Efficacy of Ultraviolet Treatments for the Inhibition of Pathogens on the Surface of Fresh Fruits and Vegetables

by

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Abstract

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Two studies investigating the use ultraviolet light at a wavelength of 253.7nm (UVC) into the inhibition of *Salmonella* spp. and *Escherichia coli* O157:H7 were conducted. The objectives of these studies were: to determine the rates for the destruction of *Salmonella* and *Escherichia coli* O157:H7 on the surface of agar and to investigate its effectiveness on the surface of fresh produce. Multiple replications of different doses and cocktail concentrations were performed and resulted in a 5 log reduction of *Escherichia coli* O157:H7 at doses exceeding 8.4 mW / cm^2, while a 5 log reduction for *Salmonella* spp. was observed at doses exceeding 14.5 mW / cm^2. Samples of Red Delicious apples, green leaf lettuce and tomatoes were subjected to different doses ranging from 1.5 – 24 mW / cm^2 of UVC to determine effective log reductions of microbial populations. UVC applied to apples inoculated with *E. coli* O157:H7 resulted in the highest log reductions of approximately 3.3 logs at 24 mW/cm^2. Lower log reductions (2.19 logs) were seen on tomatoes inoculated with *Salmonella* spp. and leaf lettuce (2.65 and 2.79) inoculated with both *Salmonella* spp. and *E. coli* O157:H7 respectfully. Due to the low capital involved in initiating a UVC system, the use of ultraviolet energy may prove to be a beneficial mechanism to decrease pathogens on fresh produce if used in conjunction with strict

adherence to a sanitation program, Good Manufacturing Practices and Good Agricultural Practices in ensuring the safety of fresh produce.

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Introduction and Justification

The produce industry has witnessed an explosion of consumption and sales in the last decade. Total per capita consumption from 1987 – 1997 increased 9.5% and consumption of fresh vegetables increased by 14.3%. Sales of fresh fruits and vegetables in the United States increased by $36.2 billion in 1997 (*44*). With the growing demand for fresh fruits and vegetables, the Centers for Disease Control and Prevention (CDC) reported an increase in the frequency of produce associated foodborne disease outbreaks. Between 1973 and 1987, approximately 2% of foodborne disease outbreaks were traced to fresh fruits and vegetables (*4*). In contrast, by 1991 fresh fruits and vegetables accounted for 8% of outbreaks (*78*). From 1993 – 1997 the CDC documented 66 outbreaks that were traced to fresh fruits and vegetables. These 66 outbreaks involved approximately 12,000 cases and resulted in 2 fatalities (*22*). Major outbreaks in fresh produce have already been associated with common foodborne pathogens such as *Salmonella*, *Listeria monocytogenes*, *Shigella* spp., and *Escherichia coli* O157:H7 (*6, 39*). In September 1997, an EPA Scientific Advisory Panel specifically identified *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 as pathogens of public health concern on produce (*32*).

Due to seasonal demand and variable growing seasons, fresh produce generally comes from many geographic areas. The combination of a short shelf life, quick sales and foreign origins makes it difficult to trace back sources of contamination. Examples of recent multi-national outbreaks have occurred in Guatemalan raspberries contaminated with *Cyclospora*

(*17*), Hepatitis A isolated from strawberries produced in Mexico and *Shigella* contaminated lettuce produced in Spain that caused an outbreak in Norway and Sweden (*6*). Recent outbreaks in the United States and Canada involving fresh produce include *Salmonella* Poona in cantaloupes (*40*), *Salmonella* Kottbus in alfalfa sprouts (*24*), *Salmonella* Baildon in tomatoes (*28*) and *Salmonella* Enteritidis phage type 913 in mung bean sprouts (*15*). These examples of outbreaks serve as an indication of the ever-increasing global food market and implicate produce as a vector for foodborne disease.

A strategy to minimize the risks involved with the consumption of fresh fruits and vegetables involves either reducing or eliminating surface contamination of pathogens. Effective surface decontamination techniques could be employed to reduce the surface load of foodborne pathogens. Simply washing fresh produce with water may remove pathogens and other spoilage organisms. Traditional detergents are known to be partially effective in removing pathogens (*2, 7, 39*), however each type of disinfectant varies both in efficiency and in allowable maximum concentration. In contrast to chemicals, the use of an alternative treatment for the destruction of pathogens on the surface of fresh fruits and vegetables would be desirable. One such alternative process is the use of germicidal ultraviolet light at a wavelength of 200 – 280 nm (UVC).

UVC is already used in some areas of produce processing. Currently, some companies employ the use of UV treated water in the decontamination of fresh produce such as shredded lettuce. Application of UVC has been shown to be effective against common foodborne pathogens such as *Campylobacter, Salmonella,* and *Escherichia coli* (*26, 29, 72,*

75). UVC has been shown to have little penetration onto the surface of samples. Therefore the use of UV should only be considered for types of produce that have a smooth tough skin free from indentations that may harbor bacteria and provide a shelter for pathogens.

Recent Hazard Analysis and Critical Control Points (HACCP) regulations require a five log performance standard in the reduction of the pertinent pathogen in juice products (*37).* The regulations do not specifically mention any particular method for achieving this, which allows for the use of alternative processes. In certain products, the five-log reduction standard must be applied in a single processing facility. For some fruit, interventions may be limited to the surface. The use of UVC may prove to be useful as a prerequisite step in HACCP protocols if it is effective at reducing microbial numbers on the surface of fresh fruits and vegetables. In order to apply this technology studies need to be undertaken to determine the efficacy of direct UVC radiation on the surface of fresh fruits and vegetables as a means of ensuring their safety.

Review of Literature

Section 1: *Salmonella* spp.

Characteristics

Every year, approximately 40,000 cases of salmonellosis are reported to the Centers for Disease Control and Prevention. Due to the relatively mild cases that are not reported, total estimates have reached anywhere up to 20 times that number (*18*). The disease is generally more prevalent in the summer than in the winter and children are more often infected than adults. The genus *Salmonella* contains over 2300 serotypes, all of which are believed to be capable of causing human disease (*18*). Recently, each of the 2324 *Salmonella* serovars has been placed into one of two species: *S. enterica* or *S. bongori*. The majority of serotypes fall under the *S. enterica* family. *S. enterica* consists of the former groups II (*S. enterica* subsp. *salamea*), IIIA (*S. enterica* subsp. *arizonae*), IIIB (*S. enterica* subsp. *diarizonae*), IV (*S. enterica* subsp. *houtenae*), and group VI (*S. enterica* subsp. *indica*). The former group V organisms are now considered *S. bongori* (*46*).

Salmonella belongs to the family *Enterbacteriaceae* which is characterized by gram-negative, non-sporeforming aerobic rods. *Salmonella* are generally motile by means of a flagellum. However, non motile strains do exist. Most strains are lactose negative and all strains are believed to be potentially pathogenic to humans and vertebrates. Additionally, most strains are able to utilize citrate as a carbon source. One of the most notable exceptions to these general characteristics is *S.* Tyhpi, which is unable to ferment glucose,

utilize citrate or ferment rhamonose (*67*). Most of the cases of salmonellosis reported in the United States are due to infections of *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Hidelberg (*1*).

Illness

The transmission of the bacterium may occur between human – human interaction or human – animal interaction (*3*). There are four main syndromes of salmonellosis: the asymptomatic or carrier state, enteric fever, gastroenteritis, and septicemia (*3, 13*). Infection by the organism can manifest itself in two ways. The first manifestation of salmonellosis is a generalized infection that characteristically invokes a high fever with diarrhea appearing late in the infection. The second expression is in the form of an enteric fever that involves an acute gastrointestinal disorder and severe diarrhea and is usually the result of food poisoning (*50*). Other symptoms may include nausea, abdominal cramps, vomiting, chills, and headaches. *Salmonella* begins to show its symptoms within 12 – 72 hours post infection. The illness may last anywhere from 4 – 7 days (*18, 38*). Hospitalization is generally rare and the use of antibiotics is generally not recommended. *Salmonella* is known to become resistant to antibiotics and outbreaks of *S.* Typhimurium DT104 have been documented at veterinary hospitals (*23*). DT104 isolates have been found to be highly resistant to antibiotics. *S.* Typhimurium is resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (*S.* Typhimurium DT104 R-type ACSSuT) (*23*).

Sources of contamination

Salmonella is generally considered a ubiquitous organism as it is an enteric pathogen and is commonly isolated from horticultural crops or wash water (*82*). It may be found in soil, water, sewage, feed, equipment and plant products. Most foods associated with outbreaks are those of animal origin or those that may have been cross contaminated with foods of animal origin. Primary hosts for *Salmonella* are the intestinal tract of birds, reptiles and mammals (*50*). As intestinal organisms, they are excreted in feces and may be transmitted to a food source by insects, soil or water (*46*). Agriculture practices such as processing, distribution and storage operations may be factors in the dissemination of *Salmonella* and have the potential to cross contaminate produce which may lead to outbreaks. Amphibians are also recognized as sources of *Salmonella*. Epidemiological links to frogs in a processing facility have been implicated as a potential source for contamination of unpasteurized orange juice (*29*).

Meat and eggs are generally the most commonly implicated foods involving outbreaks of *Salmonella*, however almost any food can be considered a secondary source if cross contamination occurs. Disease transmission is generally from foods of animal origin to human ingestion as *Salmonella* is excreted in the waste of infected animals. As a result, the use of this waste as a soil or fertilizer on crops aids in the dissemination of the pathogen. Rain also creates runoff that may contaminate irrigation waters or the reintroduction of the organism to a host who drinks the water (*3*).

Infective Dose and Susceptible Populations

The number of cells necessary to cause infection varies greatly upon the particular strain, the host characteristics and the food composition. Different food matrices may lead to survival and a lower infective dose. Products high in lipids such as chocolate and cheese may protect the bacteria from gastric acid, thereby lowering the infective dose (*50*). Competition with other bacteria, especially lactic acid bacteria may also result in the inhibition of *Salmonella*. Death is extremely rare in *Salmonella* infections where the host is considered in general good health. If morbidity occurs it is generally in those with weakened immune systems such as the elderly and infants. Death most often occurs as a result of dehydration, septicemia or other complications (*50*). The infective dose is generally considered to be about 10^6 cfu / g in healthy individuals. Reiter's syndrome is an uncommon sequale that is associated with a small number of those infected. Symptoms include painful urination, eye irritation and joint pain. Reiter's syndrome may last for months or even years and chronic arthritis may develop.

Factors Affecting Growth and Survival

Salmonella can grow at a pH range of 4.5 – 9.0. Optimum growth for *Salmonella* occurs at a pH of 6.6 – 8.2 and a water activity greater than 0.94. Salt concentrations greater than 9% inhibit *Salmonella* (*67*). Of the approximately 2300 strains of *Salmonella*, *S.* Senftenberg is generally considered the most heat resistant serovar (*46*). Research by Golden et al. (*42*), demonstrated that the flesh of melons can support the rapid growth of *Salmonella* at room temperature. Other studies have shown that *Salmonella* can grow and survive on the

surface of tomatoes if held at 20 - 30°C (*87*). *Salmonella* has been known to survive for 20 hours on the surface of tomato skins (*79*). The pH inherent to the skin of the tomato proved to be ineffective at inhibiting bacterial growth. Multistate outbreaks of *Salmonella* in ice cream have suggested survival during freezing. Studies by Parish et al. (*62*) indicated that *S.* Gaminara, *S.* Hartford, *S.* Rubislaw and *S.* Typhrimirum are capable of survival at a pH of 3.5 for 27 days and at a pH of 4.1 can survive for 60 days.

Fresh Produce Foodborne Outbreaks

Occasional reports of multi-state outbreaks associated with fresh fruits and vegetables have been on the rise due to increased consumption of fresh produce, the elimination of seasonality, consumer preference for healthy foods, and wider distribution of product (*82*). *Salmonella* induced foodborne diseases are often more prevalent in other products such as dairy, meat and poultry, however outbreaks have been associated with fresh produce. Outbreaks in the United States have mainly involved melons (*6*). According to the FDA, five outbreaks of *Salmonella* have been attributed to melon sources since 1950 (*77*). Most recently an outbreak of *S.* Poona in the US and Canada in May 2002, has been traced to a Texas distributor of cantaloupe (*40*). As this investigation is ongoing, no data is currently available on the outbreak. In the summer of 1991, CDC documented over 400 cases of *S.* Poona that occurred in 23 states. The source of the outbreak was traced to contaminated cantaloupe produced in Texas and distributed throughout the Midwest and Canada (*16*). Additional outbreaks in 1990 were attributed to *S.* Chester in cantaloupes and *S.* Javiana in tomatoes (*16*).

Due to the high visibility of the 1991 *Salmonella* outbreak in Texas traced to cantaloupe, much research has been done on the survival and growth of *Salmonella* in melons. *Salmonella* Chester and *S.* Poona have been responsible for outbreaks associated with precut cantaloupe (*42*). It has long been known that the contamination of interior watermelon tissue is possible if a tainted rind or knife is present in the slicing of the fruit (*41*). In 1990 and 1991, the FDA conducted field surveys on imported melons to eliminate practices responsible for rind contamination. The results of the study found that only about 1% of melon rinds were contaminated with *Salmonella* spp. The association of foodborne disease originating in salad bars leads to the suspicion of contaminated rinds and the subsequent growth of the bacteria during the display stage. Research by Golden et al. (*42)* found that *Salmonella* can grow rapidly in the interior tissue of unrefrigerated melon. This is of particular importance when fresh fruit is sold outdoors, especially during the summer (*34*). Wells and Butterfield reported a strong correlation (68%) of *Salmonella* contamination in fruits that exhibited bacterial soft rot (*82*). Bacterial soft rot is generally due to poor handling, inadequate sanitation or improper storage of produce.

Other types of produce associated with *Salmonella* outbreaks have included celery, watercress, watermelon, salads, and cabbage (*82*). Tomatoes have been implicated in two outbreaks of *S.* Javiana (1990) and *S.* Montevideo (1993), which were traced to the same processor. A water bath used to wash the tomatoes was identified as the likely source of contamination in both outbreaks (*78*). The acidic environment of orange juice (3.4 – 4.0) was previously thought to inhibit growth of *Salmonella*. However, recent research and

outbreaks demonstrate its survival and proliferation at low pH. In 1996, unpasteurized orange juice was identified as a source for an outbreak of *S.* Hartford, *S.* Gaminara and *S.* Rubislaw at a Florida theme park (*78*). Additionally, an outbreak of *S.* Muenchen was traced to unpasteurized orange juice in 1999 (*20*).

Section 2: *Escherichia coli* O157:H7

Characteristics

Salmonella and *Escherichia* as genera are closely related, the two share approximately 45 – 50% of their DNA sequences (*13*). *E. coli* as a species is very common. The bacterium is often found in the digestive tract of mammals and generally lives in symbiosis with its host. It should also be noted that Outbreaks of *E. coli* O157:H7 have been linked to consumption of ground beef, lettuce and raw cider (*1*). Infection often leads to bloody diarrhea, and occasionally to kidney failure. Usually little or no fever is present, and the illness resolves in 5 to 10 days. In 1982 *E. coli* O157:H7 was identified as a human pathogen after being linked to extreme and unusual gastroenteritis from patients that consumed beef (*31*). There are currently four classes of enterovirulent *E. coli* bacteria that are known to cause gastroenteritis in humans. This group is collectively referred to as the enterovirulent *Escherichia coli* group (EEC). The EEC group is comprised of enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC). *E. coli* O157:H7 is in the EHEC group. Toxins produced by *E. coli* O157:H7 attack the intestinal lining and may cause severe damage. These toxins are similar to those produced by *Shigella dysenteriae* and are considered

Shiga-like toxins (*18*, *33*). The CDC has estimated that approximately 73,000 cases occur involving *E. coli* O157:H7 annually in the United States. Outbreak data presented by the CDC in 1999 represents 1897 cases in thirty states. Of these cases, 11% required hospitalization, 2% developed HUS and death occurred in 0.2% of the cases. These deaths that were attributed to contaminated ground beef and drinking water (*19*, *21*). CDC preliminary data for 2000 indicates 631 cases of *E. coli* O157:H7 in eight states (*25*).

Due to changes in the way food is processed and handled, new challenges are being introduced to the way that foodborne surveillance programs track data (*6*). PulseNet and FoodNet are two programs within the CDC that help track down outbreaks associated with *E. coli* O157:H7. FoodNet provides active surveillance for foodborne diseases among nine states and works in collaboration with the U.S. Department of Agriculture (USDA) and the Food and Drug Administration (FDA). PulseNet utilizes Pulsed –Field Gel Electrophoresis (PFGE) to collect and store DNA "fingerprints" for rapid comparisons of isolates. PulseNet therefore is capable of linking outbreaks of foodborne disease that occur at the same time and may span over several states.

Illness

Cases of *E. coli* O157:H7 were first seen in Oregon and Michigan in 1982 when victims suffering from similar complications had all eaten undercooked ground beef from fast food restaurants. Groups with the highest susceptibility include the immunocompromised, the elderly and children (*57*). Foodborne illness attributed to EHEC can manifest itself in three

ways: hemorrhagic colitis, thrombotis thrombocytopenic purpura (TTP) and hemolytic uremeic syndrome (HUS). Currently, the infective dose of EHEC is unknown with speculation that as few as 10 cells may cause illness.

Hemorrhagic colitis (HC)

Symptoms normally begin with intestinal cramping followed by a watery diarrhea which then turns grossly bloody. A fever is generally absent, and the duration of the illness lasts anywhere from two to nine days (*31*). Although most outbreaks have been attributed to *E. coli* O157:H7, there have been instances where HC has been linked to *E. coli* O6:NM (*46*).

Hemolytic Uremic Syndrome (HUS):

HUS infections caused by *E. coli* O157:H7 are the leading cause of acute kidney failure in children in the United States (*1*). This disease usually begins with bloody diarrhea as well as a host of other renal problems. Individuals who develop HUS may require blood transfusions and dialysis. Sequale that develop may include a disease of the central nervous system that is characterized by seizures and may result in a coma (*31*). This disease may lead to permanent loss of kidney function (*38*). The CDC estimates the mortality rate of patients developing HUS at 3% –5 % (*18*). HUS seems to be a result of antibody response to three specific types of body cells: the platelets, kidney cells and erythrocytes (*50).*

Thrombotis Thrombocytopenic Purpura (*TTP):*

The elderly are generally at greater risk for developing TTP, which is characterized by a decrease in the amount of platelets as well as tissue hemorrhaging. Symptoms of TTP are generally similar to HUS except that the effect on the central nervous system is generally more pronounced. Patients may develop blood clots in the brain that can cause strokes and potentially death (*31*). The mortality rate in the elderly may be as high as 50% (*38*).

Sources of contamination

Dairy cattle are the primary source linked to outbreaks of *E. coli* O157:H7, although other animals are known as carriers. The bacterium has also been isolated from pigs, sheep and deer (31). The original outbreaks in 1982 that resulted in the diagnosis of *E. coli* O157:H7 as a human pathogen were due to the isolation of the bacteria from ground beef (*31*). Since then, products which have been implicated include fresh fruits and vegetables, apple cider, and other foods of animal origin (*57*). *E. coli* O157:H7 is generally more resistant to acid conditions than other strains of *E. coli* and can therefore survive in both acidic foods and beverages (*57*).

Factors affecting growth and survival

E. coli is well known as a common cause of traveler's diarrhea. This symptom is most often due to the influence of new microflora to the intestinal tract of visitors. It is possible that contaminated fruits and vegetables could be a factor in the dissemination of *E. coli* to the host and as a result of the action of the foreign strain of *E. coli* on the intestinal system may provoke diarrhea. The possibility of using contaminated water in a wash step may lead to the inoculation of produce with this pathogen. Outbreaks have been traced to

unpasteurized apple cider, however these incidents are declining due to recent legislation for the labeling of juices and requirements for HACCP implementation. Recently, many studies have been undertaken to study the prevalence and control measures of *E. coli* O157:H7 in apple cider and apple juice. Studies by Wright and others (84) demonstrated the reduction of *E. coli* O157:H7 on apples using different wash or chemical sanitizer treatments. *E. coli* O157:H7 has been also shown to survive and grow in cantaloupe and watermelon (*30*). Due to the agronomic practices of fertilizing with manure or using water possibly contaminated with manure, it is quite possible that this method of fertilization may adulterate the surface of the rind and possibly leads to outbreaks of *E. coli* O157:H7 (*30*). Acid and salt tolerance studies have indicated the survival of *E. coli* O157:H7 at pH ranges below 3.6 and salt concentration up to 6.5%, although generation time was slow and follows a long lag phase (*46*).

Fresh produce outbreaks

It should be noted that not all outbreaks of enterohemmorrhagic *E. coli* (EHEC) are attributed to *E. coli* O157:H7. Other members of the EHEC group associated with foodborne outbreaks include: O104:H21 (pasteurized milk outbreak in 1994), O11:NM (fermented sausage in 1995) and O6:NM (salads in 1993) (*46*). An outbreak in 1999 implicated a salad bar and ice as the likely vehicles for *E. coli* O11:H8 at a cheerleading camp in Texas (*21*).

Buchanan et al. (*14*) has demonstrated uptake of the pathogen into intact apples during immersion. This may be due to a slight pressure differential that occurs on the surface of fresh fruits when immersed in water that is slightly cooler than the produce itself. The resulting partial pressure may lead to internalization of bacteria into the fruit. Natural barriers inherent to fruit generally inhibit the colonization by pathogenic bacteria. The acidic conditions of the flesh and the skin and rind generally can serve to inhibit contamination. However, *E. coli* O157:H7 has been shown to survive well in acidic environments. Epidemiological associations of apple cider and outbreaks of *E. coli* O157:H7 serve as good examples of tolerance to acidic conditions. An outbreak in 1991 of *E. coli* O157:H7 in Massachusetts was linked to dropped apples used in the production of apple cider (*78*). This was an important outbreak because it demonstrated the acid tolerance of the organism. Zhao et al. (*86*) demonstrated that *E. coli* O157:H7 was capable of growth in apple cider (pH 3.6 – 4.0) that was held for 12 days at 8°C. Lettuce has been linked to outbreaks with *E. coli* O157:H7 and it has been suggested that leakage from the cellular structure of the leaves may provide nutrients for the growth and survival of *E. coli* O157:H7 (*8*). Lettuce leaves have been shown to absorb *E. coli* O157:H7 through stomata and cut surfaces when suspended in a broth (*71*). Studies by Li et al. (*51*) demonstrated the survival of *E. coli* O157:H7 on lettuce samples treated with mild heat and 20 ppm of chlorine. Attachment of *E. coli* O157:H7 to lettuce as demonstrated by confocal scanning laser microscopy by Takuchi et al. (*75*) showed a preferential attachment to cut edges. In 1995, an outbreak involving 40 cases was epidemiologically linked to the consumption of leaf lettuce in Montana, as was an outbreak at a Boy Scouts camp in Maine. In both cases,

there was no clear evidence of where contamination occurred, but speculation was through cross-contamination of the lettuce leaves in wash water (*77*).

Section 3: Surface Decontamination of Fresh Fruits and Vegetables

In the past, fresh fruits were rarely attributed as the cause for foodborne illness which is likely due to the natural intrinsic barriers that are part of the food itself. These include the skin, the rind and the generally acidic pH of the fruit itself (*30*). Attempts by processors to maintain a healthy food supply usually revolve around adherence to standard sanitation operating procedures (SSOPs). Sanitation is important to the produce industry for three main reasons: necessity of meeting buyer specifications, maintaining product quality and ensuring product safety (*12*). The most commonly implicated foods are those that are either temperature abused or those that may have been cross contaminated (*70*).

Methods of Contamination

Contamination of fresh produce can occur in several ways. Root vegetables that grow in the soil are in constant contact with microorganisms. Advances in agronomic practices, processing and distribution, have allowed for the extension of global trade. As a result of this increase in global trade, outbreaks have occurred due to the introduction of foreign bacteria or other viral parasitic organisms (*6, 7, 63*).

Contamination can occur during harvesting, handling, processing, storage, or distribution (*9*). Generally, fresh produce has a good track record for being one of the safest products

on the market; however contaminated produce has been implicated in foodborne disease (*12, 77*). In 1995, *Salmonella* Stanley was linked to alfalfa sprouts, *Salmonella* Hartford to unpasteurized orange juice, *Escherichia coli* O157:H7 to lettuce, and *Shigella* spp. to green onions and lettuce (*78*). Two outbreaks of *Shigella sonnei* were linked to contaminated lettuce served on college campuses (*6*). These recent outbreaks have lead to an increase in the public awareness of foodborne disease and research into the survival of these pathogens in produce. Studies by Wells and Butterfield (*82*) demonstrated the increased susceptibility of contamination due to the presence of soft rot in fruits and vegetables, including cantaloupe, carrots, lettuce, peppers and tomatoes. In addition to the possibility of infection bacterial soft rot and the resultant vegetable exudates were shown as a source of nutrients that may encourage microbial growth (*73*). This ready source of nutrients may allow excellent growth conditions for pathogens should produce become cross contaminated.

It is generally recognized that prevention of contamination is the best way of ensuring food safety. As a result, steps are taken throughout the processing of fruits and vegetables to minimize the risks associated with contamination. One of the main concerns to the fruit and vegetable industry is that the product is grown either close to or in the soil, a well-known vector for many pathogens (*5, 7*). Organisms that exist in the soil are easily transferred to water sources by runoff and rain, which in turn may transfer bacteria to the surface of fresh produce. Close proximity to the soil may be related to the increased risk of contamination of fruits and vegetables that are now becoming associated with more cases of foodborne disease. Additional concerns for the processing of fresh fruits and vegetables

include temperature abuse and partial processing that may introduce pathogens via cross contamination to an environment with less competition (*45*).

The surface decontamination of fruits and vegetables can be achieved through a number of methods. Currently, the most widespread methods include the use of chemicals such chlorine, bromine, iodine, trisodium phosphate, quaternary ammonium compounds (quats), hydrogen peroxide, ozone and irradiation (*7, 39*). Some of the non-chemical alternative processing methods that may be utilized include the use of ultraviolet light, microwaves, pulsed electric fields, ultrasound and high pressure processing (*69*). All of these methods are designed to reduce the number of pathogenic microorganisms on the surface of fruits and vegetables. It is not possible to rely entirely on one particular method to eliminate all the bacteria from the surface of fresh produce. For surface disinfection, these alternative methods would likely be used in conjunction with chemical treatments such as sodium hypochlorite, acetic acid, peroxyacetic acid or hydrogen peroxide. Since fruits and vegetables often eaten raw, it becomes increasingly important to remove bacterial populations that can cause foodborne diseases. Surface treatments are proven effective at reducing the numbers of microorganisms and hopefully may reduce the numbers of pathogens.

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is currently classified as Generally Recognized as Safe (GRAS) for use in certain food products. Acceptable uses include that of an oxidizing / reducing

agent, bleaching agent and as an antimicrobial. Hydrogen peroxide has been approved by the FDA in use for certain cheese treatments, preparation of modified whey and of thermophile free starch. It is also regulated for usage in other food products as long as residual H_2O_2 is removed during processing. Removal of residual H_2O_2 can be done by the presence of catalase, the use of heat or by post treatment rinses. Specific regulations for the use of hydrogen peroxide as a food additive can be found in the Code of Federal Regulations, Title 21 Part 184.1366.

Experimental applications of H_2O_2 as an antimicrobial have been performed on fresh fruits and vegetables with varied results. The use of 200 ppm chlorine proved more effective than the use of 0.2 and 1.0 % H_2O_2 in the reduction of *Salmonella* and *E. coli* O157:H7 on asparagus (*63*). In contrast, research studies by Sapers and Simmons (*68*) on other products found that the use of 5% H_2O_2 was more effective than using a 200 ppm chlorine solution in extending the shelf life of zucchini and cantaloupe. Additionally, H_2O_2 vapor treatments reduced the incidence of soft rot in different fresh cut products including cucumber, bell peppers and zucchini. However, the vapor treatments were ineffective on carrots, broccoli, cauliflower, strawberries and raspberries. Hydrogen peroxide treatments at a concentration of 6% have been shown to reduce the numbers of *Salmonella* Chester by 3 to 4 logs on the skin of apples, but lead to organoleptic changes in the food (*52*).

Chlorine

Chlorine has long been used as a sanitizer in the food industry. Several researches have evaluated its effectiveness for the decontamination of fresh produce. Concentrations of approximately 50 – 200 ppm with a contact of time from 1 – 2 minutes are generally used on produce. In the fresh produce industry, routine usage of chlorine can be found in flumes, wash and spray waters. Concerns for the use of chlorine include pH, temperature and the amount of organic matter present. The effectiveness of chlorine in killing microorganisms depends highly on the amount of free chlorine that is available once the chlorine dose and demand of the water is satisfied. At low pHs, metal containers and equipment may corrode when in the presence of hypochlorous acid (*7*). It should be noted that high concentrations of chlorine can cause skin irritation and may result in the formation of noxious odors. Some of the potential problems with using chlorine for the surface disinfection of produce depends on the topography of the food. Cracks, crevices, pockets and other natural openings may result in hypochlorous acid not effectively accessing microbial cells. Another cause for concern is the waxy cuticle surface and the hydrophobic effect it has on the efficacy of the chlorine reaching the microorganisms (*7*).

Others have performed studies investigating the efficacy of chlorine on the surface of fruits and vegetables. Weissinger and Beuchat (*81)* compared the effectiveness of different chemical treatments to eliminate *Salmonella* on alfalfa seeds. The use of 20,000-ppm free chlorine was effective at reducing populations by approximately 2 logs. Research by

Zhuang et al. (*87*) found no statistical difference in the log reduction of *Salmonella* on the surface of tomatoes with chlorine solution treatments of 110 and 320 ppm. Dipping in a chlorine solution of 320 ppm resulted in approximately 1.5 log reduction of *Salmonella* on the surface.

The National Advisory Committee on Microbial Criteria for Food recommend the use of 20,000 ppm of $Ca(OCl_2)$ for treatment of sprouts to eliminate pathogens (*61*). Studies have also indicated that the use of commercial produce wash products are just as effective as 20,000 ppm $Ca(OCl_2)$ for reducing *Salmonella* from the surface of alfalfa seeds (*10*). Produce washes therefore appear to be a viable alternative to the use of chlorine for washing fresh produce.

Hot Water Immersion

Studies by Pao et al. (*64*) demonstrated the effectiveness of reducing microbial populations of *Salmonella* and *E. coli* by more than 5 logs by submersing oranges in water at 80°C for 1 or 2 minutes. Additional studies have shown that the use of hot water immersion at temperatures of 80°C and 95°C were effective in reducing microbial populations of *E. coli* O157:H7 on apples by more than 5 logs (*35*). However, substantial internalization of the pathogen into the interior of the product did occur. The use of an immersion technique may be a critical step in the processing of fruits and vegetables. Under the right conditions, water flume systems may lead to the pathogens entering the flesh of fresh fruits. If warm

produce is submersed in a medium at a lower temperature, the fruit will try to equilibrate the temperature differential by allowing the water to enter the fruit (*64*).

Acidic Electrolyzed Water (AcEW)

The use of AcEW in agriculture is a relatively new phenomenon. Acidic electrolyzed water is produced by electrolysis of an aqueous sodium chloride solution between an anode and a cathode. AcEW generally has a pH below 2.7, a high oxidation – reduction potential and 30 – 50 ppm of free chlorine. Studies by Koseki and Itoh (*47*) demonstrated that its use was just as effective when compared to 150 ppm chorine. AcEW has been shown to reduce the population of naturally occurring microflora on the surface of lettuce by 2 logs and was more effective than the use of a solution containing 5 ppm ozone (*48*).

Section 4: Ultraviolet Light at 253.7 nm (UVC)

Ultraviolet (UV) light ranging from 200-280 nm is classified as UVC. This range of the UV spectrum has a germicidal effect on bacteria and viruses (*33, 69*). UV does not affect moisture or temperature of food and is economical (*83*). UV treatments have the advantage in that no excessive protection for workers is necessary and that no residual radioactivity occurs, even at high levels of exposure (*60*). The exposure of bacteria, viruses and spores to UV rays alter bonds within the DNA double helix that results in either mutation or lethality to cells (*60*). UV is effective in air, liquid media or on surface treatments (*83*). UV is commonly used to disinfect surfaces on packaging or in food processing environments (*27*).

The wavelength of UV light ranges from 100 – 400 nm. UVA (315 – 400 nm) is generally the wavelength that is absorbed by human skin resulting in sun tans. UVB (280 – 315 nm) has been linked with sunburns and may eventually lead to skin cancer. UVC (200 – 280 nm) is considered the germicidal range of ultraviolet light. The lower wavelengths of UVA and UVB have significantly less bactericidal activity as compared to UVC (*59*). The majority of UVC is absorbed in the stratosphere before reaching the surface of the Earth. The germicidal effect is generally due to the absorption of UV and the damaging effect it produces on DNA molecules (*69*). Most germicidal lights operate at a wavelength of 253.7 nm, which is the resonant band of mercury. Using this band as a standard, sensors can be easily calibrated.

UV dose is determined by the intensity of the light source and the time that the light is in contact with the surface and may be calculated from the following formula:

$$D = L\,(T)$$

Where D = dosage, L = applied intensity, and T = time of exposure. The intensity of the light is affected by the distance the source is from the sample. As a result, the overall dose can be dramatically increased by placing the source closer to the sample (*2*).

Factors Affecting Efficiency

The success of UV is dependent of the design of a system that is capable of delivering the necessary dose to the surface of food or food contact surfaces. Some of the critical design

factors that effect the rate of destruction of cells by UVC include: power, wavelength, the physical shape of the product, reflection, and distance between the light source and the target (*69*). DNA has a maximum absorbance of UV in the range of 100 – 290 nm, which corresponds to the UVC region. Cell damage is due to the absorption of UVC light, which causes the DNA pyrimidine bases of cytosine and thymine to form cross-links. This mutation impairs formation of hydrogen bonds with the purine base pair on the complimentary strand of DNA (*33, 69*). Cellular death occurs after the threshold of cross linked DNA molecules is exceeded (*69*). Cell death is due to the inhibition of cellular transcription and translation. Transcription is the process by which a messenger RNA (mRNA) molecule is synthesized from a DNA template. This mRNA molecule then transports cellular information to ribosomes and ribosomal RNA (rRNA) translates the code into amino acids used in protein synthesis.

The effectiveness of UVC for microbial disinfection depends on several factors. The form of bacteria and whether it exists in a spore or a vegetative state can alter the organism's resistance to UVC. Spores in general will be more resistant to the application of UV. The environmental stress that surrounds the growth of the organism as well as the growth stage of the organism will also come into play with the relative susceptibility or resistance to UV exposure. Cells in exponential growth are generally less resistant than cells in stationary phase due to the rapid growth that occurs (*33, 69*).

UVC radiation is produced by electricity powering UVC lights. The lamps operate similar to fluorescent lamps. An electron flow through ionized mercury vapor is used to produce the radiation. UV lamps do not have the coating and therefore emit only UV radiation (*33*). Pulsed power sources which emit high intensity light emissions as described by MacGregor et al. (*55)* have been shown to reduce populations of *E. coli* O157:H7 and *L. monocytogenes* in approximately 4 – 6 times less exposure time when compared to continuous UVC sources (*55*).

UVC has been commonly used on processing equipment and packaging material, but has had limited application to foods. This may be due to its low penetration into products and the fact that shadows and crevices on the sample itself may lead to harboring bacteria from the harmful wavelengths (*27*). However, UVC is more effective for the disinfection of smooth surfaces due to the direct path of the beam and the absence of light scattering.

Microbial Susceptibility

The shape of the microbial inactivation curve is sigmoidal (*33, 69, 85*). Injury to cells begins with the initial dose of radiation. Additional levels surpass cellular injury and begin lethal destruction of cells. As the curve proceeds, cellular death begins to tail off. This might be explained by UV resistance or the presence of suspended solids in aqueous systems that may interfere with the transmission of light (*69*).

Information regarding the use of UV radiation for the destruction of pathogens on produce has not been well documented. Lu et al., (*54*) studied the effect of low levels of UVC radiation on the shelf life of peaches and tomatoes. They found that the use of these low levels reduced the post-harvest rots as well as delayed ripening. This work also suggested a close correlation of resistance to decay and delayed ripening. These results may be of an advantage by extending the shelf life of fresh fruits and vegetables. Studies by Liu et al. (*53*) also looked at the effect of UV on inoculated tomatoes for the inhibition of black and gray mold formation. Dose levels of 1.3 – 40 KJ/m^2 (1300 — 40000 μW / sec /cm^2) were applied to the surface of the fruit. Results from this study supported the previous work of Lu et al. (*54*) and found that ripening was delayed. Studies by Piga et al. (*65*) found the UV exposure did not affect fruit weight loss in pears.

Many studies have been performed on its effect on agar plates and on other food surfaces (*26, 49, 75, 79, 83, 85*). A study by Chang et al. (*26*) found that doses for inactivation of vegetative bacteria required for a 3-log reduction to be similar among pathogens. Studies by Kuo et al. (49) resulted in approximately a 6 log decrease of *Salmonella* t*yphimurium* on the surface of brilliant green agar with doses exceeding 37 mW/cm^2. Sumner et al. (*75*) reported that doses at 1.5 – 9.3 mW /s/cm^2 eliminated 99.9% of *Salmonella typhimurium* on BHI plates supplemented with nalidixic acid. Wong et al. (*83*) reported that doses of 100μW / cm^2 or greater were most effective on fresh pork muscle for 1.5 to 2 log reductions. The greatest logarithmic reduction in his study was seen at 1000μW / cm^2 for *E. coli* on pork skin. Wong also reported that, "in all cases, *E. coli*

appeared to be more resistant to UV treatment compared to *Salmonella seftenberg*". Studies have also found that the use of UVC in carrot sensory samples did not show any difference in taste between the treated and control samples (*58*). Research by others demonstrated that UV did not adversely effect the color or general appearance of beef (*74*) and poultry (*79*).

UV Repair

As a result of low doses and UV damage, cellular mutations may arise. Two major mechanisms of DNA repair in bacteria are classified as "Light" and "Dark" repair mechanisms. Light repair is also known as photodimerzation and requires visible light (380 – 430 nm) as well as the enzyme photolyase to reverse some DNA damage. Photolyase has to be activated in the near-UV or violet blue spectral range (*69*). Light repair mechanism only occur with the presence of light and typically cannot repair all UV damage (*59*). For fresh fruits and vegetables, light repair mechanisms are of more importance due to the general storage or retail sale under lights. Dark repair occurs in the absence of visible light. Three main methods are invoked by bacteria, nucleotide excision repair, SOS-error prone repair and post-replication recombinational repair (*59*). The SOS regulatory system is a complex cellular mechanism that initiates the DNA repair processes. The lack of a template for SOS repair generally results in mutations (*13*).

Section 5: Government Regulations

Produce is subjected to a wide variety of conditions throughout processing. By breaching the natural protective barrier of the fruit or vegetable the possibility of contamination

increases. This has been documented in several cases in which the surface of a cantaloupe melon was contaminated because the physical act of cutting the product introduced *Salmonella* to the flesh of the melon (*42, 63*). Research by Golden et al. (*42*), demonstrates that *Salmonella* is capable of rapid growth on the surface of cantaloupe, honeydew melon and watermelon. Over 185 cases of *Salmonella* were confirmed from one outbreak in the United States in July of 1991 which led the FDA to instruct food retailers to wash melons prior to cutting, remove the rind from cut melons, and to keep the melons on display for less than 2 hours after cutting (*16, 56*). As a result of this outbreak and research data, steps were taken by the FDA in an attempt to identify points of contamination that may occur during the agricultural and processing phases of the operations (*56*).

In response to foodborne outbreaks in fresh produce, the Food and Drug Administration (FDA) has established guidelines for the industry. Good Agricultural Practices (GAPs) generally are non-specific in nature and can therefore apply to a wide variety of fruits and vegetables. The main impact of GAPs is seen at packinghouse operations, where large volumes of produce are washed, packed, sorted and trimmed. Sanitation and employee hygiene are important areas specifically addressed by GAPs. In addition to GAPs, the FDA also has published Good Manufacturing Practices (GMPs), which are defined in the Code of Federal Regulations, Title 21 Part 110.1 – 110.99. In contrast to GAPs, which apply to worker hygiene and generally sanitary practices, GMPs specifically apply to processing facilities which have well defined areas (*39*). The International Fresh Produce Association has published a work entitled, “Food safety guidelines for the fresh-cut

produce industry" which involves a mixture of GMPs, GAPs and sanitation programs as well as other methods to ensure product safety (*43*).

HACCP

Hazard Analysis and Critical Control Point Programs (HACCP) focus on preventing biological, chemical and physical hazards that may affect food safety by controlling critical parts in the food production process. HACCP programs are already mandatory in the processing of seafood (1995) and meat and poultry processing plants (1998) and most recently in juice (2001). A voluntary dairy HACCP pilot program is currently under review by the National Council of Interstate Milk Shippers.

The juice industry is the most recent group to adopt HACCP standards. Juice has been identified as the vehicle of transmission in several outbreaks in the United States. Outbreaks in juice have been attributed to *E. coli* O157:H7, *Cyrptosporidium parvum,* and *Salmonella* (*36*). Prior to 1998, FDA referred to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) for recommendations to guarantee the safety of juices. NACMCF recommended a 5-log performance standard to ensure the safety of juices (*37*).

In April 1998, FDA proposed to implement a HACCP program to ensure a 5-log reduction in microorganisms in juices. Shortly after in July 1998, FDA published a final rule requiring producers not using a process specifically designed to destroy bacteria bear a warning label about the risk of foodborne illness associated with the product. A final rule

for the implementation of HACCP for juice processors was published in January 2001 requiring large processors to apply HACCP control programs within one year. Under the new regulations, processors are required to achieve a 5-log reduction of the most resistant pathogen in their product. The pertinent pathogen of interest in apple cider is *E. coli* O157:H7 while *Salmonella* is linked with orange juice. Provisions allow for the use of alternative processing or a combination of techniques for the reduction of microbial numbers (*37*).

In December of 2001, FDA proposed additional guidance material for the juice industry. In it, proposed changes exclude the use of GMPs and GAPs as counting towards the proposed 5 log reduction standards. Instead, the entire reduction should be performed under the control of one processing firm and within one processing facility. As a result, the final rule calling for a five-log reduction of the pertinent pathogen in apple juice must be applied to the finished product. However, and exemption was set up by FDA stating that surface treatments on citrus fruits are considered acceptable for counting towards the 5 log performance standards (*37*).

HACCP focuses on juice processors and does not directly apply directly to fresh produce processors. The regulations do apply to those who make juice or concentrate for beverage use. Application of HACCP to the fresh processing industry in general would depend largely on the type of facility and products processed therein. The application to small

farm and packinghouse operations would likely involve documentation and standardized product testing (*66*).

Conclusion

There is no research to date on the use of UVC to inactivate foodborne pathogens on the surface of fresh fruits and vegetables. HACCP regulations for the juice industry do not apply directly to fresh produce processors. HACCP was never intended to ensure the safety of raw products, however adoption of the HACCP principles to raw commodity products may help to ensure the safety of fresh fruits and vegetables. Alternative techniques to reduce the number of bacteria and pathogens on the surface of fresh fruits and vegetables could be used as a pre-requisite to a HACCP program. The low initial cost as well as the lack of extensive safety equipment may be of benefit to those with little capital to invest. UVC has been proven to reduce microbial numbers on smooth surfaces. The use of UVC should only be considered for types of produce that have a smooth skin free from indentations that may harbor bacteria and provide a shelter for pathogens. The current lack of research into the use of UVC on fresh produce should be investigated as a means of extending the shelf life and ensuring the safety of fresh fruits and vegetables.

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Response of *Salmonella* spp. and *Escherichia coli* O157:H7 to Ultraviolet Energy

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Abstract

To determine the efficacy of the UVC treatment on microbial growth, Tryptic Soy Agar + 50 ppm nalidixic acid (TSAN) plates were inoculated with known concentrations of five strain cocktails of *Salmonella* and *Escherichia coli* O157:H7 and subjected to different UV treatments. The concentration of the cocktail inoculum was determined on TSAN agar prior to inoculation. Serial dilutions were performed and inoculation levels tested included $10^0 – 10^8$ cfu /ml for each pathogen. Multiple replications of different doses of ultraviolet light ranging from 1.5 – 30 mW / cm^2 were applied to different cocktail concentrations and resulted in a 5 log reduction of *Escherichia coli* O157:H7 at doses exceeding 8.4 mW / cm^2, while a 5 log reduction for *Salmonella* spp. was observed at doses exceeding 14.5 mW / cm^2. Results for both organisms yielded sigmoidal inactivation curves. The use of UVC is effective at reducing microbial populations of pathogens on the surface of agar.

Ultraviolet (UV) light ranging from 200-280 nm is classified as UVC. This range of the UV spectrum has a germicidal effect on bacteria and viruses (6, *9*). UVC is effective in air, liquid media or on surface treatments (*13*). The success of UVC is dependent of the design of a system that is capable of delivering the necessary dose to the surface of food or food contact surfaces. UVC has been commonly used on processing equipment and packaging material, but has had limited application to foods. This may be due to its low penetration into products and the fact that shadows and crevices on the sample itself may lead to harboring bacteria from the harmful wavelengths (*4*). However, UVC is more effective for the disinfection of smooth surfaces due to the direct path of the beam and the absence of light scattering.

Bachmann reported a large variation in different bacteria to their susceptibility to UVC (*1*). Previous studies by other researchers have produced mixed results in respect to doses required for inactivation of pathogens. Studies by Kuo et al. (*7*) resulted in approximately a 6 log decrease of *Salmonella typhimurium* on the surface of brilliant green agar with doses exceeding 37 mW/cm^2. Yousef and Marth (*14*) reported a three log kill of *Listeria monocytogenes* on the surface of tryptose agar at a dose of approximately 9 mW /cm^2 and a 7 log kill at doses exceeding 36 mW / cm^2. Sumner et al. (*12*) indicated that doses at 3.1 mW /cm^2 resulted in up to a 7 log kill of a nalidixic acid resistant strain of *Salmonella typhimurium* on brain heart infusion plates. Wong and others (*13*) reported a greater than 5 log reduction of *E. coli* on the surface of tryptic soy agar with doses exceeding 12 mW / cm^2.

Research by Sommer et al. (*11*) indicated a significant difference among three strains of *E. coli* O157:H7 that were inactivated by UV light in a water system. The most UV susceptible strain (CCUG 29199) resulted in a 6 log decrease when exposed to 12 J/m^2, whereas the most resistant strain (CCUG 29193) required greater than 50 J / m^2 to achieve a 4 log kill. Data represented in that study demonstrates that the use of a single strain of a particular species is not adequate for determining a specific dose for a determined log reduction. In September 1997, an EPA Scientific Advisory Panel specifically identified *Salmonella, Listeria monocytogenes,* and *Escherichia coli* O157:H7 as pathogens of public health concern. The panel also recommended testing five outbreak-related strains in a cocktail for each pathogen (*5*). In light of these previous experiments and recommendations, the overall objective of this study was to define the UVC dose required to effectively reduce the numbers of multi-strain cocktails of *Salmonella* and *E. coli* O157:H7 on the surface of agar.

Materials and Methods

Preparation of Inoculum

A total of five strains each of *Salmonella* and *Escherichia coli* O157:H7 were used in this study. Three of five strains of *E. coli* O157:H7 and five strains of *Salmonella* used were isolated from outbreaks associated with raw vegetables or unpasteurized fruit juices. *E. coli* O157:H7 (H1730) from a lettuce associated outbreak, *E. coli* O157:H7 (F4546) from an alfalfa sprout associated outbreak, *E. coli* O157:H7 (cider) from a cider related outbreak, *E. coli* O157:H7 (E0019) from a beef outbreak and *E. coli* O157:H7 (994) from a salami outbreak. *Salmonella* Montevideo was isolated from a tomato associated outbreak, *S.*

Agona from an alfalfa sprout related outbreak, *S.* Baildon from a lettuce and tomato associated outbreak, *S.* Michigan from a cantaloupe associated outbreak and *S.* Gaminara from an orange juice associated outbreak. All serotypes were obtained from the University of Georgia from Dr. Larry Beuchat at the Center for Food Safety and Quality Enhancement, (Griffin, GA). All strains were resistant to 50 ppm nalidixic acid.

Cultures were maintained at -80°C in Tryptic Soy Broth (TSB) (Difco, Detriot, MI) supplemented with 50 ppm of nalidixic acid (ICN Biomedicals, Aurora, OH) (TSBN). Prior to use, cultures were grown in TSB at 35°C and transferred three times at consecutive 24 hr intervals prior to their use in the inoculation. Incubation for 24 hr allowed the respective bacteria to approach the stationary phase of growth at a concentration of approximately 10^8 cfu /ml. Equal aliquots of each individual strain were vortexed and then aseptically combined into a sterile dilution blank to produce a cocktail of five strains. Serial dilution of the inoculum enumerated after 24 hr incubation at 35°C was reported as the inoculum concentration.

Plate Inoculation

Inocula were serially diluted in 9.0 ml of sterile 0.1% peptone (Difco, Detriot, MI). Duplicate spread plates were performed on TSA + 50 ppm nalidixic acid (TSAN) for each dilution ranging from 10^{-1} to 10^{-8}. Spread plates were chosen in order to get the highest possible concentration of cells on the surface of the agar. After inoculation, plates were allowed to dry for at least 30 minutes prior to treatment.

Ultraviolet Chamber

The chamber utilized for the UVC irradiation of plates was fabricated in the Virginia Tech Department of Food Science and Technology. The chamber is approximately 40" long and contains a single G36T6 Model 4136 germicidal light unit that emits 253.7 nm UV light. (Fuller Ultraviolet, Frankfort, IL) The light source is suspended on a chain and may be moved to either increase or decrease intensity as desired by the operator. The interior is lined with a highly reflective material designed to increase the UVC intensity and to minimize any shadowing effect on irregular shaped samples. Access is through a hinged bi-fold door. The UVC dose was measured using a dosimeter calibrated to read specifically at 253.7 nm (Spectronics, Westbury, NY). The meter was calibrated and standardized before the study.

Ultraviolet Treatment

Inoculated plates were randomized and individually subjected to different doses of UVC light. UVC intensity was determined prior to treatment by measuring the output of the light (mW / sec /cm^2) and the applied dosage was calculated from D = L (T) where D = applied dosage, L = applied intensity in mW / sec / cm^2 and T = irradiation time in seconds. Variable exposure times were then employed to allow for different doses of 1.5 – 30 mW / cm^2 to be applied to the surface of the agar plate.

Enumeration

UVC treated plates were incubated at 35°C for 24 hr. Random colonies were selected and confirmed after each trial. Confirmation was performed for *Salmonella* on Xylose Lysine

Deoxycholate Agar (XLD) (Difco, Detroit, MI) and on API 20E test strips (Biomerieux, Hazelwood, MO). *E. coli* O157:H7 was confirmed on Sorbitol MacConkey Agar (Difco, Detroit, MI) and with the use of a Visual Immunoprecipitate Assay (Biocontrol, Bellevue, WA).

Statistical Analysis

Experiments were replicated more than 10 times with multiple dilutions for each ultraviolet dose tested. Data presented is the average recovery from treated plates with the standard error of the mean. Means and standard errors were calculated from a commercial spreadsheet (Microsoft Excel, Redmond, WA). Survival data were treated according to Chick's Law as log (N_s/N_o) where N_s is the density of survivors and N_o is the initial concentration of bacteria, which was calculated as the inoculum concentration.

Results and Discussion

Ultraviolet light (UVC) was effective at reducing microbial populations of both *Salmonella* spp. and *E. coli* O157:H7 on the surface of TSAN. *Salmonella* cocktail cultures averaged 2.6×10^9 cfu / ml inoculum. Concentrations of *Escherichia coli* O157:H7 cocktail cultures averaged 2.0×10^9 cfu / ml inoculum. Figure 1 depicts the average log reductions of both *Salmonella* and *E. coli* O157:H7 on the surface of TSAN. The overall UVC dose required to reduce an equivalent microbial population of *Salmonella* spp. was higher than that required to achieve the same reduction in *E. coli* O157:H7. By plotting the equation of the best-fit line, it is possible to predict at which point a five-log reduction is achieved. For *E.*

coli O157:H7, a five log reduction is achieved at a dose exceeding 8.4 mW / cm^2. In contrast, *Salmonella* spp. required a dose exceeding 14.5 mW / cm^2.

Results of this study agree with other research in which similar log reductions on different agar surfaces were seen (*3, 7, 13*). However, data is in contrast to a study in which the data supported *E. coli* as being more resistant to UV than *S.* Senftenberg (*13*). This may be attributed to the use of *S.* Senftenberg, which is generally considered the most heat resistant strain of *Salmonella* and is often used in thermal destruction tests. A study by Chang (1985) found that doses for inactivation of vegetative bacteria required for a 3 log reduction was similar among pathogens (*3*). The cocktail mixture of the five *E. coli* O157:H7 strains studied required approximately half of the dose required to achieve a 5 log reduction when compared to the five strain cocktail of *Salmonella*.

Other researchers have described the effect of UVC on inactivating microorganisms as sigmoidal (*1, 10, 14*). The initial exposure of bacteria to UVC is believed to injure cells. As increasing doses of UV are received, mutations arise in the DNA code where neighboring pyrimidine bases begin to form cross linkages that impede cellular replication. Cellular death occurs after the threshold of cross-linked DNA is exceeded (*6, 10*). Similar results were received in this study where the use of UVC to reduce microbial populations led to the formation of a sigmoidal curve. The tail of the inactivation curves has been explained by multiple hit phenomena (*14*) the lack of a homogenous population (*2*) and the presence of suspended solids (*10*). It is possible that the use of multiple strains that may vary in susceptibility to UVC produced the tailing effect as demonstrated by Sommer et al. (*11*).

Other explanations that may explain a sigmoidal curve include varying ability among cells to repair DNA mutations through either light or dark pathways (*8*) or the shadowing effect that may have been produced by the edge of the petri dish.

In summary, UVC was seen as an effective way of reducing microbial populations on the surface of agar. Logarithmic reductions of greater than 6 logs are possible with an appropriate dose of radiation. In this study, the use of a multiple strain inoculum demonstrated that *E. coli* O157:H7 was more susceptible to UVC than *Salmonella*.

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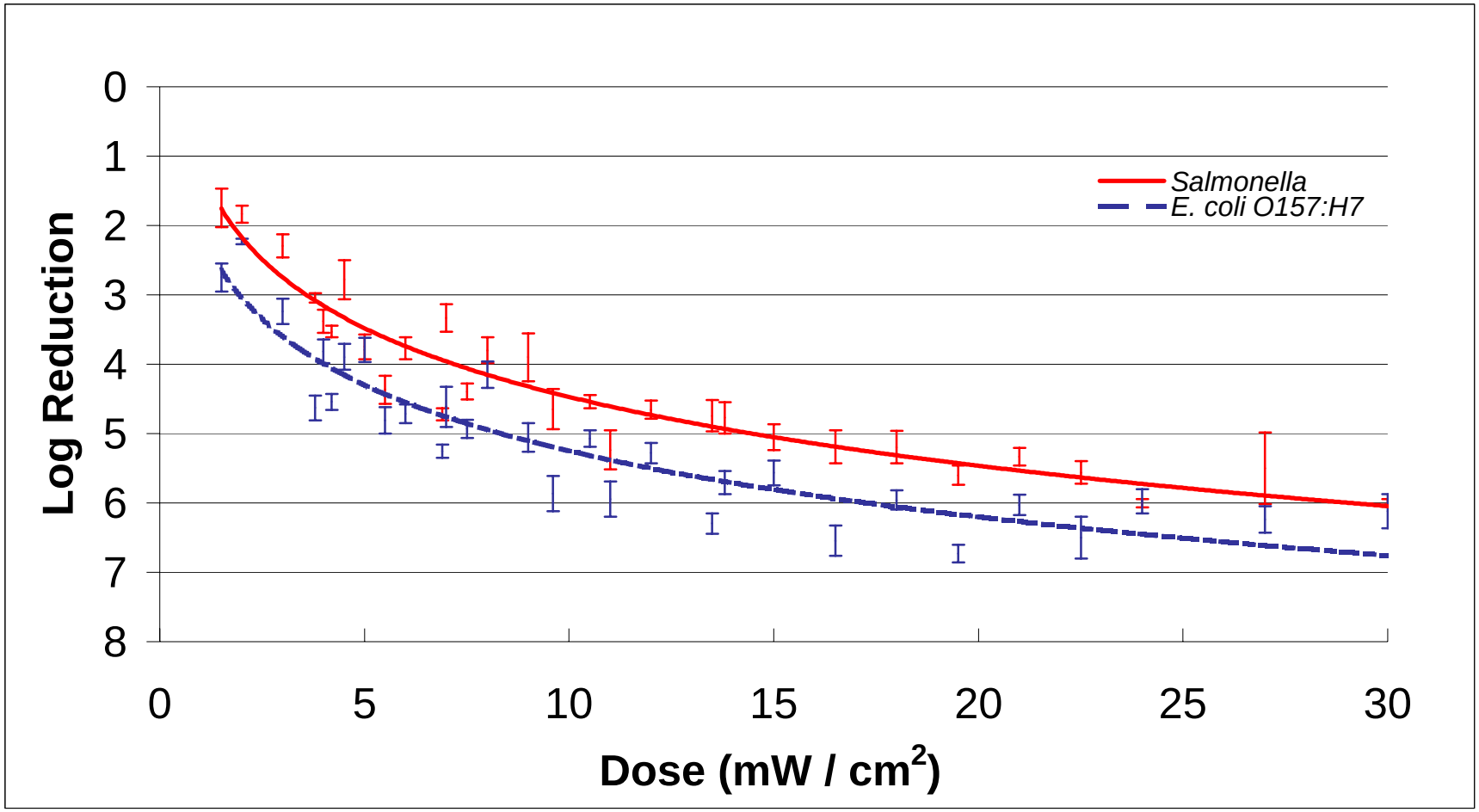

Figure 1: Mean Log Reductions and Standard Error of *Salmonella* spp. and *Escherichia coli* O157:H7 to Ultraviolet Light at 253.7 nm (UVC). *Salmonella* $R^2 = 0.91$ (n = 335); *E. coli* O157:H7 $R^2 = 0.85$ (n = 343).

Inhibition of Pathogens on Fresh Produce by Ultraviolet Energy

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Abstract

Ultraviolet energy at a wavelength of 253.7nm (UVC) was investigated for its bactericidal effects on the surface of Red Delicious apples, leaf lettuce and tomatoes inoculated with cultures of *Salmonella* spp. and *E. coli* O157:H7. Inoculated samples were subjected to different doses ranging from 1.5 – 24 mW / cm^2 of UVC to determine effective log reductions of microbial populations and enumerated on tryptic soy agar plus 50 ppm nalidixic acid. UVC applied to apples inoculated with *E. coli* O157:H7 resulted in the highest log reduction of approximately 3.3 logs at 24 mW/cm^2. Lower log reductions were seen on tomatoes inoculated with *Salmonella* spp. (2.19 logs) and green leaf lettuce inoculated with both *Salmonella* spp. and *E. coli* O157:H7 (2.65 and 2.79) respectively. No significant statistical difference ($p > 0.05$) was seen in the ability of UVC to inactivate a higher population of either *Salmonella* spp. or *E. coli* O157:H7 on the surface of green leaf lettuce. No significant difference was seen among the use of different doses applied to the surface of fresh produce for reduction of *E. coli* O157:H7 or *Salmonella* spp. ($p > 0.05$). Fresh produce processors do not fall under mandatory HACCP requirements, however the use of UVC may prove to be effective in conjunction with Good Agricultural Practices and Good Manufacturing Practices in ensuring the safety of fruits and vegetables.

Documented cases of foodborne illness associated with fresh fruits and vegetables have risen in the last few years (*11, 12*). Major outbreaks with fresh produce have been associated with common foodborne pathogens such as *Salmonella, Listeria monocytogenes*, *Shigella* spp., and *Escherichia coli* O157:H7 (*5*). In September 1997, an EPA Scientific Advisory Panel specifically identified *Salmonella, Listeria monocytogenes*, and *Escherichia coli* O157:H7 as pathogens of public health concern on produce. The panel also recommended testing five outbreak-related strains in a cocktail for each pathogen (*14*).

A strategy to minimize the risks involved with the consumption of fresh fruits and vegetables involves either reducing or eliminating surface contamination. Previous attempts used to reduce microbial numbers and prolonging shelf-life of fresh produce include modified atmosphere packaging (*2, 3, 16*), partial processing using chemical sanitizers, (*4, 6, 7, 8, 9, 17, 31*) low temperature storage (*24, 35*) and the use of edible films (*36*). Effective surface decontamination techniques could be employed to reduce the surface load of pathogens. Simply washing fresh produce with water may remove pathogens and other spoilage organisms (*7*). Traditional detergents are known to be partially effective in removing pathogens, however each type of disinfectant varies both in efficiency and in allowable maximum concentration (*4, 6*). The use of a non-selective treatment for the destruction of pathogens on the surface of fresh fruits and vegetables would be desirable. One such alternative process is the use of germicidal ultraviolet light at a wavelength of 200 – 280 nm (UVC). Treatment with ultraviolet energy offers several

advantages to food processors as it does not leave a residue, does not have legal restrictions and does not require extensive safety equipment to utilize (*33, 34*).

Information regarding the use of UV radiation for the destruction of pathogens on produce has not been well documented. Studies by Liu and others (*21*) analyzed the effect of UV on inoculated tomatoes for the inhibition of black and gray mold formation. Dose levels of 1.3 – 40 KJ/m^2 were applied to the surface of the fruit. Results from this study supported the previous work of Lu et al. (*22*) and found that ripening was delayed which in turn extended shelf-life. Studies by Piga et al. (*24*) found the UV exposure did not affect fruit weight loss in pears.

Recent Hazard Analysis and Critical Control Points (HACCP) regulations require a five log performance standard in the reduction of the pertinent pathogen in juice products. The use of UVC may prove to be useful as a prerequisite step in HACCP protocols if it is effective at reducing microbial numbers on the surface of fresh fruits and vegetables. The overall objective of this study was to define the UVC dose required to effectively reduce the numbers of antibiotic resistant strains of *Salmonella* and *E. coli* O157:H7 on the surface of apples, lettuce and tomatoes.

Materials and Methods

Preparation of Inoculum

A total of five strains each of *Salmonella* and *Escherichia coli* O157:H7 were used in this study. Three strains of *E. coli* O157:H7 and five strains of *Salmonella* that were isolated from outbreaks associated with raw vegetables or unpasteurized fruit juices were used. *E.*

coli O157:H7 (H1730) from a lettuce associated outbreak, *E. coli* O157:H7 (F4546) from an alfalfa sprout associated outbreak, *E. coli* O157:H7 (cider) from a cider related outbreak, *E. coli* O157:H7 (E0019) from a beef outbreak and *E. coli* O157:H7 (994) from a salami outbreak. *Salmonella* Montevideo was isolated from a tomato associated outbreak, *S.* Agona from an alfalfa sprout related outbreak, *S.* Baildon from a lettuce and tomato associated outbreak, *S.* Michigan from a cantaloupe associated outbreak and *S.* Gaminara from an orange juice associated outbreak. All serotypes were obtained from the University of Georgia from Dr. Larry Beuchat at the Center for Food Safety and Quality Enhancement, (Griffin, GA). All strains are resistant to 50 ppm nalidixic acid.

Cultures were maintained at -80°C in Tryptic Soy Broth (TSB) (Difco, Detriot, MI) supplemented with 50 ppm of nalidixic acid (ICN Biomedicals, Aurora, OH) (TSBN). Prior to use, cultures were grown in TSB at 35°C and were transferred three times at 24 hr intervals prior to their use in the inoculation. Incubation for 24 hr allowed the respective bacteria to approach the stationary phase of growth at a concentration of approximately 10^8 cfu /ml. Equal aliquots of each individual strain were vortexed and then aseptically combined into a sterile dilution blank to produce a cocktail of five strains.

Preparation of produce samples:

Unwaxed Red Delicious apples were obtained from Virginia Tech's Kentland Research Farm in Blacksburg, Virginia. Tomatoes were obtained from a local distributor. Leaf lettuce was obtained from a local grocery store in Blacksburg, Virginia. Red Delicious apples and tomatoes were respectively of uniform size, shape and free of visual defects

such as cuts, abrasions and bruises. Apples and tomatoes were stored at 4°C until use. Approximately 1.5" x 1.5" outer leaves of green leaf lettuce were excised from a single head of lettuce and transferred to a sterile petri dish prior to inoculation. Produce was allowed to equilibrate to room temperature (22°C) for 18 – 24 hr prior to inoculation.

Produce inoculation

Produce was placed on a plastic dish in a laminar flow biosafety hood and 100 µl of inoculum at approximately 10^7 cfu / ml was applied in multiple spots around the calyx of the apple and blossom stem scar of the tomato taking care not to inoculate either area. The surface of outer leaves of green leaf lettuce was inoculated without placing inoculum onto the torn edge of the leaf. Produce was allowed to dry under the laminar flow hood for a minimum of 30 min prior to UVC treatment.

Ultraviolet Chamber

The chamber utilized for the UVC irradiation of plates was fabricated in the Virginia Tech Department of Food Science and Technology. The chamber is approximately 40" long and contains a single G36T6 Model 4136 germicidal light unit that emits 253.7 nm UV light. (Fuller Ultraviolet, Frankfort, IL) The light source is suspended on a chain and may be moved to either increase or decrease intensity as desired by the operator. The interior is lined with a highly reflective material designed to increase the UVC intensity and to minimize any shadowing effect on irregular shaped samples. Access is through a hinged bi-fold door. The UVC dose was measured using a dosimeter calibrated to read specifically at

253.7 nm (Spectronics, Westbury, NY). The meter was calibrated and standardized before the study.

Ultraviolet Treatment

Samples were randomized and individually subjected to different doses of UVC light. UVC intensity was determined prior to treatment by measuring the output of the light (µW / sec /cm^2) and the applied dosage was calculated from D = L (T) where D = applied dosage, L = applied intensity in mW / sec / cm^2 and T = irradiation time in seconds. Variable exposure times were then employed to allow for different doses ranging from 1.5 – 24 mW / cm^2 to be applied to the surface of the produce.

Enumeration

UVC treated produce was aseptically transferred to a sterile sampling bag and rinsed with 20 ml of 0.1% Sodium Lauryl Sulfate (Sigma Chemical Co, St. Louis, MO). Serial dilutions in 0.1% peptone (Difco, Detroit, MI) were pour plated with Tryptic Soy Agar (TSA) (Difco, Detroit, MI) supplemented with 50 ppm of nalidixic acid (ICN Biomedicals, Aurora, OH) (TSAN) or Xylose Lysine Deoxycholate Agar (Difco, Detroit, MI) supplemented with 50 ppm nalidixic Acid (XLDN). Plates were incubated at 35°C for 24 hr. TSAN was chosen in order to aid in the recovery of injured cells and was used for all products except tomatoes. Sufficient background microflora on tomatoes necessitated the use of a selective and differential media. As a result, XLDN was used for enumeration of *Salmonella* ofrom the surface of tomatoes. Confirmation was performed for *Salmonella* on XLD agar and on API 20E test strips (Biomerieux, Hazelwood, MO). *E. coli* O157:H7

was confirmed on Sorbitol MacConkey Agar (Difco, Detroit, MI) and with the use of a Visual Immunoprecipitate Assay (Biocontrol, Bellview, WA).

Statistical Design

Experiments were replicated at least 5 times with two samples for each ultraviolet dose plus two positive and one uninoculated control which were analyzed in duplicate at each sampling interval. Survival data were treated according to Chick's Law as log (N_s/N_o) where N_s is the density of survivors and N_o is the initial concentration of bacteria, which was calculated from the average recovery on the positive control samples. Data presented is the average log reduction of greater than 10 trials with the standard error of the mean. Means and standard error were calculated from a commercial spreadsheet (Microsoft Excel, Redmond, WA). Data were subjected to Tukey's Honestly Significant Difference in SAS, version 8 (SAS Institute, Cary, NC) to determine if there were significant differences ($P < 0.05$) between mean log reductions for each treatment.

Results and Discussion

All experiments resulted in at least a 99% reduction of the selected pathogens on the surface of Red Delicious apples, green leaf lettuce and tomatoes. Cellular concentrations for studies inoculated onto lettuce averaged 5.51 $\log_{10}$ cfu / lettuce for *E. coli* O157:H7 and 5.39 $\log_{10}$ cfu/lettuce for *Salmonella* spp. As seen in Figures 1 and 2, both *Salmonella* and *E. coli* O157:H7 show similar logarithmic reductions when treated with the same doses of ultraviolet light. Both organisms required a dose of approximately 6 mW / cm^2 to achieve a two log reduction in initial numbers on the surface of leaf lettuce. Maximum log

reductions on green leaf lettuce for *Salmonella* and *E. coli* O157:H7 seen at a dose of 24 mW/cm^2 were 2.65 and 2.79 logs respectfully. Figure 3 depicts the log reductions of both organisms on the surface of green leaf lettuce. Takeuchi and others used confocal scanning laser microscopy to determine that *E. coli* O157:H7 has a preferential attachment to cut edges of lettuce, whereas *S. typhimurium* attached equally to either the cut edge or the intact surface (*29*). From the data presented, there is no statistical difference exhibited in the log reduction between *Salmonella* or *E. coli* O157:H7 ($p > 0.05$) on the surface of green leaf lettuce. Although this study did not investigate preferential attachment, results suggest that the equivalent doses of UVC will inactivate similar numbers of both *Salmonella* and *E. coli* O157:H7 cocktails regardless of their location. Further, there is no statistical difference among the doses applied for significant reduction of *Salmonella* and *E. coli* O157:H7 on the surface of leaf lettuce ($p > 0.05$). The use of UVC was seen to be more effective at reducing microbial populations of *E. coli* O157:H7 than the use of 20 ppm chlorine (*19*), 200 ppm chlorine (*7*) and acidic electrolyzed water (*17*).

Cellular concentrations for studies inoculated onto tomatoes averaged 3.32 log_{10} cfu / tomato for *Salmonella* spp. UVC was less effective at reducing populations of *Salmonella* on the surface of tomatoes when compared to the other produce types. Figure 4 depicts maximum log reductions of 2.19 log_{10} cfu / tomato at doses exceeding 24 mW / cm^2. No significant statistical difference was seen among doses applied for reduction of *Salmonella* on the surface of tomatoes ($p > 0.05$). Initial experiments indicated that TSAN would be insufficient to exclude the normal background flora on tomatoes. Additional studies were performed on XLD before settling on XLDN to account for the high level of background

contaminates. All *Salmonella* cultures utilized in these experiments were positive for hydrogen sulfide production which aided in colony identification. Atypical isolates which were hydrogen sulfide negative colonies were identified as *Pseudomonas aeroginosa*. A possible explanation for the lower recovery on tomatoes may be due to a food grade wax that was present on the sample or to the competition by resident microflora. UVC was more effective at reducing numbers of *Salmonella* on tomatoes than 320 ppm chlorine (*35*) and 2000 ppm chlorine (*7*). However it was less effective than the use of a produce wash (*15*) or coating with an edible film of hydroxypropyl methylcellulose (*36*).

Cellular concentrations for studies inoculated onto apples averaged 4.07 $\log_{10}$ cfu / apple for *E. coli* O157:H7. The effect of UVC on *E. coli* O157:H7 cells inoculated onto the surface of unwaxed Red Delicious apples is depicted in Figure 5. In contrast to the results from the lettuce samples, UVC was more effective at reducing microbial populations of *E. coli* O157:H7 on the surface of apples. A 3 log reduction was seen at doses exceeding 9 mW / cm^2 whereas the same dose on lettuce only resulted in approximately a 2.2 log kill. A maximum log reduction was seen at 24 mW / cm^2 with approximately a 3.3 log reduction in cellular numbers. However, no significant difference was seen in the doses applied to the surface of Red Delicious apples for the reduction of *E. coli* O157:H7. Alternative techniques utilized by other researchers on apples have resulted in similar log reductions. Dips in acetic acid resulted in a 3 log reduction of *E. coli* O157:H7 (*32*). A solution of 6% hydrogen peroxide reduced number of *S.* Chester by 3 – 4 logs from the surface of apples (*20*), and a 3 log reduction was achieved by exposure to 1.1 mg / L of free chlorine (*25*).

For all studies, there is a well-defined tail to the inactivation data, which is supported by other researchers that have described the shape of the UVC microbial inactivation curve as sigmoidal (*1*, *26*, *34*). The initial exposure of bacteria to UVC is believed to injure cells and is often seen as the formation of a shoulder. The initial shoulder response evident in other research is not apparent in this study. This may be due to the fact that the minimum dose utilized exceeded that of initial cellular injury. Additional levels of UVC surpass cellular injury and begin lethal destruction of cells. As the curve proceeds, cellular death begins to tail off. This may be explained by the presence of suspended solids in a fluid system that may interfere with the transmission of light (*26*). Yousef and Marth (*34*) suggested that the exponential inactivation may be due to a single target theory in which the cell is not inactivated by contact with a single photon of energy. Additionally, a tail in the inactivation curve may be the result of single hits on multiple targets or multiple hits on a single target. Another possible explanation of a sigmoidal curve may be due to non-homogenous microbial populations that differ in their susceptibility to UVC radiation. In this scenario, the less resistant cells are inactivated first, leaving the more resistant cells to form a tail (*10*).

In order to account for background microflora, the pathogenic strains utilized in this study were resistant to nalidixic acid. A study by Nissen and Holck (*23*) demonstrated that antibiotic resistant and antibiotic susceptible strains of *Listeria, Salmonella* and *E. coli* grew identically under laboratory conditions when variables such as pH, water activity and temperature were altered. The use of UVC was seen to be effective at reducing microbial

loads of pathogens on fresh fruits and vegetables. UVC more effective at reducing microbial populations of *Salmonella* spp. and *E. coli* O157:H7 on fresh produce than on other types of surfaces (*13, 18, 27, 28, 30, 33*). The produce evaluated in this study generally required lower doses of UVC than surfaces utilized in other studies, which may be attributed to the overall smoother surface of the samples analyzed. UVC was more effective at reducing bacterial populations on the surface of apples than on tomatoes and lettuce. This may be due to the fact that the wax applied on the surface of the tomatoes shielded bacteria from the UV rays or due to the topography of the sample. No significant difference was seen in the use of UVC at inactivating equivalent populations of *E. coli* O157:H7 or *Salmonella* spp. on the surface of green leaf lettuce ($p > 0.05$). Fresh produce processors do not fall under mandatory HACCP requirements, however the use of UVC may prove to be effective if used in conjunction with Good Agricultural Practices and Good Manufacturing Practices to increase the safety of fruits and vegetables. Due to the low cost as well as the lack of extensive safety equipment, UVC may be of benefit to those with little capital to invest as a means of ensuring product safety.

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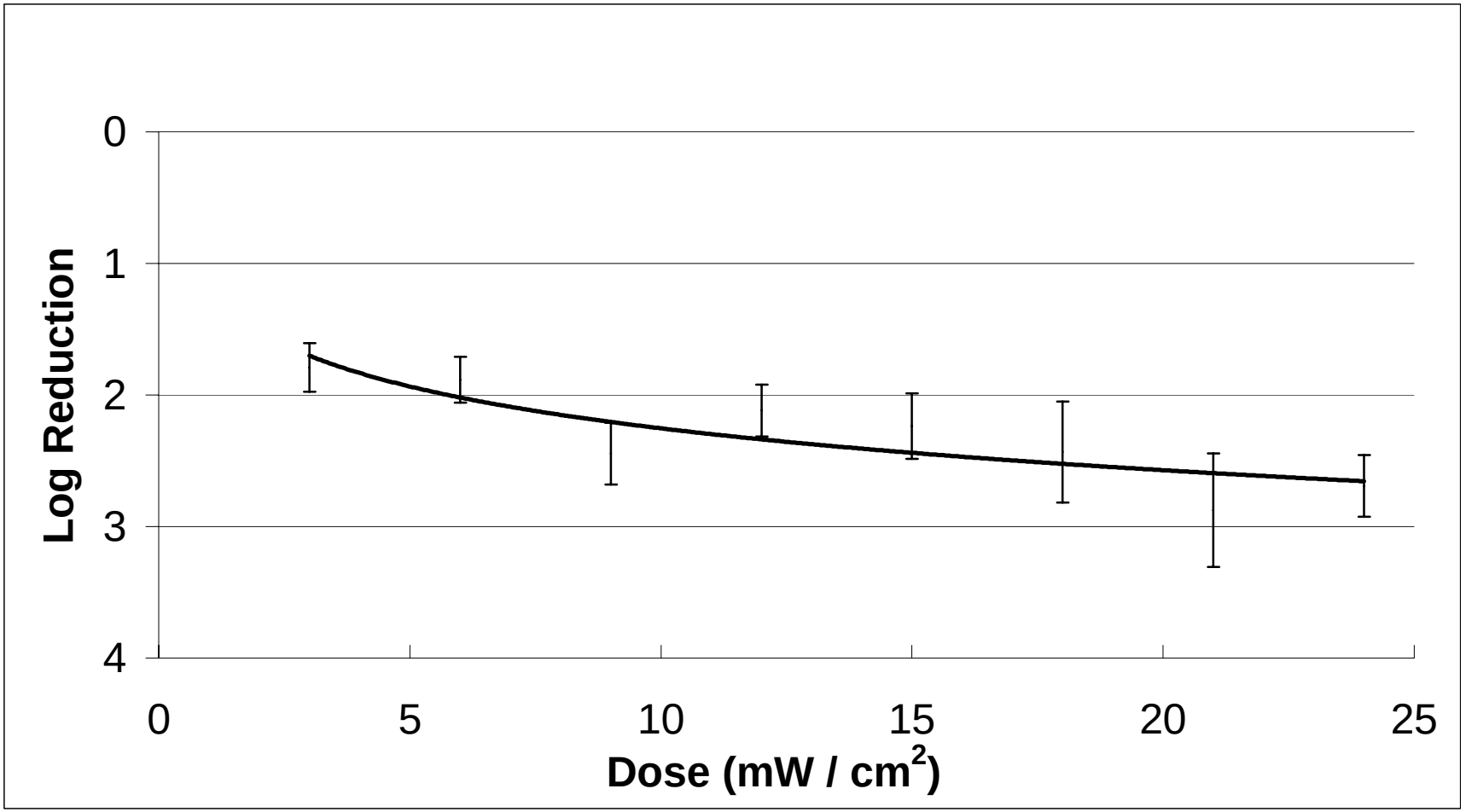

Figure 1: Mean Log Reductions and Standard Error of *Salmonella* spp. on the Surface of Leaf Lettuce by Ultraviolet Light at 253.7 nm (UVC). $R^2 = 0.74$ (n = 80).

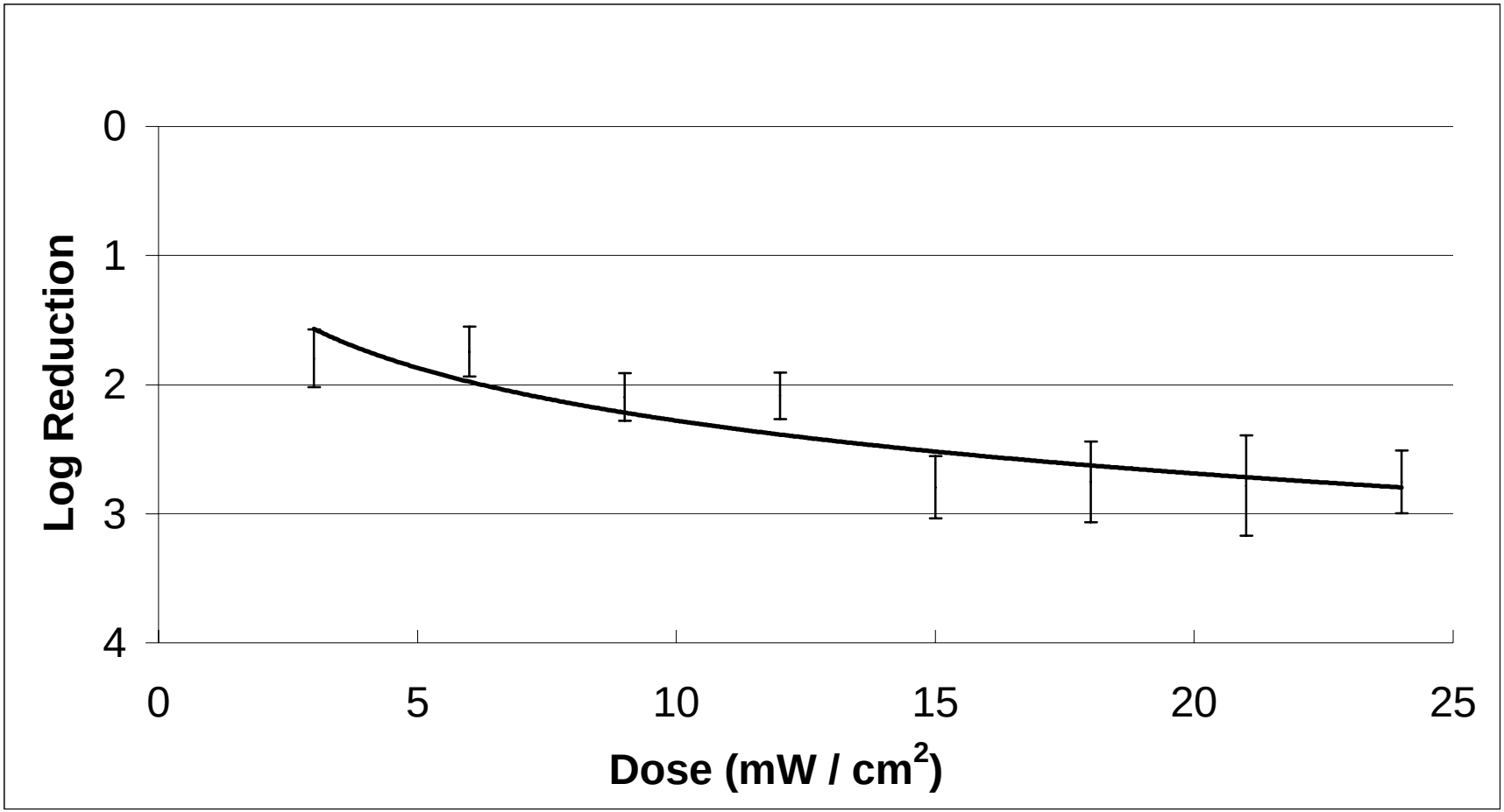

Figure 2: Mean Log Reductions and Standard Error of *E. coli* O157:H7 on the Surface of Leaf Lettuce by Ultraviolet Light at 253.7 nm (UVC). $R^2 = 0.79$ (n = 84).

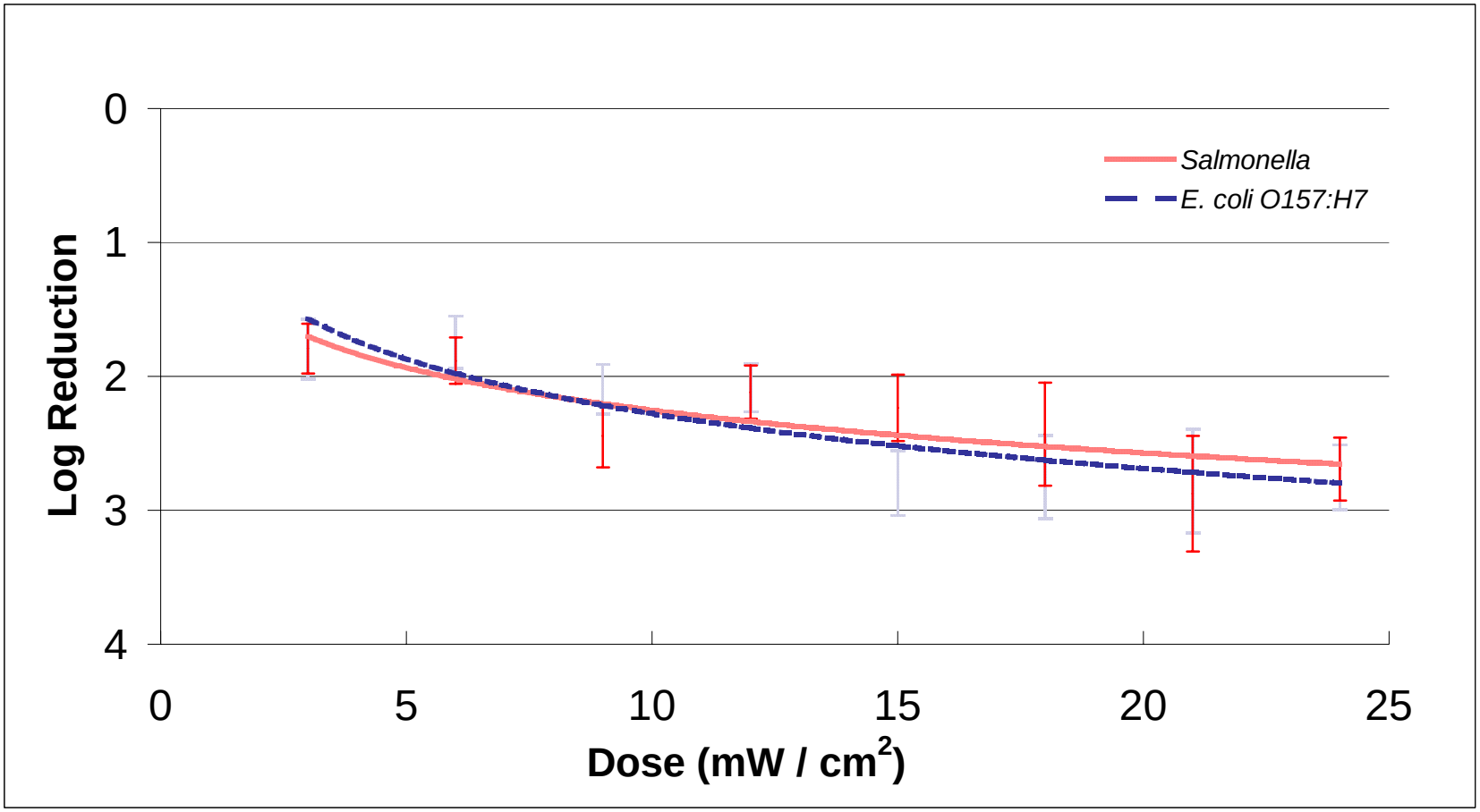

Figure 3: Mean Log Reductions and Standard Error of *E. coli* O157:H7 and *Salmonella* spp. on the Surface of Leaf Lettuce by Ultraviolet Light at 253.7 nm (UVC). *E. coli* O157:H7 $R^2 = 0.79$ (n = 84); *Salmonella* spp. $R^2 = 0.74$ (n = 80).

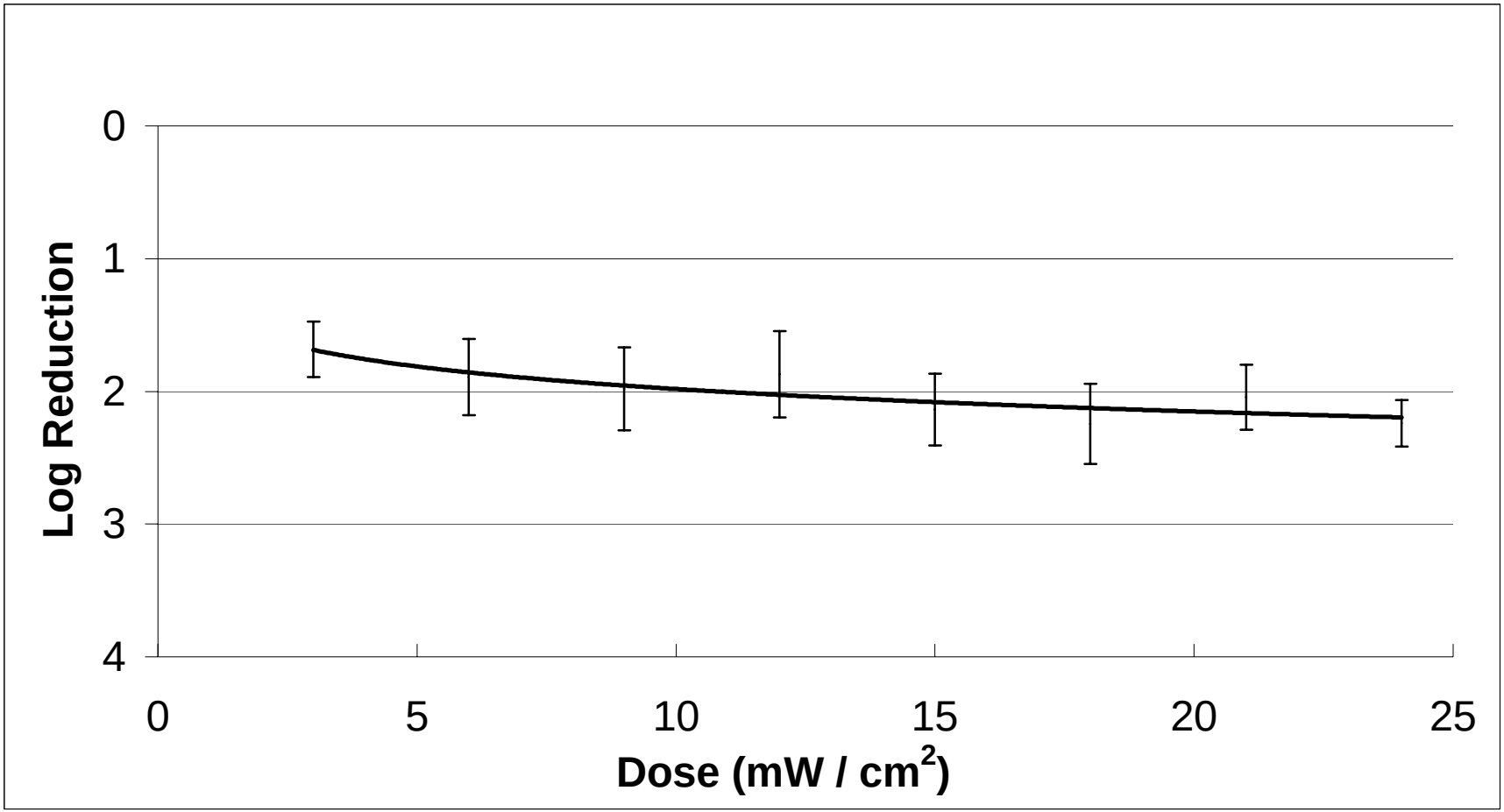

Figure 4: Mean Log Reductions and Standard Error of *E. coli* O157:H7 on the Surface of Tomatoes by Ultraviolet Light at 253.7 nm (UVC). $R^2 = 0.77$ (n = 96).

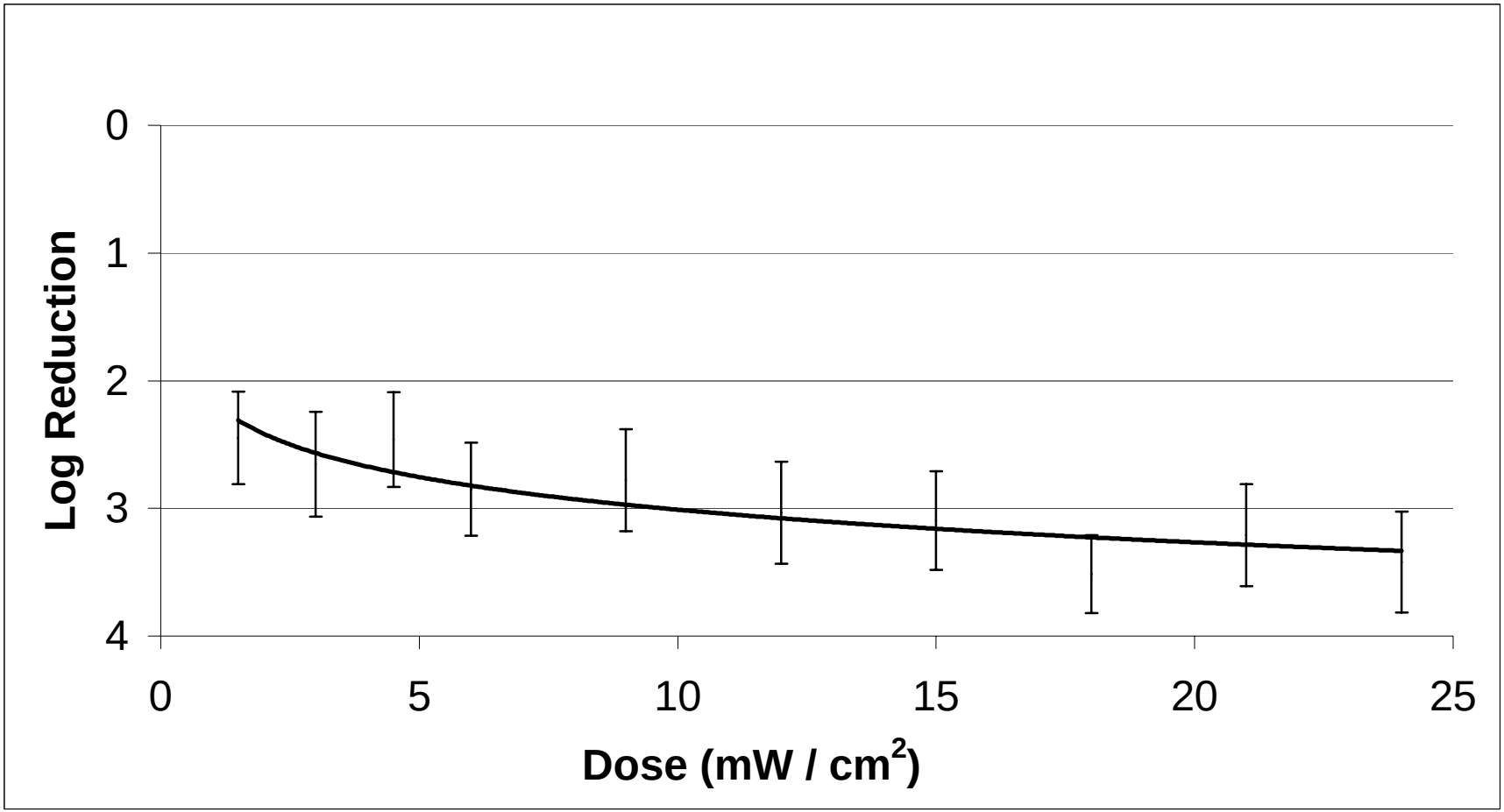

Figure 5: Mean Log Reductions and Standard Error of *E. coli* O157:H7 on the Surface of Red Delicious Apples by Ultraviolet Light at 253.7 nm (UVC). $R^2 = 0.82$ (n = 116).

FUTURE RESEARCH NEEDS

A five-year contract between the FDA and the Institute of Food Technologists (IFT) was signed in 1998 to investigate certain issues involving food safety. In September, 2001 in conjunction with FDA presented a response to Task Order No. 3: Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce[1]. Included in the document were certain research needs including:

- Determine the effect of environmental factors (for example, ultraviolet irradiation) on the survival and growth of pathogens of concern.
- Investigate traditional and non-traditional sanitizers on specific pathogen / produce combinations.
- Develop new sanitizers and innovative technologies for sanitation treatment of produce.
- Investigate alternative technologies for the safety of whole and cut produce.

Research presented in this study focuses on the last point, investigating alterative technologies to ensure the safety of whole and cut produce. Future research studies that may be generated from this work include:

1. Combination treatments of UVC with chemical treatments such as hydrogen peroxide or ozone.
2. Application of UVC to other pathogens, specifically *L. monocytogenes* and to other smooth surfaced fruits and vegetables
3. Development of a model system testing pathogen surrogates, for example *E. coli* ATCC 25922 for *E. coli* O157:H7.

[1] Food and Drug Administration. 2001. September 2001. Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce. [Internet, WWW], ADDRESS: http://www.cfsan.fda.gov/~comm/ift3-toc.html

VITAE

Brian Yaun was born in Winston-Salem, NC then spent most of his life in Blacksburg, Virginia where he graduated from Blacksburg High School. Following high school, he attended Virginia Polytechnic Institute and State University and received his Bachelor's degree in Biology and a Minor in Chemistry in 1996.

Following graduation, Brian accepted a position at Silliker Laboratories in Chicago Heights, Il where he served as a microbiologist. Two years, thousands of assays and a few audits later, Brian decided that the pursuit of a higher degree would be in his best interests and applied to Virginia Tech's Department of Food Science and Technology for admission for the Fall of 1998. While at Virginia Tech, he was a member of the Institute of Food Technologists and the International Association for Food Protection.