IMPROVING THE MICROBIOLOGICAL QUALITY AND SAFETY OF FRESH-CUT TOMATOES BY LOW DOSE ELECTRON BEAM IRRADIATION

A Thesis

by

HEATHER MARTIN SCHMIDT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Food Science and Technology

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Approved as to style and content by:

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ABSTRACT

Improving the Microbiological Quality and Safety of Fresh-Cut Tomatoes by Low Dose Electron Beam Irradiation. (August 2004)

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The effect of electron beam irradiation upon microbiological quality and safety of fresh-cut tomatoes was studied. Preliminary studies were conducted to ensure reliability of the rifampicin-resistant strain versus the parent strain of *Salmonella* serovar Montevideo for use in this study. Growth curve, heat tolerance and lactic acid resistance studies were performed, all of which showed no differences in behavior between the organisms. Fresh tomatoes were obtained from a local supplier and then cut into cubes with stem scars being separated. Both cubes and stem scars were inoculated with a rifampicin- resistant strain of either *Salmonella* Montevideo or *Salmonella* Agona, separated into treatment groups and treated by electron beam irradiation at 0.0 kGy (control), 0.7 kGy or 0.95 kGy. The effect of electron beam irradiation was determined for *Salmonella*, yeast, mold, and lactic acid bacteria (LAB) populations as well as pH on tomato cubes and stem scars over a 15-day storage period at 4ºC.

Results indicated that while irradiation treatment significantly reduced most microbial populations on tomato samples, there were no differences in the microbial populations between treatments of 0.7 kGy or 0.95 kGy. Irradiation at either dose resulted in a

significant reduction of *Salmonella* Montevideo when compared to the control, with an initial reduction of 1.8 and 2.2 $\log_{10}$ CFU/g on tomatoes for 0.7 kGy and 0.95 kGy, respectively. LAB, yeasts and molds were more resistant to the treatment than *Salmonella*. Populations present on stem scars and tomato cubes did experience some differences in log reductions, possibly due to the protective effect of the stem scar on microorganisms. However, no differences were detected between the two *Salmonella* serotypes in response to irradiation treatment. This study indicates that doses of irradiation greater than 1 kGy should be used in fresh-cut tomatoes to eliminate significant populations of pathogens, as well as to ensure the microbial quality of the product. Additional studies also need to be conducted to examine the effects of higher irradiation doses on the sensory qualities of fresh-cut tomatoes.

DEDICATION

This thesis is dedicated to my mother, Patricia Treadaway Martin, whose incredible strength and optimism through adversity has taught me how to survive anything. Thank you for your unending support, friendship and love.

To my husband, Jonathon Schmidt, who has stood by my side throughout this journey, and was willing to make sacrifices in his own life so that I might realize my dreams.

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INTRODUCTION

Fresh-cut fruit and vegetable consumption has been on the rise in the last several years, from sales of $5 billion in 1994 to $10-12 billion currently, comprising 10% of total produce sales *(27)*. These products are gaining popularity due to their convenience for consumers, increases in organic sales, changes in dietary habits and health trends, and their availability due to increased importation. These factors, along with the increased consumption of minimally processed fruits and vegetables, have enhanced the potential for incidence of foodborne illness. The number of documented outbreaks of human infections associated with consumption of raw fruits and vegetables has increased in recent years due to pathogenic organisms such as *Salmonella*, *Shigella* and *Escherichia coli* O157:H7 *(35)*. Since 1990, many different types of produce have been linked with outbreaks of foodborne illness in the United States. Tomatoes, in particular, have been associated with several multi-state *Salmonella* outbreaks in the last decade, such as a 1998 outbreak involving diced tomatoes contaminated with *Salmonella* serovar Baildon, raising concerns over industry decontamination practices *(14, 15, 51)*. Therefore, a new focus of food safety has been developing efficient methods of decontamination for such produce.

There are many difficulties associated with the microbiological safety of tomatoes. The tomato is a commodity with a variably low pH (3.4-4.7) depending on variety and state of ripeness *(31)*, so it is often believed that many pathogens associated with produce

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This thesis follows the style of the *Journal of Food Protection*.

cannot survive on this product. Salmonellae, however, can adapt to a reduced pH and subsequently exhibit tolerance to stress environments and survive on tomatoes *(6)*. As a preventive measure, the produce industry uses water washes and chlorine rinses to provide a decontamination step; however, researchers have had mixed results with the use of chlorine rinses in experimental studies to reduce log values on contaminated produce. Chlorine is also known to have an affinity for organic matter and some fruit and vegetable products have a high organic loading. Therefore, the effectiveness of chlorine as a sanitizer for such products can be limited due to the consumption of chlorine by organic matter. Common produce industry practices are in great need of revisions to ensure consumer food safety.

The application of electron beam irradiation is a promising alternative to other methods of microbial destruction for fresh-cut produce. Ionizing radiation has been proven to be effective in destroying *Salmonella* at low doses (under 1 kGy) as well as in preventing growth of spoilage microorganisms *(38)*. Irradiation can therefore enhance the shelf life and safety of fruit and vegetable products, such as fresh-cut tomatoes. This research will determine effective irradiation parameters for optimal microbiological quality and safety of chopped tomatoes.

OBJECTIVES

It was hypothesized that irradiation doses under 1 kGy would produce a significant destruction of both *Salmonella* serovar Montevideo and *Salmonella* serovar Agona in inoculated fresh-cut tomatoes.

The specific objectives of this research were to evaluate the microbial safety and quality of diced tomatoes inoculated with *Salmonella* and treated with electron beam irradiation. This was achieved by:

- Comparing a rifampicin-resistant mutated strain of *Salmonella* Montevideo to the parent strain to ensure accuracy of experimental results
- Evaluating microbiological quality of irradiated samples by yeast, mold and lactic acid bacteria counts
- Determining irradiation effects upon two serotypes of *Salmonella* using two irradiation doses
- Comparing effects of irradiation on tomato stem scars versus tomato cubes
- Observing effects of storage on microbial counts and pH over 15 days

REVIEW OF LITERATURE

Incidence of *Salmonella* in Fresh Produce

In the past two decades, there has been a noticeable increase in the consumption of fresh fruits and vegetables in the United States as well as a marked increase in the global distribution of produce *(35)*. Consumer demand for convenience has increased the desire for produce that has undergone some kind of minimal, non-thermal processing such as fresh-cut fruits and vegetables *(35)*. The International Fresh-cut Produce Association defines fresh-cut products as fruits or vegetables that have been trimmed, peeled, or cut into a 100% usable product that is bagged or prepackaged to offer consumers high nutrition, convenience and flavor while still maintaining freshness *(27)*. The fresh-cut industry has experienced exponential growth in the last 10 years, comprising an increasing percentage of total fruit and vegetable sales. However, along with this rise in demand and consumption has been an increase in foodborne outbreaks associated with produce.

Data from the CDC foodborne disease outbreak surveillance system from 1973 to 1992 suggest at least a doubling in the annual number of reported produce associated outbreaks, although the etiologic agent reported to the CDC during this time period was unknown for more than 50% of such outbreaks *(35)*. It is well known, however, that a diverse group of bacteria, viruses, and protozoa, such as *Shigella*, *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*, Hepatitis A and *Cyclospora* have been found to cause foodborne illness linked to the consumption of fresh produce. For example, *E. coli* O157:H7 has been the causative agent of illness associated with several products,

including alfalfa sprouts, lettuce and radish sprouts *(35)*. Alfalfa sprout-associated infections with *E. coli* O157:H7 occurred in 1997 in two states, with a total of 148 cases being reported; in this outbreak, all sprouts were traced back to two farmers who utilized alfalfa seeds from the same distributor lot *(35)*. In addition, two midwestern United States outbreaks of *S. flexneri* infection have been implicated with the consumption of fresh green onions *(5)*. Infections from Hepatitis A virus have also been linked to the consumption of green onions, lettuce, raspberries, frozen strawberries and sliced tomatoes *(35)*. Nevertheless, while there have been reports of contamination of produce with a wide variety of pathogens, the frequency of *Salmonella* outbreaks has proved that this microorganism is of great concern for the produce industry.

According to the CDC, *Salmonella* was the most commonly reported pathogen in 1973-87 and 1988-92 for fresh produce-associated outbreaks *(35, 3, 14)*. *Salmonella* spp. are estimated to cause approximately 1.5 million cases of foodborne infection each year in the United States, with more than 15,000 hospitalizations and 500 deaths *(32)*. In 1999, the Food and Drug Administration conducted a survey to determine the percentage of produce imported into the United States that was contaminated with *E. coli* O157:H7, *Salmonella* or *Shigella*. Alarmingly, about 3.5% of imported produce was contaminated with *Salmonella* spp. *(22, 42)*. The FDA initiated a follow-up survey of domestic produce and reported that 6 samples of 1,028 (0.58%) were contaminated with *Salmonella (23)*. Outbreaks of salmonellosis have been attributed to consumption of products such as cantaloupe, bean sprouts, watermelon and tomatoes. Large outbreaks of salmonellosis caused by ingestion of fruits and vegetables have been documented

since 1955 *(30)*. For example, *Salmonella* Poona was reported to cause infection associated with cantaloupe consumption in 1991, 2000, 2001 and 2002, and was linked to cantaloupes imported from Mexico *(11, 12, 16)*. In 1990, an outbreak of 25,000 cases was reported from consumption of cantaloupe contaminated with *Salmonella* Chester *(11, 43)*. Additionally, unpasteurized orange juice was the vehicle of transmission for *Salmonella* Hartford, *Salmonella* Gaminara and *Salmonella* Rubislaw among 62 unrelated travelers in Orlando, Florida in 1995. More than 500 cases of *Salmonella* Montevideo and over 100 cases of *Salmonella* Meleagridis infection occurred in California in 1996, which were associated with the consumption of alfalfa sprouts from a single sprouter *(13, 35)*.

Fruits and vegetables can become contaminated with pathogenic microorganisms while growing in fields, orchards, vineyards, or greenhouses, or during harvesting, post-harvest handling, processing, distribution, and preparation in food service or home settings *(6)*. Many consumers prefer 'natural' and 'organically' cultivated produce, which could result in the increased use of manure rather than chemical fertilizers in food production *(35)*. Consequently, contamination of vegetables by foodborne pathogens frequently occurs through agricultural practices such as irrigation with polluted water or fertilization with improperly composted manure or sewage sludge *(36)*. In fact, it is not uncommon for fruits and vegetables to contain total microbial populations of 10^4 to 10^6 CFU/g when they arrive at the packinghouse or processing plant *(5, 10)*. Trends toward greater geographic distribution of minimally processed fruits and vegetables from central

processing facilities and subsequent storage and handling practices may also be contributing to an increased frequency of produce associated infections *(6)*.

Aside from handling practices, intrinsic qualities of fruits and vegetables such as the nature of the epithelium and protective cuticle, tissue pH, and the presence of antimicrobials dictate which groups of produce may be more likely than others to harbor certain types of microorganisms in damaged tissues *(6)*. Fresh-cut products may be susceptible to proliferation of pathogens due to the release of nutritious internal tissue fluids that accelerate growth and spoilage; cutting also provides more surface area on which the microorganisms can grow *(9)*.

Tomatoes are of particular interest because of their extensive use, handling practices, and the general misconception that they do not support pathogen growth *(34)*. As a whole product, tomatoes maintain a delicate tissue structure that is extremely susceptible to chilling injury, mechanical damage and the presence of microorganisms; dicing tomatoes prior to distribution reduces the shelf life of the product to 10 days from the time of manufacturing *(40)*. In 1990, an outbreak of *Salmonella* Javiana infections involving 176 cases in Illinois, Michigan, Minnesota and Wisconsin, was epidemiologically linked to consumption of fresh tomatoes *(51)*. There was also an outbreak of 100 cases of salmonellosis caused by *Salmonella* Montevideo in 1993 in Illinois, Michigan, Minnesota and Wisconsin; both of these outbreaks were traced back to a packer in South Carolina where a water-bath appeared to be a likely source of tomato contamination *(35)*. In 1998, there was another outbreak in geographically separate areas of the United States, associated with consumption of diced tomatoes that

were contaminated with *Salmonella* Baildon *(25)*. According to the CDC, an investigation concluded that the tomatoes were most likely contaminated at the farm or even during packing rather than during the dicing operation *(15)*.

Survival of *Salmonella* on Tomatoes

Tomatoes are comprised of about 94% water, with a pulp pH below 4.5 for most cultivars. The high acid content of tomatoes is dominated by citric and malic acid. While the low pH of tomatoes is commonly mistaken to not support pathogen growth or survival, a study conducted by Chung and Goepfert revealed that salmonellae initiated growth in citric acid at pH values as low as 4.05 while under optimal laboratory conditions *(17)*. In addition, the acidic pH of tomatoes does not hinder the growth of yeasts and molds. Some yeasts and molds will utilize organic acids, leading to a reduced acidity, which would increase the pH and provide a more hospitable environment for survival of other microorganisms *(6)*. As tomatoes develop, the amount of sucrose decreases while starch and reducing sugars increase; this change in composition favors nutrient availability for growth of pathogens such as *Salmonella (25, 29)*. Although *Salmonella* is a human pathogen, studies have revealed its ability to survive on or in tomato fruits throughout the course of plant growth, flowering, and fruit development and maturation (*25)*. Entrance of *Salmonella* into the tomato stem may introduce the pathogen into the xylem, which has the principal role of transporting water and nutrients from the root to the extremities of the plant *(25)*.

The survival and growth of *Salmonella* on the surface and stem scar of tomatoes has also been observed. Laboratory studies using a strain of *Salmonella* Montevideo

isolated from an infected patient revealed that the pathogen can grow on the surface of tomatoes stored at 20ºC and in chopped tomatoes (pH 4.1±0.1) stored for 96 or 22 hours at 20 or 30ºC, respectively *(54)*. Weissinger et al. reported that *Salmonella* Baildon could readily grow in diced tomatoes, with an initial population of 0.79 $\log_{10}$ CFU/g increasing to 5.32 $\log_{10}$ CFU/g within 24 hours of storage at 21ºC (*49)*. In another study by Wei et al., stem scars and growth cracks provided a greater protective environment for bacteria than tomato skin against washing with tap water or aqueous chlorine *(48)*. *Salmonella* Montevideo, when added to the stem scar, can also be introduced and/or transferred into the interior of tomatoes by the physical action of cutting with a knife at levels as low as <10 CFU *(30)*.

Another interesting study examined the association between bacterial soft rot and *Salmonella* contamination of tomatoes. Bacterial soft rot is a leading source of post-harvest losses in tomatoes and is caused by a group of plant pathogens including *Erwinia carotovora*, *Pseudomonas fluorescens* and *P. viridiflava (50)*. Wells and Butterfield found that the incidence of *Salmonella* on produce affected by bacterial soft rot was twice that of healthy samples; also, controlled experiments with inoculated tomato tissues demonstrated that bacterial soft rot infection increased multiplication of *Salmonella* by at least three to 10-fold compared with multiplication on uninfected tissues *(50)*. As a possible explanation for this phenomenon, the pectolytic breakdown of tissues caused by bacterial soft rot could provide a more suitable environment for pathogen growth.

Infiltration and internalization of pathogens into tomatoes is another issue of great microbiological significance. Tomato stems and fruits are subject to mechanical injury in the field and during post-harvest handling, which makes tomatoes more susceptible to internalization of bacteria *(25)*. Infiltration of pathogens into fruit and vegetable tissues is dependent upon temperature, time and pressure, and only occurs when the water pressure on the produce surface overcomes internal gas pressure and the hydrophobic nature of the produce surface *(6)*. More specifically, research has shown infiltration to be associated with a negative temperature differential between wash water and tomatoes. In other words, if the wash water is colder than that of the tomatoes, pathogens such as *Salmonella* are rapidly taken up through the stem scar *(2, 54)*. Also, hyperchlorinated water will reduce *Salmonella* on the external surface, but is less effective on the inside of the tomato *(49)*. These two factors have influenced the recommendation that rinse waters be hyperchlorinated and also 5.5ºC warmer than the tomatoes *(54, 47)*. However, if infiltrated, bacterial cells may establish microcolonies that are extremely difficult to reach with aqueous chemical solutions *(6)*. In addition, colonization of spoilage and non-spoilage microorganisms of fruits, vegetables, and post-harvest contact surfaces can provide a protective environment for pathogens, reducing the effectiveness of sanitizers and other inhibitory agents *(6)*.

Decontamination Methods

The Food and Drug Administration proposed that treatment of fruits, vegetables and commercial fruit juices should be capable of reducing pathogen loads by a minimum of 5 log cycles *(21, 47)*. This leaves the produce industry faced with the challenge of

implementing decontamination techniques that are effective upon microorganisms, yet not detrimental to the sensory aspects of the product. Chlorine is the most commonly used sanitizing agent available for fresh produce, typically applied at concentrations no greater than 200 ppm and a pH of 6.5 to 7.5 *(44)*. While chlorine is often used to decontaminate produce, elimination of pathogenic microorganisms from the surface of vegetables by chlorine is limited or unpredictable *(36)*. This is due in part to the inaccessibility of attached microorganisms and resistance of bacteria within biofilms but also to the rapid breakdown of chlorine in the presence of organic matter *(44)*. In fact, the organic matter present in dump tanks or on tomatoes could protect *Salmonella* or other pathogens attached onto the fruit from the bactericidal effect of aqueous chlorine *(48)*. Wei et al. also reported that treatment with chlorine at 100 ppm for 2 minutes failed to kill all bacteria added to stem scars at 3.98 $\log_{10}$ CFU/g and tomato skin at 3.25 $\log_{10}$ CFU/g (*48)*. In another study, diced tomatoes inoculated with a 0.86 $\log_{10}$ CFU/ml culture of *Salmonella* Baildon did not experience a complete destruction of the pathogen when treated with chlorine at 200 ppm *(49)*. It would not be reasonable to expect treatment with chlorine at concentrations not compromising sensory qualities and perhaps health aspects of raw produce to reduce populations of naturally occurring microflora or potential human pathogens by significant amounts *(8)*. In addition, failure to maintain adequate chlorine in wash water can actually lead to increased microbial populations on produce *(4)*. When considering this phenomenon, it is apparent that decontamination methods besides chlorine rinses are needed to ensure the safety of tomatoes post-harvest.

Other methods of decontamination, such as trisodium phosphate, detergents, organic acids, ozone and hydrogen peroxide have been studied. Like chlorine, none has been shown to eliminate more than 2 $\log_{10}$ CFU/g of bacterial populations at concentrations not detrimental to sensory quality *(8)*. In a 2003 study by Raiden et al., two detergents, sodium lauryl sulfate and Tween 80, were evaluated for their effectiveness in removing *Salmonella* and *Shigella* from the surfaces of different types of produce; neither rinse was more effective than water and did not result in enhanced removal of pathogens from produce surfaces *(42)*. Tap water has limited or no effect on killing microorganisms that occur at populations ranging from 10^3 to 10^9 CFU/g on raw and minimally processed produce and can actually contribute to cross-contamination of food preparation surfaces and other food items *(4, 8, 9, 36)*. Venkitanarayanan et al. observed that a water wash treatment reduced populations of *E. coli* O157:H7, *Salmonella* Enteritidis and *L. monocytogenes* by only 1.5 to 2 log cycles on tomatoes, and a substantial population of the pathogens survived in the wash water *(47)*. These data clearly indicate a need for new techniques in fresh fruit and vegetable decontamination.

Irradiation

As an alternative, irradiation can be used for controlling disease and deterioration of fruits and vegetables. Sources of irradiation are gamma rays (with Co-60 or Cesium-137 radioisotope), machine generated electron beams and X-rays. Electron beams are produced by linear accelerators, with a standard energy limit of 10 MeV set as a safety precaution. Energy from ionizing radiation is absorbed by food material as it passes

through. This absorbed energy or dose leads to ionization or excitation of atoms and molecules of the matter *(39)*. Free radicals are then produced as a result of these primary effects, leading to secondary effects that may interact, causing radiolysis of water in food products. As a result, ionizing radiation may directly damage the genetic material of the living cell, leading to mutagenesis and eventually to cell death *(1)*. DNA base damage, single-strand and double-strand DNA breaks, and crosslinking between bases are the main effects of irradiation *(38)*. In the food industry, irradiation can be promising for the purpose of destruction of spoilage organisms as well as foodborne pathogens. Several extrinsic factors are important in the application of irradiation, including radiation dose, dose rate, temperature and atmosphere during irradiation, and temperature and atmosphere during storage, all of which can affect the outcome of specific foods *(45)*. One must also consider the water content of the product as well as size of the initial microbial population when evaluating the effectiveness of this technology.

On April 18, 1986, the Food and Drug Administration approved ionizing radiation dosages of up to 1 kGy to be applied to fresh fruits and vegetables for the purpose of disinfestations and ripening delay (*38)*. Research has also shown positive results for the use of irradiation as a decontamination step of the food chain. Irradiation doses lower than 2 kGy have been reported to be more efficient than chemical disinfections, total counts generally being reduced by 3 to 4 log cycles *(36)*. Differences in bacterial counts of minimally processed vegetables disinfected with chemicals typically disappear rapidly over time compared to controls. In contrast, lower counts achieved through irradiation persist during storage *(36)*.

The primary benefit of irradiation is in increasing the shelf-life of those products where shelf-life is limited by microbial action. The shelf-life of fresh-cut vegetables is generally 10-14 days and the use of irradiation at dose levels of less than 2 kGy can extend shelf-life by 2-12 days *(41)*. It was reported that aerobic mesophilic, heterofermentative and total lactic microflora were reduced during storage of pico de gallo containing red, ripe tomatoes that had been irradiated at a dose of 1 kGy *(26)*. Irradiation at 0.5 kGy can reduce microbial counts in diced tomatoes substantially to improve microbial shelf life without adverse effects on sensory qualities; also, it was found that tissue firmness does decrease with increasing dose, but not with storage time *(40)*. Low doses of irradiation have been found to be the most effective for produce because they typically do not cause damage to fruits and vegetables. Irradiation dosages that cause injury to produce may initially reduce populations of bacteria and fungi, but those populations will regenerate very quickly because the weakened plant tissues, an appropriate substrate for growth, are readily available *(53)*. In 2002, Prakash et al. investigated the growth of yeast and mold counts on diced tomatoes and found that they were significantly reduced at doses 0.5 kGy, 1.24 kGy and 3.70 kGy for 12 days of storage; however, after storage for 15 days, microbial counts were comparable to the control *(40)*.

Salmonella has the highest resistance to irradiation of all the non-sporeforming pathogens, with a D_{10} value (dose required to reduce the microorganisms present by 1 log cycle) of about 0.6 kGy when present in chicken *(38)*. However, D_{10} values for *Salmonella* as well as other pathogens must be treated on a product-to-product basis; the

differing composition of food products will greatly affect the outcome of response to irradiation by pathogens. In contrast to the extensive studies on irradiation to control pathogens on meat and poultry products, there are very few studies on the value of ionizing radiation for the elimination of foodborne pathogens on or in fruits and vegetables *(46)*. The present study focuses on exploring the use of irradiation as a decontamination technique for fresh-cut tomatoes. The effectiveness of low dose electron beam irradiation on two *Salmonella* serotypes as well as other native microflora of tomatoes was studied.

MATERIALS AND METHODS

Media Preparation Methods

Tryptic soy agar (TSA; Difco, Detroit, MI) + rifampicin (Sigma, St Louis, MO) + cycloheximide (Sigma) plates were prepared by adding a solution of 0.1 g of rifampicin dissolved in 5 ml methanol as well as 0.1 g of cycloheximide dissolved in 5 ml sterile water to 1 L of autoclaved and cooled TSA. Rifampicin was used as a selective agent for the rifampicin-resistant marker salmonellae, and cycloheximide was also added for inhibition of yeasts and molds.

Bacterial Cultures

Salmonella enterica subsp. *enterica* serotypes Montevideo and Agona and rifampicin-resistant mutants derived from these organisms were provided by Dr. Linda Harris, University of California at Davis. Cultures were stored at –80ºC on Protect™ Bacterial Preservers (Key Scientific Products, Round Rock, TX). Working cultures were made from these stocks and maintained on TSA slants at 4ºC. Prior to tomato inoculation, rifampicin-resistant cultures were confirmed by streaking onto TSA + rifampicin and incubating at 37ºC for 24 h. Characteristic colonies were then streaked onto TSA slants and incubated at 37ºC for 24 h.

Preliminary Experiments

Growth curves. Growth curves were conducted to establish similar growth patterns between the *Salmonella* Montevideo strain and its rifampicin-resistant derivative. Cultures of both organisms were grown on TSA slants for 24 h and then transferred into 9.9 ml tryptic soy broth (TSB; Difco) and incubated at 37ºC over a 24 h

period. The populations reached by *Salmonella* Montevideo and the rifampicin-resistant derivative under these incubation conditions were previously determined to be 10^8-10^9 CFU/ml in 24 h. Based on this information, serial dilutions were prepared in sterile 0.1% peptone water (Difco) for each overnight culture to obtain a concentration level of approximately 10^5 cells, and 0.1 ml of this culture was subsequently added to each TSB tube. The tubes were then incubated at 37ºC. At hourly intervals, triplicate tubes were separated for each organism and plated onto TSA, using the appropriate serial dilutions with sterile 0.1% peptone water. All plates were incubated at 37ºC for 18-24 h prior to counting and converting to log CFU/ml values.

Heat tolerance. The parent strain and the rifampicin-resistant derivative of *Salmonella* Montevideo were analyzed for their resistance to heat at 60ºC. Both strains were inoculated into 9.9 ml TSB and incubated at 37ºC for 24 h. The following day, cultures were transferred to sterile tubes and centrifuged at 2500 rpm for 10 min in a Jouan Centrifuge Model B4 (Winchester, VA). After discarding the supernatant, the resulting pellets were re-suspended in 9.9 ml 0.85% sterile saline, vortexed and re-centrifuged. This process was completed twice before dispensing 0.5 ml of the resulting culture into 17 x 60 mm screw cap vials containing 4.5 ml 0.85% sterile saline. Vials containing the organisms were heated to 60ºC in a water bath, removed at random at 0, 15, 30, 45, or 60 sec and rapidly cooled in ice water prior to spread plating on TSA. Temperature was verified by monitoring a non-inoculated vial containing 5 ml 0.85% sterile saline using a K-type thermocouple connected to a Traceable® 2-channel hand-held digital thermometer (Control Company, Friendswood, TX). Three control vials

were plated to determine the original population of both strains. All plates were incubated at 37ºC for 18-24 h prior to counting. Survivor curves at 60ºC were determined from the heating time intervals.

Lactic acid tolerance. A rifampicin-resistant derivative of *Salmonella* Montevideo and the parent strain were exposed to lactic acid to determine similarities in tolerance to a stress environment. Both strains were inoculated into TSB and incubated at 37ºC for 24 h. The following day, cells were washed and harvested by centrifugation using the procedure described above, using 0.1% sterile peptone to wash the cells as opposed to the saline solution. Serial dilutions were made to obtain a culture concentration of 10^6 CFU/ml. A 0.5% lactic acid solution was prepared using 88% L-lactic acid (Purac, Lincolnshire, IL) and dispensed in 9 ml increments into glass tubes. One ml of culture was added to each tube, resulting in a 10^5 concentration of cells. At times 0, 30, 60, 90 and 120 sec of exposure to 0.5% lactic acid, the samples were spread plated using 0.1% sterile peptone water onto TSA as well as TSA + 2% NaCl (Sigma) for enumeration of sublethally injured cells. Controls were also plated to determine the original populations of both organisms. All plates were incubated at 37ºC for 18-24 h prior to counting.

Effect of Irradiation upon Microbial Survival in Fresh-cut Tomatoes

Experimental design. Chopped tomato samples (cubes and stem scars) were inoculated with rifampicin-resistant *Salmonella* Montevideo or rifampicin-resistant *Salmonella* Agona, prior to irradiation treatment of either 0 kGy (control), 0.7 kGy or 0.95 kGy using dual electron beam at the National Center for Electron Beam Food

Research located at Texas A&M University. Samples were then packed into cardboard boxes and transported back to the Food Microbiology Laboratory, located in a building less than one mile from the food irradiation facility. In the laboratory, triplicate samples were separated and analyzed for initial microbiological counts. The remaining tomatoes were stored at 4ºC and sampled after 3, 6, 9, 12 and 15 days of storage for microbiological analysis.

Inoculum preparation. One day before tomato inoculation, 24-h cultures of rifampicin-resistant *Salmonella* Montevideo and *Salmonella* Agona were transferred into separate flasks containing 300 ml of TSB and grown overnight at 37ºC. Twenty-five ml of these cultures were transferred to sterile tubes, centrifuged and harvested using the same procedure described previously. This procedure was repeated two times. The resulting suspension was used for inoculating the chopped tomatoes.

Tomato preparation. Whole, ripe Roma tomatoes were purchased from a local supplier in Bryan, Texas. The tomatoes had not received any kind of prior sanitizing or waxing treatment. Tomatoes were randomly assigned a treatment group prior to chopping. Stem scars were removed using a knife by excising an area of tissue 1.5 to 2 cm deep; the remainder of the tomato was chopped into pieces approximately 6 mm thick and 12 mm wide. The tomato cubes were then placed in one single layer in an appropriately labeled 17.8 x 22.9 cm clear, shallow hinged polyethylene terephthalate (PETE) deli container (Genpak, Glen Falls, NY), approximately 50 g of cubes per box, with stem scars boxed separately from the other samples. Each sample box was inoculated with 2 ml of *Salmonella* suspension containing ca. 8.0 log CFU/ml of

Salmonella Montevideo or *Salmonella* Agona culture by evenly dispensing the inoculum over the tomato pieces. Subsequently, the samples in each box were mixed by hand to ensure a homogenous distribution of the organism over the tomato pieces and stored at 4ºC until irradiation treatment on the next day. A preliminary trial confirmed the efficacy of this inoculation method for the homogeneous distribution of organisms. Secondary packaging, consisting of placing the boxes inside Whirl-pak® bags (Nasco, Fort Atkinson, WI), was used on all samples to prevent any contact of pathogens with surfaces inside the irradiation facility.

Dosimetry. Dose mapping was completed at the National Center for Electron Beam Food Research using alanine pellets (Harwell Dosimeters, United Kingdom). Dosimeters were placed on top and bottom of tomato pieces in the upper left corner, center and lower right corner of the PETE containers to quantify absorption throughout the sample. Consistent thickness of tomato samples was made to assist in the efficiency of electron beam dose absorption. If thickness of samples were to exceed 6 mm, then under-processing may occur, leading to elevated microbial survival. Dual electron beam was used to achieve a greater penetration of samples. Absorbed dose of the dosimeters was calculated by comparison with a standard curve using an electron paramagnetic resonance instrument (Bruker EMS 104 EPR Analyzer, Bruker Instruments, Germany).

After extensive precision dose mapping, appropriate attenuation setups were determined. To achieve a target dose of 0.7 kGy, (9) 0.5 cm high-density polyethylene (HDPE) sheets were stacked on top of the PETE containers containing tomato samples. Nine 0.5 cm sheets plus (1) 0.3 cm sheet were set beneath the containers to absorb

Compton electrons at a conveyor speed of 60 feet per minute. Wooden planks were placed between HDPE sheets to prevent the sample boxes from being smashed or damaged. To achieve a target dose of 0.95 kGy, (9) 0.5 cm HDPE sheets were set on top and (9) 0.5 cm sheets plus (1) 0.16 cm sheet were placed on bottom of the containers and samples were run at a conveyor speed of 59.3 feet per minute. Based on dosimetry values, the max/min ratios were 1.34 and 1.26 for the 0.70 kGy and 0.95 kGy target doses, respectively. These values were used on the day of processing to calculate the average absorbed doses achieved.

Application of irradiation treatment. Inoculated tomato samples were placed in a single layer in cardboard boxes on a conveyer and treated with an average absorbed dose of either 0.7 kGy or 0.95 kGy of electron beam irradiation. Control samples remained in storage at 4ºC at the Food Microbiology Laboratory. Appropriate attenuation and rate of process were used for each dose as per dose mapping completed prior to the experiment described above. Bare standards were measured on the processing day at the beginning, middle and end of treatment of samples to verify consistent energy output by the electron beam. To minimize variations in dose absorption, all samples were placed in the cardboard trays in an identical attenuation configuration and geometry. Immediately following treatment, all irradiated samples were transported to the Food Microbiology Laboratory at Texas A&M University for proper storage at 4ºC and analysis at 0, 3, 6, 9, 12 and 15 days.

Sampling of tomatoes for microbial enumeration. On each day of analysis, three boxes for each treatment group were separated and sampled for count of

Salmonella. Twenty-five-gram samples of cubes were taken from each tomato box with sterile forceps, weighed and placed in stomacher bags, then combined with 225 ml sterile 0.1% peptone water and pummeled with a Stomacher-400 laboratory blender (Seward Scientific, London, England) for 60 sec. For stem scar samples, 10-g samples were taken and combined with 90 ml sterile 0.1% peptone prior to mixing using the same procedure as above. Appropriate serial dilutions were made and spread-plated onto TSA + rifampicin + cycloheximide and incubated at 37ºC for 18-24 h.

The sample suspensions described above were also used for yeast, mold and lactic acid bacteria (LAB) enumeration. Appropriate dilutions were dispensed onto Yeast and Mold Petrifilm™ (3M™, St. Paul, MN) and incubated at 25ºC for 5 days. LAB were enumerated by spread plate using deMan, Rogosa and Sharpe agar (MRS; Difco) with an overlay of All Purpose Tween agar (APT; Difco) adjusted to pH 4.0 ± 0.1 with 10% tartartic acid (Mallinckrodt Chemical Works, St. Louis, MO). These plates were incubated at 35ºC for 3-5 days *(20)*.

Confirmation of isolates. For each day of analysis, ten characteristic *Salmonella* colonies were randomly chosen and streaked onto TSA slants and incubated for 24 h. They were then confirmed in triple sugar iron (TSI; International Bioproducts, Bothell, WA) and lysine iron (LIA; International Bioproducts) agar slants as well as *Salmonella* O Poly A-I and Vi antiserum (Becton Dickinson, Sparks, MD). Typical biochemical reactions for *Salmonella* in TSI were an alkaline slant (red) and acid butt (yellow) with H_2S production causing a black color in the slant. In LIA, *Salmonella* had an alkaline reaction (purple) with H_2S production as well. To conduct the antiserum test,

a drop of 0.85% saline solution was added to a glass slide. A loopful of bacteria was emulsified with the saline and one drop of *Salmonella* O Poly A-I and Vi antiserum was added and mixed. A positive reaction was rapid and complete agglutination of the culture.

A pure culture of *Lactococcus* was maintained on TSA slants and plated onto MRS + APT to aid in identification of LAB colonies. For each day of analysis, fifteen characteristic LAB colonies were randomly picked and transferred to TSA slants for further confirmation as LAB by gram stain, catalase and fermentative metabolism (O-F Glucose; O-F Basal Medium + Glucose + 0.1%Yeast Extract, Difco) test *(24)*.

Measurement of pH. Surface pH was measured on samples from each treatment group prior to microbiological analysis. A Markson Model 612 portable pH meter (Markson Science, Inc., Phoenix, AZ) was used with a flat bulb design electrode (Markson Science, Inc). The pH meter was properly calibrated and sanitized prior to use on each day of analysis. This measurement was conducted in triplicate.

Statistical analysis. All experiments were conducted in triplicate. Microbiological data were transformed logarithmically before statistical analysis. For all microbiological data, when the counts obtained were lower than the detection limit, a number half way between 0 and the detection limit (10 CFU/g) was used to facilitate the analysis of data. Means for each parameter in the 3 trials were compared by analysis of variance using general linear model (GLM) procedures of the Statistical Analysis System (SAS; version 8.2, SAS Institute, Cary, N.C.). Least square means were

determined and standard error/percent difference was used to determine mean differences at $p<0.05$.

RESULTS AND DISCUSSION

Preliminary Experiments

Growth curves. The growth rate of the rifampicin-resistant derivative *Salmonella* Montevideo showed no significant differences ($P<0.05$) in comparison to the growth of the parent strain incubated in TSB at 37ºC over a 24 h period (Figure 1). The generation time of each organism was calculated based on data taken during the log phase of microbial growth from three replicates. *Salmonella* Montevideo had a generation time of 23.5 min and a lag phase consisting of 115 min, while the rifampicin-resistant strain had a generation time of 22 min and a lag phase of 125 min. Both organisms entered the stationary phase after approximately 9 h of incubation in TSB at 37ºC. These growth patterns indicated no significant differences in growth behavior by the two strains.

Heat tolerance. As demonstrated in Figure 2, *Salmonella* Montevideo and the rifampicin-resistant strain exhibited a similar death trend over a 60 sec period of heat treatment at 60ºC. These results were not significantly different ($P<0.05$). The initial differences in control population account for the slightly lower counts of the parent strain over the course of treatment. After 60 sec of treatment, the parent strain population was reduced by 3.1 $\log_{10}$ CFU/ml, while the rifampicin-resistant strain experienced a 3.4 $\log_{10}$ CFU/ml reduction. These results indicate a similar response by both organisms to exposure to high temperatures.

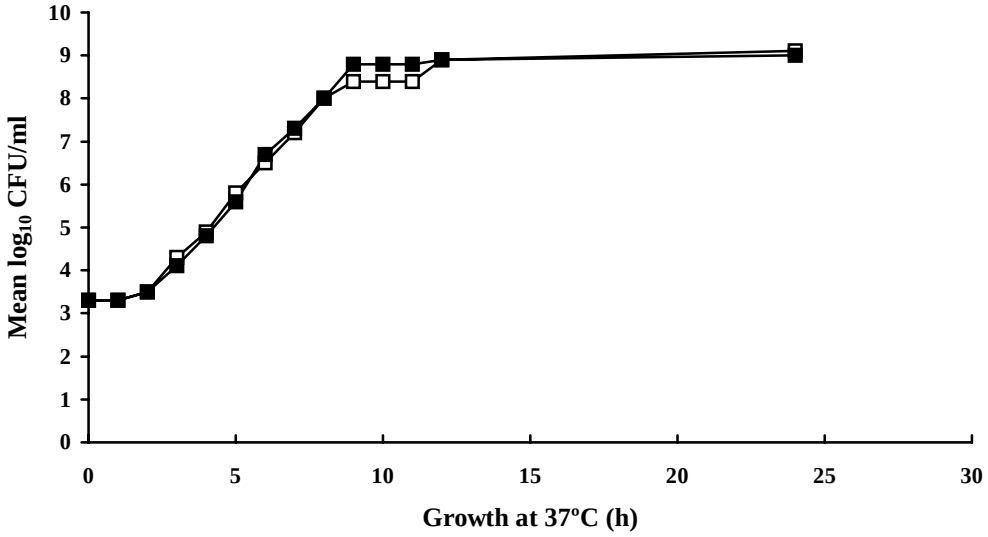

FIGURE 1. *Comparison of growth of* Salmonella *Montevideo (□) and a rifampicin- resistant derivative (■) over a 24-h period during incubation at 37°C.*

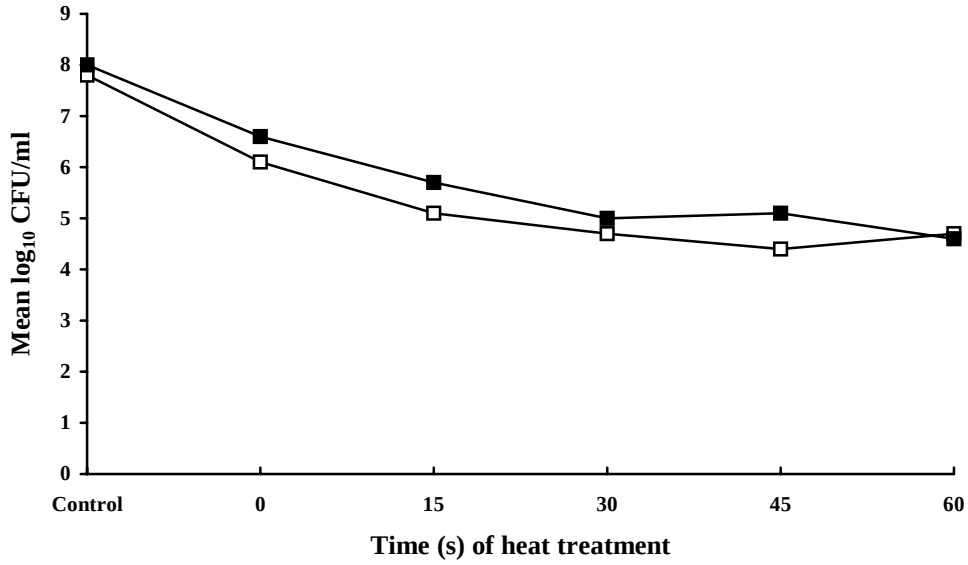

FIGURE 2. *Survival of* Salmonella *Montevideo (□) and a rifampicin-resistant derivative (■) during heating in a water bath at 60°C for 60 sec.*

Lactic acid tolerance. *Salmonella* Montevideo and the rifampicin-resistant derivative were exposed to 0.5% lactic acid to determine similarities in response to acids as well as subsequent sublethal injury of the organisms (Figure 3). A population of bacteria after exposure to a stress environment contains three physiologically different types of cells: the uninjured cells that are capable of growth and multiplication equally well in selective and nonselective culture medium, the injured cells that are capable of multiplication in a non-selective medium but not in a selective medium, and the dead cells, which are incapable of growth under any condition *(51)*. Sublethally injured cells were enumerated by plating treated samples on TSA + 2% NaCl. The resulting differences in counts between TSA plates and TSA + 2% NaCl specify bacterial cells with sublethal injury. The cell death of both organisms over 120 sec of contact with 0.5% lactic acid was not significantly different ($P<0.05$), with a 1.5 and 1.6 $\log_{10}$ CFU/ml reduction for the rifampicin-resistant strain and the parent strain, respectively. The rifampicin-resistant strain initially exhibited a lesser stress response than the parent strain, meaning that microbial counts were not immediately lower for samples plated on TSA + 2% NaCl. However, both organisms plated on TSA + 2% NaCl did decrease to counts below the detectable limit of 1 $\log_{10}$ CFU/ml after 120 sec of exposure to the lactic acid solution. This trial indicates that the two *Salmonella* strains exhibit similar responses when in contact with lactic acid; however, the sublethal injury of the organisms was not comparable.

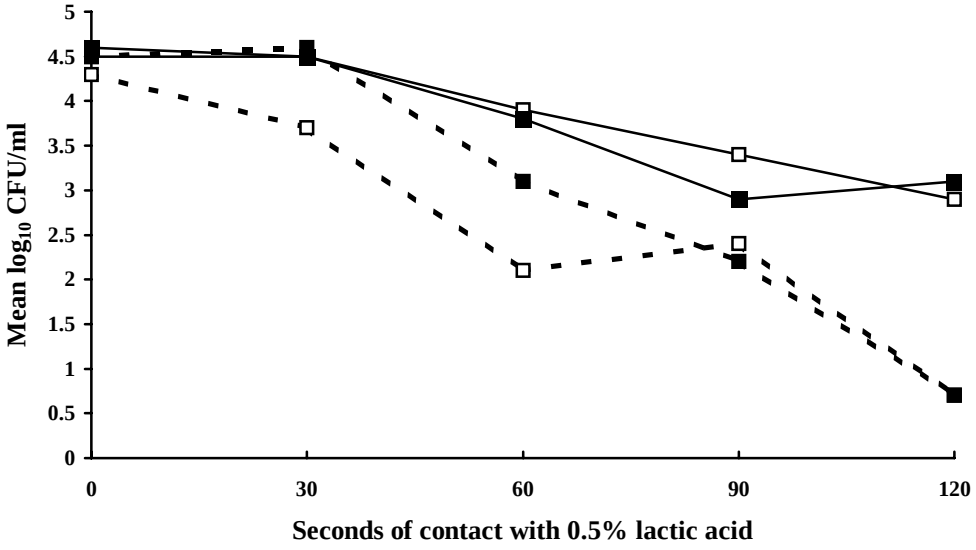

FIGURE 3. *Death and stress determination of* Salmonella *Montevideo on TSA (-□-) or TSA + 2% NaCl (- -□- -) and a rifampicin-resistant derivative on TSA (-■-) or TSA + 2% NaCl (- -■- -) following exposure to 0.5% lactic acid for 120 sec.*

Effect of Irradiation upon Microbial Survival in Fresh-cut Tomatoes

Initial effects of irradiation upon microbial populations. The initial effects of irradiation treatments on tomato cubes and stem scars are shown in Table 1. E-beam irradiation treatment of tomato cubes resulted in a 1.3 and 2.8 $\log_{10}$ CFU/ml reduction of lactic acid bacteria (LAB) for 0.7 kGy and 0.95 kGy doses, respectively. However, these two doses of irradiation did not differ statistically in their effect; this may be attributed to the high standard deviation within the 0.7 kGy treatment group. Yeast and mold counts on tomato cubes fell below the lowest detectable limit of 10 CFU/g after treatment with irradiation. Interestingly, yeasts were not as affected by the higher dose of irradiation as the lower dose. This could be due to a greater initial yeast load on the samples evaluated.

Based on the premise that stem scars have the capability to harbor and protect microorganisms from sanitizing treatments, stem scars were treated and analyzed separately from cubes to explore any protective elements displayed by this region of the tomato. Yeasts were significantly reduced, but again, there was not a difference between the irradiation doses. LAB and mold counts were not affected by irradiation treatment, with counts actually being higher on samples treated with either dose for molds and the 0.7 kGy dose for LAB. These results are not uncommon. It has been reported that yeasts, molds, and gram-positive spoilage organisms, such as lactobacilli and lactococci, are more resistant to irradiation than are gram-negative organisms, such as *Salmonella (33)*. This greater resistance may result in a drastic change in the dominant microflora in a food product treated with a low dose of irradiation. The size of the initial population

TABLE 1. *Comparison of microbiological effects of electron beam irradiation upon fresh-cut tomato cubes versus stem scars*

		Log CFU/g after dose application:		
		0.0 kGy	0.7 kGy	0.95 kGy
LAB	Cubes[a]	3.9 ± 1.11 AY[cd]	2.6 ± 1.86 ABY	1.1 ± 0.67 BY
	Stem scars[b]	4.0 ± 0.48 AY	4.3 ± 1.13 AY	2.9 ± 0.80 AY
Yeasts	Cubes	2.3 ± 0.60 AY	<1.0 BY	1.3 ± 0.24 BY
	Stem scars	4.8 ± 0.67 AZ	1.7 ± 0.87 BY	2.5 ± 0.36 BZ
Molds	Cubes	1.6 ± 0.15 AY	<1.0 BY	<1.0 BY
	Stem scars	1.5 ± 0.15 AY	2.0 ± 0.61 AZ	2.0 ± 0.52 AZ

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g, counts reported as <1.0 $\log_{10}$CFU/g were below the detectable limit of 10 CFU/g.
[d] Means within rows for each treatment group (0 kGy, 0.7 kGy, 0.95 kGy) followed by same letter (A, B) are not significantly different (P>0.05); means within columns for each treatment group (tomato cubes, stem scars) followed by same letter (Y, Z) are not significantly different (P>0.05).

of fungi, whether in the form of spores or mycelial cells, may also greatly influence the radiation dose required to inactivate most of a population of identical cells *(1)*. Therefore, if the fresh-cut tomato cubes or stem scars already had a high load of spoilage organisms prior to treatment, the effectiveness of irradiation could have been limited.

LAB counts were actually higher on stem scars for all treatment groups, although statistically there were no differences in counts between sample types for each irradiation treatment group. Yeast counts were significantly greater for control stem scar samples and those treated with 0.95 kGy when compared to tomato cube populations. Mold counts for stem scars did not decrease to a level below the detectable limit, as did the tomato cube mold populations at 0.7 and 0.95 kGy, despite both sample types having a similar mold population on controls. Overall, fresh-cut tomato cubes treated with low dose irradiation experienced greater reductions in microbial counts than stem scars and there were differences in some microbial populations between the sample types for both doses. Differences in the effects experienced on tomato cubes versus that of stem scars became more common with increasing doses of irradiation. These results support the likelihood that the stem scar region of tomatoes may provide a protective effect for microorganisms on tomatoes. The water content of stem scars may be lesser than that of tomato tissues, which in turn could have limited the secondary effects of irradiation. Native microflora present on tomato stem scars, such as LAB, yeasts and molds, could also have been protected from irradiation by the formation of biofilms.

Irradiation effects upon *Salmonella* serotypes. It is known that serotypes of *Salmonella,* as well as other pathogens, can behave differently when exposed to ionizing

radiation, resulting in a broad range of D_{10} values. The effective dose is also largely dependent on the food product itself. Data in Table 2 shows the counts of *Salmonella* Montevideo and *Salmonella* Agona on tomato cubes and stem scars as affected by the dose of e-beam irradiation. *Salmonella* Montevideo counts on tomato cubes were significantly reduced ($P<0.05$) by 1.8 log cycles after treatment with 0.7 kGy, while 0.95 kGy reduced the population by 2.2 log cycles. The difference between these log reductions was significant ($P<0.05$). *Salmonella* Montevideo counts did not differ between treatments with either dose of irradiation on stem scars, although they were significantly different from the control by 2.4 $\log_{10}$ CFU/ml. *Salmonella* Agona counts on stem scars were significantly reduced by 1.3 and 2.2 log cycles for treatment with 0.7 and 0.95 kGy, respectively. However, log reductions of *Salmonella* Agona populations on tomato cubes, while different from the control, were not significantly different between the two irradiation doses. The log reductions of both *Salmonella* serotypes were not as microbiologically significant as expected. It is possible that this is due to the large initial *Salmonella* populations. Inoculating the tomato samples with a *Salmonella* population of 8.0 log CFU/ml may have inhibited microbial destruction, whereas a lesser population may have been more greatly reduced by irradiation.

The statistical comparison of irradiation effects on samples inoculated with *Salmonella* Montevideo and *Salmonella* Agona is also present in Table 2. Although the initial *Salmonella* populations on untreated tomato cubes differed for the two serotypes, the populations on irradiated tomato cubes were not significantly different after treatment with either dose of irradiation. Stem scars also had slightly differing control

TABLE 2. *Comparison of effects of electron beam irradiation on populations of* Salmonella *Montevideo and* Salmonella *Agona on fresh-cut tomatoes*

	Cubes[a]			Stem scars[b]		
	0 kGy	0.7 kGy	0.95 kGy	0 kGy	0.7 kGy	0.95 kGy
Salmonella Montevideo	7.2 ± 0.23 AY[cd]	5.4 ± 0.21 BY	5.0 ± 0.17 CY	7.1 ± 0.02 AY	4.7 ± 0.10 CY	4.7 ± 0.13 CY
Salmonella Agona	6.7 ± 0.11 AZ	5.4 ± 0.09 BY	5.2 ± 0.07 BY	6.8 ± 0.11 AZ	5.5 ± 0.16 BZ	4.6 ± 0.38 CY

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g.
[d] Means within rows followed by same letter (A, B, C) are not significantly different (P>0.05); means within columns followed by same letter (Y, Z) are not significantly different (P>0.05).

populations and exhibited a significant difference in pathogen counts at 0.7 kGy. *Salmonella* Montevideo populations on tomato stem scars experienced a 2.4 $\log_{10}$ CFU/g reduction after treatment with 0.95 kGy, while the reduction of *Salmonella* Agona was of 2.2 $\log_{10}$ CFU/g.

Data analysis also revealed that although tomato cubes and stem scars inoculated with *Salmonella* Montevideo contained similar populations on control samples, there were significant differences in reductions experienced at 0.7 kGy for the two sample types, with stem scars receiving a greater log reduction. For samples inoculated with *Salmonella* Agona, pathogen populations on stem scars differed significantly from tomato cubes after treatment with 0.95 kGy; however, all other sample groups responded similarly to irradiation treatment.

Inoculated fresh-cut tomatoes were sampled over 15 days of storage at 4ºC after irradiation treatment (Table 3). Samples inoculated with *Salmonella* Montevideo showed more differences in microbiological counts between irradiation doses than samples inoculated with *Salmonella* Agona. However, these differences were not likely due to greater log reductions by the 0.95 kGy irradiation dose. Rather, a larger *Salmonella* population was frequently recovered from the 0.95 kGy treated cubes and stem scar samples than the 0.7 kGy samples. It is possible that this phenomenon occurred due to the greater destruction of the native tomato microflora at 0.95 kGy, thereby reducing any potential antagonistic effect against the pathogen. Some foodborne organisms produce substances that are either inhibitory or lethal to others microbes *(28)*. LAB are known for their antagonistic effect by the production of bacteriocins, pH

TABLE 3. *Mean populations of* Salmonella *Montevideo and* Salmonella *Agona over 15 days of storage at 4ºC after treatment with different doses of electron beam irradiation on fresh-cut tomato cubes and stem scars*

Sample Type	Day of Storage	*Salmonella* Montevideo			*Salmonella* Agona		
		0.0 kGy	0.7 kGy	0.95 kGy	0.0 kGy	0.7 kGy	0.95 kGy
Cubes[a]	0	7.2 A[cd]	5.4 B	5.0 C	6.7 A	5.4 B	5.2 B
	3	6.2 A	3.8 B	4.8 C	6.5 A	5.0 B	4.7 B
	6	5.7 A	3.6 B	4.5 C	6.4 A	4.5 B	4.7 B
	9	5.9 A	3.3 B	4.3 C	6.4 A	4.4 B	4.3 B
	12	5.7 A	3.4 B	3.7 B	6.2 A	4.1 B	3.6 C
	15	5.4 A	2.9 B	3.5 C	5.9 A	3.8 B	3.7 B
Stem scars[b]	0	7.1 A	4.7 B	4.7 B	6.8 A	5.5 B	4.6 C
	3	6.7 A	3.8 B	4.6 C	6.6 A	4.9 B	4.9 B
	6	5.9 A	2.7 B	4.6 C	6.4 A	4.7 B	4.3 C
	9	5.8 A	2.8 B	4.0 C	6.6 A	4.2 B	4.3 B
	12	5.5 A	2.9 B	3.5 C	6.4 A	3.8 B	3.7 B
	15	4.9 A	2.5 B	3.6 C	5.6 A	3.6 B	3.5 B

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g.
[d] Means within rows for each sample type (cubes or stem scars inoculated with *Salmonella* Montevideo or *Salmonella* Agona) followed by same letter (A, B, C) are not significantly different (P>0.05).

depression, organic acids, H_2O_2, diacetyl, and nutrient depletion. These bacteria have been shown to inhibit proliferation or survival of pathogens *(28)*. E-beam irradiation at 0.95 kGy reduced a greater population of LAB than the 0.7 kGy dose, thereby lowering any inhibitory effects *Salmonella* may have received as a result of LAB proliferation on the samples treated with the higher dose. Few studies have been conducted on the effect of low radiation doses on the microbial ecology of indigenous microflora with respect to inoculated pathogens; loss of rare individuals within bacterial populations, effect on species present in low densities, or other undefined changes in bacterial population structure and function are typical concerns when considering the impact of antimicrobial measures *(37)*. In fact, there is concern regarding irradiated foods that the suppression of indigenous microflora by irradiation could lead to increased pathogen growth during storage *(37)*.

Salmonella Agona was significantly reduced on inoculated tomatoes by irradiation in comparison to non-treated tomatoes regardless of sample type throughout storage. However, there were significant differences between the doses of 0.7 kGy and 0.95 kGy at only a few storage days, although in these instances the counts for the higher irradiation dose were less than that of the lower dose, contrary to the effect observed for *Salmonella* Montevideo. It cannot be concluded, however, that the differences observed for the two organisms over the storage period, although statistically significant, represent a biological phenomenon of variation in response to ionizing radiation. The variation in the natural microbial load present on tomato samples may have altered the effects of irradiation upon *Salmonella* populations.

The survival of the *Salmonella* serotypes over the storage period is of microbiological significance. As a possible explanation, sublethal injury should be considered when examining the resulting microbial populations of irradiated samples over a storage period. Irradiation at low dose levels might not kill all cells but instead cause injury. In fact, the injured population can constitute a very high proportion of the total-surviving bacteria, up to 99% and more *(52)*. Given the appropriate conditions, these microorganisms can repair themselves and multiply *(41)*. However, all samples in this study were subject to a storage temperature of 4ºC, and due to the mesophilic nature of *Salmonella*, is was not possible for the cells to multiply; however, there was a microbiologically significant population of both serotypes still surviving on tomato samples after 15 days of storage. The weakening of plant tissues, as well as the availability of nutrients from chopped tomatoes, likely aided in cell survival.

Another mechanism for the survival of pathogens over storage at 4ºC in this study is hypothesized. Yeasts and LAB occur simultaneously in many natural food habitats because they have many common ecological determinants *(19)*. Yeasts are normally not as important in the spoilage of vegetables as LAB, although activity of yeasts becomes apparent when environment conditions are favorable, such as during the lactic acid fermentation of vegetables *(19)*. Yeasts presumably use by-products of LAB metabolism as energy sources, while bacteria depend on several growth factors supplied by yeasts. Spoilage by yeasts results from fermentative activity; in turn, molds, many of which can utilize ethanol and simple sugars as sources of energy, then grow and eventually degrade structural polysaccharides *(6)*. The growth of yeasts and molds on

tomato tissues may result in an increased pH due to their utilization of organic acids, such as citric and malic acids.

Yeasts and molds have a competitive advantage over bacteria on bruised tissues of acidic vegetables because they are able to grow at a lower pH range (2.2-5.0) *(6)*. In this study, the presence of LAB and molds caused shifts in pH, with LAB providing a more acidic environment, presumably from lactic acid production, while molds would subsequently raise the pH of the tomatoes. This competition between microbes kept the pH of the samples from much variation, with the exception of control stem scars, with values not rising far above or below the normal range for tomatoes. This result coincides with studies implying that there is a metabiotic relationship between molds and bacteria *(34)*. *Salmonella* exhibited tolerance to an acidic environment, while mold proliferation maintained a steady pH on which the pathogens could survive.

Effects of storage on microbial counts and pH over 15 days. LAB counts showed great variability throughout storage (Table 4). The most erratic LAB counts were found on samples treated with 0.7 kGy, regardless of sample type. The statistical analysis revealed that the initial LAB populations (Day 0) were not affected by irradiation as significantly as those enumerated throughout storage. This outcome could be due in part to sublethal injury of LAB cells. Similar to the results found for *Salmonella,* there were more significant differences found between control populations and those that had been irradiated, regardless of dose, than differences between doses themselves. Stem scars treated with 0.95 kGy had greater LAB counts than those treated

with 0.7 kGy on analysis days 3, 6, 12 and 15. Overall, reductions in counts did persist throughout storage for irradiated samples when compared to the control.

These results coincide with those reported by Howard et al. on irradiated pico de gallo containing chopped tomatoes, onions and jalapeno peppers *(26)*. Irradiation at 1 kGy suppressed the growth of total lactobacilli enumerated using MRS agar for up to 4 weeks of storage at 2ºC. Rather than crediting the consistently low LAB counts in the study to sublethal injury effects, the authors hypothesized that the low populations of lactobacilli were attributed to the gas atmosphere in the container, which had oxygen levels of 14-18% and carbon dioxide at 3.5-5.6%. This is based on the preference of lactobacilli for much greater carbon dioxide levels *(26, 10)*. Although the gas composition of the packaging used in this experiment was not quantified, these theories may be applicable for the samples used in this study. The wounding of tomatoes by chopping as well as irradiation must have increased the respiration rate, thereby providing a more hospitable environment for LAB proliferation. However, the oxygen transmission rate of the PETE containers may have been high, allowing for a greater concentration of oxygen to remain in the sample container that was not being consumed by the microorganisms present. In addition, the low concentrations of carbon dioxide could have stimulated yeast growth *(19)*.

Data in Table 5 shows the yeast counts of tomato cubes and stem scars during refrigerated storage, with a steadily increasing yeast population regardless of irradiation dose. Storage temperature did not suppress yeast proliferation since the minimum growth temperature for most yeast genera is 0ºC *(19)*. Mean populations for control

TABLE 4. *Mean populations of lactic acid bacteria (LAB) over 15 days of storage at 4ºC after treatment with different doses of electron beam irradiation on fresh-cut tomato cubes and stem scars*

		LAB CFU/g after dose application:		
Sample type	Day of Storage	0.0 kGy	0.7 kGy	0.95 kGy
Cubes [a]	0	3.9 A[cd]	2.6 A	1.1 B
	3	3.5 A	1.5 B	1.6 B
	6	3.1 A	2.1 AB	1.1 B
	9	6.0 A	3.5 B	2.1 B
	12	5.7 A	3.5 B	1.2 C
	15	5.0 A	2.3 B	2.3 B
Stem scars [b]	0	4.0 A	4.3 A	2.9 A
	3	3.2 A	<1.0 B	1.6 B
	6	2.5 A	<1.0 B	1.6 AB
	9	4.0 A	3.6 AB	2.4 B
	12	5.1 A	1.4 B	1.8 B
	15	4.1 A	1.8 B	2.9 AB

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g, counts reported as <1.0 $\log_{10}$CFU/g were below the detectable limit of 10 CFU/g.
[d] Means within rows for each sample type (cubes or stem scars) followed by same letter (A, B, C) are not significantly different (P>0.05).

samples reached 8.9 and 9.2 $\log_{10}$ CFU/g by day 15 of storage. While LAB are usually the main cause of spoilage in ready-to-eat vegetable products, it is known that yeasts also increase in numbers with storage time *(19)*. Yeast growth could visually be observed on control tomato cube and stem scars samples by day nine of storage. Irradiated samples did not present visible growth until 15 days of storage (Figure 4), indicating that irradiation may be used for preserving product quality as well as safety. Viable cell counts on irradiated samples were significantly different than control samples, regardless of sample type. However, similar to results for *Salmonella* and LAB, differences between effects caused by the two doses of irradiation occurred only twice, on storage days 9 and 15. For all irradiated samples, yeast counts reached levels of at least 6.9 $\log_{10}$ CFU/g by day 15 of storage. These results are similar to those reported in a study by Beuchat and Brackett *(7)* where chopped tomatoes were analyzed for total populations of yeasts and molds over 8 storage days at 10ºC. Samples that were packaged under ambient air atmospheres experienced an increase in yeast and mold populations by at least 3.5 log cycles by day 8 of storage.

Overall, yeast counts were initially reduced but quickly recovered, continuing on an upward trend for the remainder of the study. Deak et al. reported a similar result on irradiated whole cob sweet corn. They demonstrated that doses of 1 kGy or less reduced the total aerobic count on whole cob sweet corn more than it did yeasts; in addition, populations of yeasts recovered sooner than bacterial populations *(9, 18)*. The initial reduction of yeasts was greater for stem scars than for tomato cubes, with cubes

TABLE 5. *Mean populations of yeasts over 15 days of storage at 4ºC after treatment with different doses of electron beam irradiation on fresh-cut tomato cubes and stem scars*

		Yeast CFU/g after dose application:		
Sample type	Day of Storage	0.0 kGy	0.7 kGy	0.95 kGy
Cubes [a]	0	2.3 A[cd]	<1.0 B	1.3 B
	3	3.3 A	2.4 B	2.1 B
	6	5.5 A	3.7 B	3.2 B
	9	7.8 A	5.2 B	5.8 B
	12	8.3 A	6.1 B	5.3 B
	15	8.9 A	7.0 B	7.0 B
Stem scars [b]	0	4.8 A	1.7 B	2.5 B
	3	6.2 A	2.7 B	2.4 B
	6	8.0 A	3.8 B	4.4 B
	9	8.3 A	5.1 B	7.1 C
	12	9.0 A	6.9 B	6.4 B
	15	9.2 A	6.9 B	7.8 C

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g, counts reported as <1.0 $\log_{10}$CFU/g were below the detectable limit of 10 CFU/g.
[d] Means within rows for each sample type (cubes or stem scars) followed by same letter (A, B, C) are not significantly different (P>0.05).

A.

B.

FIGURE 4. *Control tomato cubes (A) and tomato cubes treated with 0.7 kGy electron beam irradiation (B) after 12 days of storage at 4°C.*

experiencing a reduction of only 1.6 and 1.0 log cycles for 0.7 kGy and 0.95 kGy, respectively. For both tomato sample types, a peak in yeast populations occurred at day 9 followed by a drop in counts for day 12. This peak on storage day 9 coincides with a rise in LAB counts and pH as well as a drop in mold populations.

The mean mold populations for samples irradiated at 0.0 kGy (control), 0.7 kGy and 0.95 kGy over 15 days of storage at 4ºC can be observed in Table 6. Although there was much variability in counts over time for both tomato cubes and stem scars, the counts did not differ by more than one log at any time between irradiation doses for cubes. Stem scar samples, however, had a much greater amount of variability, which could be due in part to the differences in the initial microbial load of each individual sample. As mentioned previously, the initial population size of mold on samples has been shown to cause great discrepancy in the radiation dose required to inactivate all or most of a population *(1)*.

Stem scar samples also had more significant differences in populations when compared to the control than tomato cubes, however, a variation was present between irradiation doses 0.7 and 0.95 kGy for stem scars on storage days 6 and 15 only. These and other statistical data for mold counts should not be overly interpreted, however, considering the small range in log values between samples. Mold growth for control samples and those irradiated at 0.7 and 0.95 kGy did not follow a steady upward trend, as did yeasts, although the mold observed visually on the tomato pieces increased with storage day. Starting on storage day 12, control samples and those treated with 0.7 kGy were covered in black and yellow mold. However, samples treated with 0.95 kGy did

TABLE 6. *Mean populations of molds over 15 days of storage at 4°C after treatment with different doses of electron beam irradiation on fresh-cut tomato cubes and stem scars*

		Mold CFU/g after dose application:		
Sample type	Day of Storage	0.0 kGy	0.7 kGy	0.95 kGy
Cubes [a]	0	1.6 A[cd]	<1.0 A	<1.0 A
	3	<1.0 A	<1.0 A	<1.0 A
	6	1.7 A	1.0 A	1.2 A
	9	<1.0 A	<1.0 A	<1.0 A
	12	<1.0 A	1.6 B	<1.0 A
	15	1.0 A	<1.0 A	<1.0 A
Stem scars [b]	0	1.5 A	2.0 A	2.0 A
	3	2.8 A	2.0 B	2.0 AB
	6	<1.0 A	1.2 A	2.4 B
	9	<1.0 A	1.6 B	1.4 AB
	12	<1.0 A	1.8 B	1.8 B
	15	1.3 A	<1.0 A	2.6 B

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] All microbiological counts reported as $\log_{10}$ CFU/g, counts reported as <1.0 $\log_{10}$CFU/g were below the detectable limit of 10 CFU/g.
[d] Means within rows for each sample type (cubes or stem scars) followed by same letter (A, B, C) are not significantly different (P>0.05).

not present any mold growth visually even after 15 days of storage. Overall, mold counts were not reduced on either type of tomato sample over storage, regardless of irradiation dose. This coincides with reports that mold has a greater resistance to irradiation than that of bacteria *(33)*. For example, the "black yeast", *Aureobasidium pullulans*, is a radioresistant fungus, whose importance considerably increases after irradiation of fruits and vegetables. This radio-resistance is attributed to its polymorphism; in its older stages and under starvation conditions, it produces radio-resistant chlamydospores and a resting mycelium *(1)*.

The pH of all samples was measured prior to microbiological sampling on each analysis day (Table 7). Significant differences in pH values occurred only after 9 days of storage at 4ºC. As mentioned previously, these values had a direct correlation with the proliferation of spoilage organisms. Some variability did occur between samples of each treatment group, with tissue closer to the stem scar consistently giving a higher pH reading than the flesh of the tomato samples. The pH measurements taken were a helpful tool in resolving issues between varying counts of spoilage organisms over the storage period.

TABLE 7. *Mean pH measurements of tomato cubes and stem scars inoculated with* Salmonella *Montevideo or* Salmonella *Agona over 15 days of storage at 4ºC after treatment with different doses of electron beam irradiation*

Sample Type	Day of Storage	*Salmonella* Montevideo			*Salmonella* Agona		
		0.0 kGy	0.7 kGy	0.95 kGy	0.0 kGy	0.7 kGy	0.95 kGy
Cubes[a]	0	4.44 A[c]	4.42 A	4.53 A	4.47 A	4.48 A	4.43 A
	3	4.14 A	4.28 A	4.36 A	4.32 A	4.27 A	4.29 A
	6	4.06 A	3.99 A	4.12 A	4.04 A	4.10 A	4.06 A
	9	4.23 A	3.81 B	4.01 AB	4.05 A	4.16 A	3.93 A
	12	3.66 A	3.90 AB	3.94 B	4.21 A	4.27 A	3.92 B
	15	4.43 A	4.07 B	4.21 AB	4.31 A	4.25 A	4.12 A
Stem scars[b]	0	4.67 A	4.60 A	4.46 A	4.45 A	4.40 A	4.55 A
	3	4.17 A	4.19 A	4.21 A	4.32 A	4.20 A	4.25 A
	6	4.00 A	3.98 A	4.05 A	3.74 A	3.94 A	3.96 A
	9	5.75 A	4.04 B	3.99 B	4.43 A	3.95 B	3.93 B
	12	6.13 A	4.58 B	4.25 C	4.15 A	3.98 A	4.14 A
	15	7.51 A	4.92 B	4.24 C	4.51 A	4.67 A	4.24 B

[a] Fresh tomatoes cut into pieces approximately 1.5 cm per side.
[b] Stem scar areas of fresh tomatoes cut to a depth of approximately 1.5 cm.
[c] Means within rows for sample types (cubes or stem scars inoculated with *Salmonella* Montevideo or *Salmonella* Agona) followed by same letter (A, B, C) are not significantly different (P>0.05).

CONCLUSIONS

Low dose electron beam irradiation reduced populations of two *Salmonella* serotypes in fresh-cut tomatoes. The differences observed in irradiation effects between stem scars and tomato cubes coincide with theories that tomato stem scars can harbor and protect microorganisms. LAB, yeasts and molds all exhibited a greater resistance to irradiation than *Salmonella*, which was consistent with results found by other researchers. The lack of difference in microbial log reductions between the irradiation doses of 0.7 kGy and 0.95 kGy proved to be the most unexpected result in this study.

The similarities in microbial reductions by the irradiation doses raise an interesting question. How could an increase of 0.25 kGy in the irradiation dose not produce a greater level of microbial destruction? There are several possible answers to this question, none of which can be proved without further research. Firstly, the opportunity for error in the treatment of samples with electron beam irradiation is great. There could possibly be inefficiencies in the power being distributed by the linear accelerators themselves. This is accounted for by dosimetry, but how reliable are the alanine pellets used in dosimetry for low doses of irradiation? Also, it is concluded that there are some biological effects taking place during the irradiation of fresh-cut tomatoes, as well as other fresh produce, that are not fully implicit. Relationships between the microorganisms quantified in this research raise important questions about irradiation treatment on fresh-cut produce. It is suspected that higher doses of irradiation inhibit antagonistic behavior of tomato microflora upon pathogens.

This study demonstrated the potential of ionizing radiation for decontaminating fresh-cut produce. It was hypothesized that the higher dose of irradiation would produce a greater kill of microorganisms that the 0.7 kGy dose. However, neither dose used achieved more than a 2.4 $\log_{10}$ CFU/g reduction of *Salmonella* on fresh-cut tomato cubes and stem scars. A higher dose is needed to achieve larger reductions of pathogen populations and would subsequently increase shelf life through a greater reduction in spoilage microflora. However, along with higher dose is the possible reduction in sensory quality of produce. Further studies are needed to determine if the higher doses required for decontamination will also negatively affect objective parameters such as color and texture.

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