To the Graduate Council:

I am submitting herewith a thesis written by Amelia J. Evans entitled "Survival of *Listeria*, *Staphylococcus* and *Salmonella* on Sliced Cantaloupe and Tomatoes Treated with 1.0 and 3.0 kGy Using a 5 MeV Electron Beam Accelerator." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Greg J. Hulbert, Major Professor

We have read this thesis
And recommend its acceptance:

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Survival of *Listeria*, *Staphylococcus*, and *Salmonella* on Sliced Cantaloupe and Tomatoes Treated with 1.0 and 3.0 kGy Using a 5 MeV Electron Beam Accelerator

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Abstract

This research was conducted to determine microorganism survival and shelf life extension for sliced cantaloupe and tomatoes treated with electron beam irradiation. In the first study, the survival of *Listeria* and *Staphylococcus* in sliced cantaloupe held at 4 °C and treated with 1.0 and 3.0 kGy was examined along with the shelf life extension of irradiated cantaloupe slices. Serial dilutions were plated on standard methods agar (SMA) for aerobic plate count, RoseBengal agar with supplement C (RBA-C) for yeast and mold, violet red bile agar (VRB-MUG) for coliforms, Baird Parker agar with egg yolk telurite (BPA) for *S. aureus,* and PALCAM with Ceftazidmine for *Listeria*. Samples (25 g) were enriched in *Listeria* enrichment broth and streaked onto PALCAM agar. The shelf life study involved two experienced panelists using a visual rating scale. *Listeria* and *Staphylococcus* survival was significantly ($P<0.05$) inhabited by 3.0 kGy. Additionally, 3.0 kGy increased the shelf life of cantaloupe slices while not effecting texture quality.

In the second study, the survival of *Listeria* and *Salmonella* on tomato slices stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation was examined. Additionally, the shelf life extension of irradiated tomato slices was also examined utilizing two experience panelist using a visual rating scale. Samples (25 g) were enumerated on six different media. Samples were also enriched for *Listeria* on PALCAM agar and *Salmonella* on Hektoen Enteric and Bismuth Sulfite agar. The survival of Listeria and Salmonella was significantly

($P<0.05$) inhibited by 3.0 kGy. Positive confirmation of *Listeria* and *Salmonella* at the 3.0 kGy treatment during enrichment indicate that enrichment procedures must also be used to ensure the destruction of microorganisms. Additionally, 1.0 and 3.0 kGy irradiation can extend the shelf life of tomatoes.

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Part I

Literature Review

Introduction

Food processors want to increase the shelf life and safety of their products. Changes in processing, packaging, and shipping technologies have enabled an increase in consumer access to otherwise seasonal or regional products. Associated with the increase in availability of food products is an increase in the risk of contamination (Beuchat et al., 1997; Satin, 1996). Pathogenic microorganisms can contaminate food in the field, during slaughter or harvest, and during processing and packaging. The USDA ranked food related illnesses due to foodborne pathogens as the number one problem associated with food while economic losses are in the billions. In the United States alone, foodborne infections cause millions of illnesses and an estimated 9,000 deaths annually. Foodborne diseases are considered a global health and economic problem (FAO/WHO, 1983;Tauxe, 1997).

On May 6, 2000, President Clinton initiated an aggressive strategy to combat *L. monocytogenes* in ready to eat foods due to a high fatality rate associated with listeriosis with approximately 20 % fatality out of 2500 cases (Clinton, 2000). *Listeria spp.* and *Toxoplasma* are responsible for 75% of deaths from pathogens while the most frequent cause of intoxication is *Staphylococcus aureus* (Mead et. al., 1999). Additionally, the Center for Disease Control, the USDA, and the FDA have identified *Salmonella spp.*, *L. monocytogenes,* and *Campylobacter* to be the three most common infectious foodborne pathogens (USDA/DHHS, 1998).

Ionizing radiation can be used to increase shelf life (Loaharanu, 1994). With fresh fruits and vegetables, radiation can provide an alternative to chemical additives while controlling disease by eliminating many microorganisms that may be present (Monk, 1995). While irradiation of foods does offer the advantage of reducing disease causing microorganisms and increasing shelf life, limitations on use of irradiation must be imposed due to the susceptibility of fruits and vegetables to free radical formation during irradiation (Kader, 1986).

Food irradiation is not a new concept but consumer perception has made the food industry hesitant to try the technology. However, irradiation has received increasing interest from food processors in recent years due to the demand for pathogen free foods (Pohlman et al., 1994). In reality, ionizing energy has been used for over thirty years as an alternate preservation technique for spices and other foods, especially outside the U.S. (Monk et al., 1995). Exposing food to this ionizing energy serves to destroy bacterial cells resulting in a reduction of post harvest spoilage (thus extending shelf life) and improve by reducing pathogenic microorganisms while providing an alternative to chemical treatments (Loahranu, 1994). Physical processes such as heat and cold treatments have many limitations (Loaharanu, 1994). Irradiation provides a viable alternative to food processors for foods which are not heated or frozen for preservation (Kader, 1986).

Food Irradiation

Two primary forms of irradiation are currently used on food. Traditionally, gamma irradiation has been used due to dose uniformity, availability of cheap gamma sources, ability to penetrate into thick pallets of food since the energy is high. Typically, the irradiation is delivered by a x-ray machine or by a fixed isotopic source in the form of Cobalt-60 or Cs-137. Electron beam accelerators deliver beta rays as a stream of electrons and are less penetrating but lower in cost and have less safety and regulatory concerns (Newsome, 1987; Kader, 1986).

Both types of irradiation have advantages and disadvantages. Gamma irradiation is much more penetrating than beta irradiation and so can be used for thicker food products and to deliver a more uniform dose. Unfortunately fixed isotopic sources present many safety and regulatory challenges because they must be constantly shielded and monitored when not in use. X-ray machines have the advantage of being able to be turned on and off, but are relatively inefficient at delivering radiation doses to the product, when compared to electron beam accelerators. Electron beam accelerators may have safety and process advantages, but produce the least penetrating type of radiation and so may only be used for relatively thin products. Additionally, it is more difficult to get a uniform dose within the product using the electron beam accelerator. Nevertheless, for many applications where safety is a concern and the irradiated product is thin, electron beam irradiation may be the most suitable choice.

Ultimately irradiation by gamma rays, X-rays, and high-energy electrons accomplishes the same goal of killing food-borne pathogens by attacking them at a molecular level (Elias and Cohen, 1977). The effect on food is essentially the same for both types of irradiation given that the food receives the same dose. The biggest difference in the two types of irradiation is the method in which the dose is delivered. Gamma rays are massless, neutral particles that are extremely penetrating and can be effective for delivering a dose deep into a food product. Accelerated electrons have a negative charge and have mass making them interact with the food physically. This means less penetration, resulting in a smaller maximum food thickness suitable for irradiation. This is the primary factor limiting the use of electron beam accelerators for food irradiation. The penetration issue also makes it more difficult to achieve good dose uniformity within a food product when irradiating foods using electron accelerators. Figure 1 shows the process by which the initial electrons create secondary electrons to deliver a dose within the product. Ionizing energy is capable of knocking electrons out of their normal orbits in atoms and molecules creating secondary electrons (Satin, 1996). Secondary electrons deliver large portions of the dose thus, the dose delivered just below the surface of the food will always be higher than the dose at the food surface. The other factor contributing to this effect is that electrons deliver more dose per unit length as they slow down until they lose all penetrating strength.

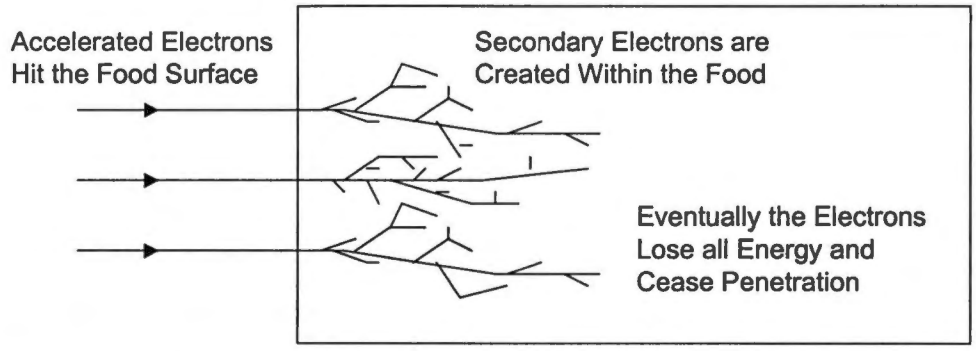

Figure 1. Electron interaction with food.

The penetration issue limits dose uniformity as secondary electrons deliver higher portions of the dose to the product. Ionizing energy used in food products is measured in kGy and consists of three general categories describing the application of irradiation. Low dose is measured up to 1.0 kGy and is used for sprout inhibition and insect desinfestation; medium dose from 1.0 to 10.0 kGy is used to reduce microorganisms; and are used for high dose ranging from 10.0 to 50.0 kGy (Elias and Cohen, 1977; Satin, 1996). The dose issue is significant in that it determines the ideal thickness of a product with the max/min ratio being the limiting factor. Legally, refrigerated meat must be irradiated to a dose ranging from 3.0 to 4.5 kGy allowing for a max/min ratio of only 1.5 (FDA, 1997). Additionally, fruits and vegetables may not exceed 1.0 kGy in total absorbed dose.

Fruits and Vegetables

The consumption of fruits and vegetables has increased with the international trend of consuming fresh food products (Wills et al., 1998). Fruits

and vegetables are susceptible to contamination with pathogenic microorganisms during both pre and post harvest growth on the plant or through the processing stages and transportation as they undergo long journeys to reach market (Beuchat et al., 1995; Mitra, 1997). Microorganisms are of primary concern in the spoilage of fruits and vegetables. Fresh fruit and vegetables are responsible for an estimated 6 % of all foodborne disease outbreaks (Tauxe, 1997). Loss of world wide agricultural production is approximately one-fourth of total global agricultural production due to spoilage by microorganisms (Woods, 1994). In developing countries the food loss reaches in the billions (Satin, 1996). Soil contains many nutrients providing a vehicle of contamination while processing steps also increase exposure of produce to microorganisms. *Listeria monocytogenes* and *Salmonella spp.* are of concern with minimally processed foods (Brackett, 1992). *L. monocytogenes* and *L. innocua* are present in conjunction with feces; *L. ivanovii* and *L. seeligeri* are the most common in soil (Jay, 1996; Petran et al., 1988).

At low levels, gamma irradiation has been shown to improve shelf life of tropical fruits such as mangoes and papayas by delaying the ripening process and destroying Mediterranean fruit flies and larvae (Gholap et al., 1990; Mansour and Franz, 1996). At higher levels, irradiation has been linked to softening of fruits (Spalding et al., 1988). Pectic polysaccharides, cellulose and glucoproteins comprise the major components of the cell walls of fruits and vegetables. Polyscaccharides have been shown to break down after irradiation causing

tissue softening in strawberries and pears (d'Amour, 1993). Gamma irradiation has been linked to the breakdown in cellulose and the hydrolysis of polygalacturonides. This process has also been associated with softening of tissue (d'Amour, 1993). Ultimately, the effect of irradiation on the rate of softening is different for different fruits and vegetables (d'Amour, 1993).

Most research performed with irradiated strawberries was done with gamma rays up to 2.0 kGy (Yu et al., 1995). Electron beam irradiated strawberries at 1 and 2 kGy dosage showed textural softening but an increase in shelf life due to reduction in yeast and mold (Yu et al., 1996). Color of irradiated strawberries tended to be lighter with acidity remaining the same as non irradiated strawberries (Yu et al., 1995)

Irradiation of mangoes is typically used for disinfestation and shelf life extension (Mitchell et al., 1990). Mangoes irradiated at 0.75 and 0.3 kGy were found to have little or no changing chemical composition (Mitchell et al., 1990). Mangoes subjected to hot water treatments plus irradiation at levels of 0.3, 0.6, and 1.0 kGy showed significantly less rotting. This treatment preserved quality and extended shelf life by delaying ripening (FAO/IAE, 1995). The shelf life of mangoes has been shown to increase by 6 days at room temperature when exposed to irradiation at low dosage levels (Gholap et al, 1990). From a quality perspective, irradiated mangoes were judged to be sweeter and more tender while maintaining their vitamin A and vitamin C content when exposed to low dose irradiation (FAO/IAEA 1995). Additionally, microorganisms at 6.0 kGy were

were eliminated, while at 4.0 kGy microorganism were slowed but still maintained minimal growth (FAO/IAEA 1995).

Oranges are subjected to irradiation at treatment doses of 0.30, 0.53, and 1.0 kGy in order to destroy Mediterranean fruit fly larvae and to provided an alternative to the fumigant ethlene dibromide. Results indicated that at levels exceeding 0.15 kGy, changes occurred in texture, aroma, and flavor but no changes occurred in soluble solids (Nagai and Moy, 1985). Below 1.0 kGy in oranges no major effects on appearance were found but at levels exceeding the 1.0 kGy, browning of the skin and major textural and flavor changes do occur in oranges (Elias and Cohen, 1977).

Shredded carrots were exposed to irradiation at 0.5, 1.0, 2.0, and 3.0 kGy. Results revealed an extension of shelf life and an increase in sensory scores, nutritional and microbial quality (Chervin, 1994). The prevention of sprouting is the major concern for potato producers. Potatoes irradiated at the legal maximum of 1.0 kGy showed a reduction in total glycoalkaloids, which indicates sprouting (Mondy and Seetharaman, 1990).

Cantaloupe

Cantaloupe or muskmelons are an orange fleshy melon belonging to the genus of *Cucumis melo* of which quality is measured by sugar content (Hulme, 1971). It is essential to harvest cantaloupes at the full slip stage and to avoid possible compression injuries with treatment. Ethylene can be applied to increase the ripening process (Wills et al, 1998).

Cantaloupes have been associated with numerous outbreaks of salmonellosis in recent years and also suffer form infestation of whiteflies (Castle, et al 1996; Golden, 1993). The rind or outer skin lies directly on the ground and is subject to contamination by animal waste and the soil itself. In California during May 2000 there were 19 reported cases of contamination by *Salmonella Poona*. Irradiation of muskmelons for purposes of quarantine treatment has been widely studied and accepted. Whole melons irradiated at 1.0 kGy showed a decrease in ripening and no injury due to irradiation was found (Lalaguna, 1998).

Tomatoes

Tomatoes, a fleshy berry or swollen ovule, belongs to the genus *Lycopersicon*. Tomatoes are comprised of approximately 95% water, 1% protein, 2% sugars, and 2% fat, potassium, calcium, and dietary fiber (Hulme, 1971; Wills et al., 1998). Ethylene can be used to increase ripening in melons and bananas but tomatoes are not as sensitive (Wills et al., 1998). Delay in ripening has been associated with gamma irradiation treatment due to impairment of cellular function. Radiation induced changes in the ACC metabolism of cherry tomatoes at the green mature stages occurred at 1.0 and 5.0 kGy (Larrigaudiere et al., 1991). Electron beam irradiated tomatoes harvested at the mature green stage showed an initial decrease in firmness at 1.0 and 2.0 kGy with the firmness of the pericarp affected (Assi et al., 1997). At 2 kGy gray mold has been shown to be inhibited (Wills et al., 1998).

General Microorganisms

Foodborne disease causes from 6.5 to 12.6 million illnesses and numerous deaths each year. Estimates for economic loss range from 24 to 81 million dollars (Bean et al., 1990). In the United States alone the estimated annual cases of illness from *Salmonella spp.* (excluding *S. typi*) is 800,000; from Staphylococcus aureus caused 600,000 cases; and from *Listeria monocytogenes* is 25,000. Globally the estimates are higher (Todd, 1989). Sara Lee issued a recall in 1999 (costing $76 million) while Apple Valley estimates its recall due to *L. monocytogenes* to have cost 1 to 7 million (Kuhn, 1999).

Numerous outbreaks of gastroenteritis associated with fresh vegetables and fruits have occurred due to consumption of salads. Listeriosis has been linked to illness from consumer consumption of fresh cabbage, tomatoes, celery, and lettuce (Berrang et al, 1989) while salmonellosis has been attributed to consumption of tomatoes, cantaloupe and watermelon (Beuchat et al., 1995). Salmonellosis has been an increasing health problem and is responsible for approximately 1,000 deaths annually (Tauxe, 1991). Five strains of Listeria spp. (*L. moncytogenes, L. ivanovii, L. seeligeri, L. innocua, and L. welshire*), *Staphylococcus* aureus and two *Salmonella spp. (S. Typhimuriumi*, S. *anatum*) were chosen for the experiment. All of these pathogens are found in fruits and vegetables.

Listeria are gram-positive, nonsporeforming rods (Jay 1997). Listeria can grow within a range from 4.1 to 9.6 pH and at temperatures from 1 to 45C (Jay,

1997). It is capable of surviving in soil for up to 1 year. Thus it has the capability to contaminate fruits and vegetables in the field. Listeria monocytogenes is associated with several forms of illness including limited flu like symptoms, and more series illnesses such as meningitis, miscarriages, and neonatal fatality (Tauxe, 1991). Since *Listeria* has the ability to grow at a wide range of temperatures and pH's, and can survive for extended periods of time on fresh vegetables it was chosen for this study.

Staphylococcus aureus is a gram-positive spherical, coagulase positive bacterium with a diameter of 0.5 to 1.5 mm. It can grow at temperatures from 7 to 47.8 °C (making it mesophilic) and at a pH from 4.0 to 9.83. Entertoxins are produced between 10 and 46 C (Jay, 1996). Due to the high acidity of tomatoes, *Staphylococcus aureus* was inoculated onto cantaloupe only.

Salmonella are widely distributed gram-negative nonsporing rods typically found in the intestinal tract of animals and thus can contaminate soil by way of animal feces (Jay, 1996). The optimum pH range is 6.6 to 8.2 with an expanded range form 4.05 to 9.0 (Jay, 1996). Traditionally, Salmonella has been associated with undercooked meat, poultry or seafood. Even unpasterized milk has been a carrier. In 1992, an outbreak involving apple cider showed that even low acid foods are susceptible (Tauxe, 1997). Additionally, fruit inoculated with Salmonella spp. showed that it could grow well at 22-27 °C (Fernandez et a., 1989). Due to Salmonella's wide growth range and its association with low acid foods, two strains were used with the tomato study.

Purpose of the Study

Consumer demand for produce has increased in recent years. Linked to the increase is an increase in contaminated produce related foodborne pathogen outbreaks. Additionally, food processors such as Strickland's Produce are looking for a convenient way to minimally process sliced fruits and vegetables. Traditionally, studies preformed on produce have focused on whole produce (Brackett, 1999). Irradiation provides for a safe post harvest and post packaging technique to eliminate or reduce bacteria, decontaminate, and extend the shelf life of produce (Kader, 1986). The objective of this study was to determine the effect treatment using that a 5 MeV electron accelerator would have on quality and survival of pathogens in cantaloupe inoculated with 5 strains of *Listeria spp.* and *Staphylococcus aureus.* A second objective was to determined survival of pathogens on sliced green tomatoes inoculated with 5 strains of *Listeria spp.* and 2 strains of *Salmonella spp.* Additionally, the shelf life of the products was determined.

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Part II
Survival of Listeria and Staphylococcus on Cantaloupe Slices Stored at 4 °C and Treated with 1.0 and 3.0 kGy Electron Beam Accelerator

Abstract

Cantaloupes can be contaminated with pathogenic bacteria through contact with the soil and during post-harvest and pre-harvest handling. The purpose of this study was to examine the survival of *Listeria* and *Staphylococcus* on sliced cantaloupe stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation. A second part of this study was to examine if 1.0 and 3.0 kGy irradiation would affect the shelf life of sliced cantaloupes. Serial dilutions of 0.1% peptone water were plated on five different types of agar to enumerate microorganisms. Samples were enriched in Listeria Enrichment Broth (UVM) for 48 hours at 35 °C and then streaked on PALCAM agar and incubated at 35 °C for 24 to 48 hours. *Staphylococcus aureus* and *Listeria* was significantly reduced by treatment with 3.0 kGy. Aerobic plate counts and yeast and mold counts were not affected. The shelf life of sliced cantaloupes was increased by at least 10 days when treated with 3.0 kGy. Irradiation can provide an alternative for food processors wanting to reduce pathogens and extend the shelf life of produce. Regulatory action is needed to permit irradiation of minimally processed fruits at levels required for sufficient destruction of pathogenic bacteria.

Introduction

Fruits and vegetables are highly susceptible to contamination with pathogenic microorganisms during the pre-harvest and post-harvest period or during processing and transportation to reach markets (Beuchat, 1997; Mitra, 1998). Skins and rinds may lie directly on the ground exposing them to bacterial

contamination by animal waste and soil contact. Although skins and rinds of fruits provided an initial barrier to such contamination, rinds of fruits may carry contamination through to the interior during slicing. Sliced cantaloupes provide a perfect medium for foodborne pathogens due to a neutral pH and high water activity. Cantaloupes have been associated in recent years to numerous outbreaks of salmonellosis including *Salmonella Poona*, *Salmonella Chester*, and *Salmonella Javiana.*

Food irradiation is not a new concept. Military foods and the spice industry have used irradiation as an alternative preservation technique for over thirty years (Monk, 1995). In recent years, irradiation has received increasing interest from food processors looking to extend shelf life and comply with zero tolerance policies for certain foodborne pathogens such as *Listeria* and *E. coli* O157H7 (Pohlam, 1994). Traditionally gamma irradiation has been used due to dose uniformity, ability to penetrate thicker food products, and availability of cheap long lasting energy sources. Electron beam accelerators are an available alternative for food processors since they are less expensive and have fewer regulatory problems than gamma irradiation (Newsome, 1987; Kader, 1986).

Listeria spp., especially *Listeria monocytogenes,* are associated with several forms of illness including flu like symptoms, meningitis, miscarriages and neonatal fatalities (Tauxe, 1991). *Listeria* can survive for extended periods of time on fresh fruits and vegetables. *Staphylococcus aureus*, a common cause of food poisoning, can contaminate fruits and vegetables when workers handle the

product since over 50% of humans carry *S. aureus* on their skin or in the upper respiratory system (Jay 1997).

Although studies on have been performed on whole cantaloupes to reduce fruit flies and reduce microorganisms, no studies have been performed on sliced cantaloupes with the purpose of reducing microorganisms and extending shelf life. The purpose of this study was to determine the survival of *Listeria spp* and *S. aureus* on sliced cantaloupe that had been irradiated at 1.0 and 3.0 kGy by a 5 MeV electron accelerator and to determine the effect of irradiation at 1.0 kGy and 3.0 kGy on shelf life extension.

Materials and Methods

Culture maintenance and inoculum preparation

L. monocytogenes (ATCC 7644), *L. seeligeri* (ATCC 35967), *L. ivanovii* (ATCC 19119), *L. innocua* (ATCC 33090), *L. welchiii* (ATCC 35897), and *S. aureus* (ATCC 49444) were used. Cultures were stored refrigerated on tryptose soy agar slants with yeast extract (Difco, Detroit, MI) at 4 °C and were tested for purity before each inoculum preparation inoculation. To prepare inoculum, test strains of bacteria were inoculated into Brain Heart Infusion broth and incubated at 35 °C for 24 hours. Separate cultures were subjected to three successive 24-hour transfers prior to use. Cantaloupe slices were surface inoculated by a syringe with a 5 ml cocktail of all cultures.

Cantaloupe samples

Cantaloupes were purchased from a local retailer. Each cantaloupe was processed and handled individually. Cantaloupes were cut in half along the longitudinal axis. After removing the seed with a spoon, each half was sliced longitudinally by an electric slicer (Rival, Kansas City, MO) into 1 cm slices (O'Conner-Shaw, 1996).

Preparation of cantaloupe samples

Six treatments were performed using a commercial 5 MeV electron accelerator (Biosterile; Ft Wayne, IN). Treatments consisted of 1cm slices of uninoculated and inoculated cantaloupe held as controls which were not irradiated, uninoculated and inoculated slices irradiated at 3.0 kGy, and uninoculated and inoculated cantaloupe slices irradiated at 1.0 kGy. These doses represent doses measured at the surface of the irradiated product. The actual dose within the product will vary with depth. Locations within the product will receive doses higher or lower than the measured surface dose. Three replications were preformed for all experiments. All irradiated samples were irradiated with a 5 MeV self-shielding electron beam food system (BioSterile Technologies, Ft Wayne, IN).

Microbiological analysis

Samples were taken at day 0 and day 10 from all six treatments. For analysis, each sample (approximatly half of each slice) of cantaloupe (25g) was placed in 225 ml of 0.1% peptone water (Difco; Detroit, MI). Each sample was

mixed in sterile stomacher bags (Fisherbrand, Fabrique, Canada) using a Model 400® Lab Blender (Tekmar, Cincinnati, OH) for 60 seconds. Serial dilutions of 0.1% peptone water were plated on five different types of agar to enumerate microorganisms. Standard method agar (SMA) (Difco; Detroit, MI) was used for aerobic plate count, RoseBengal agar with supplement C (RBA-C) (BBL, Sparks, MD) was used for yeast and molds, violet red bile agar (VRB-MUG) (Difco, Detroit, MI) was used for coliforms, Baird Parker agar with egg yolk telurite (BPA) (BBL, Cockeysville, MD) was used for *S. aureus*, and PALCAM with supliment Ceftazidime (Difco, Detroit, MI) was used for *Listeria spp*. Direct plating was performed using the spread plate method of 0.1 ml of the initial dilution. All plates were incubated at 35 °C for 24-48 hours; RBA-C plates were placed in the dark at room temperature for 5 days. *S. aureus* was confirmed after isolates from Baird Parker Agar were placed on tryptose soy agar with yeast extract (TSA-YE) (BBL, Sparks, MD) using coagulase plasma (Difco, Detroit,MI) (FDA, 1997).

Cantaloupes were cut into 25-gram samples and blended in Fisherbrand stomacher bags for 60 seconds in Listeria Enrichment Broth (UVM) (Oxford, Hampshire, England) for 48 hours at 35 °C. Enriched samples were streaked onto PALCAM agar and incubated at 35 °C for 24 to 48 hours. Three suspect colonies per treatment were then placed on TSA-YE for 24 hours at 35 °C. The following biochemical confirmation test were performed according to FDA, 1997: D-Manitol (Difco, Detroit, MI), L-rhamnose (Difco, Detroit, MI), D-Xylose (Difco,

Detroit MI) for fermentation tested at 35 °C, CAMP test and hemalyses on sheep's blood agar at 35 °C, and Motility Medium S (Difco, Detroit MI) for motility tested at room temperature and 37 °C. Three replications were preformed.

Shelf Life Study

One cm slices of cantaloupes sealed in plastic sandwich bags were irradiated at 0, 1.0 kGy, and 3.0 kGy were visually inspected by two experienced panelists at days 1, 6, 11, 16 and 21. A 9 point scale was implemented with bench marks being 9 = excellent just sliced, 7 = very good, 5 = good but limit of marketability, and 1 = poor and inedible. (Gorney et al., 1999, Gorny et al., 1998). Two samples per each of three replications were inspected and all treatments were inspected in triplicate.

Physical parameters

At day 1, and day 11 texture analysis was performed using a texture analyzer (Texture Technologies Corp., Scarsdale New York). Three treatments of 1 cm slices were analyzed: irradiated at 0 kGy, 1.0 kGy and 3.0 kGy. Two 2.5 cm core samples per 1 cm thick slices were compressed measuring force verses time measured in grams per second. Three replications were performed.

Experimental Design and Statistical Analysis

The design of this experiment was a randomized block design blocked on replication. Data was analyzed using Proc GLM (SAS version 8.1; Cary, N.C.).

Results and Discussion

Microbial analysis

Changes in aerobic plate counts of 1 cm thick cantaloupe slices stored in plastic bags and treated with 1.0 and 3.0 kGy of electron beam irradiation are shown in Figure 1. Initially, treatments had approximately 5.0 log CFU/g. Recovery of microorganisms ranged from 1 to 3 log CFU/g lower over all treatments however; uninoculated and inoculated samples did not change significantly ($P>0.05$) with in treatments.

Cantaloupe provides a perfect medium for the growth of *Listeria* with a pH approaching neutrality and a nutritionally favorable environment (Jay, 1996, Hulme, 1971). Both 1.0 and 3.0 kGy treatments in Figure 2 significantly ($P\leq0.05$) reduced *Listeria* 5 log CFU/g levels by 2 to 5 log CFU/g in inoculated and uninoculated samples at both day 0 and at day 10. Uninoculated and nonirradiated samples showed growth of *Listeria*-possibly due to contamination of soil or through handling practices (Beuchat, 1995). The amount of irradiation required to reduce *Listeria spp.* by 5 log CFU/g is reported to be 2.5 kGy in a phosphate buffer solution (Jay, 1996; Monk, 1995). The inoculated and irradiated treatment of 3.0 kGy significantly reduced *Listeria* levels compared to treatment with 1.0 kGy, indicating that 3.0 kGy is more effective at reducing *Listeria* levels. Although 3.0 kGy over each treatment and day indicated that enumeration and direct plating detected no Listeria, enrichment methodology in Table 1 indicated the survival of *Listeria spp. L. innocua* was isolated from all 3.0

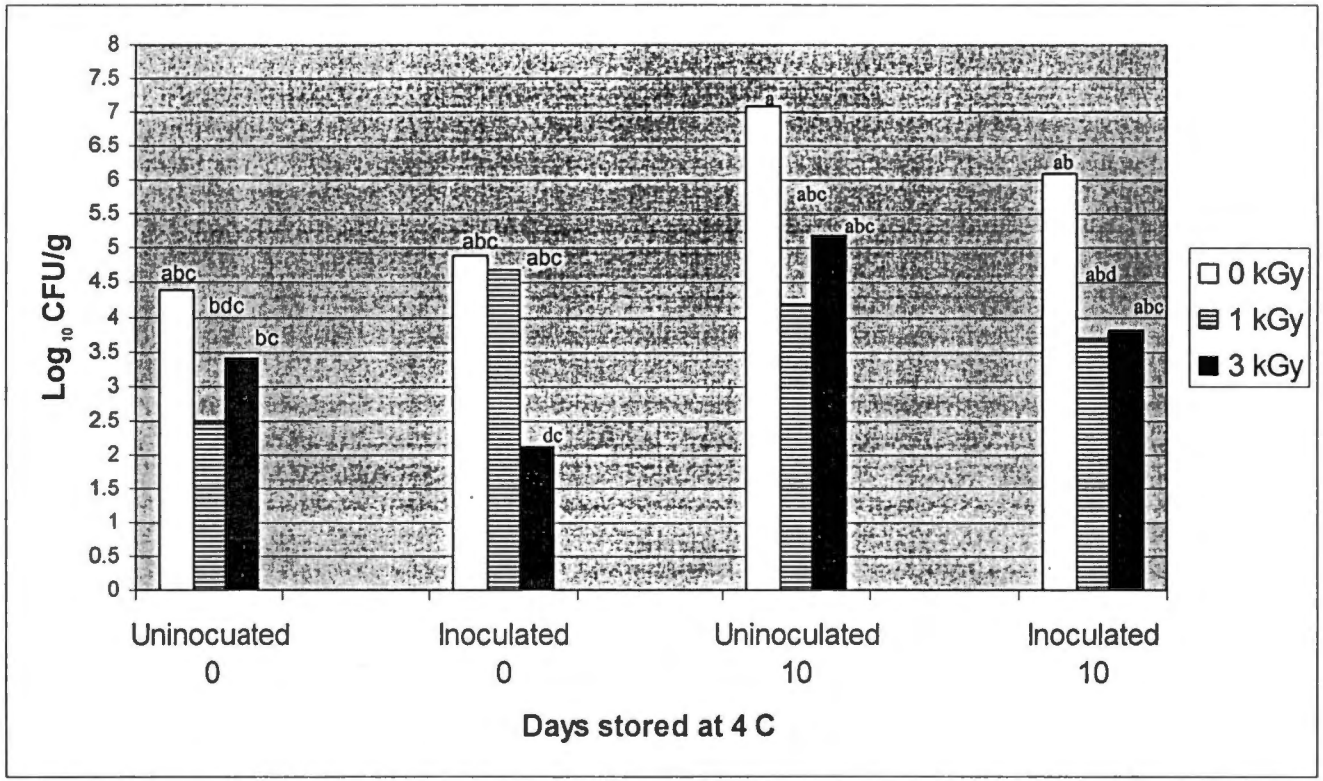

Figure 1. Changes in aerobic plate counts of 1 cm thick cantaloupe slices stored in plastic bags at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation. (Treatments with the same letter are not significantly different at the P<0.05 level)

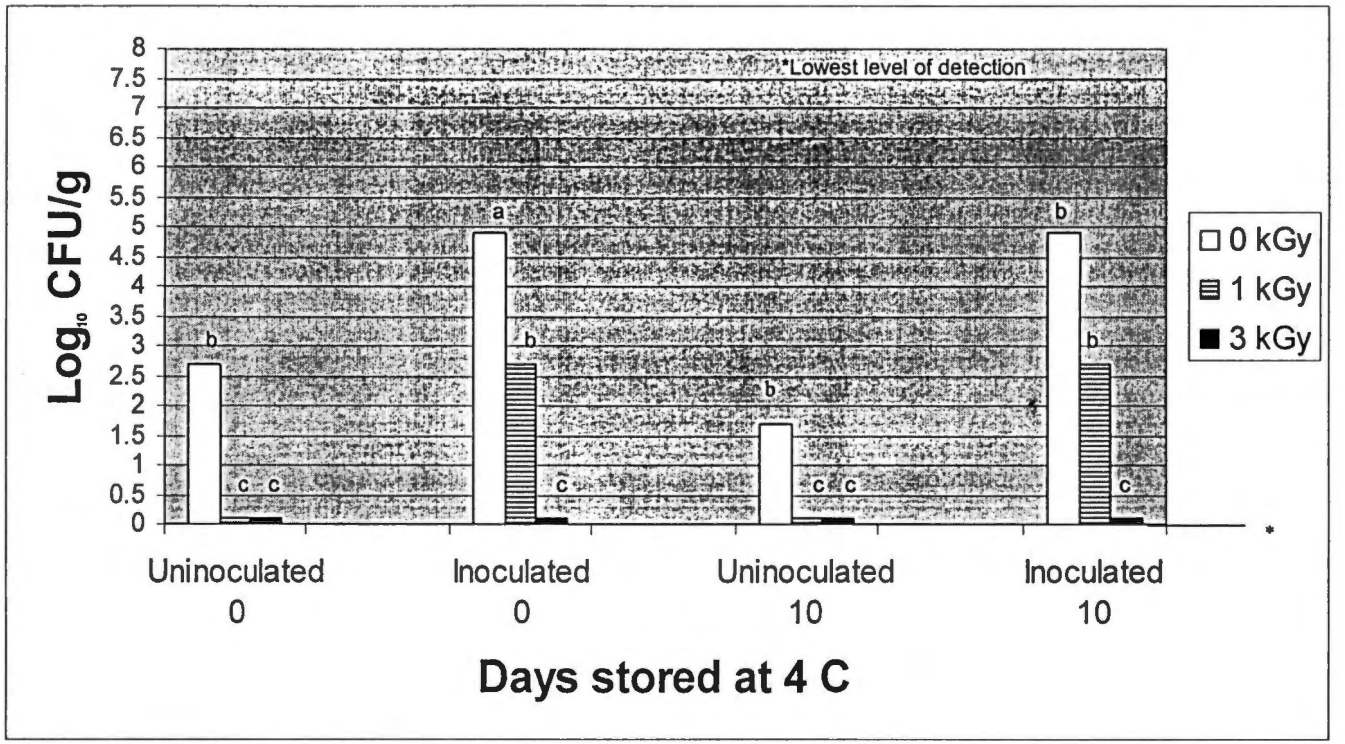

Figure 2. Isolation of *Listeria* from 1 cm thick cantaloupe slices stored in plastic bags at 4 C and treated with 1.0 and 3.0 electron beam irradiation. (Treatments with the same letter are not significantly different at the $P<0.05$ level)

Table 1. Confirmed *Listeria spp.* and *Listeria monocytogenes* positives in cantaloupe slices stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation.

	Listeria spp.	*L. monocytogenes*	*Listeria spp.*	*L. monocytogenes*
	Confirmed positives/sample number			
	Day 0	Day 0	Day 10	Day 10
Inoculated 0 kGy	9/9[a]	5/9	9/9[b]	7/9
Inoculated 1 kGy	9/9[a]	5/9	9/9[c]	6/9
Inoculated 3 kGy	6/6[d]	0/6	3/3[e]	0/3
Irradiated 0 kGy	9/9[f]	0/9	9/9[g]	1/9
Irradiated 1 kGy	9/9[h]	0/9	9/9[i]	0/9
Irradiated 3 kGy	6/6[j]	0/6	3/3[f]	0/3

[a] *L. innocua* was isolated from 4 samples (4/9).
[b] *L. innocua* was isolated form 2 samples (2/9).
[c] *L. innocua* was isolated from 3 samples (3/9).
[d] *L. innocua* was isolated from 6 samples (6/6).
[e] *L. innocua* was isolated from 3 samples (3/3)
[f] *L. innocua* was isolated from 9 samples (9/9).
[g] *L. innocua* was isolated from 8 samples (8/9).
[h] *L. innocua* was isolated from 7 samples (7/9), *L. ivanovii* was isolated from 2 samples (2/9).
[i] *L. innocua* was isolated from 9 samples (9/9).
[j] *L. innocua* was isolated from 6 samples (6/6).

kGy samples over time and *L. innocua* and *L. monocytogenes* was isolated from uninoculated samples irradiated at 1.0 kGy at day 0 using enrichment methodology even though no *Listeria* were detected by enumeration. This indicates that *Listeria* cells may become injured but it is not enough to rely on enumeration of irradiated samples to check for *Listeria.* Enrichment procedures are required to ensure recovery of *Listeria* when evaluating the safety of the product.

In unirradiated inoculated samples Staphylococcus recovered initially at 5 log CFU/g. From cantaloupe slices treated with 1.0 and 3.0 kGy electron beam irradiation as shown in Figure 3. *Staphylococcus* was recovered from uninoculated samples possibly due to post harvest contamination from handling but tested negative with the coagulase test. Therefore these were not *S. aureus* but could have been one of numerous staphylococcal spp. including *S. epidermidis* and *S. sciuri.* The amount of irradiation required to destroy 5 log CFU/g *S. aureus* is 1.6 kGy in beef (Jay, 1996). Counts in irradiated 3.0 kGy samples were significantly ($P<0.05$) lower than nonirradiated and 1.0 kGy treatments at day 0 and 10 indicating that irradiation at 3.0 kGy can be used to decrease staphylococcal levels. *S. aureus* was confirmed in Table 2. for inoculated samples irradiated at 0 and 1.0 kGy across all inoculated treatments. Irradiated samples indicated Staphylococcus spp. was present at day 0 for nonirradiated treatments and at day 10 for 1.0 and 3.0 kGy. Although samples

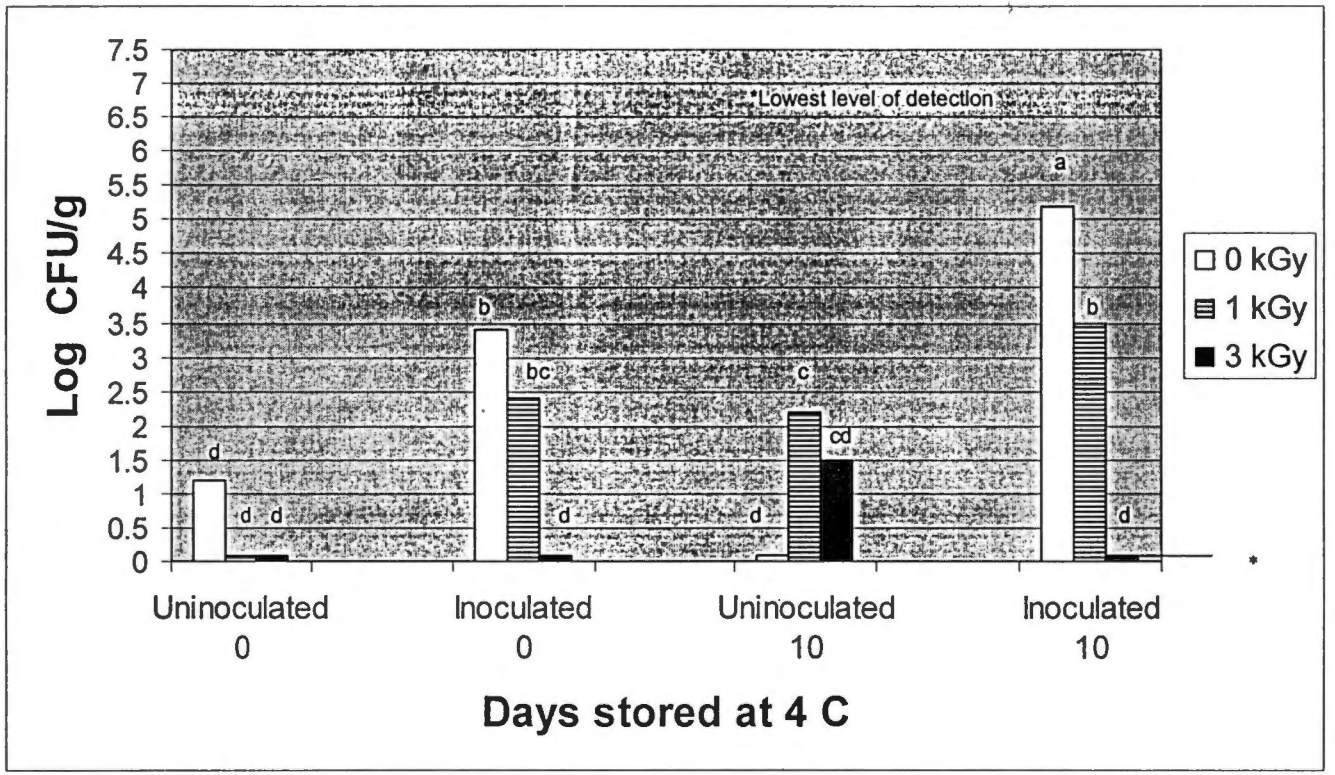

Figure 3. Recovery of *Staphylococcus* from 1 cm thick cantaloupe slices stored in plastic bags at 4 C and treated with 1.0 and 3.0 electron beam irradiation. (Treatments with the same letter are not significantly different at the P<0.05 level)

Table 2. Confirmed coagulase positive *Staphylococcus aureus* samples in sliced cantaloupe stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation.

	Number of positives/sample number	
	Day 0	Day 10
Inoculated 0 kGy	9/9	9/9
Inoculated 1 kGy	9/9	9/9
Inoculated 3 kGy	--*	--*
Irradiated 0 kGy	0/3	--*
Irradiated 1 kGy	--*	0/3
Irradiated 3 kGy	--*	0/3

*Non detected at the lowest detection level.

were only inoculated with *S. aureus*, other strains may be present due to contamination from people handling and cutting the product.

Yeast and mold growth from cantaloupe slices (Figure 4) did not significantly ($P>0.05$) change over treatment and time. Yeasts and molds are known to be more resistant to irradiation than many bacteria (Jay, 1996; Monk, 1995).

Shelf life and texture

Texture analysis in Figure 5 revealed irradiation levels of 1.0 and 3.0 kGy did not significantly affect the firmness of texture of cantaloupe slices. Although not significant, texture measurements for firmness were half that of nonirradiated samples indicating further research is needed. This indicates that irradiation will not have an effect on the texture and thus the quality of cantaloupe slices.

Initial shelf life scores for all treatments shown in Figure 6 ranged from excellent to good with 3.0 kGy having a significantly ($P<0.05$) higher score. Scores for nonirradiated samples decreased significantly ($P<0.05$) from good to poor and inedible. Figure 1 indicates that nonirradiated samples reached spoilage levels of 7 log by day 10. Treatments at 1.0 and 3.0 did not differ until day 16. At day 16 and 21 irradiated treatments at 3.0 kGy rated very good while the other treatments rated poor and inedible. This indicates that 3.0 kGy can extend the shelf life of cantaloupe slices.

Our study presents interesting results that may have a significant impact on the produce and food irradiation industries. The survival of *Listeria* and *S.*

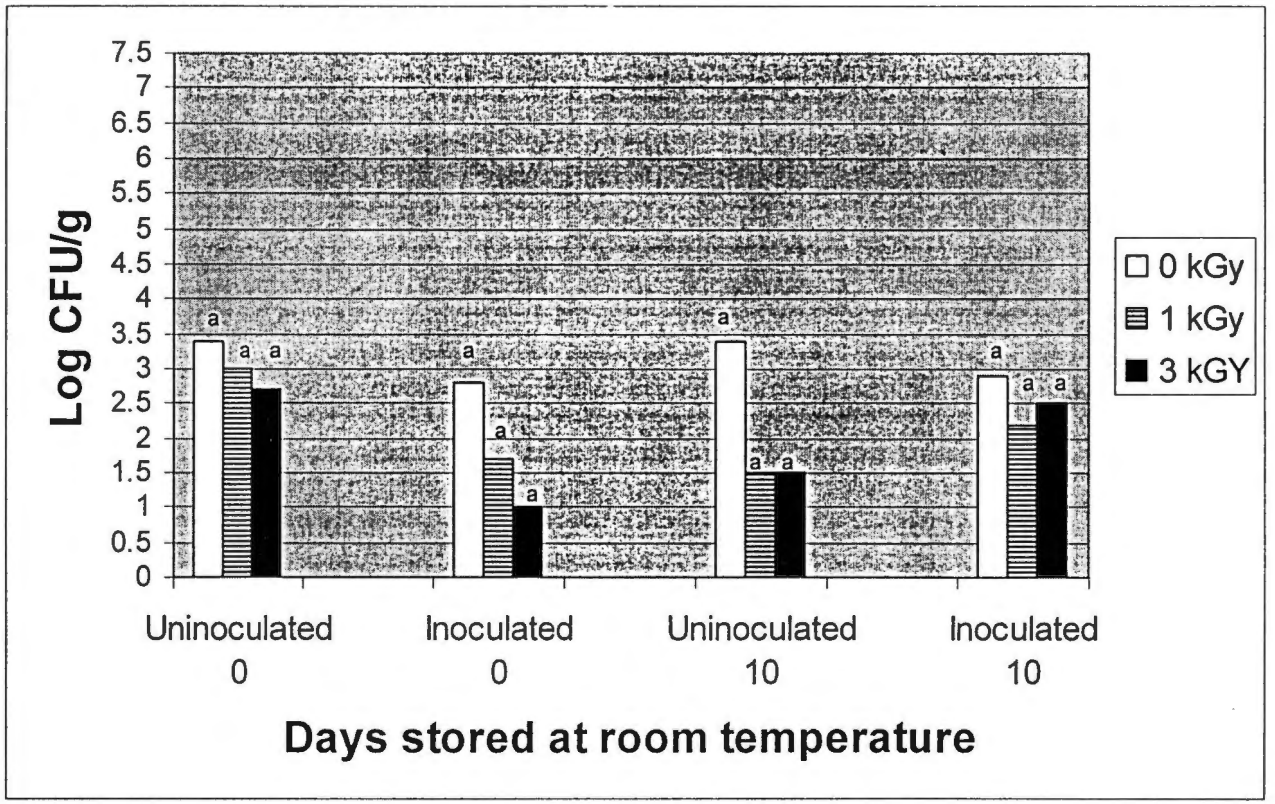

Figure 4. Changes in yeast and mold growth from 1 cm thick cantaloupe slices stored in plastic bags at 4 C and treated with 1.0 and 3.0 kGy electron beam irradiation.(Treatments with the same letter are significantly different at the $P<0.05$ level)

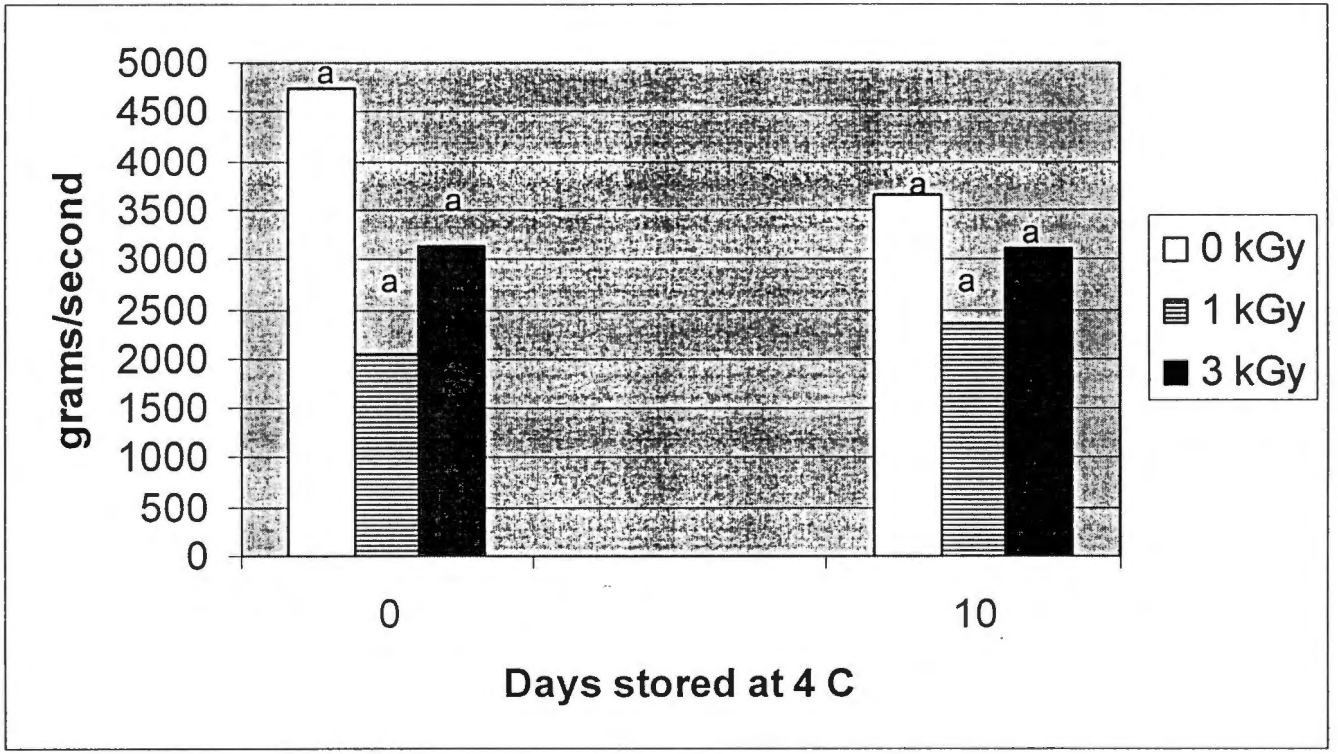

Figure 5. Textural changes in cantaloupe stored at 4 C and treated with 1.0 and 3.0 electron beam irradiation. (Treatments with the same letter are not significantly different at the P<0.05 level)

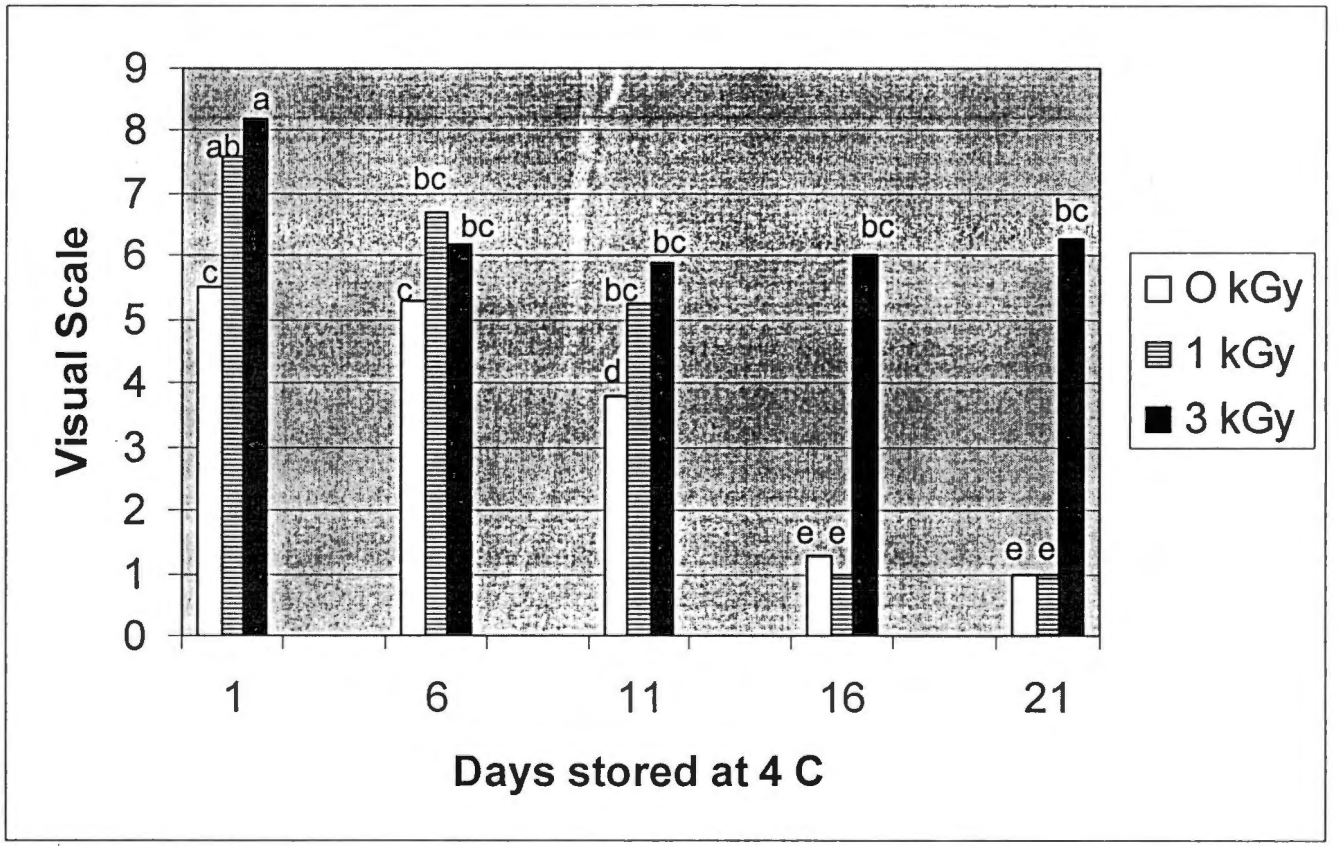

Figure 6. The change in shelf life of sliced cantaloupes stored at 4 °C and treated with 1.0 and 3.0 electron beam irradiation.(Treatments with the same letter are not significantly different at the P<0.05 level)

aureus was reduced or eliminated by irradiation treatments at 3.0 kGy. Additionally, irradiation at 3.0 kGy extended the shelf life of cantaloupe slices by at least 10 days while not affecting the firmness of the product. Shelf life visual inspections were confirmed by aerobic plate counts that were 2 log CFU/g lower at day 10 for treatments of 1.0 and 3.0 kGy. Electron beam irradiation at 3.0 kGy can provide an alternative for otherwise minimally or non-processed fruits and vegetables.

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Part III
Survival of Listeria and Salmonella on Tomato Slices Stored at 4 °C and Treated with 1.0 and 3.0 kGy Electron Beam Irradiation

Abstract

Tomatoes can be contaminated with pathogenic bacteria in the field and during post- and pre-harvest handling. The purpose of this study was to examine the survival of *Listeria* and *Salmonella* on sliced green tomatoes stored at 4 °C and treated with 1.0 and 3.0 kGy by electron beam irradiation. Additionally, the shelf life extension of slices exposed to 1.0 and 3.0 kGy by electron beam irradiation was explored. Enumeration was used to determine the aerobic plate, *Listeria*, *Salmonella*, and yeast and mold count. Enrichment was also used to confirm the presence of *Listeria* and *Salmonella*. Tomatoes irradiated at 3.0 kGy showed significant reduction in *Listeria* and Salmonella survival. Enrichment samples indicated that cells survived indicating confirmation must be performed on both 1.0 and 3.0 kGy treatments. Treatments at 1.0 and 3.0 kGy extended the shelf life of tomato slices to 10 days. If approved by the FDA for tomatoes at levels above 1.0 kGy, electron beam irradiation provides a method to increase both the safety and shelf life of green tomatoes.

Introduction

Numerous outbreaks of gastroenteritis associated with fresh vegetables and fruits have occurred due to salads containing bacterial pathogens. Food processors are not only interested in overall bacterial contamination but reduction in pathogens. Fruits are easily contaminated in the field at during growth due to contact with soil or flooding and can be contaminated through processing.

Tomatoes have been associated with outbreaks of listeriosis and salmonellosis (Brackett, 1998).

Irradiation provides a viable alternative to food processors for foods which are not heated or frozen for preservation (Kader, 1986). Exposing food to ionizing energy serves to destroy bacterial cells resulting in a reduction of post harvest spoilage (thus extending shelf life) and reducing microorganisms while providing an alternative to chemical treatments (Loahranu, 1994). Two main types of irradiation exist; gamma irradiation and electron beam irradiation.

Electron beam irradiated whole tomatoes harvested at the mature green stage showed an initial decrease in firmness at 1.0 and 2.0 kGy with the firmness of the pericarp affected (Assi et al., 1997). While tomatoes have been irradiated at low levels to delay ripening, sliced tomatoes have not been studied. The purpose of our study was to determine the survival of *Listeria spp*. and *Salmonella* in green tomatoes and to evaluate the effect of irradiation at 1.0 and 3.0 kGy on the shelf life of sliced tomatoes.

Materials and Methods

Culture maintenance and inoculum preparation

L. monocytogenes (ATCC 7644), *L. seeligeri* (35967), *L. ivanovii* (19119), *L. innocua* (ATCC 33090), *L. welchii* (ATCC 35897), and *S. typhimurium* (ATCC 14028), and *S. anatum* (ATCC 9270) were used. Test strains of bacteria were inoculated into brain heart infusion (BHI) broth (Difco, Detroit, MI) and incubated at 35 °C for 24 hours. Separate cultures were

subjected to three successive 24-hour transfers prior to use. Tomato slices were surface inoculated with a 5 ml cocktail of all cultures. Cultures were kept refrigerated on tryptose soy slants with yeast extract (Difco, Detroit, MI) and were tested for purity prior to use.

Tomato samples

Green tomatoes were purchased from a local retailer in Knoxville, TN. Each tomato was processed immediately after purchase and handled individually. Tomatoes were cut in half along the longitudinal axis into 1 cm slices using a sanitized electric slicer (Rival, Kansas City, MO) (O'Conner-Shaw, 1996).

Preparation of tomato samples

Six treatments were performed using a commercial 5 MeV electron accelerator (Biosterile; Ft. Wayne, IN). Treatments consisted of 1 cm, slices of uninoculated and inoculated tomatoes held as controls which were not irradiated, uninoculated and inoculated slices irradiated at 3.0 kGy, and uninoculated and inoculated slices irradiated at 1.0 kGy. These doses represent doses measured at the surface of the irradiated product. The actual dose within the product will vary with depth. Locations within the product will receive doses higher or lower than the measured surface dose. All treatments and tests were performed in triplicate. All irradiated samples were irradiated with a 5 MeV self shielded electron beam food system (BioSterileTechnologies, Ft. Wayne, IN).

Microbiological analysis

Samples were taken at day 0 and day 10 from all six treatments. For analysis, each sample of tomato (25g) was placed in 225 ml of 0.1% peptone water (Difco; Detroit, MI). Each sample was mixed in sterile stomacher bags (Fisherbrand, Fabrique, Canada) using a Model 400® Lab Blenlder (Tekmar, Cincinnati, OH) for 60 seconds. Serial dilutions of 0.1% peptone water were plated on five different types of agar to enumerate microorganisms. Standard method agar (SMA) (Difco; Detroit, MI) was used for aerobic plate count, RoseBengal agar with supplement C (RBA-C) (BBL, Sparks, MD) was used for yeast and molds, violet red bile agar (VRB-MUG) (Difco, Detroit, MI) was used for coliforms, hektoen enteric (HE) (Difco, Detroit, MI) and bismuth sulfite agar (BSA) (Becton Dickinson, Sparks, MD) was used for *Salmonella spp.* and PALCAM with supplement Ceftazidime (Difco, Detroit, MI) was used for *Listeria spp.* Direct plating was performed using the spread plate method of 0.1 ml of the initial dilution. All plates were incubated at 35 °C for 24-48 hours; RBA-C plates were placed in the dark at room temperature for 5 days. HE and BSA plates were placed at 37 °C for 24 hours.

Treatments were cut from tomato slices into 25-gram samples and blended in Fisherbrand stomacher bags for 60 seconds in Listeria Enrichment Broth (UVM) (Oxford, Hampshire, England) for 48 hours at 35 °C. Enriched samples were streaked onto PALCAM agar and incubated at 35 °C for 24 to 48 hours. Three suspect colonies per treatment were then placed on TSA-YE for 24

hours at 35 °C. The following biochemical confirmation tests were performed according to FDA, 1995: D-Manitol (Difco, Detroit, MI), L-rhamnose (Difco, Detroit, MI), D-Xylose (Difco, Detroit MI) for fermentation tested at 35 °C, Camp and hemalyses test on sheep's blood agar at 35 °C, and Motility Medium S (Difco, Detroit MI) for motility tested at room temperature and 35 °C. Tomatoes were cut into 20 g samples which were mixed in a stomacher bag for 60 seconds in tetrathionate broth (Difco, Detroit, MI) and incubated at 37 °C for 24 hours. Enriched samples were streaked onto HE and BSA agar. Suspect colonies were stabbed into triple sugar iron agar (TSI) (Difco, Detroit, MI) test and confirmed by urease and serology (FDA, 1995). Three replications were performed per test.

Shelf-life study

Tomatoes sliced into 1 cm slices and sealed in zip lock sandwich bag were irradiated at 0, 1.0 kGy, and 3.0 kGy. Slices were visually inspected by two experienced panelists at days 1, 6, 11, and 16. A 9 point scale was implemented with benchmarks being 9 = excellent just sliced, 7 = very good, 5 = good but limited of marketability, and 1 = poor and inedible. (Gorney et al., 1999, Gorny et al., 1998). Two samples per each of the three replications were inspected.

Experimental Design and Statistical Analysis

The design of the experiment was a randomized block design, blocked by replication. Data were analyzed using Proc GLM (SAS version 8.1; Cary, N.C.)

Results and Discussion

Microbial analysis

Changes in aerobic plate counts of 1 cm thick tomato slices stored in plastic bags and treated with 1.0 and 3.0 kGy of electron beam irradiation are shown in Figure1. Initial treatments had approximately 3.0 log CFU/g over all treatments however; uninoculated and inoculated treatments did not change significantly over time. This could be due to the natural microflora of tomatoes being resistant to irradiation treatment levels or due to the low pH of tomatoes inhibiting growth (Jay, 1996; Hulme, 1971; Satin, 1996).

Isolation of Listeria from sliced tomatoes in Table 1 indicate 3.0 kGy significantly ($P<0.05$) reduced *Listeria* levels for inoculated treatments by 2 to 3 log CFU/g across time. Additionally, no *Listeria* were detected for uninoculated treatments. Although, *Listeria* was not detected in inoculated 3.0 kGy treatments by enumeration, enriched samples tested positive for *L. innocua*. *L. innocua* was also identified from samples irradiated at 0 and 1 kGy (Table 2). Positive identifications indicate that enumeration of samples for *Listeria* is not sufficient, enrichment must be done to ensure products are not contaminated with *Listeria*. Additionally, green tomatoes are more acidic than ripe tomatoes providing a more harsh environment for *Listeria* growth and may explain why isolation was low (Hulme, 1971).

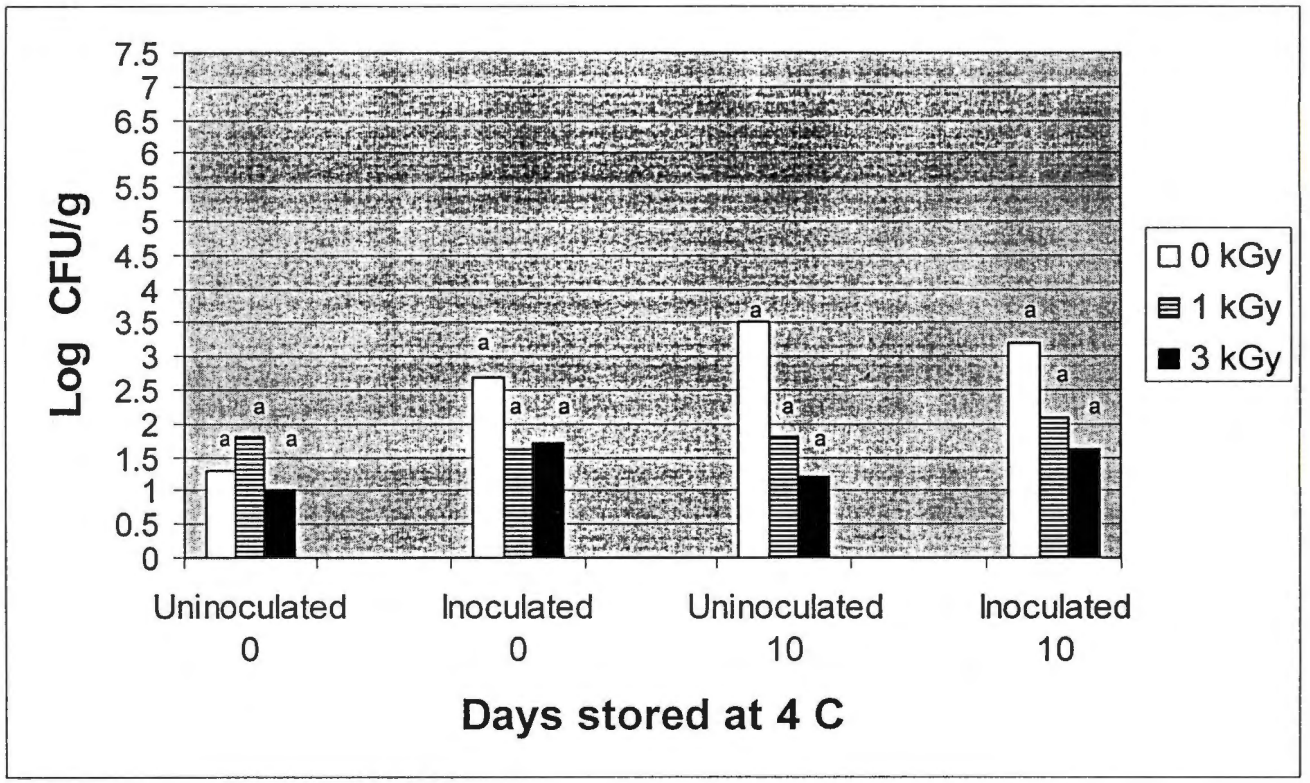

Figure 1. Changes in aerobic plate count of sliced tomatoes stored at 4 C and treated with 1.0 and 3.0 by an electron beam irradiator.(Treatments with the same letter are not significantly different at the $P<0.05$ level)

Table 1. Isolation of *Listeria* from sliced tomatoes stored at 4 °C and treated with 1.0 add 3.0 electron beam irradiation.(Treatments with the same letter are not significantly different at the P<.0.05 level)

	Log CFU/g	
Treatment	Day 0	Day 10
Inoculated 0 kGy	2.0[ab]	3.0[a]
Inoculated 1.0 kGy	ND*	1.0[ab]
Inoculated 3.0 kGy	ND*	ND*
Uninoculated 0 kGy	ND*	ND*
Uninoculated 1.0 kGy	ND*	ND*
Uninoculated 3.0 kGy	ND*	ND*

*Not detected

Table 2. Confirmed *Listeria spp.* and *Listeria monocytogenes* positives in tomato slices stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation.

	Listeria spp.	*L. monocytogenes*	*Listeria spp.*	*L. monocytogenes*
	Confirmed positives/samples number			
	Day 0	Day 0	Day 10	Day 10
Inoculated 0 kGy	9/9[a]	2/9	9/9[b]	4/9
Inoculated 1 kGy	9/9[c]	2/9	9/9[d]	2/9
Inoculated 3 kGy	3/3[e]	0/9	--	--
Irradiated 0 kGy	3/3[f]	0/9	--	--
Irradiated 1 kGy	3/3[g]	0/9	3/3[g]	0/9
Irradiated 3 kGy	--	--	--	--

[a] *L. innocua* was isolated from 5 samples (5/9), *L. seeligeri* was isolated from 2 samples (2/9.)
[b] *L. innocua* was isolated from 4 samples (4/9), *L. seeligeri* was isolated from 1 sample (1/9).
[c] *L. innocua* was isolated from 7 samples (7/9).
[d] *L. innocua* was isolated from 7 samples (7/9).
[e] *L. innocua* was isolated from 3 samples (3/3).
[f] *L. innocua* was isolated from 3 samples (3/3).
[g] *L. ivanovii* was isolated from 3 samples (3/3).

Data for *Salmonella* from sliced tomatoes indicated no Salmonella were detected from uninoculated treatments (Table 3). Irradiation at 1.0 and 3.0 kGy significantly ($P<0.05$) lowered *Salmonella* from inoculated treatment targets of 3 log CFU/g by 2 to 3 log CFU/g across time and media. Table 4 indicates that enrichment procedures must be used to determine if *Salmonella* is present in green tomatoes, as enumeration was not sufficient. The low pH of green tomatoes can also effect the growth of *Salmonella* which has an optimum growth range of 6 to 7 and green tomatoes pH is approximately 4 (Hulme, 1971; Jay, 1996).

Changes in yeast and mold in Figure 2 showed no significant reductions occurred over treatment and time. Electron beam irradiation levels of 1.0 and 3.0 kGy did not affect the growth of yeast and mold. Yeast and molds are more resistant to irradiation than many bacteria (Jay, 1996; Monk, 1995).

Shelf-life evaluation

Shelf life evaluations in Figure 3 showed a decrease from excellent to poor and inedible over time with all treatments. The treatments Irradiated with 3.0 kGy rated significantly higher at day 6 and day 11. On day 11, 1.0 kGy rated as good but limited marketability and 3.0 kGy was significantly better ($P<0.05$) at good. The nonirradiated treatment was rated as poor and inedible at day 1 while 1.0 kGy and 3.0 kGy indicating that irradiated treatments at 1.0 and 3.0 kGy are useful in extending the shelf life of sliced green tomatoes.

Table 3. Isolation of *Salmonella spp* from sliced tomatoes stored at 4 °C and treated with 1.0 and 3.0 electron beam irradiation.

	Log CFU/g			
	HE Day 0	BSA Day 0	HE Day 10	BSA Day 10
Inoculation	3.2	2.4	2.4	2.8
Inoculation 1.0 kGy	ND*	ND*	1.0	1.0
Inoculation 3.0 kGy	ND*	ND*	ND*	ND*
Irradiated 0 kGy	ND*	ND*	ND*	ND*
Irradiated 1.0 kGy	ND*	ND*	ND*	ND*
Irradiated 3.0 kGy	ND*	ND*	ND*	ND*

*Non detected at the lowest detection level

Table 4. Confirmed serological positives of *Salmonella spp.* samples in sliced tomatoes stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation.

	Number of positives/sample number	
	Day 0	Day 10
Inoculated 0 kGy	9/9	9/9
Inoculated 1 kGy	9/9	9/9
Inoculated 3 kGy	1/3	--*
Irradiated 0 kGy	--*	--*
Irradiated 1 kGy	--*	--*
Irradiated 3 kGy	--*	--*

*Non detected at the lowest detection level.

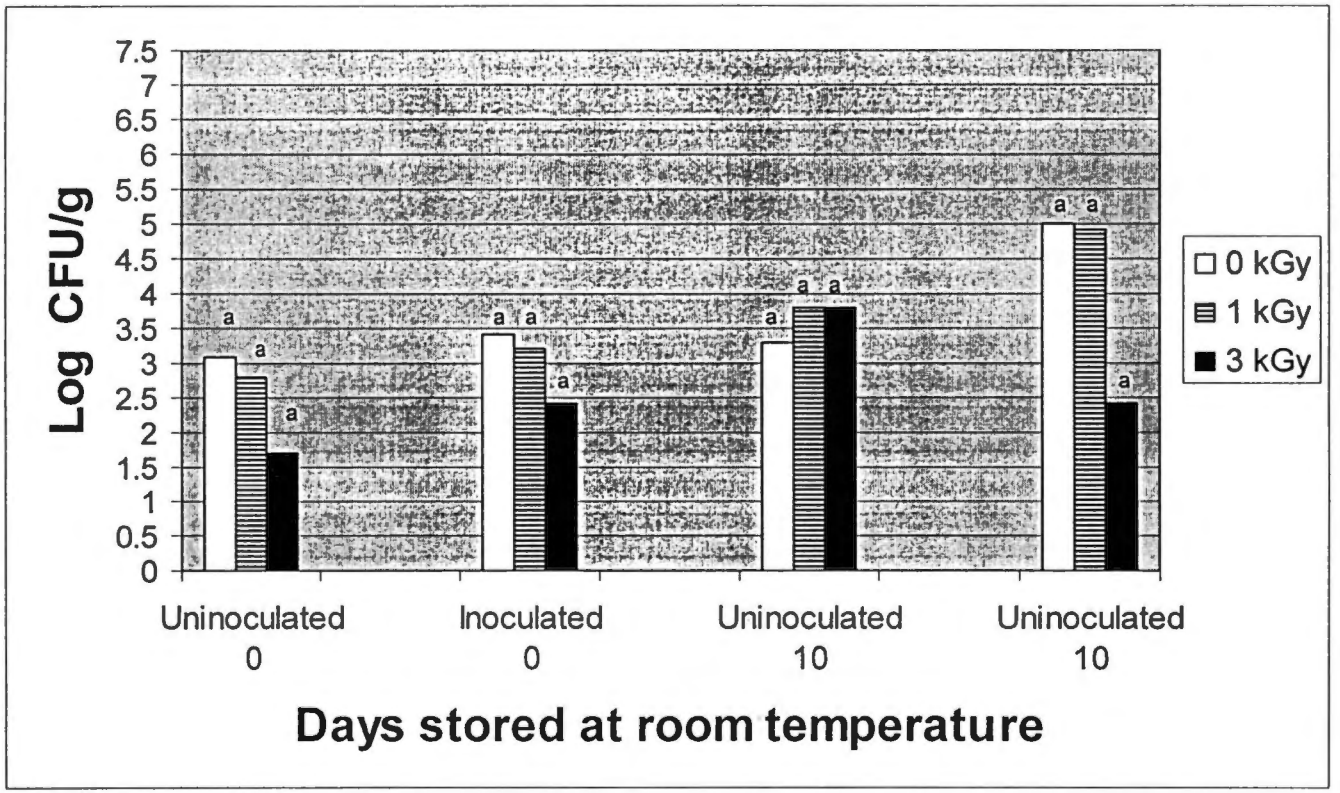

Figure 2. Changes in yeast and mold counts of 1 cm thick sliced tomatoes stored at room temperature and treated with 1.0 and 3.0 kGy by electron beam acceleration.(Treatments with the same letter are not significantly different at the $P<0.05$ level)

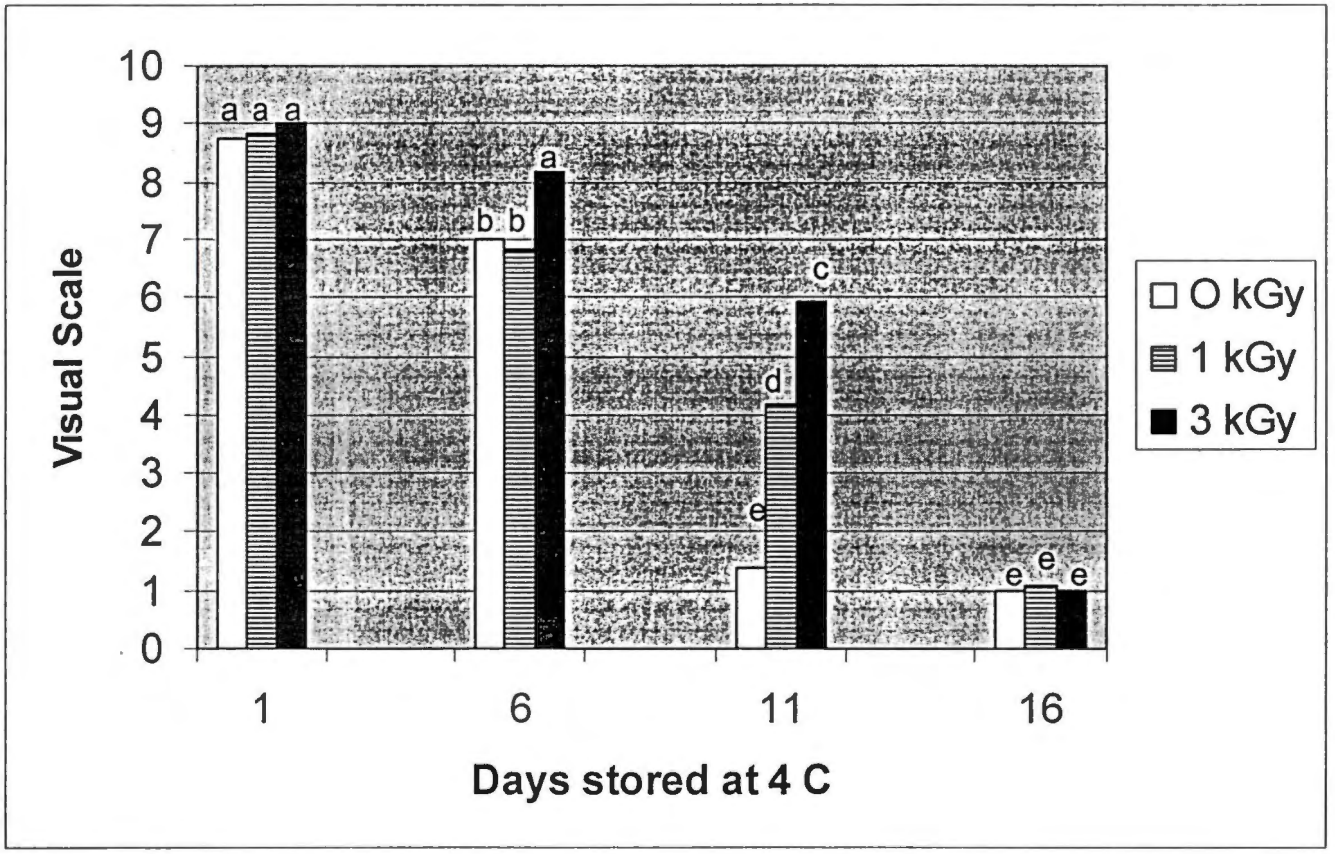

Figure 3. The shelf life of tomatoes stored at 4 °C and treated with 1.0 and 3.0 electron beam irradiation. (Treatments with the same letter are not significantly different at the P<0.05 level)

Our study produced some interesting results. A treatment of 3.0 kGy was effective in reducing *Salmonella* and *Listeria* although enumeration must be followed by enrichment to insure destruction of microorganisms. Additionally, irradiation at 1.0 and 3.0 kGy can extend shelf life of tomatoes. Visual inspections confirm the extension of shelf life as a 2 log CFU/g reduction in microorganisms was noted at day 10 for 1.0 and 3.0 kGy on aerobic plate counts. Shelf life extension and reduction in pathogens make irradiation a viable alternative for the produce industry if the FDA for use with minimally processed or fresh vegetables approves levels above current approved levels.

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APPENDIX

Statistical Analyses

Part II: Survival of *Listeria* and *Staphylococcus* on Sliced Cantaloupe stored at 4 C and treated with 1.0 and 3.0 kGy electron beam irradiation.

Statistical analysis was conducted using SAS version 8,1 (SAS Instiute; Cary, NC) to determine the differences in microbial growth, texture analysis, and shelf life extension. This experiment was a randomized block design with replication. Data was analyzed using GLM and SNK with Proc Means.

SAS program for inhibition effect of irradiation treatments:

```
Options ls = 72;
data micro;
Input inoc $ irr day rep treat $ 1-6;
cards;;
run;
proc glm;
classes treat rep day;
model treat rep day/snk;
run;
proc means;
run;
```

Dependant variable: Aerobic Plate Count

Source		Mean Square	P>F
Treat	11	10.1204977	0.0002
Rep	2	6.6073583	0.0340
Error	22	1.6691402	

Dependant variable: *Listeria*

Source		Mean Square	P>F
Treat	11	9.8975869	0.0001
Rep	2	0.1610778	0.7475
Error	22	.5461263	

Dependant variable: *Staphylococcus*

Source		Mean Square	P>F
Treat	11	8.37152929	0.0001
Rep	2	0.44008611	0.2566
Error	22	.30398005	

Dependant variable: Yeast and Mold

Source		Mean Square	P>F
Treat	11	2.05103308	0.2374
Rep	2	7.10658611	0.0176
Error	22	1.45639823	

Dependant variable: Texture

Source		Mean Square	P>F
Treat	2	2599099.64	0.4870
Rep	2	9284908.85	0.1054
Day	1	8628759.38	.1372
Error	12	3400530.87	

Dependant variable: Shelf life of cantaloupe

Source		Mean Square	P>F
Treat	14	18.5154762	0.0001
Rep	2	0.0541667	0.8891
Error	28	0.4589286	

Part III: Survival of Listeria and Salmonella on Sliced Tomatoes stored at 4 °C and treated with 1.0 and 3.0 kGy electron beam irradiation.

The design of the experiment was a randomized block design blocked by replication. Data were analyzed using GLM, SNK, and Proc Means.

SAS program for inhibition of microorganisms from irradiation treatment

```
options ls = 72;
data micro;
input irr rep treat $ 1-6;
cards;
run;
proc glm;
classes treat rep;
model @ = treat rep;
mean treat rep/snk;
run;
```

Dependant variable: Aerobic Plate Count of Tomatoes

Source		Mean Square	P>F
Treat	11	2.46928990	0.0002
Rep	2	2.05381944	0.0340
Error	22	1.18696187	

Dependant variable: *Listeria*

Source		Mean Square	P>F
Treat	11	3.14085152	0.0002
Rep	2	2.30047500	0.0279
Error	22	0.54357197	

Dependant variable: *Salmonella*

Source		Mean Square	P>F
Treat	11	2.73397247	0.0001
Rep	2	0.48128611	0.0764
Error	22	0.16609823	

Dependant variable: Yeast and Mold

Source		Mean Square	P>F
Treat	11	2.82079293	0.0680
Rep	2	8.48291944	0.0069
Error	22	1.34953157	

Dependant variable: Shelf Life of Tomatoes

Source		Mean Square	P>F
Treat	11	33.6494634	0.0001
Rep	2	0.7413194	0.0653
Error	22	0.2394255	

Vita

Amelia Evans was born in Lewisburg, KY on April 3, 1973. She graduated from Logan County High School in Russellville, KY in May 1991. Afterwards she enrolled at Cumberland College, Williamsburg, KY and later transfer to Middle Tennessee State University, Murfreesboro, TN of which she graduated from in December 1997. In August of 1998, she started her graduate study for a Master's Degree in the Department of Food Science and Technology. There she pursued a Master of Science degree, with an emphasis in Food Microbiology.