect surfaces of meat products have also been evaluated. Lyon et al. *(69)* observed a 2 log reduction in the population of *L. monocytogenes* on broiler breast fillets when treated with UV-C light (254 nm) at an intensity of 1,000 µW/cm^2 for 5 min. Wong et al. *(100)* found that *E. coli* and *S.* Senftenberg populations on pork muscle was reduced by 1.5 and 2.0 logs, respectively, when treated with ultraviolet light. *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium on chicken meat with or without skin showed a log reduction of 0.36 to 1.28 after UV treatment of 500 µW/cm^2 for 3 min *(55)*.

UV treatment is also effective in reducing various bacterial populations on eggshell surfaces. Kuo et al. *(60)* reported a 2.9-4.6 log reduction of *S.* Typhimurium after 1-7 min of UV treatment at 620 µW/cm^2. They also evaluated the effectiveness of different UV treatment times (0, 15 and 30 min) at 620 µW/cm^2 and different intensities (620, 1,350, and 1,720 µW/cm^2) for 15 min on the populations of aerobic bacteria and molds on eggshell. A 99% reduction of aerobic bacteria/egg and <1 CFU mold/egg was observed for all UV treatments. Study done by Rodriguez-Romo and Yousef *(77)* showed that UV treatment significantly reduced *S.* Enteritidis populations on shell eggs by 2.6 and 2.0 logs after exposed to UV at 100 µW/cm^2 for 2 and 4 min, respectively. Higher UV intensity (1,500 to 2,500 µW/cm^2) for 5 min decreased *Salmonella* populations by 4.3 logs.

The effectiveness of UV radiation as a surface treatment of fresh produce has also been demonstrated. Not only is it effective in reducing microorganisms, but it can also prolong shelf life and improve product quality. Yaun et al. *(103)* investigated the effectiveness of UV-C light on reducing *Salmonella* spp. and *E. coli* O157:H7 contamination on the surface of Red Delicious apples, leaf lettuce, and tomatoes. Apples inoculated with *E. coli* O157:H7 showed an approximately 3.3 log reduction in the population at UV dose of 24 mJ/cm^2. Meanwhile, UV at the same dose reduced 2.19 logs of *Salmonella* spp. inoculated on tomatoes. On the other hand, green leaf lettuce inoculated with both *Salmonella* spp. and *E. coli* O157:H7 showed 2.65 and 2.79 log reductions, respectively, when treated with UV at 24 mJ/cm^2 *(103)*. Sommers et al. *(84)* reported that UV-C dose of 0.5 J/cm^2 reduced the populations of *Salmonella* spp., *S. aureus*, and *L. monocytogenes* by 2.6-3.1 logs on the surface of Roma tomatoes, while a higher dose, 4 J/cm^2, reduced the populations of the three pathogens by 3.6-3.8 logs. Chun et al. *(21)* found that *E. coli* O157:H7 and *L. monocytogenes* populations on ready-to-eat salad were reduced by 2.16 and 2.57 logs, respectively, when irradiated with 800 mJ/cm^2. Research done by Fonseca and Rushing *(31)* indicated that exposing packaged watermelons cubes to UV-C light at 410 mJ/cm^2 reduced microbial populations by more than 1 log without affecting juice leakage, color, and overall visual quality. Lamikanra et al. *(61)* compared the effect of processing cantaloupe melon under UV-C light on storage properties of the cut fruit with post-cut UV-treated fruit. The result showed that the populations of aerobic mesophilic and lactic acid bacteria were lower on fruit cut under UV light than post-cut UV-treated fruit and the control. While UV applied after post-cut improved shelf life, cutting fruit under UV improved product quality. UV light has also been used to stimulate beneficial responses called hormesis on fruits and vegetables. Hormesis is a stimulation of beneficial response in a host by low doses of an agent *(68)*. Application of UV light has been shown to induce resistance in fruits and vegetables to postharvest storage rots *(65, 85, 86)* and to extend the shelf life of fruit by delaying ripening *(65)*.

Water treatment is the most successful application of UV disinfection *(79)*. UV light has been used for several years to disinfect water as it is effective in eliminating a variety of microorganisms *(3)*. The application includes disinfection of sewage effluent, drinking water, and water for cosmetics industry and swimming pools *(101)*. UV light has been considered as alternative to chlorine for the disinfection of wastewater *(64)*. The advantage of using UV as disinfection treatment is that it does not produce changes in color, flavor, odor or pH *(7)*. The application limitations are the lack of penetration and the presence of suspended solids and salts of calcium, magnesium, iron, and manganese in the water *(82)*. In order to effectively disinfect water, the water must have a high transmission for UV and be free of suspended solids *(56)*. Any suspended solids must be filtered out prior to UV application *(11)* because they may provide a site for the bacteria to aggregate *(57)* and shield the bacteria from radiation; thereby, reducing the germicidal effect *(56)*.

UV light can also be used to disinfect other liquids besides water. UV light has potential promise as an alternative to thermal pasteurization to reduce microbial contamination for a variety of liquid foods and beverages (e.g., fresh juices, soft drinks, raw milk, liquid eggs, liquid sugars and sweeteners, etc.) *(57)*. Disinfection of liquid foods using UV light may be a little challenging as physical properties such as liquid density and viscosity must be considered in meeting the required standard of a 5-log reduction of microbial populations in fruit juices. The presence of color compounds, organic solutes, and suspended matter must also be considered since those may lower the efficiency of UV pasteurization process *(58)*.

Wright et al. *(102)* observed a reduction of *E. coli* O157:H7 populations in unpasteurized cider treated with UV light. Keyser et al. *(54)* found that UV-C light was successful in reducing the microbial load in apple juice, guava and pineapple juice, mango nectar, strawberry nectar, and two different orange and tropical juices without changing taste and color profiles of the juices. Unluturk et al. *(97)* examined the efficacy of UV-C light as a non-thermal process to inactivate *E. coli* (ATCC 8739) in liquid egg products (liquid egg yolk, liquid egg white, and liquid whole egg). They reported that a greater than 2 log reduction of *E. coli* (ATCC 8739) populations was achieved in liquid egg white, while maximum inactivation in liquid egg yolk and liquid whole egg was 0.675 and 0.316 log CFU/ml, respectively. The results indicated that UV-C light may not be practicable for inactivation for liquid whole egg and liquid egg yolk. However, since UV systems are less costly than thermal process, it was suggested that UV-C light can be used as a pre-treatment process or alternative method when combined with mild heat or non-thermal treatment to reduce initial populations of microorganisms and the adverse effects of thermal pasteurization of liquid egg products. Matak et al. *(70)* reported that *L.*

monocytogenes in goat's milk was reduced by more than 5 logs when exposed to UV light at a dose of 15.8 ± 1.6 mJ/cm^2.

Disinfection of air has a twofold application: (1) treating air in spaces where food and drugs are packed can reduce microbial contamination; (2) sterilizing air of occupied spaces prevents the spread of air-borne diseases *(56)*. For instance, UV-C radiation has been used as a barrier to sterilize air in hospitals. UV radiation at 254 nm and 0.25 W/m^2 has also been used in theatres in the United States since 1930s to reduce the air-borne bacteria load *(11)*. Bailey et al. *(4)* reported that air sanitization using UV light in the egg hatching cabinets effectively reduced *Enterobacteriaceae* and *Salmonella* species.

In addition, UV has also been used in combination with other disinfectants to provide a synergistic effect. The most common synergistic effect is the combination of UV light and hydrogen peroxide *(39, 79)*. This effect has been employed in the production of aseptic packaging material for food *(79)*.

Mechanisms of Microbial Inactivation by UV Light and Repair Mechanisms

Unlike chemical disinfectants which destroy and damage cellular structures of the microorganisms, UV light prevents microorganisms from replicating; thus, they are inactivated and cannot infect. UV light inactivates microorganisms by damaging their nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Nucleic acid absorbs UV light from 200 to 310 nm. This absorption damages DNA or RNA structures by inducing six types of damage, with the primary mechanism being the creation of pyrimidine dimers. Pyrimidine dimers are "bonds formed between adjacent pairs of thymine or cytosine pyrimidines on the same DNA or RNA strand" (Fig 2.2 and 2.3). These dimers prevent microbial replication *(58)*.

However, since ultraviolet radiation from the sun is present in the environment, it is not surprising that bacteria and other microorganisms have evolved natural defense mechanisms to repair the UV-induced DNA damage *(42, 75)*. Microorganisms are capable of repairing themselves after UV light exposure via repair pathways such as photoreactivation, excision or dark repair, recombinational repair, and inducible error-prone repair *(58)*. This is a major drawback of UV disinfection because these repair mechanisms may allow the inactivated microorganisms to regain viability *(33, 42)*.

Photoreactivation and nucleotide excision repair are the two major pathways to reverse UV-induced DNA damage *(88)*. Photoreactivation is a light-dependent process which involves the photoreactivating enzymes, photolyases, to repair UV-induced DNA damage by splitting the pyrimidine dimers into monomers *(33)*. It usually requires light energy in the near UV or violet-blue spectral range from 310 to 480 nm *(42)*. Photoreactivation has been observed in many prokaryotes and eukaryotes, but not in mammals. However, not all species within the mentioned taxonomic groups are able to photorepair. Organisms like *Haemophilus influenza*, *Deinococcus radiodurans*, several species of the genus *Bacillus*, and *Schizosaccharomyces pombe* lack photoreactivation *(42)*. Organisms that have been found to photorepair include total and fecal coliform, *E. coli*, *Streptococcus feacalis*, *Streptomyces*, *Saccaharomyces*, *Aerobacter*, *Micrococcus*, *Erwinia*, *Proteus*, *Penicillium*, and *Neurospora (43, 99)*.

In contrast, nucleotide excision repair or dark repair is a light-independent process which involves the excision of dimers and requires more than a dozen of proteins to remove the damaged DNA *(33)*. Nucleotide excision repair is universally distributed and highly conserved throughout evolution *(88)*.

Photoreactivation: A Concern

Photoreactivation is mainly a concern for UV disinfection used for water treatment. Since drinking water is usually stored and transported under dark conditions in containers and distribution systems, photoreactivation does not play an important role in drinking water. However, it becomes problematic for sewage water and water used for irrigation and fish farming because this water is exposed to visible light directly after UV disinfection *(83)*.

Photoreactivation can be influenced by several factors, such as initial ultraviolet dose, exposure time to photoreactivating light, temperature, type of ultraviolet lamps, the species of the microorganisms, and the wastewater quality *(49, 53, 105)*. Photoreactivation is inversely related to the applied UV dose *(64)*. The repair is generally higher with low UV dose. Reported exposure times on maximum photoreactivation have ranged from minutes *(41, 42)* to hours *(43, 99, 105)* to days *(72)*. These differences may be due to initial of pyrimidine dimers formed, the number of photoreactivating enzymes present, the temperature during the complex of photoreactivating enzyme and dimer, and the dose of photoreactivating radiation *(64)*.

Since these repair mechanisms reduce the efficiency of UV disinfection, numerous researchers have studied photoreactivation and dark repair following UV application. Many studies have demonstrated the possibility of photoreactivation or dark repair to reverse UV-induced DNA damage *(43, 89, 99)*. Most of the studies used *E. coli* as their target organism because of its well-characterized repair mechanism *(33)* and its usage as a bacterial indicator in disinfection *(75)*.

Possible photoreactivation of 3.4 logs of *E. coli* and 2.4 logs of *S. faecalis* were reported by Harris et. al *(43)*. Hoyer *(48)* found that without considering photoreactivation, a UV dose of 10 mJ/cm^2 was sufficient to reduce the population of *E. coli* ATCC 11229 by 4 logs. However, when photoreactivation was taken into consideration, the minimum dose to reduce the *E. coli* ATCC 11229 population by 4 logs was 30 mJ/cm^2 *(48)*. Tosa and Hirata *(89)* examined the susceptibility of enterohemorrhagic *E. coli* to UV radiation and photoreactivation. The results showed that photoreactivation was observed in EHEC O26, but not in EHEC O157:H7. To achieve 90 and 99% inactivation of EHEC O26 without photoreactivation, UV doses of 5.4 and 8.1 mJ/cm^2 were required, respectively. However, after photoreactivation, a higher dose of 12 mJ/cm^2 was required to achieve 90% inactivation of EHEC O26. Quek and Hu *(75)* explored the ability of various strains of *E. coli* to perform photoreactivation and dark repair. Their findings indicated that different *E. coli* strains have different repair abilities. *E. coli* strain ATCC 15597 was found to repair the fastest in the case of photoreactivation. Meanwhile, *E. coli* strain ATCC 11229 was found to repair the fastest in the case of dark repair. These strains were also confirmed to repair better than *E. coli* O157:H7. In a different study, they found that photoreactivation increased with increasing fluorescent light intensities on both of the *E. coli* strains. Photoreactivation rates were also higher when microorganisms were exposed to near optimum growth temperatures (23-37ºC), compared to exposure to temperatures which were too high (50ºC) or too low (4ºC) *(76)*. Zimmer and Slawson *(105)* demonstrated that photorepair was observed in *E. coli* following UV exposure using low-pressure UV source, but no repair was detectable when using medium-pressure UV lamp. The inactivation and photorepair ability of enteric pathogenic microorganisms (*S.* Typhimurium, *Shigella dysenteriae*, *E. coli*, and human rotavirus) with UV light was examined by Hu et al. *(50)*. Except for rotavirus, the others were found to be able to perform photoreactivation after UV light exposure. Higher UV doses significantly decreased photoreactivation. Kuo et al. *(60)* studied photoreactivation and dark repair ability of *S.* Typhimurium on shell eggs. Their study suggested that neither 1 hour of light exposure nor 1 hour of dark exposure significantly influenced photoreactivation and dark repair.

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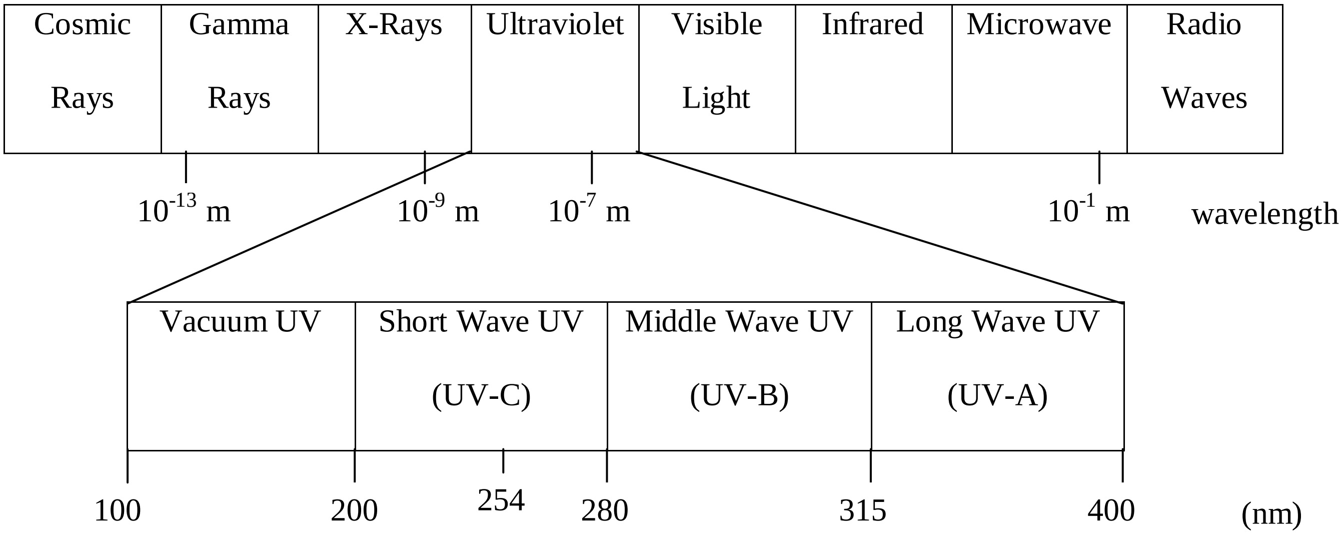


Figure 2.1. Electromagnetic radiation spectrum with division of UV light. Adapted from UltraViolet Lighting Products *(96)*.

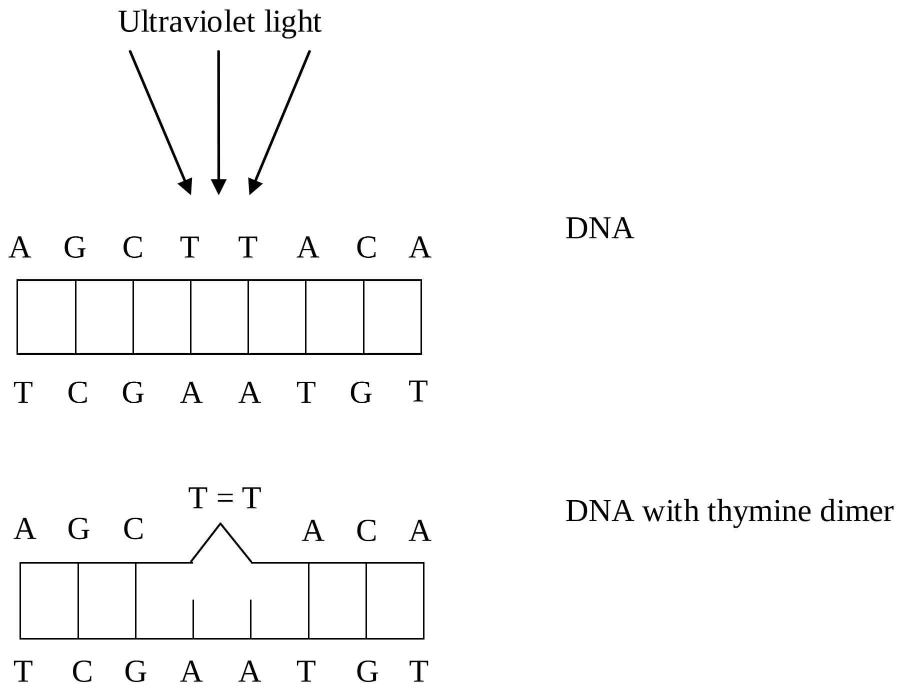
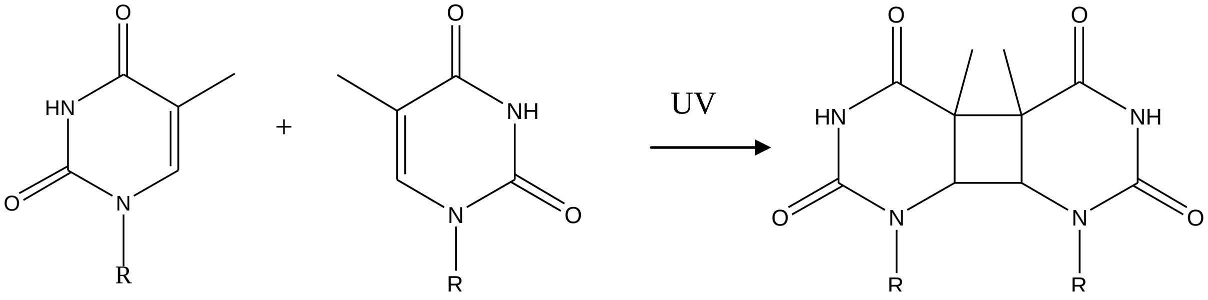


Figure 2.2. DNA structure before and after UV light absorption.



Thymine Thymine Thymine dimer

Figure 2.3. Thymine dimer

CHAPTER 3

EFFECTIVENESS OF UV LIGHT AS A MEANS TO REDUCE *SALMONELLA* CONTAMINATION ON TOMATOES AND FOOD CONTACT SURFACES

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Abstract

The effectiveness of ultraviolet light at a wavelength of 254 nm to reduce *Salmonella* contamination on tomatoes and food contact surfaces was evaluated. Inoculated tomatoes were exposed to UV-C light at doses ranging from 0 to 223.1 mJ/cm^2. All UV treatments significantly reduced *Salmonella* populations ($p<0.05$). The effectiveness of UV-C light in reducing *Salmonella* contamination on different locations on tomato surfaces under various UV doses (0-117.2 mJ/cm^2) was also explored. Results indicated that regardless of the locations, UV treatment was shown to be effective in decreasing *Salmonella* populations. Subsequent studies evaluated possible photoreactivation or dark repair of injured *Salmonella* post-UV treatment. Following UV light exposure at doses of 0, 22.3, 44.6, and 89.2 mJ/cm^2, tomatoes were either exposed to visible light for 0, 3, and 5 h or stored in the dark for the same amount of time. Photoreactivation was not detected, nor was dark repair. UV light was also evaluated for its effectiveness to reduce *Salmonella* contamination on food contact surfaces (stainless steel, HDPE, waxed cardboard and PVC). Contaminated coupons were exposed to UV-C light at 0, 3.3, and 19.7 mJ/cm^2. Significant differences were observed between coupons treated with UV light and the controls ($p<0.05$). Coupons exposed for longer time had greater *Salmonella* population reductions, except for waxed cardboard coupons. Application of UV-C light to reduce *Salmonella* contamination in tomato handling facilities is feasible.

Index words: UV-C light, *Salmonella*, tomatoes, photoreactivation, food contact surfaces

Introduction

The frequency of reported foodborne illness outbreaks linked to fresh produce has increased in recent decades. This increase may be due to increased surveillance and increased consumption of fresh produce *(11)*. Since most fresh produce is consumed raw or minimally processed, pathogen contamination may be a potential threat to human health. From 1990 to 2005, produce outbreaks was responsible for 13% of all foodborne outbreaks and 21% of all foodborne illnesses *(10)*. A more recent report, analyzing outbreaks from 1998-2007, indicated that produce outbreaks caused 15% of all foodborne outbreaks and 23% of all foodborne illnesses *(7)*. Most of the produce-associated outbreaks and illnesses were associated with greens-based salads, cantaloupes, tomatoes, lettuce, and sprouts *(26)*. *Salmonella* is one of the primary pathogens associated with produce outbreaks and accounts for 17% of the produce outbreaks during 1998 and 2007 *(7)*. Produce items associated with outbreaks of salmonellosis include sprouts, greens-based salad, melon, and potatoes *(10)*.

The first large multistate *Salmonella* outbreaks related to tomatoes consumption were reported back in 1990 *(15)*. The tomato contamination source implicated in at least two outbreaks, involving *Salmonella* Javiana and *Salmonella* Montevideo, was traced to tomato packinghouses *(8)*. Asplund and Nurmi reported that *S*. Enteritidis, *S*. Infantis, and *S*. Typhimurium can grow in cut tomatoes (pH 3.99-4.37) at 22 and 30ºC, reaching populations over 10^6/g *(4)*. The growth of *S*. Montevideo isolated from an infected patient on the surface of tomatoes stored at 20ºC and in chopped tomatoes (pH 4.1± 0.1) stored for 96 h at 20ºC or 22 h at 30ºC was also observed by Zhuang et al. *(37)*. Results of a study done by Allen et al. *(1)* indicated that *Salmonella* can survive on tomato surfaces and packing line surfaces under common environmental conditions.

Interventions are needed to minimize pathogen contamination of raw produce and to eliminate them if present on produce. Normally, fresh produce receives little microbial intervention other than washing. For these reasons, alternative strategies to decrease pathogenic bacterial levels on fresh produce are needed. One of the alternatives is to use ultraviolet-C light at 254 nm.

UV light is mostly used for air, water/liquid, and surface decontamination treatments *(6)*. Its application has been limited due to the poor penetration capabilities of the UV wavelengths and shadowing effects *(28)*. UV's effectiveness to reduce bacterial contamination on produce has been documented *(9, 12, 30, 35)*. UV light is also effective in reducing black mold, gray mold, and *Rhizopus* soft rot on tomatoes. Treated tomatoes were firmer and had slower ripening, indicating extended shelf life *(21)*. UV light offers some other advantages as it does not leave any residue, it does not have legal restrictions, it does not require installation of extensive safety equipment *(34, 36)*, it is easy to use, and it is economical *(6)*. Microbial inactivation is caused by the cross-linking of pyrimidine dimers in DNA which prevents microbial reproduction *(28)*.

However, many microorganisms have developed mechanisms to repair the UV-induced DNA damage. One of the repair mechanisms is photoreactivation. Photoreactivation is a light-dependent process which involves photolyase to reverse UV-induced DNA damage *(14)*. Therefore, photoreactivation increases the possibility that microorganisms might regain viability after UV light treatment and thus raise food safety concerns. Photoreactivation of fecal coliforms and *E. coli* has been reported *(13, 29, 32, 38)*. Hu et al. *(16)* found that *Salmonella* Typhimurium, *Shigella dysenteriae*, and *E. coli* are able to photoreactivate after UV treatment *(16)*. Meanwhile, Kuo et al. *(18)* did not notice photoreactivation of *Salmonella* Typhimurium on shell eggs.

There are some uncertainties concerning the effectiveness of UV light on reducing *Salmonella* contamination on mature green tomatoes and whether photoreactivation of UV-injured *Salmonella* on tomatoes occurs. If UV light is applied to tomatoes in a processing line, it is most likely that not all spots on the tomatoes will be directly exposed to the UV light as tomatoes will be rolling on the conveyer belt. Thus, effectiveness of UV-C light in reducing *Salmonella* contaminating on different locations on tomato surfaces needs to be determined. In addition, efficacy of UV light in reducing *Salmonella* contamination on food contact surfaces commonly encountered in tomato handling facilities is also of interest. Thus, the objectives of this study were: (1) to determine the effectiveness of UV-C light in reducing *Salmonella* populations on tomatoes; (2) to evaluate the effectiveness of the treatment to reduce *Salmonella* contamination regardless of its location on the tomato surface; (3) to determine whether photoreactivation by the visible light or the dark repair mechanisms can result in the recovery of damaged *Salmonella* cells post-UV treatment; and (4) to study the effectiveness of UV light to decrease *Salmonella* contamination on food contact surfaces that could be used in tomato handling facilities.

Materials and Methods

Bacterial Cultures and Growth Media

Rifampicin resistant *Salmonella enterica* serovars Michigan, Montevideo, Newport, Poona, and Saintpaul were obtained from the Citrus Research and Education Center, University of Florida, Lake Alfred, FL (Table 3.1) and were adapted to 100 µg/ml of rifampicin. All strains were preserved on Microbank beads (Microbank; Pro-Lab Diagnostics, Austin, TX) at -80°C. Prior to use, each strain was activated by three successive transfers into 9 ml tryptic soy broth (Becton, Dickinson and Company, Sparks, MD) containing 18 µl of 5% solution of rifampicin

(100 µg/ml; Cat. # BP2679-5; Fisher Scientific, Pittsburgh, PA) for 24 h at 35°C. Rifampicin stock solution was prepared by dissolving 1 g of rifampicin in 20 ml of methanol (Cat. # A412SK-4; Fisher Scientific, Pittsburgh, PA) and filtered through 0.22 µm Millipore Express® PLUS Membrane (Cat. # SCGP00525; Millipore Corporation, Billerica, MA).

On the day of the experiment, 2 ml of each *Salmonella* serovar was combined to produce a 10-ml *Salmonella* cocktail. The 10 ml *Salmonella* cocktail was centrifuged for 10 min at a relative centrifugal force (RCF) of 2,500 x g (Model 5681; Forma Scientific, Inc., Marietta, OH) and the supernatant was decanted. The pellet was washed twice with 10 ml of 0.1% peptone water (Becton, Dickinson and Company, Sparks, MD) and resuspended in 10 ml of 0.1% peptone water for final use. Tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD) with 100 µg/ml rifampicin (TSAR) was used to enumerate *Salmonella*. Resuspended cultures were serially diluted with 0.1% peptone water and enumerated to verify the initial concentration. Resuspended cultures contained approximately 10^8-10^9 CFU/ml *Salmonella*.

UV-C Light Source

The UV-C light source (American Air & Water, Inc.; Hilton Head Island, SC) was a closed end reflector (CE) germicidal fixture, CE-36-2, with two germicidal (254 nm) slimline (GML005) lamps. The dimension of the CE fixture is 94 x 18 x 19 cm. The UV lamp fixture was mounted horizontally by adjustable, tripod style legs. The UV-C lamp apparatus was enclosed with cardboard lined with aluminum foil to increase the containment of the UV light. The surface on which the samples were placed on was covered with bench coat (Versi-Dry Lab Soakers; Fisher Scientific, Pittsburgh, PA). The intensity of the UV-C light was measured using a Newport power meter model 1928-C (Newport Corporation, Irvine, CA). UV-C light was turned on at least 30 min to warm up before use.

Effectiveness of UV-C Light (254 nm) in Reducing *Salmonella* Contamination on Tomatoes under Varying UV-C Doses

Mature green tomatoes were obtained from U.S. Foods, a local distributor in Athens, GA. Tomatoes were stored at 4°C until used and allowed to equilibrate to room temperature for approximately 1 h before inoculation. Tomatoes were spot inoculated with 100 µl of *Salmonella* cocktail within marked areas (~ 6 cm^2) on the equatorial plane of each tomato. After inoculating, tomatoes were air-dried for about 2 h in a biological safety cabinet before exposing to UV-C light. Three positive control tomatoes which were not UV treated were sampled to determine the initial *Salmonella* inoculation level. Three negative control tomatoes were not inoculated with the *Salmonella* cocktail.

The inoculated areas on 3 tomatoes were directly exposed to UV light for 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 s. The average intensity of the UV light was 743.6 $\mu W/cm^2$. The corresponding doses were 0, 22.3, 44.6, 66.9, 89.2, 111.5, 133.9, 156.1, 178.5, 200.8, and 223.1 mJ/cm^2. The distance between the center of the light and top of tomato was approximately 53.3 cm.

Treated tomatoes were placed in individual sterile stomacher bags containing 20 ml of universal preenrichment broth (Becton, Dickinson and Company, Sparks, MD). Tomatoes were manually shaken for 30 s, hand-rubbed for 30 s at the inoculation spot, and shaken for another 30 s. Serial dilutions were made using 9 ml of universal preenrichment broth and dilutions were spiral plated (Autoplate 4000; Spiral Biotech, Inc., Bethesda, MD) onto TSAR. All plates were incubated at 35°C for 24 h before enumerating surviving *Salmonella*. Negative control tomatoes were plated on both TSA and TSAR plates to check for background microflora.

Evaluation of Whether the Orientation of *Salmonella* Contaminated Tomatoes Influences the Effectiveness of UV-C Light

Inoculation of mature green tomatoes was the same as described previously. Six tomatoes with *Salmonella* inoculated on the same spot of tomato surface, were positioned differently under UV-C light to evaluate the effectiveness of the treatment to reduce *Salmonella* contamination regardless of its location on the tomato surface (Fig. A.3). The average intensity of the UV light at those 6 spots was 651 µW/cm^2. Tomatoes were exposed at doses of 0, 19.5, 39.1, 58.6, 78.1, 97.6, and 117.2 mJ/cm^2 for 0, 30, 60, 90, 120, 150, and 180 s. The distance between the center of the light and the tomato surface was kept the same as the previous study. Recovery of *Salmonella* from tomatoes was done in the same manner as mentioned previously, except that dilutions were spread plated instead of spiral plated.

Evaluation of Possible Photoreactivation of Injured *Salmonella* Post-UV-C Treatments

This study was to determine possible repair of injured *Salmonella* post-UV-C treatments with visible light. Three inoculated mature green tomatoes were exposed directly to UV-C light at one time at various doses of 0, 22.3, 44.6, and 89.2 mJ/cm^2 for 0, 30, 60, and 120 s at a distance of approximately 53.3 cm from the tomato surface. Following UV-C exposure, tomatoes were exposed to 8 fluorescent lamps in a chamber (Percival model E-41 HO; Percival Scientific, Inc., Perry, IA). The intensity of the visible light was around 10 mW/cm^2 measured with Newport power meter model 1928-C. The inoculated tomatoes were exposed to the lamps for 0, 3, and 5 h. The distance between the light source and tomato surface was approximately 43.8 cm. The temperature of the chamber was 20°C (±0.5°C).

To determine if recovery under darkness occurred, inoculated tomatoes treated after UV-C light were stored in the dark in stainless steel containers for 0, 3, and 5 h at room temperature, 19-20.5°C. Subsequent to the respective visible light and dark exposure, treated tomatoes were placed in individual sterile stomacher bags containing 20 ml of universal preenrichment broth and sampled for surviving *Salmonella* as previously described.

Effectiveness of UV-C light (254 nm) in Reducing *Salmonella* Contamination on Food Contact Surfaces under Varying UV-C Doses

Four food contact surfaces (stainless steel, high density polyethylene, polyvinyl chloride, and waxed cardboard) were chosen to simulate the contact surfaces that could commonly be used in commercial tomato facilities. Stainless steel coupons (SS; type 304, finish #4B, 0.9 mm thick) sized 30 x 50 mm with smooth edges were fabricated by University of Georgia Instrument Shop, Athens, GA. Stainless steel coupons were autoclaved before use. High density polyethylene (HDPE; 2 mm thick; United States Plastic Corporation, Lima, OH) and polyvinyl chloride (PVC) belting (3 mm thick; # 120 white; W.L. Deckert Company, Inc., Milwaukee, WI) were cut to size 30 x 50 mm using scissors and utility knife, respectively. The cut coupons were used one time and autoclaved before use. Waxed cardboard (WC; 4 mm thick; International Paper Company, Griffin, GA) was cut to size 30 x 50 mm using a scalpel. Waxed cardboard coupons were sterilized by spraying 70% ethanol (Decon Laboratories, Inc., King of Prussia, PA) over the front and back sides of the cardboard pieces. After 30 s exposure, the ethanol was wiped from coupon surfaces using a sterile Whirl-PaK® Speci-Sponge® Environmental Surface Sampling Sponge (38 x 76 mm; Nasco, Fort Atkinson, WI) wetted with sterile DI water.

All four types of coupons were placed in sterile metal pans and the surfaces were spot inoculated with 100 µl of the resuspended *Salmonella* cocktail. Inoculated coupons were air-dried in a biological safety cabinet for 90 min. Three positive UV-control coupons did not receive UV-C treatments. Three negative control coupons were not inoculated. Three inoculated coupons of each type of surface were aseptically placed under UV-C light and exposed to UV-C light at an intensity of 656 µW/cm^2 for 0, 5 (3.3 mJ/cm^2) and 30 s (19.7 mJ/cm^2) at a distance of 61.6 cm. The 5 s and 30 s treatments were done on separate days.

After the UV treatment, treated coupons were aseptically placed in individual sterile stomacher bags containing 20 ml of universal preenrichment broth. Treated coupons were hand-rubbed for 30 s. Serial dilutions were made using 9 ml of universal preenrichment broth and dilutions were spread plated onto TSAR. All plates were incubated at 35°C for 24 h. Positive UV-control coupons were plated to determine initial concentration of *Salmonella* cocktail on coupons, and negative control coupons were also plated on both TSA and TSAR plates to check for background microflora.

Statistical Analysis

Experiments with tomatoes and food contact surfaces were replicated three times, with the exception of the first experiment, in which 8 repetitions were performed. Survival of *Salmonella* (CFU/tomato or CFU/coupon) was converted to $\log_{10}$. Statistical analysis was performed using SAS 9.3 (SAS Institute, Inc., Cary, NC). Analysis of variance was carried out by the general linear models (GLM) procedure. When differences among treatments existed ($p<0.05$), Tukey's multiple comparison method was used to determine the difference.

Results and Discussion

Effect of UV-C Light in Reducing *Salmonella* Contamination on Tomatoes

The efficacy of UV-C light in inactivating *Salmonella* on green tomatoes was evaluated. *Salmonella* populations inoculated on tomatoes significantly decreased ($p<0.05$) by 3.22 logs after UV treatment for 30 s (22.3 mJ/cm^2). A maximum reduction of 4.39 log CFU/tomato was obtained when inoculated tomatoes were exposed for 240 s, a UV dose of 178.5 mJ/cm^2 (Fig.

3.1). Thirty seconds and sixty seconds of exposure were not significantly different from each other. After 90 s of exposure, there was no further significant reduction. Doyle and Erickson *(11)* stated that interventions applied to produce should at least inactivate enteric pathogens by 3 logs. The results in this study showed that a 3 log reduction was achieved even with the lowest UV dose. Therefore, UV light can be an alternative intervention to inactivate pathogens on produce.

In a study by Yaun et al. *(35),* the populations of *Salmonella* on inoculated tomatoes, which were obtained from a local distributor, were reduced by 2.19 log CFU/tomato when a maximum UV-C dose of 24 mJ/cm^2 was applied. Sommers et al. *(30)* reported that *Salmonella* spp. inoculated on the surface of Roma tomatoes (maturity level not mentioned) were inactivated by 3.1 log CFU/g at a dose of 500 mJ/cm^2. A higher log reduction, 3.8 log CFU/g, was obtained when UV-C dose of 4,000 mJ/cm^2 was used *(30)*. The authors stated that the higher log reductions were below the detection limit. Song et al. *(31)* found that UV-C at 5 kJ/m^2 (500 mJ/cm^2) significantly decreased *S*. Typhimurium on light red cherry tomatoes by 2.58 logs. Compared to other studies, the results in this study showed higher log reduction even with the lowest dose. This may be due to using different strains of *Salmonella* with varying levels of UV-sensitivity in the different studies. Shechmeister *(27)* noted that UV sensitivities of bacteria vary not only among species, but also among strains of the same species. In addition, in some cases, the initial inoculum levels were not large enough to obtain a greater log reduction calculations than those reported after UV treatment. For example, Sommers et al. *(30)* obtained a *Salmonella* log reduction of 3.08 CFU/g when exposed to UV at a dose of 500 mJ/cm^2, but even when a four-fold greater dose was applied, there was no significant increase in the log reduction. This study demonstrates that UV light had great efficiency in reducing *Salmonella* populations on tomatoes. The effectiveness of UV-C in reducing microbial growth in baby spinach, lettuce, melon, and fresh-cut apple has also been reported *(2, 3, 12, 22, 23)*.

Orientation of *Salmonella* Contaminated Tomatoes under UV-C Light

Statistically, there was no interaction between the UV treatment and the locations of *Salmonella* on tomato surfaces, meaning that UV at a certain dose exhibited the same statistical pattern regardless of *Salmonella* locations on tomatoes and vice versa. After UV exposure, *Salmonella* populations were reduced ($p<0.05$) to similar levels regardless of the location of the *Salmonella* contamination on the tomato surface (Fig. 3.2). There was no significant difference in population reduction for the 30-120 s of exposures. While a similar level of *Salmonella* inactivation was obtained for the various contamination locations on the tomatoes, the population on position 1, facing directly to the UV light source, was reduced significantly more than several of the positions (#3, 4, and 5). UV radiation was effective in reducing *Salmonella* contamination regardless of its location on tomato surfaces. Therefore, if UV treatment is applied to harvested tomatoes rolling on conveyer belts in a processing line, pathogen reduction on the tomatoes is possible. To ensure that tomatoes receive adequate UV treatments, the speed of the conveyer belt would have to be monitored. Wilson et al. *(33)* reported that postharvest decay on apples was reduced when treated with UV light on a processing line. The result in this study might show lower log reduction than the previous study, but more than 3 log reduction was still observed for UV at higher dose.

Photoreactivation of Injured *Salmonella* Post-UV-C Treatments

Statistically, there was no interaction between the UV exposure and the ability of injured *Salmonella* to recover after exposure to visible light. Therefore, significant differences were analyzed only among UV treatment and among visible treatment. *Salmonella* counts after UV radiation were significantly lower than those of controls ($p<0.05$) (Fig. 3.3). Since *Salmonella* population levels on tomato surfaces exposed to visible light for 3 or 5 h were less than those on tomatoes not exposed to visible light, no significant injury repair by photoreactivation by visible light treatments was observed. The lower *Salmonella* population on those exposed to visible light might due to the fact that there was limited nutrient availability on the tomato surfaces or the surfaces were too dry to support recovery of *Salmonella*. Lang et al. *(19)* found that air drying reduced recovery of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on the surface of tomatoes by more than 1 log, suggesting that bacteria cells may be injured or stressed during dessication.

To determine if recovery under darkness occurred, post-UV treated *Salmonella* contaminated tomatoes were stored in closed stainless steel containers (Fig. 3.4). Since there was a lack of interaction, significant differences were analyzed only among UV treatment and among dark treatment. The results were similar to those of visible light treatment. *Salmonella* levels reduced significantly after UV treatment. The treatment of 3 h and 5 h dark did not result in injury recovery.

Kuo et al. *(18)* reported similar results on the lack of repair of UV damage. Their study indicated that neither 1 h of light exposure nor 1 h of dark treatment post-UV treatment significantly influenced the photoreactivation or dark repair ability of *S.* Typhimurium on shell eggs *(18)*. However, photoreactivation of *S. dysenteriae* and *S.* Typhimurium was detected in a study done by Hu et al. *(16)*. After UV light treatment, contaminated surface water samples were exposed to a fluorescent lamp with an intensity of 0.066 mW/cm^2 at a wavelength of 360 nm for 3 h. They noticed that photoreactivation decreased significantly with higher UV doses.

Some factors may contribute to the results of the present study. Lindenauer and Darby *(20)* noted that the initial pyrimidine dimers formed, the number of photolyase present, the temperature during the complex of photoreactivating enzyme and dimer, and the dose of photoreactivating radiation could affect photoreactivation. Harm *(14)* also pointed out that "photoreactivation requires that a cell contain the photoreactivaing enzyme and that the extent of repair varies with the genetic and physiological state of the cell". At least one of these factors may have contributed to the present findings.

Effect of UV-C Light in Reducing *Salmonella* Contamination on Food Contact Surfaces

This study evaluated the efficacy of UV-C light in decreasing *Salmonella* contaminated food contact surfaces (stainless steel, HDPE, waxed cardboard, and PVC) which may be used in commercial tomato facilities. Analysis of UV light exposure for 5 s was done separately from 30 s UV exposure since the experiment was done in separate days. Statistically, there was interaction ($p<0.05$) between the UV treatment and the four food contact surfaces. However, the four contact surfaces were not statistically compared to each other because one type of material is usually used for a specific application, not interchangeably. For instance, stainless steel, a widely recognized excellent material for the food industry *(24)*, is usually used in the manufacture of dump tanks, processing lanes, and most equipment in tomato packinghouses *(1)*. Meanwhile, PVC is usually used for conveyer belts or to cover roller bars which move tomatoes along the processing lines *(25)*. Bins made of HDPE are usually used to gather tomatoes during harvest, and waxed cardboard boxes can be used as to pack tomatoes for distribution.

UV exposure for 5 s (3.3 mJ/cm^2) significantly reduced *Salmonella* populations on all food contact surfaces (Fig. 3.5). *Salmonella* was decreased by 2.75, 2.93, 1.39, and 1.91 log CFU/coupon on SS, HDPE, WC, and PVC, respectively. *Salmonella* log reduction in waxed cardboard was not as great as that on other coupons. Significant differences were also observed between the 30 s of UV treatment and the controls (Fig. 3.6). Thirty seconds (19.7 mJ/cm^2) of UV exposure, respectively, resulted in 3.51, 4.32, 1.43, and 3.51 log reductions of *Salmonella* contamination on SS, HDPE, WC, and PVC. When comparing the results of coupons treated for 5 s and 30 s of UV, coupons exposed for longer time had greater *Salmonella* population reductions. Regardless of 5 s or 30 s of UV radiation, *Salmonella* populations decreased the least on waxed cardboard. Yaun et al. *(35)* found that microbial populations on the surface of tomatoes were greater than those on the surface of unwaxed apples after UV-C exposure. They assumed that wax applied on the surface of tomatoes might have shielded bacteria from UV radiation.

Sommers et al. *(30)* obtained more than 5 log reductions of *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* on electropolated and bead blasted stainless steel coupons when inoculated coupons were exposed to UV-C at a dose of 400 mJ/cm^2. When inoculated coupons were treated with 50 mJ/cm^2, the three pathogens were reduced by 1.86-3.05 logs. Kim et al. *(17)* also reported that either intensities of 250 or 500 µW/cm^2 significantly decreased *L. monocytogenes*, *S.* Typhimurium , and *E. coli* O157:H7 populations on stainless steel chips. Intensity at 500 µW/cm^2 for 3 min (90 mJ/cm^2) reduced populations of the three pathogens by more than 4 logs. Intensity of 250 µW/cm^2 for 1 min (15 mJ/cm^2) decreased the levels of the pathogens by 2.43-4.38 logs. This result is similar to the present findings, in which a 3.51 log reduction was achieved when stainless steel was treated for 30 s at 19.7 mJ/cm^2. Bae et al. *(5)* demonstrated that UV treatment at intensity 0.236 ± 0.013 mW/cm^2 for 3 h significantly reduced *S.* Typhimurium, *L. monocytogenes*, and *S. aureus* on stainless steel surfaces by 3.06, 2.18, and 2.70 log CFU/coupon, respectively, and *S. aureus* on polypropylene by 3.11 logs. They concluded that the effectiveness of the UV treatment depends on the surface material. The overall findings of this study indicated that UV treatment is effective in reducing *Salmonella* contamination on food contact surfaces.

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Table 3.1. Cultures of *Salmonella enterica* used to evaluate UV treatment of contaminated tomatoes and food contact surfaces.

Strain	**Source**
S. Michigan	Cantaloupe isolate
S. Montevideo	Human isolate from a tomato outbreak
S. Newport	Environmental isolate from a Virginia tomato outbreak
S. Poona	Human isolate from a cantaloupe outbreak
S. Saintpaul	Orange juice isolate

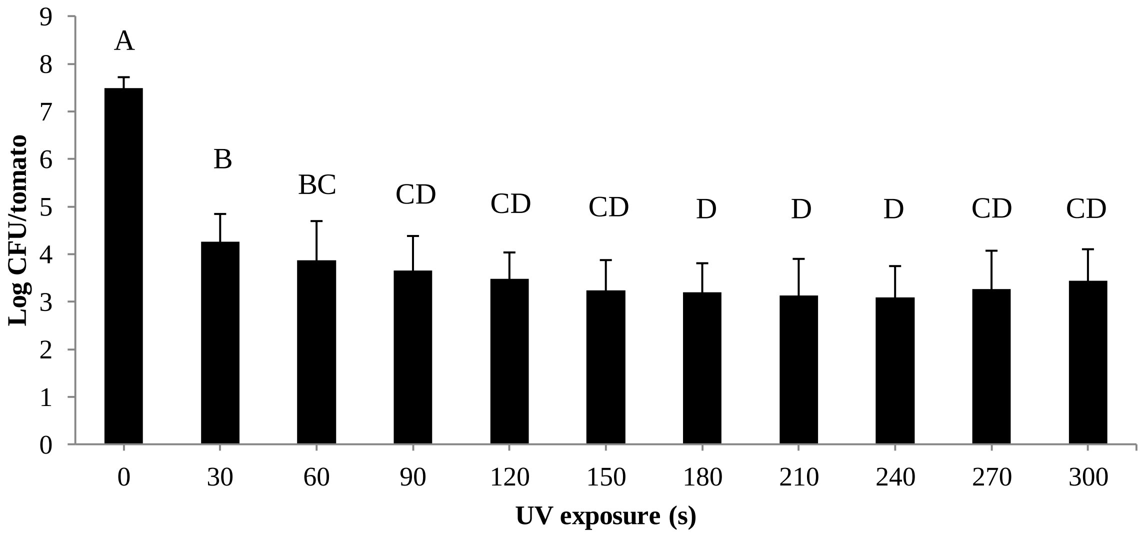

Figure 3.1. Survival of *Salmonella* on tomato surfaces after UV light treatment at an intensity of 743.6 µW/cm^2 for 0 to 300 s. Values with different letters were significantly different ($p<0.05$).

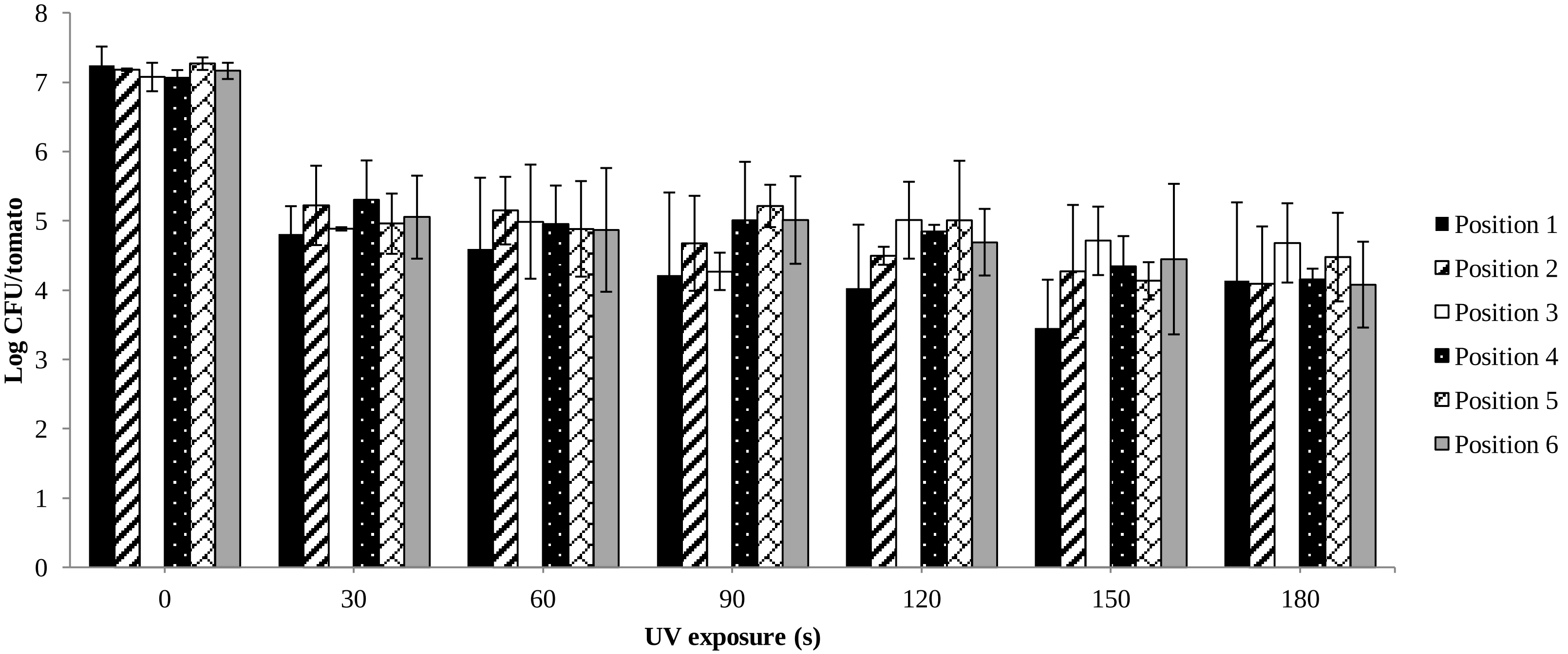

Figure 3.2. Survival of *Salmonella* on 6 different locations on the tomato surfaces after treatment of UV light at intensity of 651 µW/cm^2 from 0 to 180 s. *Salmonella* contamination positions on tomato surfaces: 1 – directly exposed to UV source; 2 and 5 – on equatorial plane facing reflective side wall of UV protective shield; 3 – on equatorial plane facing tomatoes; 4 and 6 – on equatorial plane facing reflective end walls of UV protective shield.

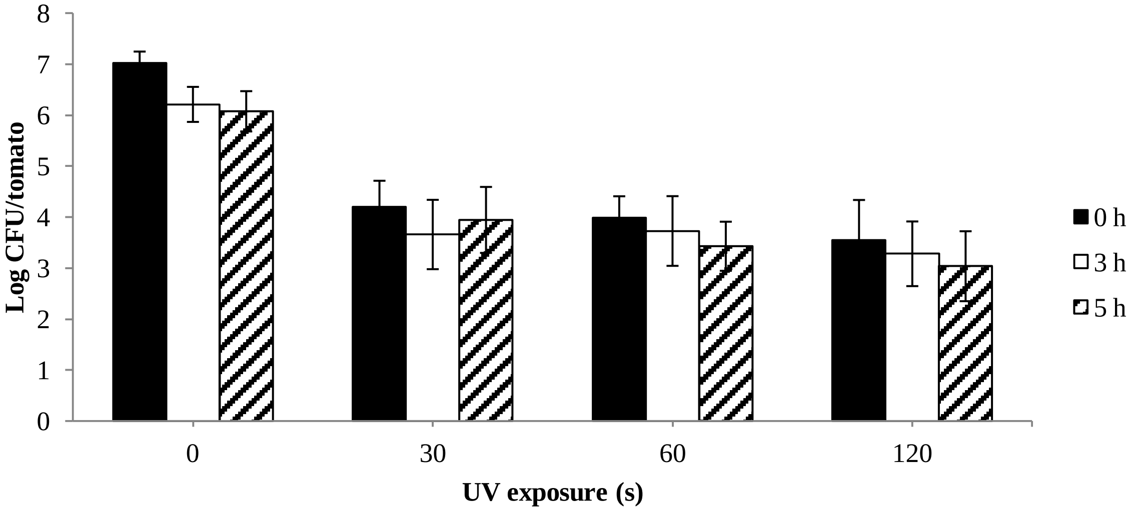

Figure 3.3. Survival of *Salmonella* on tomato surfaces after exposed to visible light for 0, 3, and 5 h at each UV light treatment of 0, 30, 60, and 120 s.

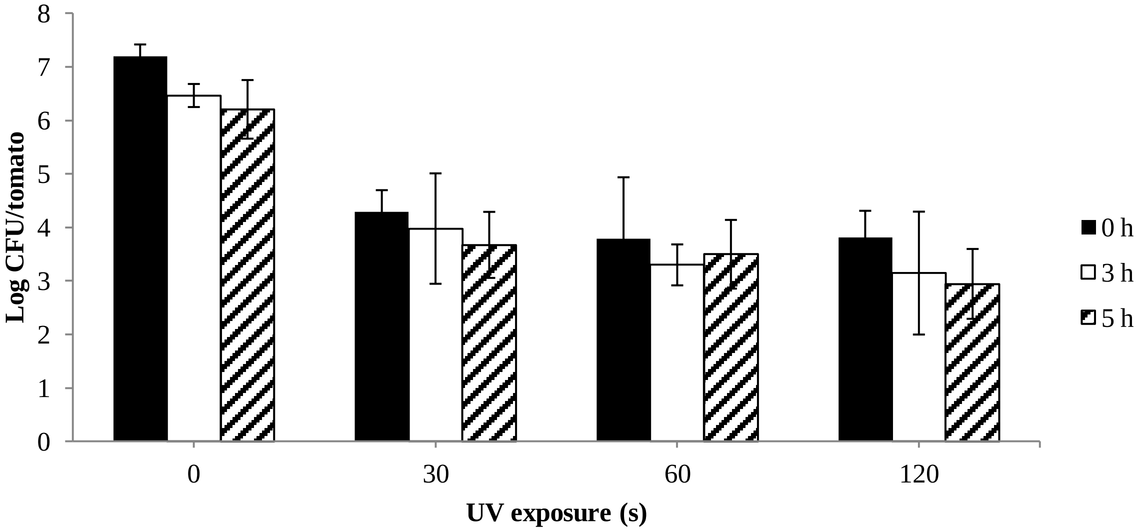

Figure 3.4. Survival of *Salmonella* on tomato surfaces after stored in the dark for 0, 3, and 5 h at each UV light treatment of 0, 30, 60, and 120 s.

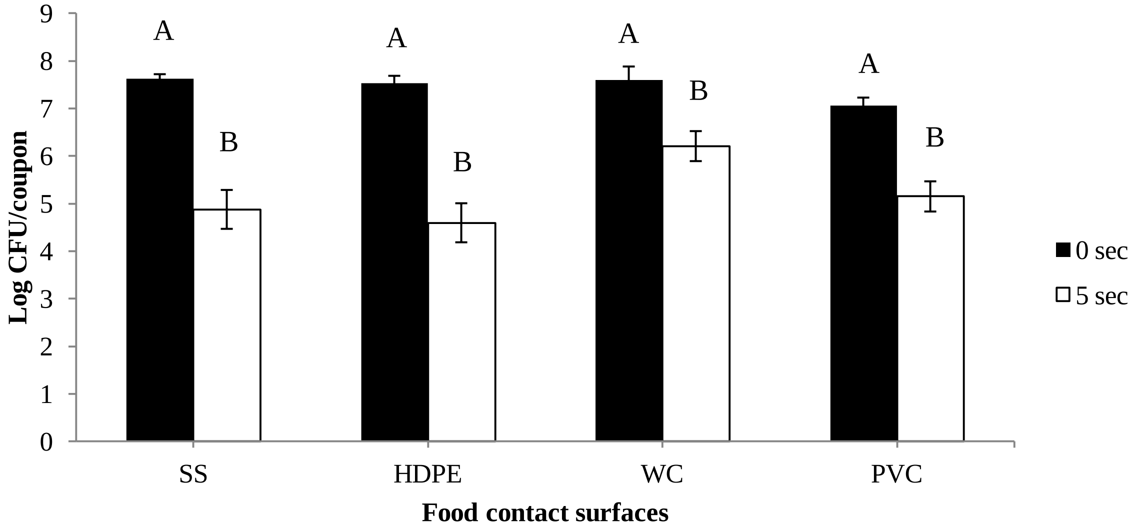

Figure 3.5. Survival of *Salmonella* on food contact surfaces after UV light treatment at an intensity of 656 µW/cm^2 for 0 and 5 s. SS: stainless steel; HDPE: high density polyethylene; WC: waxed cardboard; PVC: polyvinyl chloride. Values within each contact surface type with different letters were significantly different ($p<0.05$).

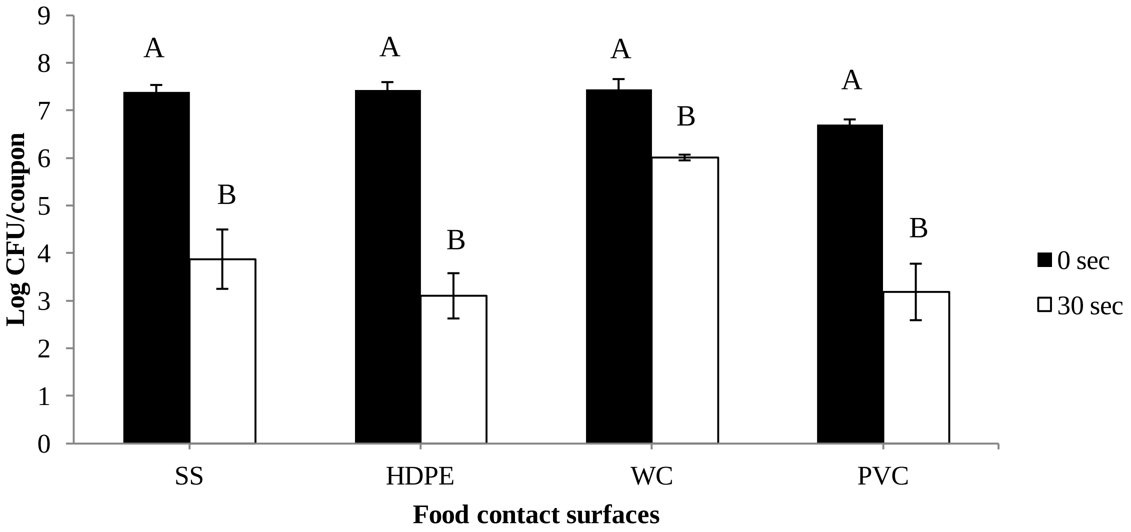

Figure 3.6. Survival of *Salmonella* on food contact surfaces after UV light treatment at an intensity of 656 µW/cm^2 for 0 and 30 s. SS: stainless steel; HDPE: high density polyethylene; WC: waxed cardboard; PVC: polyvinyl chloride. Values within each contact surface type with different letters were significantly different ($p<0.05$).

CHAPTER 4

CONCLUSION

This study indicated that UV-C light was effective in reducing *Salmonella* contamination on tomatoes. Regardless of *Salmonella* contamination on different locations on tomato surfaces, UV treatment was also shown to be effective in decreasing *Salmonella* populations. Photoreactivation was not evident in this study, nor was dark repair. UV-C light was also effective in reducing *Salmonella* contamination on food contact surfaces. Therefore, the application of UV-C light to treat tomatoes and food contact surfaces in commercial tomato handling operations is feasible.

CHAPTER 5

APPENDIX A

Figure A.1. UV lamp apparatus without the front cardboard shield (A). UV lamp apparatus covered with cardboard lined with aluminum foil during UV exposure (B).

Figure A.2. Air drying of *Salmonella* inoculated tomatoes in the biological safety cabinet (A). Three tomatoes were placed in the center of the UV lamp chamber (B).

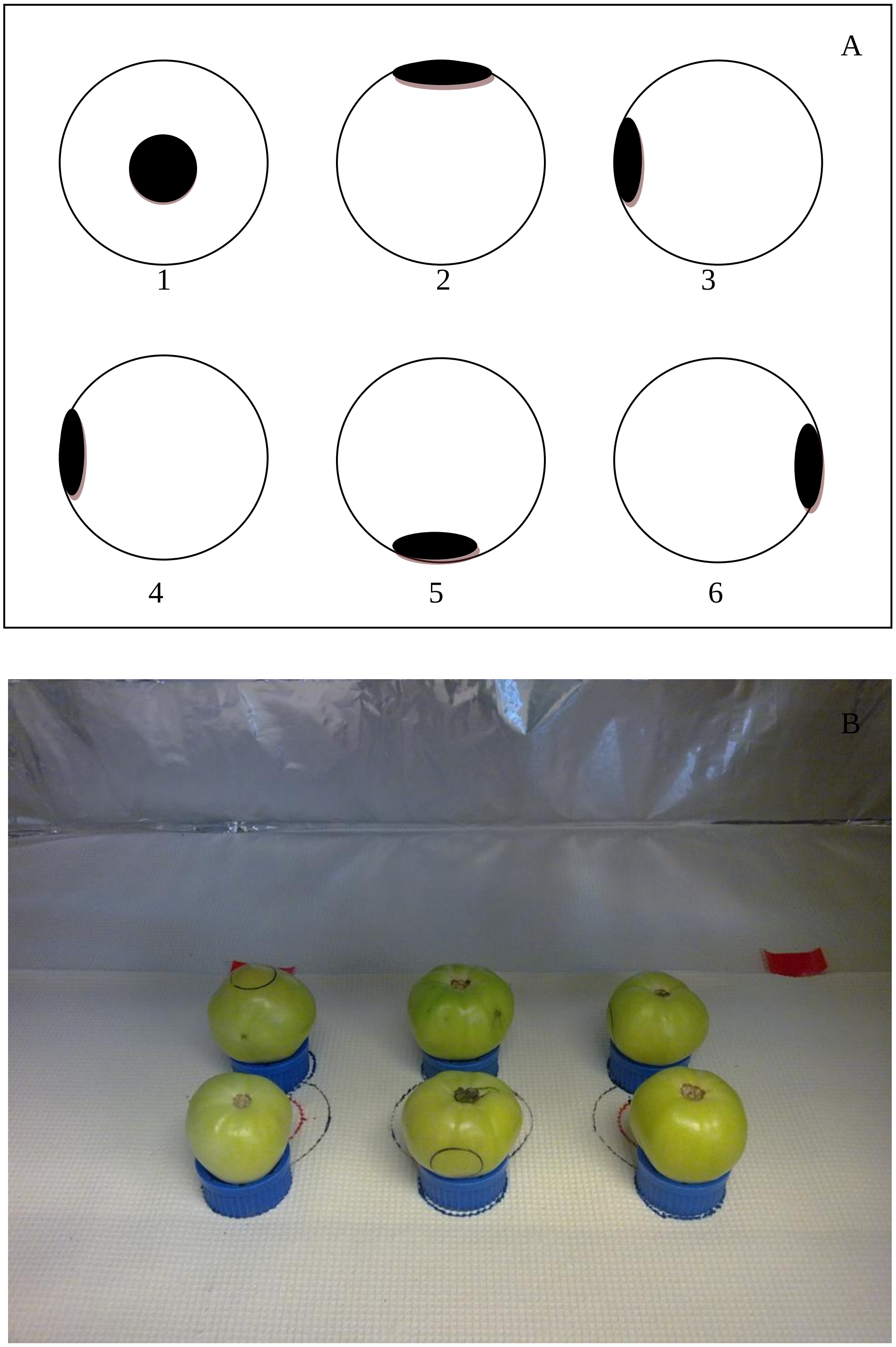

Figure A.3. The positions of marked inoculated tomatoes under UV-C light (as viewed from top). Black circles represented the inoculation sites (A). Placement of tomatoes inoculated at 6 different surface locations in the UV chamber (B).

Figure A.4. Representative of spot inoculated stainless steel coupons.

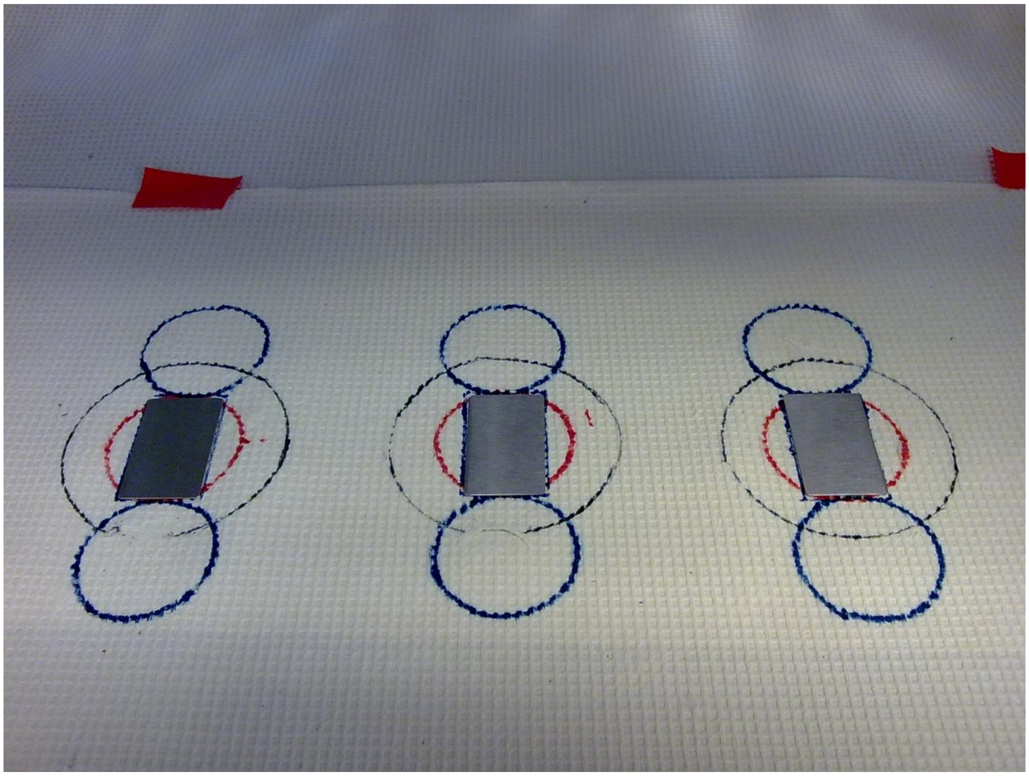

Figure A.5. Representative of three stainless steel coupons in the UV chamber.