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The fate of Salmonella Tennessee and Salmonella Typhimurium in peanut butter subjected to electron beam irradiation

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THE FATE OF *SALMONELLA* TENNESSEE AND *SALMONELLA* TYPHIMURIUM
IN PEANUT BUTTER SUBJECTED TO ELECTRON BEAM IRRADIATION

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Thesis submitted to the
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Abstract

The Fate of *Salmonella* Tennessee and *Salmonella* Typhimurium in Peanut Butter
Subjected To Electron Beam Irradiation

Andrea Lynn Hvizdzak

Peanut butter and peanut paste products were implicated as the vehicle of contamination in an outbreak of *Salmonella* Typhimurium that began in September 2008 and in the November 2006 outbreak of *Salmonella* Tennessee; therefore based on the apparent need for intervention measure, the purpose of this research was to evaluate the effectiveness of non-thermal electron beam irradiation for reduction of *Salmonella* serovars Tennessee (ATCC 10722) and Typhimurium (ATCC 14028) in creamy peanut butter. Each strain was studied independently. Peanut butter samples were inoculated with approximately 7.0 log CFU/g of *Salmonella* and exposed to e-beam doses ranging from 0-3.1 kGy. Doses were confirmed using film dosimetry. Survivors were enumerated by standard spread-plating on non-selective (tryptic soy agar; TSA) and selective (xylose lysine deoxycholate agar; XLD) media. Microbial counts (CFU/g) were log-converted and the slopes of the respective survivor curves were determined by linear regression. D_{10}-values were calculated as the negative reciprocal of the slope of the survivor curve and were reported as mean values ± standard deviation. One-way analysis of variance and Tukey's honestly significant difference test were used to determine differences. D_{10}-values show that *Salmonella* Typhimurium was more resistant (0.82 ± 0.02 and 0.73 ± 0.01 kGy on TSA and XLD, respectively) than *Salmonella* Tennessee (0.72 ± 0.02 and 0.60 ± 0.01 kGy on TSA and XLD, respectively) to e-beam irradiation ($P<0.05$). The recovery on growth and selective media was different ($P<0.05$), indicating cell injury. Microbial analyses were conducted after 4, 6, 8, and 14 d of storage. The rate of microbial decline was significant over the 14 d storage period ($P<0.05$); the greatest reductions were seen at target e-beam dose 2.5 kGy at day 14 ($P<0.05$). *Salmonella* Tennessee and *Salmonella* Typhimurium mean survivor populations exposed to e-beam irradiation in peanut butter will decline overtime. E-beam irradiation may be an effective processing step for the non-thermal inactivation of *Salmonella* in peanut butter.

DEDICATION

I wish to dedicate this thesis to my parents, Ed and Faye Hvizdzak.
Their love, encouragement, guidance, and support made this possible.

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

Each year in the United States there are approximately 76 million cases of foodborne disease, resulting in an estimated 325,000 hospitalizations and 5,000 deaths. Every year $37.1 and $23 billion are lost in production and medical expenses, respectively (CDC 2007). There are seven major foodborne pathogens responsible for these cases, and one of the seven implicated pathogens is *Salmonella*. Over 2,400 serotypes of the genus *Salmonella* are known to exist. Salmonellosis is the second most reported foodborne illness. The Outbreaks are commonly associated with foods of animal origin, including poultry, eggs, and meat (FDA 2009 [a]). The Center for Disease Control and Prevention examines aspects of production as well as food service in order to help eradicate foodborne illnesses. A standardized approach was established to food safety referred to as the Hazard Analysis and Critical Control Points. These were developed in hopes to improve the safety of food products (FDA 2009 [b]). Despite advances in food safety, foodborne pathogens continue to be of concern in the United States. The most recent outbreaks of *Salmonella* implicate peanut butter and peanut butter products (CDC 2009). Despite prescribed pasteurization, peanut butter continues to harbor infectious *Salmonella* cells. Researchers believe *Salmonella* survival in peanut butter is recognized to unique attributes such as: a low water activity (a_w), high fat content, pH, and iron composition. Distinctive features of *Salmonella* in these environments are its ability to adapt to its environment through filamentation, habituation, and protein degradation. Without an alternative method of effective microbial reduction, peanut butter and peanut butter products will continue to be a food safety concern.

As a result of the need for a more effective intervention, the objective of this thesis was to evaluate the effect of e-beam irradiation on the reduction of *Salmonella* Tennessee and *Salmonella* Typhimurium at a target dose of 10^7 log CFU/g in peanut butter, and to look at the fate of these strains over time.

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[b] Food and Drug Administration Center for Food Safety and Applied Nutrition http://vm.cfsan.fda.gov February 2009.

CHAPTER 2

REVIEW OF LITERATURE

Salmonellosis infects about 2 to 4 million people annually. In the past decade there has been a rise in S*almonella* infections. In the United States *Salmonella* is responsible for 15% of all food poisonings. Salmonellosis is also the second most common bacterial foodborne illness reported (CDC 2007). Despite scientific efforts there is still little known about how *Salmonella* cells survive in certain low water activity foods, such as, peanut butter, chocolate, and various processed foods. There does, however, appear to be evidence of a connection between heat tolerance and low water activity for *Salmonella* cells and the way they survive and grow (Mattick et al 2001). Scientific research demonstrates this connection as well as an interaction of pre-exposed cells to osmotic stress (Mattick et al 2000 [a]). *Salmonella* cells are unique particularly in the way they are able to adapt to their environment. Due to recent bacterial outbreaks, the food industry is looking for alternatives to thermal pasteurization for foods that have a low water activity and high fat content like peanut butter. Ionizing radiation may be an effective alternative. Irradiation is a process which uses ionizing radiation to reduce microorganisms present in or on food and food products. Irradiation is approved by the Federal Drug Administration for meat, spices, fresh fruits, and vegetables (FDA 2009). The bactericidal effect of irradiation for colloidal systems has yet to be studied.   Research into the effect of irradiation on *Salmonella* in peanut butter may prove to beneficial for the safety of peanut butter processing. This in turn may allow food industries to adopt a more effective process for the elimination of harmful microorganisms.

Salmonella

There are over 2,000 different species of *Salmonella*. The differences that can be identified between species are cell structure, specifically O and H antigens in *Salmonella* species. The H antigen represents the flagellar antigen while O represents the somatic antigen. O antigens are important because they occur on the surface of the outer membrane. They are determined by sugar sequence and are responsible for determining the virulence of the organism. O antigens are the body of the microorganism and are characterized by the number and type of sugars in the outer core. O antigens are also heat stable. The H antigens are heat labile and are found in the flagellar, or tail portion of the microorganism. The H antigens are unique because they have the ability to change forms. Species and serovars are categorized as A, B, C, etc. according to similarities in the content of the O antigen (Rubin and Weinstein 1977).

Salmonellosis

Salmonella infections cause salmonellosis. *Salmonella* infections begin when one of the over 2,000 species of *Salmonella* are ingested. Ingestion of *Salmonella* is typically from undercooked eggs, poultry, and meat products. The *Salmonella* bacteria are able to endure the acidity of the stomach and travel to the small intestine. Once in the small intestine they adhere to the lining of the small intestine and begin their life cycle. The *Salmonella* cells are then shed through the feces. Symptoms usually arise within 12 to 72 hours after ingestion and usually last four to seven days. The infections cause various symptoms such as, diarrhea, stomach cramps, and fever (CDC 2007).

Recent Outbreaks

Salmonellosis is an important medical problem. Every year in the United States *Salmonella* infects about 40,000 individuals. Although most cases are mild, causing only diarrhea and cramps, some cases can progress and eventually lead to death. This tends to happen in individuals with a weakened immune system such as children and elderly (CDC 2007). The Centers for Disease Control and Prevention in combination with the Associate of State and Territorial Epidemiologists has maintained watch over *Salmonella* infections in the United States since 1962. Despite their efforts little is known about the *Salmonella* outbreaks that have occurred. This is mainly because most cases are not reported, and of those that are little information is available to identify the source. *Salmonella* outbreaks have been occurring for many years despite efforts to control it, the outbreaks seem to be more numerous than ever (CDC 2009).

In November 2006, a *Salmonella* outbreak associated with peanut butter caused 483 illnesses in 43 U.S. states (CDC 2007). In January 2009, an outbreak of *Salmonella* forced companies to recall more than 3,000 products associated with peanut butter. In this outbreak 691 people were sickened, including 9 deaths, in 47 U.S. states. The cause of these *Salmonella* outbreaks is unknown. Typical pasteurization and sterilization processes should be sufficient to eliminate pathogens; however, contamination could occur after processing. It is therefore important to study the survival characteristics of *Salmonella* cells, in low water activity and high fat content environments.

Recent Peanut Butter Outbreaks

The first known and documented outbreak of *Salmonella* in peanut butter was documented in Australia in 1995 (Scheil et al. 1998). The first outbreak reported in The United States occurred in November 2006. The outbreak was associated with commercial jars of peanut butter. The most recent *Salmonella* outbreak started in November 2008 and implicated peanut butter and peanut butter paste products. In January 2009, this outbreak of *Salmonella* Typhimurium forced companies to recall more than 3,000 products associated with peanut butter as a common ingredient. These products included various cookies, crackers, cereals, candies, ice creams, pet treats, etc. The CDC considers this outbreak to be of special concern because of the wide range of food products that were affected (CDC 2009).

Peanut butter is typically consumed throughout the world. Peanut butter consumers span a wide range of ages including those individuals who may be more adapt to developing severe symptoms of Salmonellosis, such as, children, elderly, and those with weakened immune systems. Peanut butter has a long shelf life allowing it to be stocked in various kitchens, industries, and households for many weeks. Peanut butter is a colloidal system containing a high fat content as well as a low water activity. *Salmonella* is able to survive within this environment for the duration of its self life. The heating of >70**º**C may not eliminate all the *Salmonella* cells possibly present in the product (CDC 2007). *Salmonella* cells may be introduced into peanut butter at multiple points during production. It is for this reason that non thermal safety measures for peanut butter be investigated.

Water Activity

Water activity in a food is the water available for use, or free water. Water is extremely important in food industries because the lower the water activity the less likely the chance of microbial growth, thus leading to a longer shelf life and higher consumer acceptance. Most microorganisms are unable to survive and grow in low water activity environments. Yet, *Salmonella* has the ability to both survive and reproduce when water is unavailable for use (Mattick et al 2000 [a]).

Keiboom and others (2006) examined the morphological and physiological changes *Salmonella* experiences under low water activities. It was found that *Salmonella* does have the ability to form elongated cells which was not a result of surface effects or solute concentrations. The survival of *Salmonella* at low water activity increases the threat for cross contamination over a long period of time. This is mostly because these cells are capable of building a tolerance when pre exposed to certain environmental factors such as heat and solutes. These cells are then able to survive more efficiently than other cells and in turn cause more *Salmonella* contaminations (Keiboom et al. 2006). Knowing this it is important to examine the aspects and use of temperatures, specifically those used for pasteurization and sterilization, on *Salmonella* in low water activities.

Heat Tolerance

Salmonella is a rod shaped gram negative pathogenic bacterium. *Salmonella* is typically found in foods of animal origins, specifically beef, poultry, and eggs. Animals have a higher affinity for *Salmonella* species than humans. For this reason, animals are not sickened with disease the way that humans are. *Salmonella* is naturally occurring in the digestive tract of

animals. This potential for cross contamination is why animal products need to be cooked to as high as 165°F for meat products. Cooking to certain temperatures kills *Salmonella* present within the food (FDA 2009). Because of this, inactivation methods have been developed to help lessen the number of active *Salmonella* species on food sources. These methods include different variations of thermal processing that are not as effective when it comes to eliminating *Salmonella* in low water activity foods such as peanut butter.

In a 2006, Shachar and Yaron studied the effectiveness of heat tolerance on three strains of Salmonella in peanut butter. They found the peanut butter pasteurization process of temperatures at 70°C to 75°C for 20 minutes to be unsuccessful in destroying the microorganism. *Salmonella* continued to survive even at temperatures of 90°C for 50 minutes (Shachar and Yaron 2006). Thermal tolerance demonstrates problems that *Salmonella* has on increased heat resistance in foods that have low water activities and high lipid contents. *Salmonella* does not exhibit growth at temperatures below 5.3°C or above 45°C (CDC 2009).

Salmonella species are known to have greater heat tolerance at low a_w. In 2001 Mattick et al. studied the effect of temperatures on *Salmonella* at low water activity. Some studies have reported that heat tolerance of *Salmonella* can increase or decrease at low water activity (Baird-Parker 1970). While other studies argue that heat tolerance of *Salmonella* increases as the a_w decreases (Kirby 1990). Mattick et al. (2001) found that *Salmonella* cells were more heat tolerant at low water activity; whereas, heat tolerance was not seen at temperatures below 65°C. Mattick et al (2001) proposed that improved protein stability may play a large part into the mechanism. The ribosomes and proteins may exhibit increased heat stability at low water activities. The interaction between heat tolerance and water activity in *Salmonella* species proves to be unique. *Salmonella* demonstrates responsiveness when exposed to a stimulus.

Recent studies have found that *Salmonella* grow best at a water activity of 0.99. Ngwai and others studied *Salmonella Typhimuium* and *Salmonella enteritidis* under stressful growth conditions in hopes to better identify stress tolerances. They found that *Salmonella Typhimurium* did not grow well at elevated temperatures unless supplemented with iron. Peanut butter naturally has a small percentage of iron within its composition. The iron found within peanut butter may actually help to aid in the survival of *Salmonella* species enough to bypass the elevated temperatures that would typically destroy the bacteria (Ngwai et al. 2007). Unlike typical food products, in peanut butter *Salmonella* cells do not undergo protein denaturation. The environmental conditions and composition of peanut butter aid in *Salmonella* cells survival. This survival by the microorganism *Salmonella* contributes to an unsafe food product. A direct mechanism or association for the protective effects of peanut butter harboring *Salmonella* has yet to be identified.

Habituation

The habituation of *Salmonella* species at a reduced water activity and its effects on heat tolerance have been studied by Mattick et al. (2000 [a]). They found that despite past results and implications, protein synthesis is not likely to be the mechanism whereby *Salmonella* cells increase their stability. The way in which protein synthesis occurs is through the ribosome inhibitors; therefore, they theorized that the solutes used in their study held the cells at a higher osmotic stress causing them to modify their structure in order to stabilize with the environment. This, in turn, caused a higher heat tolerance (Mattick et al. 2000 [a]).

Filamentation

Filamentation is the abnormal growth of bacteria in which cells continue to elongate but do not divide. This is a defect in completing replication and usually occurs under certain conditions such as high heat or osmotic stress. *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium survive at reduced a_w for long periods and form filaments (Mattick et al 2000 [b]). Surviving filamentous cells can maintain their membrane integrity after exposure to low a_w for 21 days and are able to survive disinfection more efficiently (Kieboom et al. 2006). The filamentous cells formed as a result of a biomass increase in the absence of cell division during the low a_w stress. If filamentation is found to occur in foods, there are implications for low a_w food products. A single contaminating *Salmonella* cell could continue to replicate, in turn leading to infection after consumption (Mattick et al. 2000 [b]).

The ability of *Salmonella* to survive in low water activity foods raises specific issues of food safety; it is indicated that 3 CFU/g may be sufficient to cause illness when consumed in low a_w foods (Scheil et al. 1998). The a_w of peanut butter ranges from 0.18-0.24. Mattick et. al (2000 [a]) studied the effect of temperature (55° to 80°C) and a_w (measured by relative vapor pressure 0.65 to 0.90) on the survival of *Salmonella*. The Weibull model ($\log s = -bt^n$) was used to describe microbial inactivation, and surface presence models were developed to predict death rates for serovar *Typhimurium*. Cells suspended at low a_w and treated with temperatures $\geq 70^\circ$C were more heat tolerant than cells suspended at a higher a_w; however, below 65°C, the reverse was true. Predicted inactivation of *Salmonella* in peanut butter (65° and 74°C) was more rapid than what was actually observed in the other foods studied. At high temperatures, low a_w protected the cell against thermal death with a longer time to obtain a 3-$\log_{10}$ reduction. The

temperature dependent effects of low a_w on heat tolerance of *Salmonella* reflects different targets for death at low temperatures than at high temperatures (Mattick et al. 2001).

Irradiation

Irradiation in food is a process by which food products are exposed to ionizing radiation in order to destroy microorganisms, bacteria, viruses, or insects that might be present. Irradiation will reduce the number of probable microbiological hazards that could be present in or on a food or food product. The food or food product remains potentially unaffected in texture, taste, and nutritional value. It has been approved by the Food and Drug Administration for use on meat, poultry, spices, fresh fruits, and vegetables. The safety of irradiated foods has been well studied for many years and no documented adverse health effects have been seen (Urbain 1986).

The energy source in irradiation can be either gamma rays or electron beam. In irradiation, the dose is measured in a unit called a Gray. A Gray measures the amount of energy being used. The death rate of microbes being irradiated is measured by using D-values. D-values represent the amount of irradiation needed to eliminate 90% of a pathogen. D-values help researchers and food industry workers identify the correct dosage needed to administer in a given irradiated food or food product. The dosage of a given food or food product may vary in conjunction with finding the right D-value for a given microorganism and food or food product.

Irradiation works to eliminate organisms by moving energy waves through the molecular bonds in the DNA of an organism. The energy creates reactions that damage the DNA in the microorganism and cause water radiolysis. This in turn, causes problems in genetic instruction, and a dissociation of water molecules. Once this happens the organisms are unable to reproduce. If the organism tries to reproduce or grow it will die (Urbain 1986).

Current Studies Using E-beam

Alvarez et al. (2007) studied the effects of modeling irradiation followed by heat inactivation for *Salmonella* inoculated in liquid whole egg. They focused on *Salmonella Typhimurium* and *Salmonella Senftenberg* in liquid whole egg. The authors examined irradiation in correlation with heat treatment as a way to model D-values. They did find significant 5-$\log_{10}$ reductions in *Salmonella Typhimurium* and *Salmonella Senftenberg* with no differences in non-selective (tryptic soy agar; TSA) versus selective (tryptic soy agar amended with 3% NaCl; TSA-SC). This process allowed the authors to specify a direct irradiation dose followed by heat treatments at a set temperature and time to receive a 90% reduction of viable cells (Alvarez et al. 2007). This approach helps demonstrate the fact that irradiation is successful in reducing the number of viable *Salmonella* cells in the food product. It also shows the importance of implementing a process that focuses on more than just one process to implement the safest product possible. They found that cell injury occurred following equivalent irradiation and heat treatments. This process was more effective with respect to the corresponding heat treatments, to any temperature and irradiation dose, applied to inactivate the *Salmonella*. Cell injury following both treatments could reduce the process standard to inactive 5 $\log_{10}$ population of *Salmonella*. Irradiation with heat treatment showed a reduction in the heat treatment intensities. This may, in turn, also reduce the impact of the heat treatment on the freshness and quality of the product.

Conclusion

Salmonella proves to be a unique microorganism. It has the ability to adapt and survive in low water activity foods for long periods of time. The exact mechanism by which it is able to survive is still unknown. It is known that the way in which it interacts with its environment is

vital to its survival. Heat tolerance is of main concern in the food industry because without effective processing methods, *Salmonella* will continue to survive in low water activity, high fat foods such as peanut butter. To have the best protection against food borne pathogens, it is necessary to study them and understand their capabilities.

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CHAPTER 3

Use of Electron Beam Irradiation for the Reduction of *Salmonella enterica* Serovars Typhimurium and Tennessee in Peanut Butter

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Abstract

Peanut butter and peanut paste products were implicated as the vehicle of contamination in an outbreak of *Salmonella* Typhimurium that began in September 2008 and in the November 2006 outbreak of *Salmonella* Tennessee. Therefore, this study evaluated the effectiveness of non-thermal electron beam irradiation for the reduction of *Salmonella* serovars Tennessee (ATCC 10722) and Typhimurium (ATCC 14028) in creamy peanut butter. Each strain was studied independently. Peanut butter samples were inoculated with approximately 7.0 log CFU/g of *Salmonella* and exposed to e-beam doses ranging from 0-3.1 kGy. Doses were confirmed using film dosimetry. Survivors were enumerated by standard spread-plating on non-selective (tryptic soy agar; TSA) and selective (xylose lysine desoxycholate agar; XLD) media. Microbial counts (CFU/g) were log-converted and the slopes of the respective survivor curves were determined by linear regression. D_{10}-values were calculated as a negative reciprocal of the slope of the survivor curves and are reported as mean values ± standard deviation. One-way analysis of variance and Tukey's honestly significant difference test were used to determine differences. D_{10}-values show that *Salmonella* Typhimurium was more resistant (0.82 ± 0.02 and 0.73 ± 0.01 kGy on TSA and XLD, respectively) than *Salmonella* Tennessee (0.72 ± 0.02 and 0.60 ± 0.01 kGy on TSA and XLD, respectively) to e-beam irradiation ($P<0.05$). The recovery on growth and selective media were different ($P<0.05$), indicating cell injury. The results of this study demonstrate that e-beam irradiation may be an effective processing step for the non-thermal inactivation of *Salmonella* in peanut butter.

Introduction

In January 2009 numerous outbreaks of *Salmonella* serotype Typhimurium were attributed to the consumption of peanut butter, peanut paste, and products made with them. The source of the contamination was traced back to a facility that manufactures peanut products shipped in bulk to hospitals, nursing homes, and schools for use as institutionally-served peanut butter, and to other facilities for use as an ingredient in products such as cookies, cakes, crackers, and ice cream (CDC 2009). Several outbreaks of salmonellosis also emerged in November 2006. The outbreaks of *Salmonella* serotype Tennessee were associated with the consumption of commercial brands of peanut butter brand; the source of the peanut butter contamination was unknown. While most salmonellosis outbreaks are linked to poultry, meat, eggs, or fresh produce, the incidence of *Salmonella* in peanut butter proves to be unique because peanut butter provides an environment where organisms are able to survive because of the high fat and low water activity (CDC 2007).

Peanut butter is a semi perishable food, not readily susceptible to spoilage because of its low moisture content. The physical properties of peanut butter make it an unlikely vehicle for foodborne microbial illnesses. Peanut butter is an oil and water emulsion with low water activity (a_w). *Salmonella* cells become more heat tolerant at low a_w, and the effectiveness of heat processing is reduced when *Salmonella* is present in low a_w foods (Gill et al. 1983) (Goepfert et al. 1970) (Mattick et al. 2001) (Shachar and Yaron 2006). The high fat content and low a_w of peanut butter would likely protect *Salmonella* against typical peanut butter processing temperatures (Senhaji et al. 1977) (Burnett et al. 2000).

The manufacturing of peanut butter begins with peanuts harvested from the farm and shipped to a manufacturing facility where they are shelled and cleaned. They are then roasted in ovens at temperatures around 180°C for 50 minutes which should be adequate to kill *Salmonella* (Woodruff 1983). However, since there is no set regulation for the temperature and time in roasting, adequate temperatures or holding times may not be employed. Contamination of peanut butter may also occur after the roasting process (CDC 2007).

When roasting is complete, the peanuts are blanched, ground into a paste, and perhaps mixed with other ingredients such as salt, oil stabilizers and/or sugar. *Salmonella* can be introduced in the production environment raw peanuts or by other means such as inadequate sanitation, poor plant maintenance during production, and contaminated personnel and/or containers. The target pasteurization temperature prior to packaging is between 70°-75°C; however, *Salmonella* survives at temperatures as high as 90°C (Shachar and Yaron 2006). *Salmonella* cells demonstrate an increased heat resistance within a high lipid and low a_w environment (Juneja et al. 2001) (Mattick et al. 2001) (Shachar and Yaron 2006), making this a pathogen of concern for these types of food products. The pasteurization process is ineffective against *Salmonella* and microbial reductions are not improved by longer treatment (Shachar and Yaron 2006). In fact, higher temperatures and longer exposure times would decrease the quality of the peanut butter by eliciting taste and texture changes (Mattick et al. 2001). The recent *Salmonella* outbreaks associated with peanut butter and peanut butter products highlight the need for better process controls to ensure product safety.

Electron-beam (e-beam) radiation is a form of ionizing radiation that is generally characterized by its low penetration and high dosage rates. The beam is a concentrated highly charged stream of electrons generated by the acceleration and conversion of electricity. When a

contaminated food is exposed to e-beam radiation, energy from the electrons is absorbed

(absorbed dose) by microbial cells which alter various chemical and biological bonds, destroying

their DNA and rendering them unable to reproduce (Cleland 1993).   The main assets of this

technology include high lethality, fewer detrimental effects on food quality compared to thermal

methods, and it does not require the use of radioactive isotopes.

The effectiveness of e-beam irradiation as a non-thermal process for the reduction of

pathogenic bacteria in peanut butter has not been reported; therefore, the objective of this

research was to investigate the efficacy of e-beam for reduction of *Salmonella enterica* subsp.

enterica serotypes Tennessee and Typhimurium in peanut butter.

Materials and Methods

Bacterial Cultures

Salmonella Typhimurium ATCC 14028 and *Salmonella* Tennessee ATCC 10722 were

revived in tryptic soy broth (TSB; unless otherwise stated, all media were from Difco,

Becton Dickinson, Sparks, MD), incubated at 37°C for 18-24 h and twice transferred.

Working stocks of these cultures were spread onto sterile slants of tryptic soy agar (TSA),

incubated at 37°C for 18-24 h and stored at 4°C.

Inoculum Preparation

Each strain of *Salmonella* was twice transferred to 100 ml of TSB (tryptic soy broth)

and incubated at 37°C for 24 h in a rotating incubator (Classic C24, New Brunswick

Scientific Co., Inc., Edison, NJ, USA) at 150 rpm. Cells were harvested by twice

centrifugation at 10,000 X G for 10 min at 5°C (Sorvall RC-SB refrigerated super speed centrifuge, Du Pont, Wilmington, DE, USA). After the first centrifugation, the supernatant was poured off. The cells were washed with 100 ml of ddH2O and then re-centrifuged using the same procedure. The remaining pellet was used to inoculate the peanut butter samples.

Peanut Butter Preparation

A commercially processed creamy peanut butter was purchased at a local grocery store (Morgantown, WV). All equipment used to handle the peanut butter was sanitized by spraying with 70% ethanol, rinsed with ddH20, and dried under UV light. The inoculums of *Salmonella* were placed into separate labeled large stomacher bags (Fisherbrand, Hampton, NH, USA) each containing 375 g of peanut butter. Each bag was inoculated with washed pelleted cells and mixed for one minute to ensure even distribution of the culture. The target initial inoculum level for each strain was 10^7 CFU/g. The inoculated peanut butter was further divided into 10 g aliquots, placed into SealPAK bags (Kapak Corp, Minneapolis, MN, USA), and labeled to receive one of six different e-beam radiation doses: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy. The sample bags were individually sealed and placed into a larger SealPAK bag to reduce the possibility of leakage or contamination. The peanut butter was spread evenly in the bag to promote even e-beam absorption. The bags were packed in plastic containers (Ziploc 9 ½ cup container, SC Johnson Products Racine, WI, USA) and stored at 22°C, (classic C24, New Brunswick Scientific Co., Inc., Edison, NJ USA) until transportation to the irradiation facility.

Electron Beam Irradiation

Samples were shipped overnight to an e-beam processing facility (Sterigenics Intl., San Diego, CA, USA). Upon arrival at the irradiation facility, samples were maintained at room temperature (20°C - 24°C). The samples were treated with one sided e-beam with energy fixed at 10 MeV. The following target doses were applied: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy. The actual absorbed dose s were confirmed with film dosimetry. Immediately after e-beam treatment, the samples were repacked and shipped overnight at room temperature. Once back at West Virginia University, samples were placed and stored in an incubator at 22**°C**. The total time from inoculation of the samples until commencement of microbial analyses was 6 days (144 hours). Microbial survival over this time was confirmed with no significant reduction in control samples ($P<0.05$).

Microbiological Analysis

Microbial analyses commenced on day 2 after e-beam exposure (day 0). Samples were serially diluted and aliquots of the appropriate dilution were spread-plated in duplicate onto TSA and XLD (xylose, lysine, desoxycholate agar; Remel, Lenexa, KS, USA) agars and incubated for 18-24 hours at 35°C. Non-selective media (TSA) was used to facilitate repair of injured cells for comparison of cells recovered on selective media (XLD). Discrete *Salmonella* colonies were counted.

Statistical Analysis

The experiment was repeated three separate times. All microbiological analyses were performed in duplicate. Microbial counts (CFU/g) were converted to logarithmic values and analyzed by linear regression using Microsoft Office Excel Software (Office Excel 2007,

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Microsoft Corporation). Tukey's Honestly Significant Differences Test was used to determine

significance between the survival rates on (TSA vs. XLD). The recovered cells were expressed

as $\log_{10}$ CFU/g. The D_{10} value was calculated using the following equation: log (N/No) = -

1/D*t; where N= number of survivors at e-beam dose, No=initial microbial concentration,

D=D_{10}- value (decimal reduction dose); and t= radiation dose (Jaczynski and Park 2003).

Survival rates for each dose were analyzed by Tukey's Honestly Significant Differences Test

(P<0.05). The recovered cells were expressed as $\log_{10}$ CFU/g.

Results and Discussion

The average recovery of *Salmonella* Typhimurium and *Salmonella* Tennessee in creamy

peanut butter subjected to e-beam radiation are represented in Table 1. *Salmonella* Tennessee

was more susceptible to e-beam radiation with a 4.9 and 6.66 log reduction of cells on TSA and

XLD, respectively, at target e-beam dose of 2.5 kGy. *Salmonella* Typhimurium was reduced by

4.14 and 4.89 logs on TSA and XLD respectively at target e-beam dose of 2.5 kGy. Microbial

reductions were related to e-beam, as the e-beam dose increased, there was a reduction in the

amount of recovered *Salmonella* cells. Significant microbial reductions were seen after exposure

to 0.5 kGy e-beam dose (P<0.05). Further significant reductions occurred with increasing e-

beam doses (P<0.05). Survivor curves were calculated using actual applied e-beam doses,

confirmed using film dosimetry, and D_{10}-values (Table 2) were calculated from the respective

survivor curves (Figures 1 and 2). D_{10}-values show that *Salmonella* Typhimurium was more

resistant (0.82 ± 0.02 and 0.73 ± 0.01 kGy on TSA and XLD, respectively) than *Salmonella*

Tennessee (0.72 ± 0.02 and 0.60 ± 0.01 kGy on TSA and XLD, respectively) to e-beam

irradiation ($P<0.05$). A greater than 5-log reduction was achieved after 3.0 kGy dose when cells were recovered on TSA media; a greater than 6-log reduction was attained when recovery was on selective XLD media. The greatest significant microbial reductions in *Salmonella* were seen at the target dose of 2.5 kGy e-beam radiation ($P<0.05$). The recovery on growth and selective media were different ($P<0.05$), indicating cell injury.

E-beam treatment was effective in reducing the overall number of *Salmonella* survivors. While reductions were seen from 1 kGy to 3.1 kGy, the most significant ($P<0.0.5$) reduction in survivors was seen at e-beam dose of 2.7-3.1 kGy. *Salmonella Typhimurium* was more resistant to e-beam treatment compared to *Salmonella Tennessee* ($P<0.05$). One study by Sherry et. al. (2008) looked at the response of forty different *Salmonella* species to different doses of irradiation in order to identify the *Salmonella* species that are most likely to be radiation resistant. They examined the correlation between surviving cell counts and the recovery time of *Salmonella* at 1.5 kGy at 20°C. After the stresses of irradiation, heat, or high hydrostatic pressure were applied to *Salmonella,* the time taken to recover and grow to a defined activity level was calculated. The time taken was referred to as the TTD (Time to Detection) while the TVC (Total Viable Counts) was recorded in $\log_{10}$ CFU/ml. High numbers of survivors would be seen in resistant isolates and would result in a shorter TTD; in conjunction a longer TTD would be seen by a sensitive isolate. There was a significant correlation between the TVC and TTD ($P<0.01$), of irradiated *Salmonella* species. The TTD was inversely proportional to the TVC ($P<0.01$) (Sherry et al. 2008). After comparison to heat and high hydrostatic pressure Sherry and others (2008) determined that irradiation stresses left less cell injury. They attribute their findings to the low incubation time of 9 hours and the low dosage (1.5 kGy) of irradiation. They

did not report differences between each species of *Salmonella*, but noted that differences were seen between species.

The D-values of Salmonella in peanut butter were greater than those reported for other pathogens in higher a_w foods. The D-value of *E.coli* 0157:H7 subjected to e-beam irradiation at room temperature beef patties was 0.22 kGy (Black and Jaczynski 2006). Similar results were presented by Rochelle et al (1994) and Thayer and Boyd (2001) respectively. The differences in fat content of the beef patties influenced the effectiveness of e-beam to reduce microbial load. This may partly explain the greater D-values for *Salmonella* in peanut butter. Peanut butter shows a much higher resistance to irradiation compared to that of beef, chicken, or trout.   Peanut butter has a much higher fat content and lower a_w which would contribute to the greater D-values.

The effect of γ-radiation on bacterial reductions in butter showed that bacterial counts were reduced by 75% and 98.9% with the application of irradiation at 2.5 and 5 kGy dose. D-values were not reported (Rady and Badr 2003). Butter, like peanut butter, is an oil and water emulsion with a high fat content; butter has greater a_w than peanut butter. The difference in water activity may account for differences seen in microbial reductions in peanut butter. Differences are also likely to be attributed to the diversity of the microorganisms used (Rady and Badr 2003).

When microbes are present in the food being irradiated, the energy from the rays is transferred to the water and other molecules in the microbe. The energy damages the DNA in the microbe, causing defects in the genetic instructions. Unless it can repair this damage, the microbe will die when it grows and tries to duplicate itself (Urbain 1986). The microorganisms

are unable to multiply, thus, reproductive death is achieved. Water subjected to ionizing

radiation undergoes a radiolysis reaction, in which free radicals are generated. This is considered

an indirect mechanism of microbial inactivation because when the ionized radiation is applied,

the radiolysed water molecules disintegrate the microbial cells (Urbain 1986). It is therefore

believed that the most important contributor to the antimicrobial effect of e-beam by indirect

mechanism is the physical state of water. Peanut butter has low a_w which may provide protection

to *Salmonella* against damage by free radicals.

Two distinct characteristics make peanut butter a unique system; its high fat content and

its colloidal suspension of lipid and water. The combination of both, high fat and low water in

foods such as peanut butter might have a synergistic effect. Mattick et al. (2000 [a]) found that

although death of *Salmonella* in low a_w foods occurred at the rate predicted by their thermal

inactivation models, *Salmonella* actually survived in peanut butter for a much longer amount of

time. This demonstrates the limits of using a predictive modeling for peanut butter perhaps

because of its relatively neutral pH and high fat content They believe that pH is an important

factor influencing the survival of Salmonella at low a_w when exposed to heat (Mattick 2000 [a]).

The pH of a food is an important factor in the way in which a foodborne pathogen can survive.

A study by Ngwai et al. (2007) studied the effects of *Salmonella* Typhimurium and *Salmonella*

enteritidis in different pH levels. They found both *Salmonella* species to have more growth at a

pH of 4 compared to a pH of 2.6 (P<0.05). There were no differences between species (P<0.05).

The pH of 4 and 2.6 were chosen to represent what would typically be seen in the human

stomach. The data presented shows that *Salmonella* is easily able to survive the gastric juices of

the stomach (Ngwai et al. 2007). Peanut butter has a pH of approximately 6.3. It is not acidic,

but *Salmonella* may have more resistance and better motility at a less acidic pH. Eggs also have

a pH of approximately 6.5 and are commonly associated with *Salmonella* pathogens. It is known

that microbes are most resistant to heat at an optimum pH; this may also hold true for irradiation

(Nwagi et al. 2007). This demonstrates the importance of pH as a factor in bacterial growth.

E-beam irradiation shows positive effects for the reduction of *Salmonella* spp. in peanut

butter.   All levels of e-beam irradiation reduced initial microbial counts. Future studies to

investigate survival and growth characteristics of e-beamed *Salmonella* spp. in various

commercial brands of peanut butter and peanut butter products need to be done in order to better

understand the specific environment, quality, and safety of irradiation in peanut butter.

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Table 1. Survival of *Salmonella* Tennessee and *Salmonella* Typhimurium (mean log CFU/g ± SD; n=6) in peanut butter plated on non-selective tryptic soy agar (TSA) and selective xylose lysine deoxycholate agar (XLD).

Target E-beam dose (kGy)	*Mean Survivors (log CFU/g)*			
	Salmonella Tennessee		*Salmonella* Tyhpimurium	
	TSA	XLD	TSA	XLD
0	8.20 ± 0.09^a	8.20 ± 0.09^a	8.32 ± 0.12^a	8.25 ± 0.14^a
0.5	5.43 ± 0.05^b	4.30 ± 0.11^b	5.41 ± 0.03^b	5.32 ± 0.16^b
1	$4.26 \pm 0.16^{c, d}$	4.19 ± 0.11^b	5.35 ± 0.16^b	4.77 ± 0.17^c
1.5	4.46 ± 0.08^c	4.32 ± 0.11^b	4.49 ± 0.15^c	4.42 ± 0.07^c
2	3.84 ± 0.28^d	3.55 ± 0.18^c	4.04 ± 0.29^c	3.90 ± 0.32^d
2.5	3.30 ± 0.16^e	1.54 ± 0.15^d	4.18 ± 0.09^c	3.43 ± 0.10^d

[a,b,c,d,e] Values designated with the same letter within a column are not significantly different

Table 2. D_{10}-values of *Salmonella* serotypes Tennessee and Typhimurium in peanut butter exposed to e-beam irradiation.

	D_{10}-Value	
Salmonella **serotype**	*TSA*	*XLD*
Tennessee	0.72 ± 0.02 [1, a]	0.60 ± 0.01 [2, a]
Typhimurium	0.82 ± 0.02 [1, b]	0.73 ± 0.01 [2, b]

[1, 2] Values with different superscript numbers within rows indicate significant differences in recovery on selective verses non-selective media ($P \leq 0.05$).

[a, b] Values with different superscript lowercase letters within columns indicate significant differences in recovery between the two *Salmonella* serotypes ($P \leq 0.05$).

Figure 1. Survivor curve for *Salmonella* Tennessee in peanut butter subjected to electron beam on TSA and XLD.
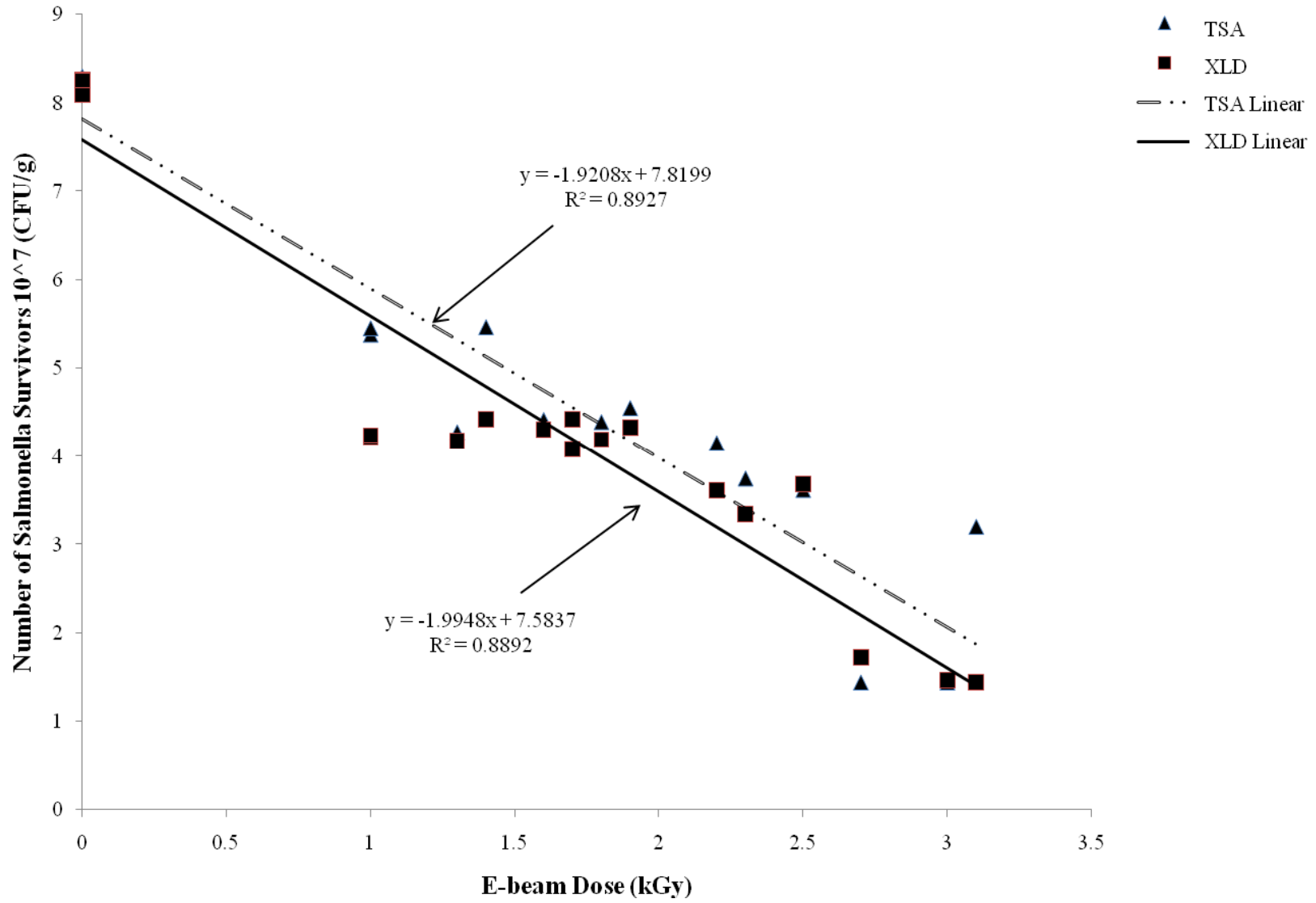
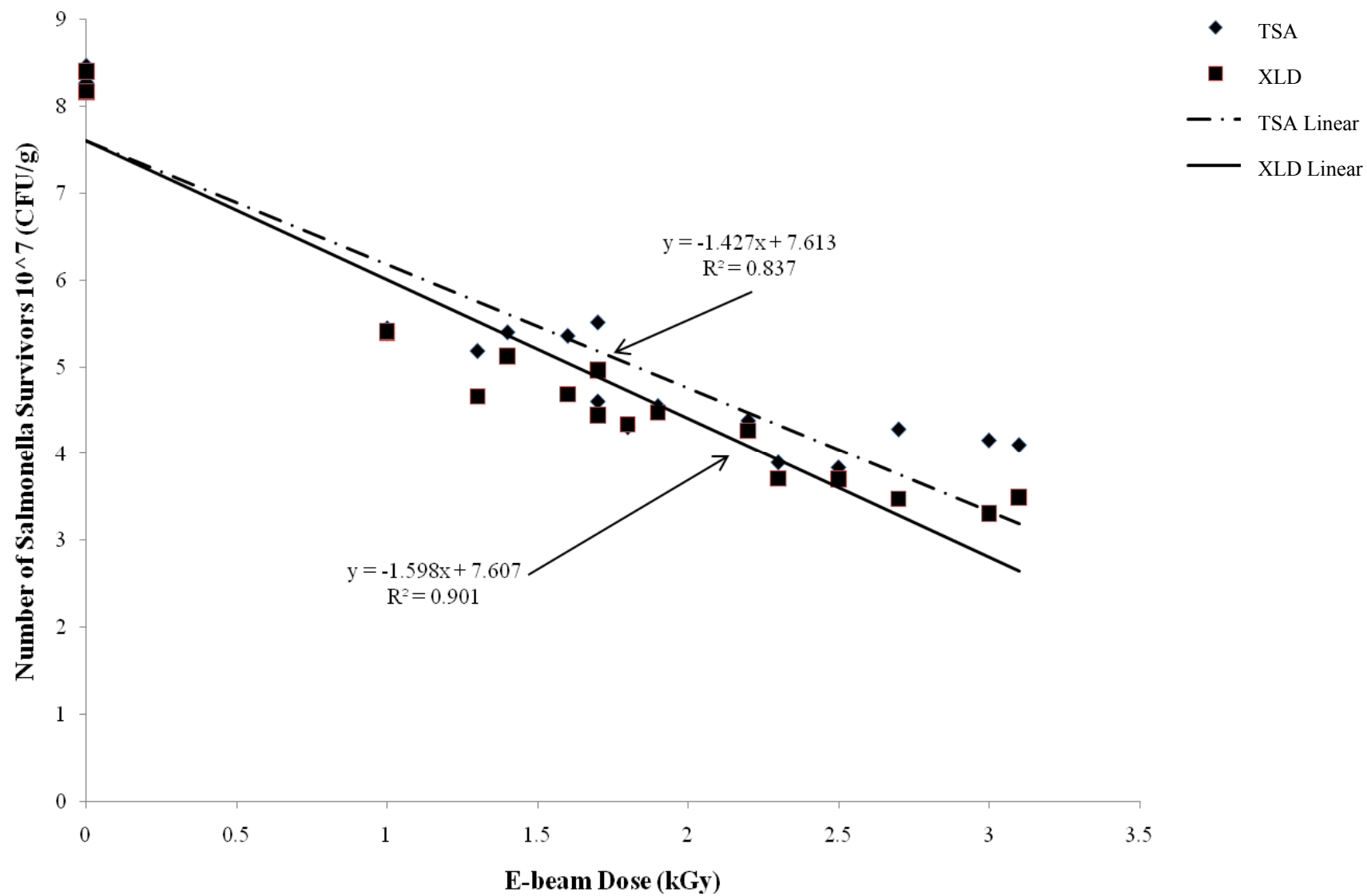**Figure 2.** Survivor curve for *Salmonella* Typhimurium in peanut butter subjected to electron beam on TSA and XLD.

CHAPTER 4

Fate of *Salmonella enterica* Serovars Typhimurium and Tennessee in Peanut Butter after Electron Beam Exposure

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Abstract

The purpose of this study was to determine the effect of electron beam irradiation on the survival of *Salmonella* serotypes Tennessee (ATCC 10722) and Typhimurium (ATCC 14028) in creamy peanut butter over a 14 d storage period at room temperature (22$^\mathbf{o}$C). Each *Salmonella* type was independently inoculated into peanut butter and subjected to the following e-beam doses: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy, which were confirmed using film dosimetry. After 2, 4, 6, 8, and 14 d of storage, microbial analyses were conducted. Survivors were enumerated on growth (tryptic soy agar; TSA) and selective (xylose lysine deoxycholate: XLD) media using a standard spread-plating method. Microbial counts (CFU/g) were log converted and differences were determined using one-way analysis of variance and Tukey's honestly significant difference test. The rate of microbial decline was significant over the 14 d storage period ($P<0.05$). The greatest reductions were seen at target e-beam dose 2.5 kGy at day 14 ($P<0.05$). *Salmonella* Tennessee was not recovered at target dose 2.5 kGy in either growth (TSA) or selective (XLD) media (<10CFU/g). *Salmonella* Typhimurium had the fewest mean survivors at the target 2.5 kGy dose with 2.12 ± 0.37 log CFU/g recovered on TSA and no recovery on XLD (<10 CFU/g). The recovery of cells on growth and selective media were different ($P<0.05$), indicating cell injury. Microbial reductions were related to e-beam dose, as the e-beam dose increased, there was a significant reduction in the amount of recovered *Salmonella* cells. The results of this study indicate that *Salmonella* Tennessee and *Salmonella* Typhimurium exposed to e-beam irradiation in peanut butter will decline overtime.

Introduction

In February 2009 the Center of Disease Control (CDC) collaborated with public health officials and the United States Food and Drug Administration (FDA) to investigate a multistate outbreak of human infections due to *Salmonella* serotype Typhimurium. The infections were related to peanut butter and peanut butter paste products, and as of March 2009 potentially contaminated products were still being recalled (CDC 2009). In November 2006, *Salmonella* Tennessee was the infectious agent in a multistate outbreak related to the consumption of peanut butter. The source of the S*almonella* is still under investigation. The ability of *Salmonella* to survive in peanut butter and peanut butter products over a period of time is one of the CDC's main concerns (CDC 2007).

Salmonella can be introduced into peanut butter manufacturing through a number of different processes including: cleaning, peeling, roasting, cooling, and packaging. The source of the *Salmonella* can come from a wide variety of possible sources such as: unsanitary workers, contaminated/unsanitary equipment, or environmental sources. Once *Salmonella* is introduced into a low water activity (a_w) food it has the ability to survive for long periods of time; likely due to an adaptive stress response. For example, filamentation occurs in *Salmonella* cells as a result of the exposure to a low a_w over time (Mattick et al. 2000 [a]) (Kieboom et al. 2006). Filamentation is an abnormal growth that occurs within bacteria in response to stress where DNA replication continues and the cells elongate, but they do not divide. When conditions become more favorable, like with an increase in a_w, rapid division of viable *Salmonella* cells resume (Mattick et al. 2000 [b]). *Salmonella* cells that exhibit filamentation are able to maintain their membrane integrity for up to 21 days (Kieboom et al. 2006). These changes will make the cells more resistant to disinfection measures like exposure to hypochlorite solutions, highlighting the

importance of proper cleaning and disinfection procedures in the processing environment (Kieboom et al. 2006). Peanut butter is a low water activity food and for that reason gives *Salmonella* an optimal environment for growth. This poses to be a risk factor for public health because the filaments of the *Salmonella* cells are able to survive for long periods of time.

Salmonella are more resistant to heat at low a_w. The a_w of peanut butter is between (0.22 – 0.29) and is considered a low a_w food. Peanut butter undergoes a typical heat pasteurization process at >70°C. Mattick et al. (2001) showed that low a_w had a protective effect on *Salmonella* cells exposed to temperatures ≥ 70°C. Cells are more tolerant to heat when suspended in low a_w environments; therefore, the heat treatment for peanut butter may not be effective for reducing the amount of viable *Salmonella* cells in peanut butter. Researchers argue whether it is the change in structure of the *Salmonella* species, or whether it is the ability of *Salmonella* to adapt that makes it more resistant to certain stresses including heat. Mattick and others (2001) studied the effects on heat tolerance at a reduced water activity state. They found that the *Salmonella* was able to adapt through habituation; however, this was not demonstrated by de novo protein synthesis or RpoS expression (Mattick et al. 2000 [a]). Recall that RpoS controls the expression of genes that respond to environmental stresses such as heat shock and osmotic stress. The ability of viable *Salmonella* cells to conform to their environment may have been due to the modification of existing cell proteins (Mattick et al. 2000 [a]). Mattick et al. (2001) identified that at temperatures > 70°C, *Salmonella* was more heat tolerant at low a_w than at higher a_w. Researchers need to come up with a reliable method of eliminating *Salmonella* in low a_w foods. This leaves many government agencies and public health officials searching for new methods and processes that may alleviate the unsafe practices now implied.

A new area of research in food safety is irradiation. Certain foods or food products are exposed to ionizing radiation in order to destroy microorganisms, bacteria, viruses, or insects that might be present. Irradiation has the ability to reduce the number of probable microbiological hazards that could be present in or on a food or food product. Many food and food products tested remain potentially unaffected in texture, taste, and nutritional value. This process has already been implemented in the food industry for products like poultry, meat, spices, fresh fruit and vegetables. Irradiation has been shown to be effective in its ability to reduce *Salmonella* species in these products. This has been demonstrated in multiple studies. A study by Sarjeant et al. (2005) showed a 4-log reduction in microbial counts of commercial chicken breasts treated with 1, 2, and 3 kGy of irradiation (Sarjeant et al. 2005). Another study by Lewis and others (2002) found boneless skinless chicken breasts free of *Salmonella* after being treated with e-beam irradiation doses of 1.0 and 1.8 kGy (Lewis et al. 2002). Its ability to be effective in low water activity high fat foods like peanut butter has not yet been studied.

To date there have been no studies that have examined the survival of *Salmonella* species in low a_w foods over time after being treated with doses of e-beam irradiation. The objective of this study was to observe the survival characteristics of *Salmonella enterica* subspecies *enterica* serotypes Tennessee and Typhimurium in e-beam irradiated peanut butter over a two week shelf life at 22ºC.

Materials and Methods

Bacterial Cultures

Cultures of *Salmonella* Typhimurium ATCC 14028 and *Salmonella* Tennessee ATCC 10722 were revived in tryptic soy broth (TSB; unless otherwise stated, all media were from Difco, Becton Dickinson, Sparks, MD), incubated at 37°C for 18-24 h and twice transferred. The working stocks were spread onto sterile slants of tryptic soy agar (TSA), incubated at 37°C for 18-24 h and stored at 4°C.

Inoculum Preparation

Salmonella Typhimurium and *Salmonella* Tennessee were respectively twice transferred into 100ml TSB (tryptic soy broth) and incubated at 37°C for 24 h in a rotating incubator (Classic C24, New Brunswick Scientific Co., Inc., Edison, NJ, USA) at 150 rpm. Twice centrifugation at 10,000 X G for 10 min at 5°C was used to harvest the cells (Sorvall RC-SB refrigerated super speed centrifuge, Du Pont, Wilmington, DE, USA). After centrifugation, the supernatant was poured off. The cells were then washed with 100 ml of ddH2O.   After being rinsed, the cells were re-centrifuged using the same procedure. The remaining cells were used to inoculate the peanut butter samples.

Peanut Butter Preparation

For the samples, regular creamy peanut butter (Peter Pan, ConAgra Foods, Omaha, NE) was used. The peanut butter was purchased at a local grocery store. During the experiment, all equipment was sanitized by spraying with 70% ethanol, rinsed with ddH20, and dried under UV light. The *Salmonella* inoculums were placed into large stomacher

bags (Fisherbrand, Hampton, NH, USA) each containing 375 g of peanut butter. Each bag was labeled and inoculated respectively. The samples were then pummeled by hand to ensure even distribution. 10^7 CFU/g was the target inoculum level for each strain. For even treatment distribution purposes, the inoculated peanut butter was further divided into 10 g aliquots, and placed into 4" × 6" SealPAK bags (Kapak Corp, Minneapolis, MN, USA). All bags were labeled to receive one of six different e beam radiation doses: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy. There were twenty bags per dose to allow for analysis over time. In order to reduce the possibility of leakage or contamination, the sample bags were individually sealed and placed into 6.5" × 8" SealPak. The peanut butter was spread out in the bag to promote even e-beam absorption. The samples were packed in plastic containers (Ziploc 9 ½ cup container, SC Johnson Products Racine, WI, USA) according to treatment and stored at 22ºC (classic C24, New Brunswick Scientific Co., Inc., Edison, NJ USA) until transportation to the irradiation facility.

Electron Beam Irradiation

Samples were shipped to an e-beam processing facility (Sterigenics Intl., San Diego, CA, USA). Samples were received at the facility overnight and were maintained at room temperature (20ºC - 24ºC). One-sided e-beam with energy fixed at 10 MeV was used for all the samples. Target doses of: 0 (control), 0.5, 1, 1.5, 2, and 2.5 kGy were applied. After e beam treatment, the samples were repacked, shipped overnight, and kept at 22ºC. Once received back at West Virginia University, the samples were stored at 22ºC. Total time from inoculation of the samples until beginning of microbial analyses was 6 days (144 hours). Microbial survival over time was confirmed with no significant reduction in control samples ($P<0.05$).

Microbiological Analysis

Microbial analyses started on day 2 after exposure to e-beam (day 0). Analysis was completed on days 4, 6, 8, and 14 to determine microbial reductions over time. Serially diluted samples and aliquots of the dilutions were spread-plated in duplicate onto TSA and XLD (xylose, lysine, deoxycholate agar; Remel, Lenexa, KS, USA) agars. The plates were incubated for 18-24 hours at 35ºC. Non-selective media (TSA) was used to facilitate repair of injured cells for comparison of cells recovered on selective media (XLD). Isolated *Salmonella* colonies were counted.

Statistical Analysis

The experiment was replicated three times, and microbiological analysis was performed in duplicate. Microbial counts (CFU/g) were converted to logarithmic values and analyzed by linear regression using Microsoft Office Excel Software (Office Excel 2007, Microsoft Corporation). The recovered cells were expressed as $\log_{10}$ CFU/g. The survival rates for each dose and for each day were analyzed by Tukey's Honestly Significant Differences Test (P<0.05).

Results and Discussion

The average recovery of *Salmonella* Typhimurium and *Salmonella* Tennessee in creamy peanut butter subjected to e-beam radiation over a period of 14 days is represented in Tables 1-4. Recovery of cells on growth and selective media were different (P<0.05), indicating cell injury. *Salmonella* Tennessee and *Salmonella* Typhimurium both had the fewest mean survivors at target e-beam dose 2.5 kGy at day 14. *Salmonella* Tennesssee was not recovered at the target e-beam dose 2.5 kGy on either selective (XLD) or non-selective (TSA) media (<10 CFU/g).

Salmonella Typhimurium had the fewest mean survivors at the target e-beam dose of 2.5 kGy with 2.12±0.37 log CFU/g recovered on TSA and no recovery on XLD (<10 CFU/g). Microbial reductions were related to e-beam dose, as the e-beam dose increased, there was a significant reduction in the amount of recovered *Salmonella* cells (Figures 1-4). Mean *Salmonella* survivors also decreased overtime. Significant reductions overtime were not seen until day 8 for *Salmonella* Typhimurium and day 14 for *Salmonella* Tennessee when recovered on TSA agar ($P<0.05$). Mean *Salmonella* populations were significantly reduced after exposure to 0.5 kGy e-beam dose, significant reductions occurred with increasing e-beam doses ($P<0.05$). Changes in the log number of *Salmonella* survivors were seen between days, but were only significantly different at day 14, specifically at target e-beam dose of 2.5 kGy ($P<0.05$). At day 14, a greater than 2-log and 0.5-log reduction was seen in *Salmonella* Tennessee recovered on TSA and XLD, respectively. A greater than 2-log reduction of *Salmonella* Typhimurium was seen over time regardless of recovery media. The greatest overall significant microbial reductions in *Salmonella* were seen at the target dose of 2.5 kGy e-beam radiation overtime ($P<0.05$).

Other recent studies on the effects *Salmonella* in peanut butter over storage time have found similar results. Burnett et al. (2000) found that *Salmonella* survives in peanut butter and peanut butter spreads for at least 24 weeks. They believe that *Salmonella* is likely to survive in peanut butters and spreads for the duration of their expected shelf life (Burnett et al. 2000). In our study, *Salmonella* was able to survive up to two weeks even when significantly injured by e-beam irradiation.

Salmonella viability is affected by the storage temperature in colloidal food products. It is more likely to survive at lower temperatures over higher ones, likely due to their reduction of

metabolic activity at the lower temperatures (Holliday and Beuchat 2003) (Holliday et al. 2003) (Park et al. 2000). This is consistent with the finding of Park et al. (2000) who looked at the effect of storage time (14 day) and temperature (22°C vs. 4°C) on the fate of *Salmonella* Tennessee in different commercial brands of peanut butter. They found that *Salmonella* death was more rapid when stored at 22°C compared to 4°C for a two week period.

Burnett et al. (2000) reported that the rate of *Salmonella* survival in peanut butter and peanut butter spread was related to differences in the size and stability of water droplets. Since *Salmonella* in peanut butter is likely to be found near the water phase, a finer emulsion would create an environment where nutrients are less available (Mattick et al. 2000 [b]). Of the five commercial brands of peanut butter studied, one brand showed significance (P>0.05) between selective versus non-selective media, while the other 4 brands showed no significant difference. Despite these differences there were no differences in the composition of the five brands studied (Park et al. 2008). Our results on the survival of *Salmonella* in peanut butter support the observations of Park and others (2008). Our results indicated that there was no significance (P>0.05) trends seen between selective (XLD) and non-selective (TSA) media. It demonstrates the ability of *Salmonella* to survive independently over time despite temperature variations. Our current study showed that under typical storage temperatures at 22°C, *Salmonella* will persist even if injured by e-beam irradiation. However, Park and others (2008) showed that *Salmonella* would likely survive for a much longer period if stored at 4°C. This highlights the critical need to find a processing treatment that would effectively kill all pathogens of concern.

In our study, all levels of e-beam treatment reduced the initial *Salmonella* populations. Regardless of dose and species, the microbial counts significantly decreased after 14 days of

storage time; however, significant reductions were seen sooner (after day 6) in samples treated with greater e-beam doses (≥ 2 kGy). *Salmonella* would survive over the shelf life of peanut butter even if the cells sustained injury. The results of this study demonstrate the need to reduce the occurrence of post-processing contamination and to find a non-thermal processing method capable of effectively eliminating pathogens from peanut butter. Future research will look at the effect of high beam dose on the functional and sensorial properties of peanut butter.

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Table 1. *Survival curves of Salmonella* Tennessee *populations (mean log CFU/g ± SD; n=6) in peanut butter plated on non-selective tryptic soy agar (TSA) with an initial inoculums of 10^7.*

E-beam Dose	Day 2	Day 4	Day 6	Day 8	Day 14
0	8.20±0.09 [1,a]	8.14 ± 0.08 [1,a]	8.12 ± 0.07 [1,a]	8.12 ± 0.12 [1,a]	8.09 ± 0.12 [1,a]
0.5	5.43±0.05 [1,b]	5.34 ± 0.18 [2,b]	5.33 ± 0.19 [2,b]	5.38 ± 0.14 [2,b]	4.82 ± 0.39 [3,b]
1	4.26±0.16 [1,c, d]	4.23 ± 0.08 [1,c]	4.30 ± 0.22 [1,c]	4.22 ± 0.06 [1,c]	3.79 ± 0.48 [2,b]
1.5	4.46±0.08 [1,c]	3.72 ± 0.19 [2,d]	3.54 ± 0.15 [2,d]	3.50 ± 0.06 [2,d]	3.28 ± 0.19 [3,c]
2	3.84±0.28 [1,d]	3.34 ± 0.10 [2,e]	3.45 ± 0.22 [2,d]	3.43 ± 0.10 [2,d]	3.31 ± 0.08 [3,c]
2.5	3.30±0.16 [1,e]	2.50 ± 0.24 [2,f]	2.51 ± 0.21 [2,e]	2.44 ± 0.20 [2,e]	$<1.0*10^2$ [3,d]

[a,b,c,d,e] Values designated with the same letter within a column are not significantly different (P<0.05).

[1, 2, 3, 4] Values with different superscript numbers within rows indicate significant differences in within days ($P \leq 0.05$).

Table 2. *Survival curves of Salmonella* Tennessee *populations (mean log CFU/g ± SD; n=6) in peanut butter plated on selective xylose lysine deoxycholate agar (XLD) with an initial inoculums of 10^7.*

E-beam Dose	Day 2	Day 4	Day 6	Day 8	Day 14
0	8.20±0.09 [1,a]	8.10 ± 0.30 [1,a]	8.13 ± 0.11 [1,a]	8.10 ± 0.08 [1,a]	8.04 ± 0.14 [2,a]
0.5	4.30±0.11 [1,b]	4.27 ± 0.29 [1,b]	4.62 ± 0.76 [2,b]	4.35 ± 0.13 [1,b]	3.87 ± 0.42 [3,b]
1	4.19±0.11 [1,b]	4.15 ± 0.10 [1,b]	4.10 ± 0.16 [1,b]	4.14 ± 0.01 [1,b]	3.86 ± 0.53 [2,b]
1.5	4.32±0.11 [1,b]	3.64 ± 0.21 [2,c]	3.64 ± 0.18 [2,c]	3.57 ± 0.07 [2,c]	3.38 ± 0.08 [3,c]
2	3.55±0.18 [1,c]	3.36 ± 0.12 [2,c]	3.46 ± 0.18 [1,c]	3.44 ± 0.06 [1,c]	3.30 ± 0.10 [2,c]
2.5	1.54±0.15 [1,d]	2.35 ± 0.14 [2,d]	2.31 ± 0.09 [2,d]	1.88 ± 0.05 [1,d]	<1.0*10^2 [3,d]

[a,b,c,d,e] Values designated with the same letter within a column are not significantly different (P<0.05).

[1, 2, 3, 4] Values with different superscript numbers within rows indicate significant differences in within days ($P \leq 0.05$).

Table 3. *Survival curves of Salmonella* Typhimurium *populations (mean log CFU/g $\pm$ SD; n=6) in peanut butter plated on non-selective tryptic soy agar (TSA) with an initial inoculums of 10^7.*

E-beam Dose	Day 2	Day 4	Day 6	Day 8	Day 14
0	8.32±0.12 [1,a]	8.27 ± 0.11 [1,a]	8.35 ± 0.07 [1,a]	8.08 ± 0.25 [2,a]	8.13 ± 0.11 [2,a]
0.5	5.41±0.03 [1,b]	5.13 ± 0.19 [2,b]	5.12 ± 0.23 [2,b]	5.16 ± 0.17 [2,b]	5.04 ± 0.11 [3,b]
1	5.35±0.16 [1,b]	4.22 ± 0.20 [2,c]	4.30 ± 0.09 [2,c]	4.27 ± 0.06 [2,c]	3.89 ± 0.33 [3,c]
1.5	4.49±0.15 [1,c]	4.29 ± 0.21 [2,c]	4.22 ± 0.25 [2,c]	4.22 ± 0.16 [2,c]	3.44 ± 0.17 [3,d]
2	4.04±0.29 [1,c]	3.8 ± 0.06 [2,d]	3.62 ± 0.16 [3,d]	3.50 ± 0.21 [3,d]	3.48 ± 0.05 [3,d]
2.5	4.18±0.09 [1,c]	3.18 ± 0.25 [2,e]	3.05 ± 0.24 [2,e]	3.00 ± 0.29 [2,e]	2.12 ± 0.37 [3,e]

[a,b,c,d,e] Values designated with the same letter within a column are not significantly different (P<0.05).

[1, 2, 3, 4] Values with different superscript numbers within rows indicate significant differences in within days ($P \leq 0.05$).

Table 4. *Survival curves of Salmonella* Typhimurium *populations (mean log CFU/g ± SD; n=6) in peanut butter plated on selective xylose lysine deoxycholate agar (XLD) with an initial inoculums of 10^7.*

E-beam Dose	Day 2	Day 4	Day 6	Day 8	Day 14
0	8.25±0.14 [1,a]	8.21 ± 0.11 [1,a]	8.31± 0.07 [1,a]	8.21 ± 0.09 [1,a]	8.07 ± 0.11 [2,a]
0.5	5.32±0.16 [1,b]	4.60 ± 0.12 [2,b]	4.62 ± 0.04 [2,b]	4.66 ± 0.06 [2,b]	4.52 ± 0.04 [2,b]
1	4.77±0.17 [1,c]	3.56 ± 0.12 [2,c]	3.68 ± 0.09 [2,c]	3.77 ± 0.33 [2,c]	3.46 ± 0.04 [3,c]
1.5	4.42±0.07 [1,c]	3.74 ± 0.24 [2,c]	3.76 ± 0.32 [2,c]	3.76 ± 0.22 [2,c]	3.41 ± 0.12 [3,c]
2	3.90±0.32 [1,d]	3.60 ± 0.15 [1,c]	3.41 ± 0.14 [2,d]	3.43 ± 0.04 [2,d]	3.33 ± 0.05 [3,c]
2.5	3.43±0.10 [1,d]	2.54 ± 0.15 [2,d]	2.61 ± 0.14 [2,e]	2.54 ± 0.12 [2,e]	<1.0*10^2 [3,d]

[a,b,c,d,e] Values designated with the same letter within a column are not significantly different (P<0.05).

[1, 2, 3, 4] Values with different superscript numbers within rows indicate significant differences in within days ($P \leq 0.05$).

Figure 1. *Salmonella* Tennessee survivors in peanut butter subjected to electron beam on TSA over a 14 day period.
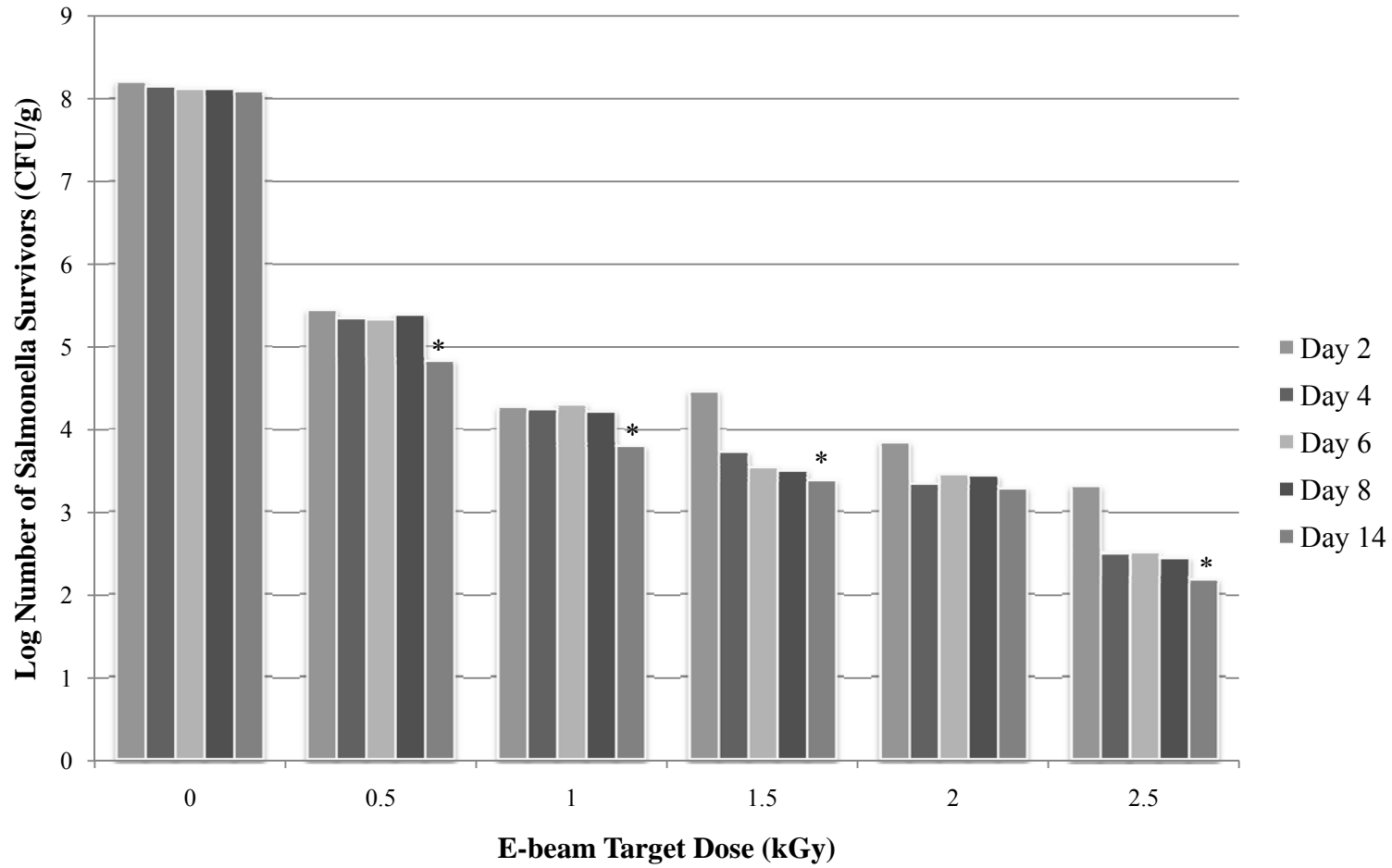
Figure 2. *Salmonella* Tennessee survivors in peanut butter subjected to electron beam on XLD over a 14 day period.
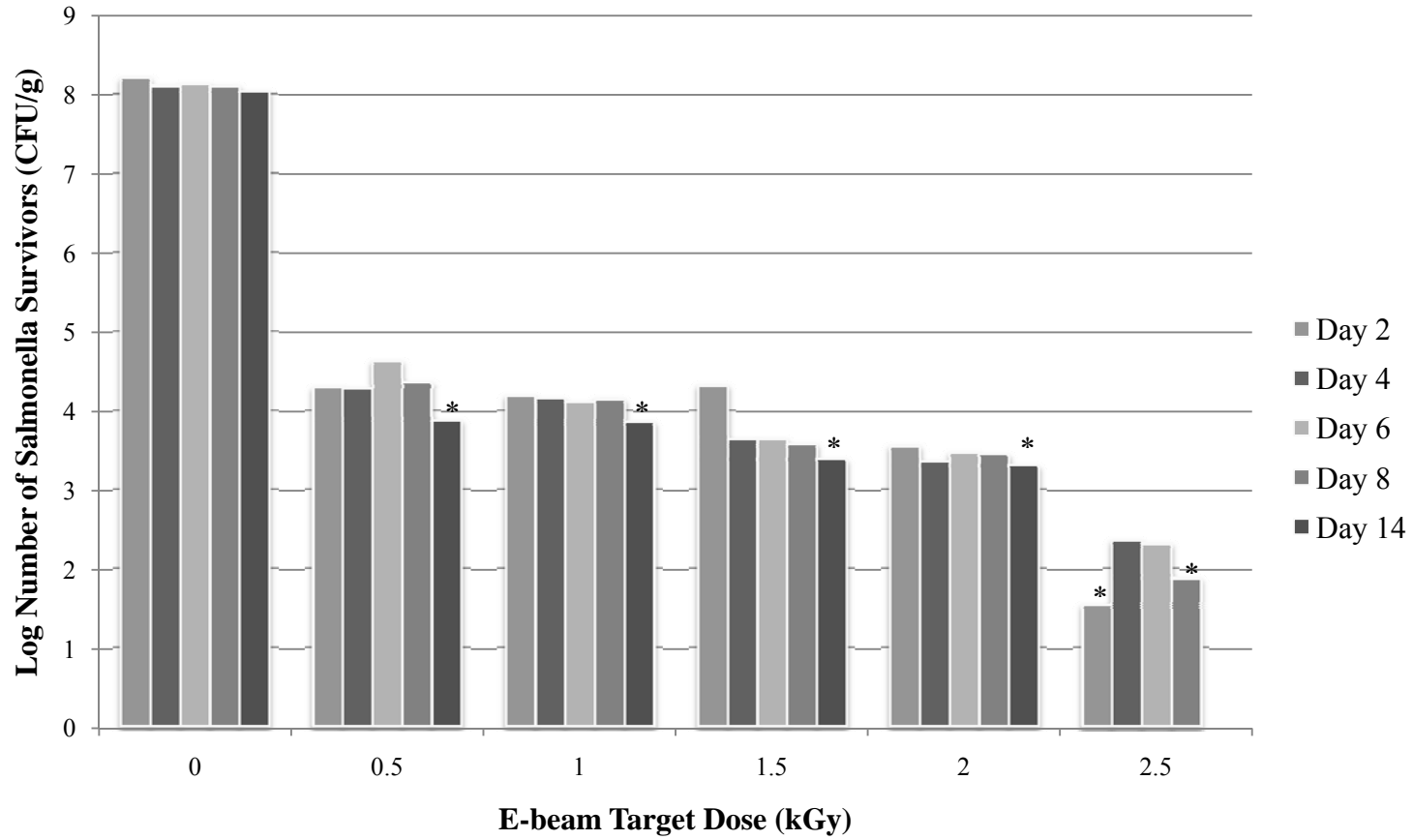
Figure 3. *Salmonella* Typhimurium survivors in peanut butter subjected to electron beam on TSA over a 14 day period.
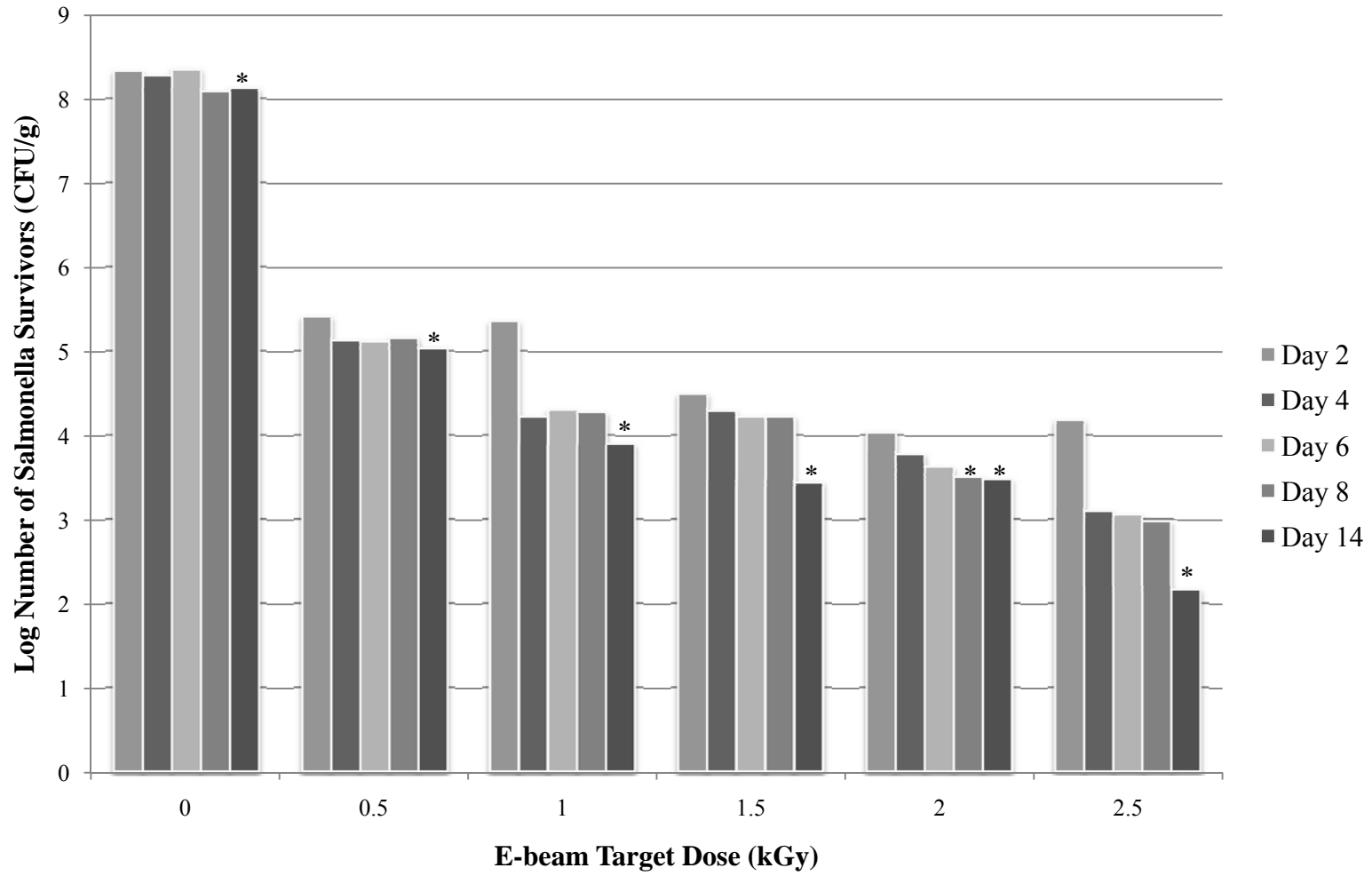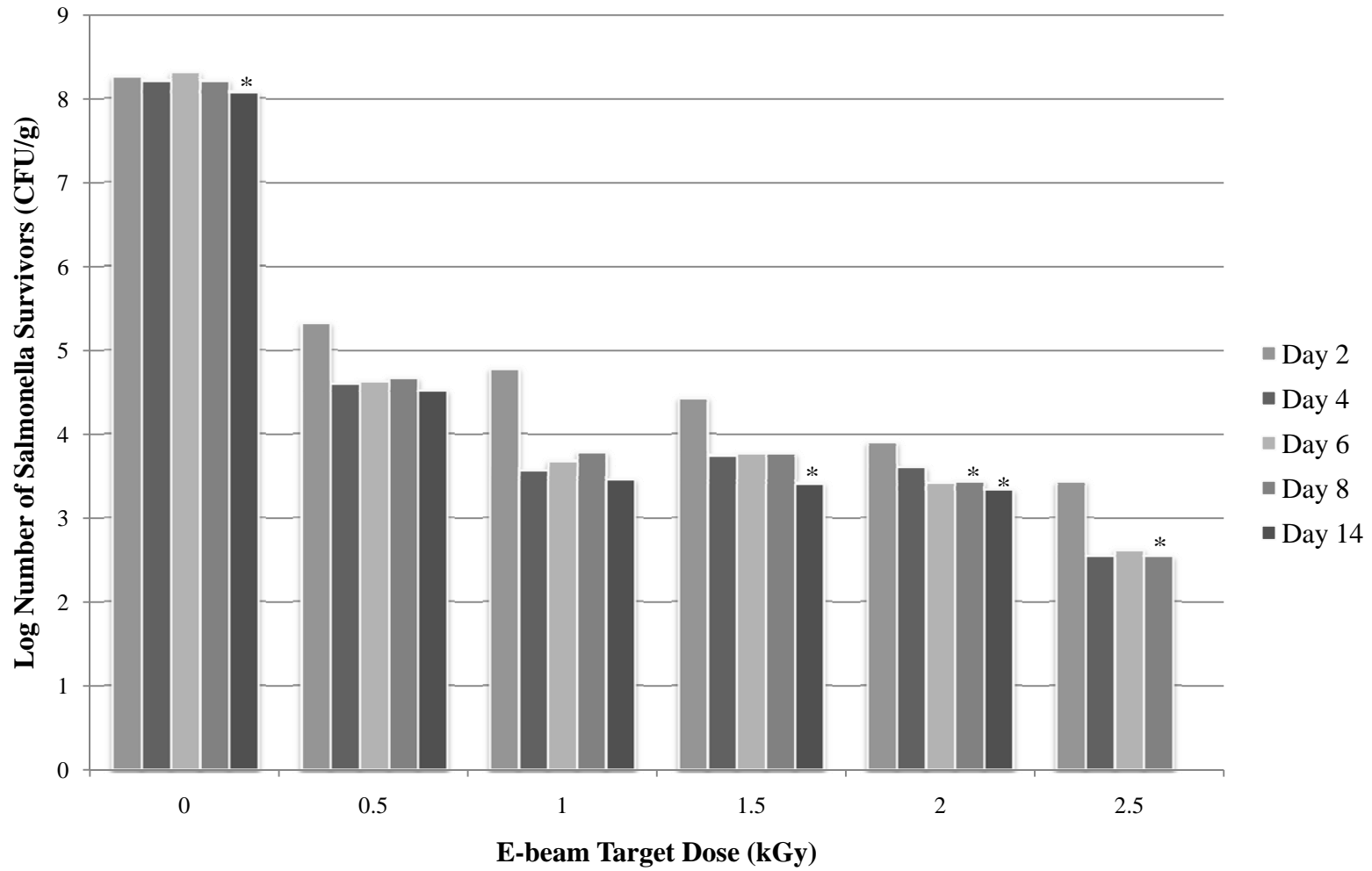
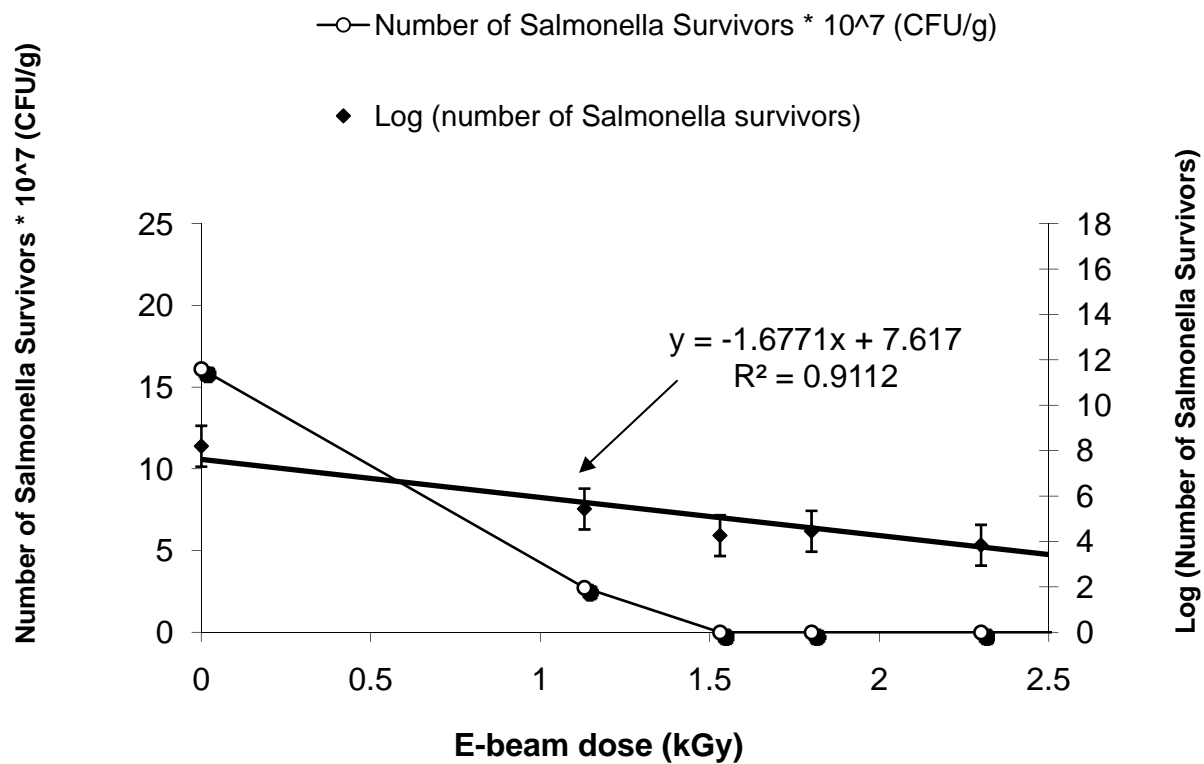**Figure 4.** *Salmonella* Typhimurium survivors in peanut butter subjected to electron beam on XLD over a 14 day period.

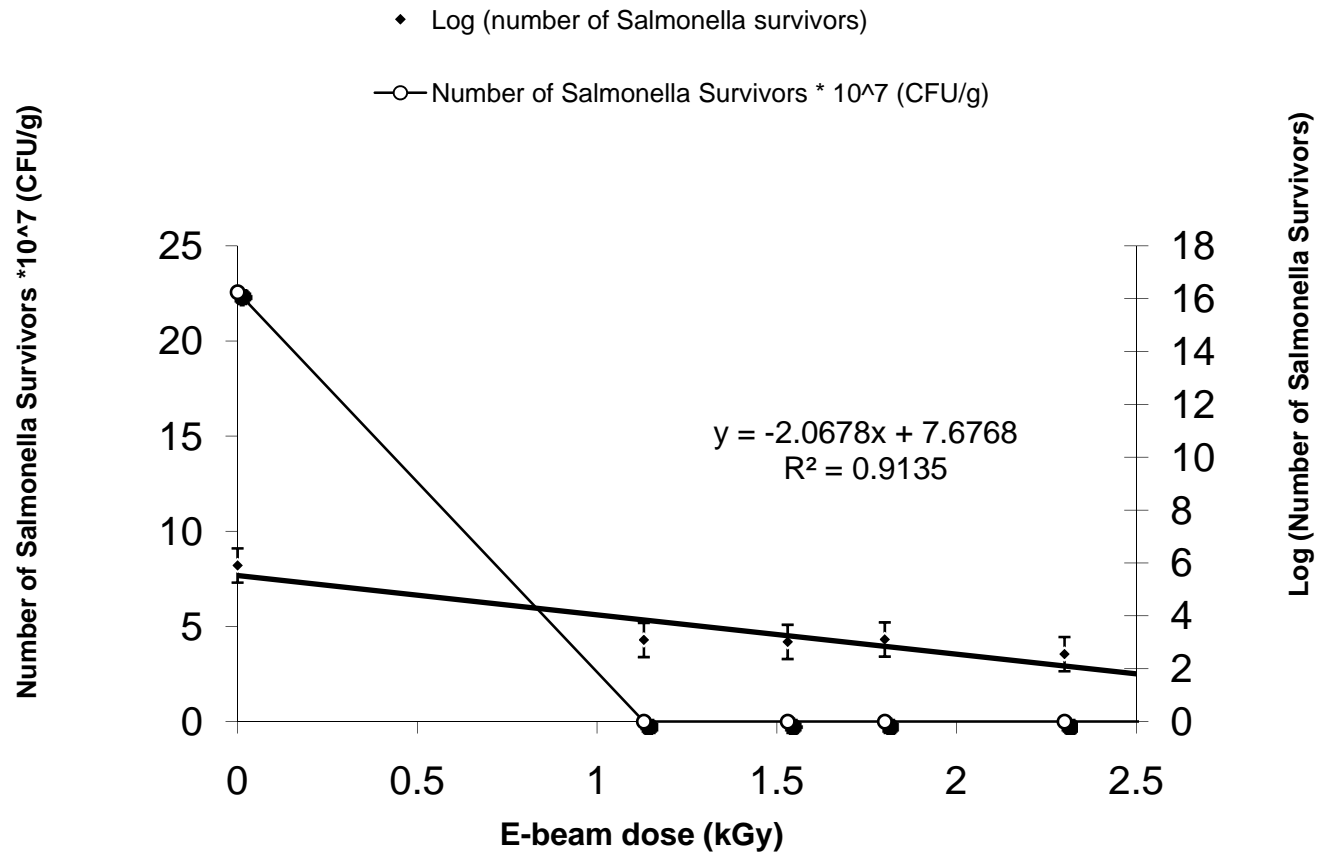Survivor curve for *Salmonella* Tennessee in peanut butter subjected to electron beam on TSA. Bars on the data points indicate standard error.
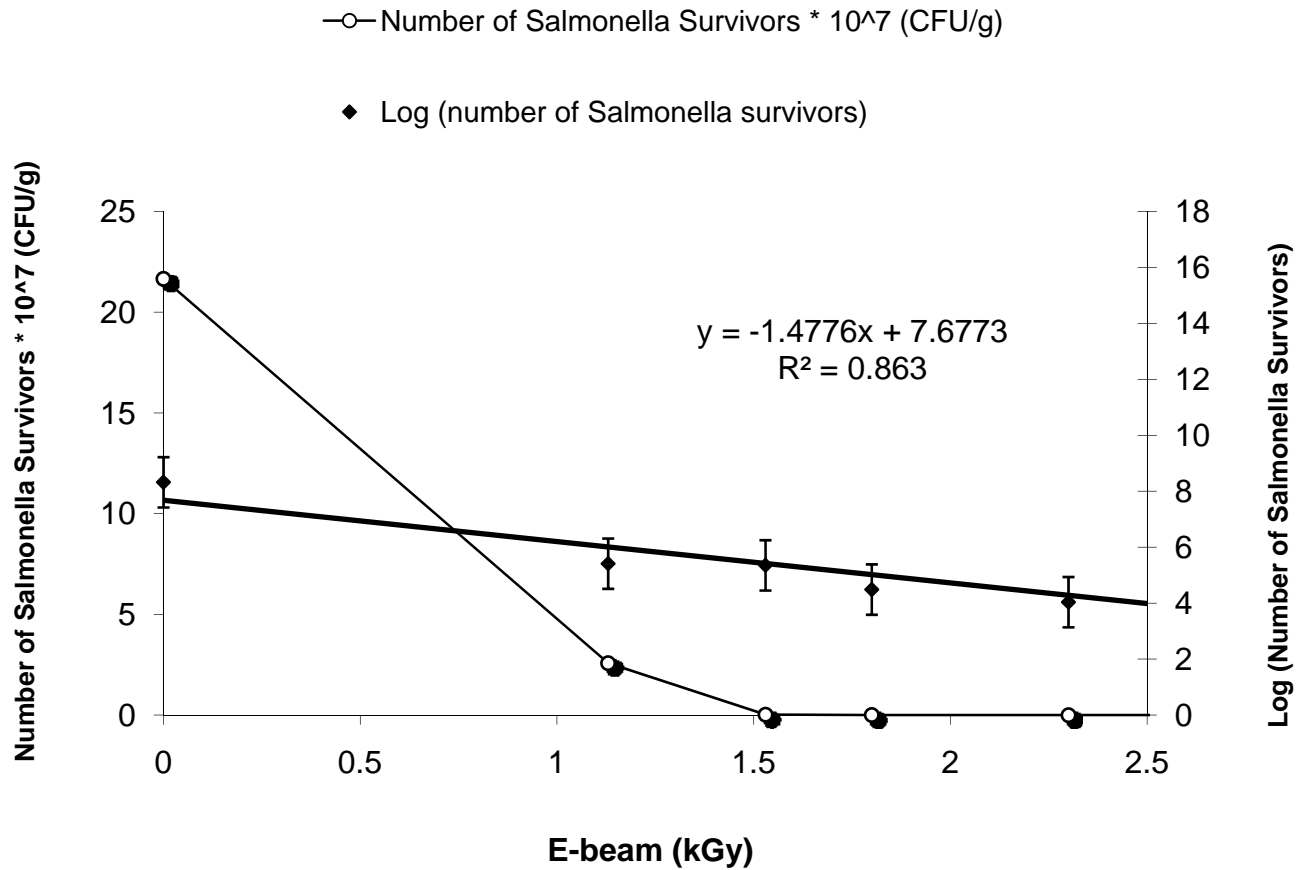
Survivor curve for *Salmonella* Tennessee in peanut butter subjected to electron beam on XLD. Bars on the data points indicate standard error.
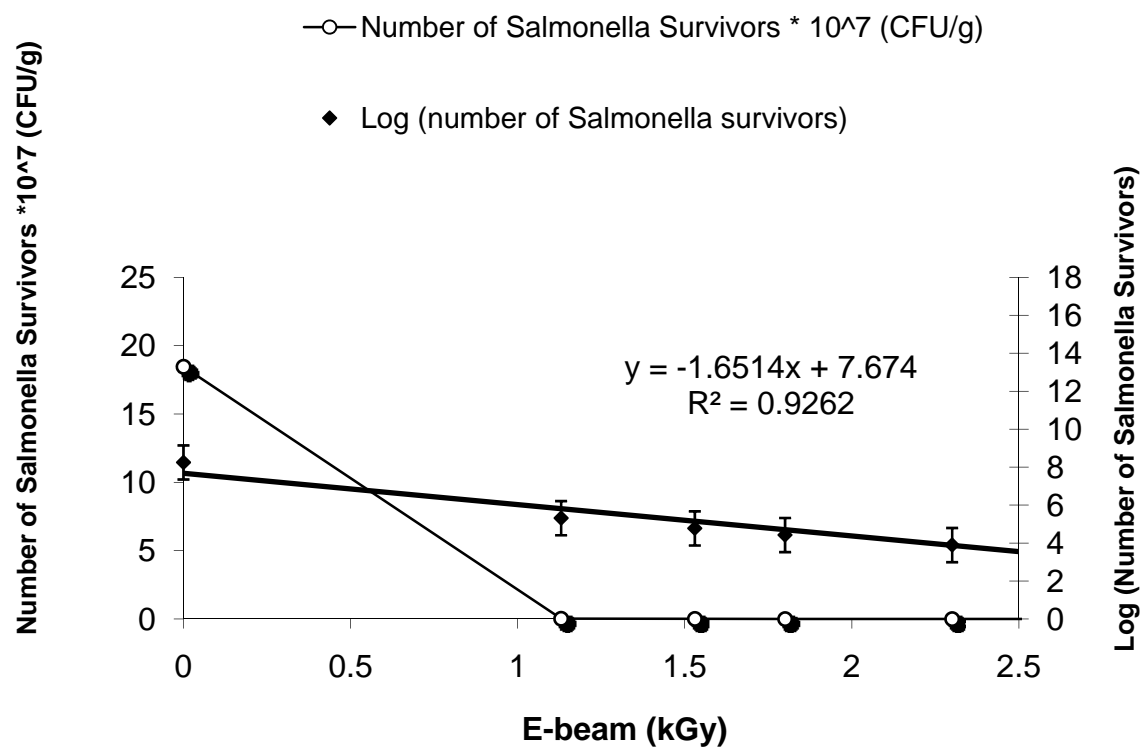
Survivor curve for *Salmonella* Typhimurium in peanut butter subjected to electron beam on TSA. Bars on the data points indicate standard error.

$y = -1.4776x + 7.6773$
$R^2 = 0.863$

Survivor curve for *Salmonella* Typhimurium in peanut butter subjected to electron beam on XLD. Bars on the data points indicate standard error.

$y = -1.6514x + 7.674$
$R^2 = 0.9262$

Vitae

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