EFFECTS OF LOW DOSE ELECTRON BEAM TREATMENT ON THE QUALITY AND SAFETY OF ALFALFA SPROUTS

A Thesis

by

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ABSTRACT

Fresh Produce provides a rich assortment of the necessary vitamins and minerals needed to sustain a healthy diet. Alfalfa sprouts provide a concentrated number of compounds that have been shown to help support the body and aid in reducing the risk of serious health issues such as cancer and heart disease. Unfortunately, Alfalfa sprouts have also been linked to many foodborne illness outbreaks. In the past 20 years there have been over 30 outbreaks in the U.S alone linked to Alfalfa sprouts. The primary pathogens associated with Alfalfa sprouts are *Salmonella* spp. along with multiple stereotypes of Toxigenic non O157 E. *coli*. One cause of these outbreaks is the lack of thermal processing or other "kill steps" taken during the manufacturing process. These steps are usually skipped to help retain the desired sensory and nutritional qualities desired by consumers. However, this lack of a "kill step" leaves the Alfalfa sprouts vulnerable to pathogen contamination. The purpose of this study was to determine the effectiveness of Electron Beam (eBeam) in lowering both the natural bioburden and inoculated pathogenic organisms in Alfalfa sprouts. Additionally, we investigated whether treatment with eBeam at a max dose of 1kGy would aid in increasing the shelf-life of the sprouts. We established a max dose of 1kGy so that we would be able to see the benefits achieved following the current standards set by the FDA today. The hypothesis of this study was that the treatment of Alfalfa Sprouts with eBeam at the current maximum dose (1kGy) approved by the FDA would result in a significant reduction of both the natural bioburden and pathogenic organisms while

increasing the shelf-life of the samples.

Results showed no statistical difference ($p<0.05$) between treated and untreated sprout samples when comparing the texture and color sensory qualities. Microbiological studies revealed a 2.03 and 2.11 log10 reduction in natural bioburden of aerobic bacterial and fungal populations. The low dose (1kGy) eBeam treatment resulted in a 4.44 log10 reduction in the cocktail of 6 stereotypes of toxigenic non O157 E. *coli* inoculum. Quantitative Microbial Risk Assessment (QMRA) analysis utilizing the reductions found revealed that the reduction potential correlates to a theoretical drop in the probability of infection from the consumption of a normal serving size of alfalfa sprouts contaminated with E. coli from 0.044 to 0.000002.

DEDICATION

I would like to dedicate this to my family, friends, and advisors. Without whom this achievement would not have been possible

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Pillai, for allowing me to be a part of his lab. His guidance, patience, and support throughout the course of this research are what made this achievement possible. I would also like to acknowledge my committee members Dr. Miller and Dr. Awika for their patience and contributions along the way to achieving my goal.

I would like to thank Mickey, Emma, and everyone at the National Center for Electron Beam Research Center whose patience and assistance throughout my project helped in the completion of this research.

In addition, I would like to express appreciation to my lab mates Chandni, Charlotte, Dave, Julie, Bianca, Shima, and Lindsay whose words of encouragement and advice helped me along the way.

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Last, but certainly not least, I would like to thank my parents for their unwavering support and love. Without their guidance I would never have been able to achieve this goal.

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by a thesis committee consisting of Professor Dr. Suresh D. Pillai [advisor] of the Poultry Science Department, Professor Dr. Joseph M. Awika of the Soil and Crop Sciences Department, and Professor Dr. Rhonda K. Miller of the Department of Animal Science.

The data analyzed for Quantitative Microbial Risk Assessment in Section 4 was assisted by Lindsay Ward of the department of Nutrition and Food Science.

All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

B/CS	Bryan/College Station
FDA	Food and Drug Administration
USDA	United States Department of Agriculture
kGy	Kilo Gray
eBeam	Electron Beam
CFU	Colony Forming Unit
MeV	Million Electron Volt
PBS	Phosphate Buffer Solution
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
PCA	Plate Count Agar

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1. INTRODUCTION

Fresh produce is an important staple of a well-balanced and healthy diet. However, it is also a growing source of foodborne illness outbreaks. As we notice an increase in consumers searching for healthier diets and growing trends in fresh produce consumption, we see an increase in the number of outbreaks of foodborne illnesses. Over the past few decades the relation of foodborne illness outbreaks linked to fresh produce has risen from <1% to 12% (1). A major reason for the risk is that fresh produce items are most commonly consumed raw. This removes the potential for pathogen reduction that is achieved through the thermal process of cooking. This step is most commonly forgone to retain the nutritional and sensory qualities of the food desired by consumers.

One of the highest risk foods associated to the outbreaks are alfalfa sprouts (4). Sprouts are most commonly grown under ideal conditions for bacterial growth. In addition, they are consumed raw, desired for their texture and nutritional qualities. This makes them susceptible for the transmission of pathogens. Since 1997 there have been over 30 reported outbreaks linked to alfalfa sprouts in the United States (1). These outbreaks have been most commonly caused by E. *coli* and *Salmonella* spp. Because of these outbreaks many retailers and restaurants have stopped selling sprouts. However, by implementing a non-thermal processing step, with significant pathogen reducing potential, we can deliver a safer product while maintaining the desired qualities. One such technology is electron beam food processing technology. Electron beam (eBeam) technology has been shown to improve the safety and quality of fresh

produce by inactivating pathogens and spoilage organisms (2,5). This technology has been used in the food industry for pasteurizing ground beef, for decontaminating spices and for phytosanitary treatment purposes (3). eBeam is a preferred method for irradiation technology due to its efficiency. FDA has approved eBeam technology for the use in phytosanitary treatment of fresh produce at a dose not to exceed 1kGy.

I hypothesize that eBeam treatment of alfalfa sprouts at a low dose (≤1kGy) will result in a significant reduction of both the natural bioburden and a six-stereotype cocktail of toxigenic non O157 E. *coli* that will be inoculated on the sprouts. And that this reduction will be achieved without the deterioration of the alfalfa sprouts sensory qualities. The overall goals of this study are to evaluate the effectiveness of low dose (≤1kGy) electron beam treatment on the reduction of both natural bioburden and a cocktail of the E. *coli* stereotypes 0111, 0121, 045, 0145, 0103, and 026 pathogen loads as well as determine what, if any, effects the treatment has on the sensory quality of alfalfa sprouts.

Specific objectives were:

1. Determine the reduction of background microbial load on alfalfa sprouts (*Medicago sativa*) after being treated with an eBeam dose below 1 kilo gray (kGy) and monitor the microbial levels over a period of 3 weeks

2. Conduct objective sensory analysis on the color and texture of eBeam treated and un-irradiated samples to determine if there are any deleterious effects to the texture and color

3. Determine whether eBeam treatment at doses below 1 kGy damages the

cellular structures of sprouts

4. Inoculate alfalfa sprout samples with a six-stereotype cocktail of toxigenic non O157 E. *coli* and subject them to eBeam doses below 1 kGy and quantify the reduction of the pathogen

5. Calculate the reduction in infection risk associated with alfalfa sprouts contaminated with toxigenic non O157 E. *coli* if eBeam technology is adopted.

2. LITERATURE REVIEW

2.1. Foodborne Illness

Foodborne illnesses are a major public-health concern in the United States (US). Each year there are over 9.1 million foodborne illnesses in the US accounting for more than 55,000 hospitalizations and 1,300 deaths (1). Out of these occurrences many were caused by Salmonella spp. and E. coli with a leading cause of death coming from Salmonella spp. Each year these illnesses cost the US roughly $157 billion dollars in health care cost. Even with an estimated spend by the CDA of around $1 billion dollars with other government agencies spending roughly $300 million dollars on education or other food safety efforts each year data on the issue has shown that the problem is not going away. In addition to the cost of health care it is estimated that losses to the economy due to foodborne illnesses are around $357 billion to $1.4 trillion dollars each year (2).

Foodborne Illness Related to Fresh Produce

Fresh produce is an important part of a healthy and well-balanced diet. Unfortunately, the consumption of fresh produce is not risk free as they most often consumed raw and have been linked to numerous foodborne illness outbreaks in the US. During a period between 1973 and 1997 a total of 190 produce-associated outbreaks were recorded in the US with 16,058 illnesses, 598 hospitalizations, and 8 deaths reported (6). During this period, it was noted that

these outbreaks are on the rise. With 1907 seeing a rise of 0.7% to 1990 seeing a rise of 6%. This increase is related to improvements in the diets of Americans and the availability of fresh produce year around with global trade. The most common products associated with outbreaks are salad, lettuce, juice, melon, sprouts, and berries. 48% of the bacterial pathogen related outbreaks were caused by Salmonella spp. with E. coli being related to a number of outbreaks.
Since these product are normally consumed raw they are susceptible to spreading pathogens as they are lacking a "kill step" in their processing. Although the source of contamination of the produce is not always known there have been many reported incidents of contamination coming from fecal matter from untreated fertilizer and wild animals along with untreated irrigation water that have been linked to outbreaks (8).

Shiga Toxin-producing E. *coli (STEC)*

E. *coli* is a major cause of foodborne illnesses with an estimated 265,000 infections annually. This Gram-negative bacterium is commonly found in the environment and can be introduced into the food system through contaminated water and fecal matter. Most strains of E. coli are non-pathogenic and cause no harm to the host when ingested. However, some such as the Shiga toxinproducing strains of E. *coli* can cause severe gastrointestinal distress and illness if consumed (9). STEC organisms have an incubation time of around 3 to 4 days following consumption with common symptoms including vomiting, bloody

diarrhea, and severe abdominal pain. In rare occurrences patients who have been infected with STEC can develop a life-threatening form of kidney failure called hemolytic uremic syndrome or HUS.

2.2. Ionizing Radiation and Electron Beam

The use of ionizing radiation as a non-thermal food processing aid has become increasingly prevalent in the industry over the past decades. It's ability to extend the shelf-life of products and improve consumer safety, while limiting the impact on the products sensory and nutritional qualities has made it a promising tool for manufacturers or producers. The most common forms of radiation used in the food industry are gamma radiation with 60Co, photon radiation with X-rays, and beta radiation with Electron Beam. All three forms of radiation work by emitting ionizing partials towards the product, weather through radioactive decay or electrical generation. When these particles collide with a target they carry enough energy to knock an electron from the its orbital. This in turn results in single and double strand breaks in the organism DNA rendering it unable to repair the DNA causing the organism to die or in sub-lethal dose ranges unable to replicate. Currently the FDA has approved radiation for use as a phytosanitary treatment of fresh produce at a dose not to exceed 1kGy.

3. MATERIALS AND METHODS

3.1. Natural Bioburden Reduction

Alfalfa Sprout Sample Preparation

Alfalfa Sprouts (Medicago sativa) were purchased from a local formers market in Bryan, Texas 24hrs prior to each run. They were transported and stored at ≈4°C until they were ready to be repackaged and prepared for treatment. The repackaging of samples for processing consisted of aseptically opening the packages and mixing multiple lots in a sterile plastic tub. Following mixing the sprouts were aseptically weighed out into samples of 20 ± 0.5 g using sterile weigh boats and packed into perforated polyethylene terephthalate (PET) plastic clam shells (Sambrailo, Santa Maria, CA). Two experimental treatment groups were divided and labeled accordingly, treated or control, then stored at ≈4°C until treatment, ≤3hrs. Each experiment consisted of 3 technical runs with 3 biological replications in each run.

Electron Beam Treatment

Samples were exposed to high energy (10 MeV) electron beam (eBeam) irradiation at the National Center for Electron Beam Research Center in College Station, TX utilizing a 15kW 10MeV linear accelerator as the irradiation source (L3 Pulse Sciences). Dose checks were carried out prior to each run to determine appropriate attenuation and conveyor belt speed to achieve a Dose Uniformity Raito (DUR) of 1 with a measured dose ≤ 1kGy. Dosimetry, the measurement of absorbed dose delivered

by ionizing radiation, was conducted using L-α-alanine pellets (Gamma-Service Produkbestrahlung GmbH, Germany) along with E-scan electron paramagnetic resonance spectroscopy (Bruker, BioSpin, Billerica, MA). 2 L-α-alanine pellets were used for each dose check, one laid flush with the top and the second laid flush with the bottom of the sprout sample. Following treatment samples were transported back to the lab and stored at ≈4°C to be analyzed.

Microbial Analysis

Microbiological analysis for fungi, mold, and viable bacterial growth were performed on the day of treatment and on days 1, 7, 14, and 21 following treatment per the standard methods outlined in the FDA's online Bacteriological Analytical Manual. Each sample, 20g of sprouts, were aseptically transferred into a sterile stomacher bag with filter (VWR International) along with 180ml of Butterfields Phosphate Buffer (Sigma Chemical, St. Louis, MO) and homogenized at normal speed for 2min using a stomacher machine (Seward 350, London, UK). Following homogenization appropriate dilutions were made with Butterflied Phosphate Buffer to achieve countable concentrations. 100µl of sample were taken from the selected dilutions and spread plated on the appropriate agar plates for enumeration. For the enumeration of aerobic bacteria samples were plated on Plate Count Agar (PCA) (VWR International) and incubated at 35°C for 24hrs after which plates were hand counted. The enumeration of fungal and mold counts was determined by plating samples on Sabouraud Dextrose Agar (SD) (Criterion). These plates were incubated at 26°C for 48hrs after which the colonies were

hand counted.

Data Analysis

All experiments were done in triplicate, where three technical experiments were performed each with 3 biological replicates. The data was analyzed statistically using Statistical Analysis System (SAS 9.3, 2001) and visually utilizing GraphPad Prism 5.0 (GraphPad Software Inc., California). For microbial analysis results were expressed in colony forming units per gram (CFU/g), these data points were then used to construct scatter plots to visually represent the population numbers over time and compare each day. A Mann-Whitney test was used to compare between treated and control samples for each time period to determine if a statistical difference exists between their observed microbial loads.

3.2. Pathogen Reduction

Alfalfa Sprout Samples

Alfalfa sprouts (Medicago sativa) were purchased from a local farmers market in Bryan, Texas 24hrs prior to each run. They were transported and stored at ≈4°C until they were ready to be repackaged and prepared for treatment.

Sample Inoculation and Preparation for Treatment

Sprouts were aseptically extracted from their original packaging and mixed together in a sterile plastic bin. Sprouts were then separated out into weigh boats to

achieve samples that were 5g each. These samples were then transferred into the biosafety hood where 0.1ml of inoculum was added to each sample. They were left to dry in the hood for 1hr after which they were placed into whirl-packs and heat sealed. All samples were triple packaged in accordance with biohazard safety standards for the transportation of pathogenic organisms.

Electron Beam Treatment

Samples were exposed to high energy (10 Mev) electron beam (eBeam) irradiation at the National Center for Electron Beam Research Center in College Station, TX utilizing a 15kW 10MeV linear accelerator as the irradiation source (L3 Pulse Sciences). Dose checks were carried out prior to each run to determine appropriate attenuation and conveyor belt speed to achieve a Dose Uniformity Raito (DUR) of 1 with a measured dose ≤ 1kGy. Dosimetry, the measurement of absorbed dose delivered by ionizing radiation, was conducted using L-α-alanine pellets (Gamma-Service Produkbestrahlung GmbH, Germany) along with E-scan electron paramagnetic resonance spectroscopy (Bruker, BioSpin, Billerica, MA). 2 L-α-alanine pellets were used for each dose check, one laid flush with the top and the second laid flush with the bottom of the sprout sample. Following treatment samples were transported back to the lab and processed immediately.

Pathogen Extraction and Enumeration

Sprout samples were aseptically extracted from the plastic whirl-packs and transferred to sterile stomacher bags. Each stomacher bag contained the transferred 5g of sprouts from 1 whirl-pack and would be treated as a single data point. 45ml of phosphate buffer solution would then be added to each stomacher bag and the sample would be homogenized utilizing a stomacher (Seward 350, London, UK) set at high speed for 2min. the resulting liquid was then aseptically transferred to a 50ml conical vial followed by creating 10-fold serial dilutions with phosphate buffer.
Enumeration was carried out by plating 0.1ml of diluted sample solution on a Modified MTEC Agar. The plates were then incubated at 35°C for 2 hours then transferred to an incubator set at 44°C and left to incubate for 24 hours. All pink colonies were counted and used for data analysis.

Data Analysis

All experiments were done in triplicate, where three technical experiments were performed each with 3 biological replicates. The data was analyzed statistically using Statistical Analysis System (SAS 9.3, 2001) and visually utilizing GraphPad Prism 5.0 (GraphPad Software Inc., California). For pathogen reduction results were expressed in colony forming units per gram (CFU/g), these data points were then used to construct bar graphs to visually represent the population numbers over time and compare each day. A Student T test was used to compare between treated and control samples to determine if a statistical difference exists between their observed pathogen loads.

3.3. Sensory Analysis

Alfalfa Sprout Sample Preparation

Alfalfa Sprouts (*Medicago Sativa*) were purchased from a local formers market in Bryan, Texas 24hrs prior to each run. They were transported and stored at ≈4°C until they were ready to be repackaged and prepared for treatment. The repackaging of samples for processing consisted of aseptically opening the packages and mixing multiple lots in a sterile plastic tub. Following mixing the sprouts were aseptically weighed out into samples of 20 ± 0.5 g using sterile weigh boats and packed into perforated polyethylene terephthalate (PET) plastic clam shells (Sambrailo, Santa Maria, CA). Two experimental treatment groups were divided and labeled accordingly, treated or control, then stored at ≈4°C until treatment, ≤3hrs. Each experiment consisted of 3 technical runs with 3 biological replications in each run.

Electron Beam Treatment

Samples were exposed to high energy (10 Mev) electron beam (eBeam) irradiation at the National Center for Electron Beam Research Center in College Station, TX utilizing a 15kW 10MeV linear accelerator as the irradiation source (L3 Pulse Sciences). Dose checks were carried out prior to each run to determine appropriate attenuation and conveyor belt speed to achieve a Dose Uniformity Raito (DUR) of 1 with a measured dose ≤ 1kGy. Dosimetry, the measurement of absorbed dose delivered by ionizing radiation, was conducted using L-α-alanine pellets (Gamma-Service Produkbestrahlung GmbH, Germany) along with E-scan electron paramagnetic

resonance spectroscopy (Bruker, BioSpin, Billerica, MA). 2 L-α-alanine pellets were used for each dose check, one laid flush with the top and the second laid flush with the bottom of the sprout sample. Following treatment samples were transported back to the lab and stored at ≈4°C to be analyzed.

Color Analysis

Color measurements were taken on the day of treatment and on days 7, 14, and 21 following treatment. The color was measured utilizing a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan) that was calibrated before each run using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan). Preliminary studies were conducted to standardize the sample size and placement to obtain consistent readings. It was found that by packing 10g of sample into the sample holder consistent readings could be achieved. Two readings per sample were taken. The parameters measured were L*(lightness), a*(red component), and b*(yellow component), these measurements were used to compare treated and control samples to determine if any change due to treatment was caused.

Texture Analysis

Texture analysis of control and treated sprout samples was conducted on the day of treatment and days 7, 14, and 21 following treatment. Texture analysis of the sprout samples were carried out using a model TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Survey, UK), which was

equipped with 75 mm aluminum compression plate (P/75) probe for compression. The instrument was calibrated prior to each run. Preliminary studies were conducted to standardize the sample size and placement to obtain consistent readings. It was determined that 6 alfalfa sprouts would be used per single compression run, and that the placement of sprouts had no effect on the consistency of the readings. Three readings were taken for each data point. The instrument settings are presented in table 3.1 For each sample a reading of the modulus of deformation (N/mm), force (N), distance (mm), and work to rupture (N.mm) were recorded. An explanation of these parameters is shown in table 3.2 as described by Smith 2013(9).

Table 3.1 Texture Analysis Setup

Pretest Speed	Reversing Speed	Trigger Force	Compression Travel Speed	Hold Time	Travel Distance
5mm s^{-1}	10mm s^{-1}	10g-Force	0.5mm s^{-1}	0.01s	1mm

Table 3.2 Texture Analysis Parameters Measured

Parameter	Units	Description
Deformation of Modulus	N/mm	Slope of the Curve
Maximum Force	N	Firmness, Hardness of the Sample
Work	N.mm	Measure of Energy, Area Under the Curve Until Rupture
Distance to Rupture	Mm	Measure of the Samples Extensibility

3.4. Quantitative Microbial Risk Assessment

QMRA was used to determine the reduction of infection risk is eBeam were to be introduced into the processing of Alfalfa Sprouts. The data obtained from the pathogen reduction portion of this study were used as concentrations for running the analysis. The risk of infection from the E. coli cocktail was estimated using an exponential distribution model. The formula used is show below.

$$Pi = 1 – e\text{-}k * N$$

Where Pi is the probability of infection, k is the probability of survival of the organism to reach and infect the individual, and N is the number of organism ingested. Due to a lack of literature of the natural presence of the 6 strains of E. coli used in this study an initial concentration of 103CFU/g was assumed. K was determined to be 2.18E-4 from previous studies conducted by Cornick and Helgerson. A Monte Carlo technique was used with 10,000 iterations to construct the model using Oracle Ball Software (V. 11.1.2.4.600, Redwood City, Calif.).

4. RESULTS

4.1. Natural Bioburden Reduction

Low dose eBeam treatment of ≤ 1kGy was able to achieve a significant reduction in natural bioburden of Alfalfa Sprouts. For aerobic bacteria that were plated out on PCA following treatment a reduction of 2.03 Log10 was noticed. The reduction of mold and fungus had a similar reduction level of 2.11 Log10 follow treatment at ≤ 1 kGy. Follow treatment under refrigerated conditions the reduction levels remained consistent over the 21 days of observation.

Figure 4.1 Effects of eBeam on Alfalfa sprouts at day 1 following treatment

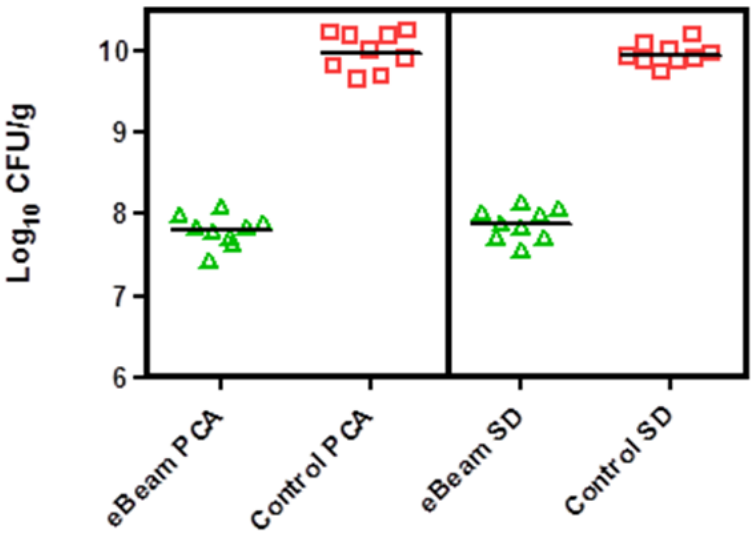


Figure 4.2 Effects of eBeam on Alfalfa sprouts at day 7 following treatment

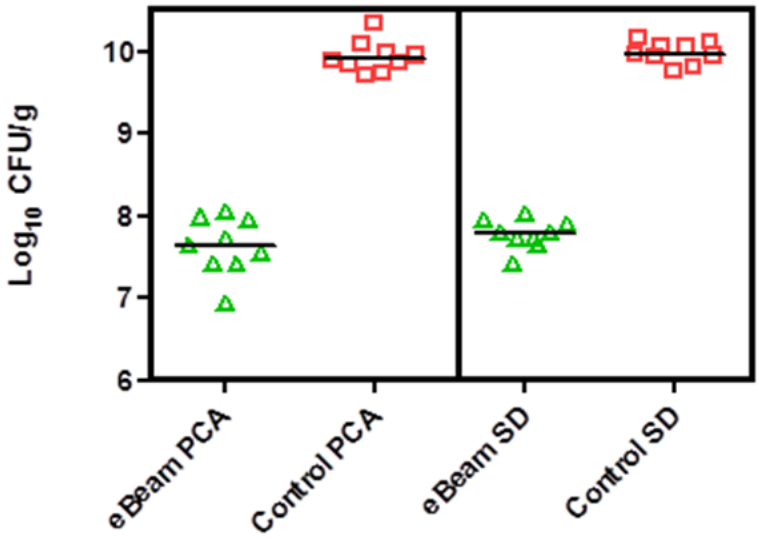


Figure 4.3 Effects of eBeam on Alfalfa sprouts at day 14 following treatment

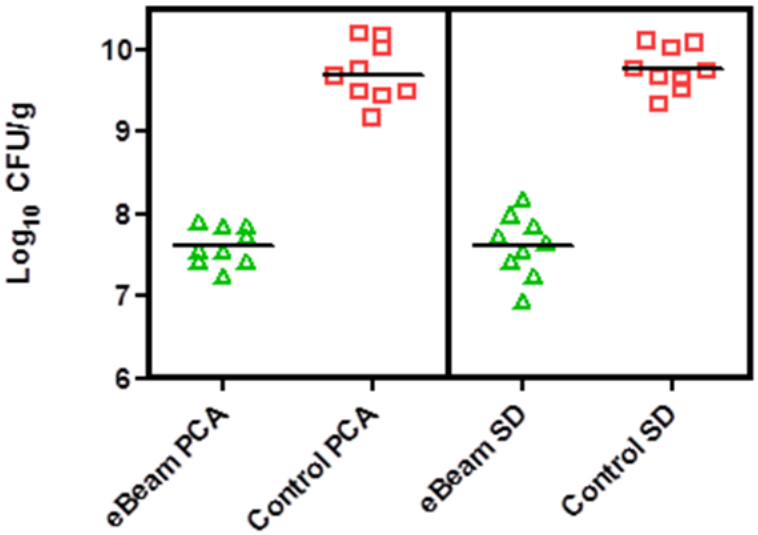


Figure 4.4 Effects of eBeam on Alfalfa sprouts at day 21 following treatment

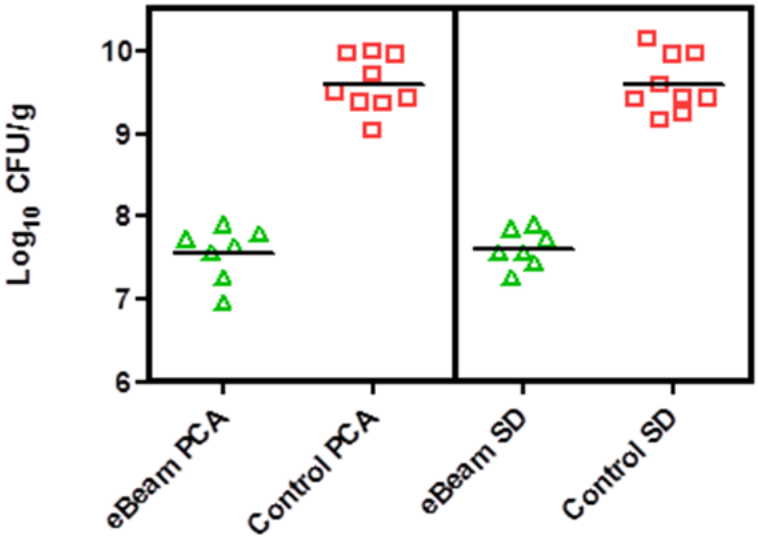
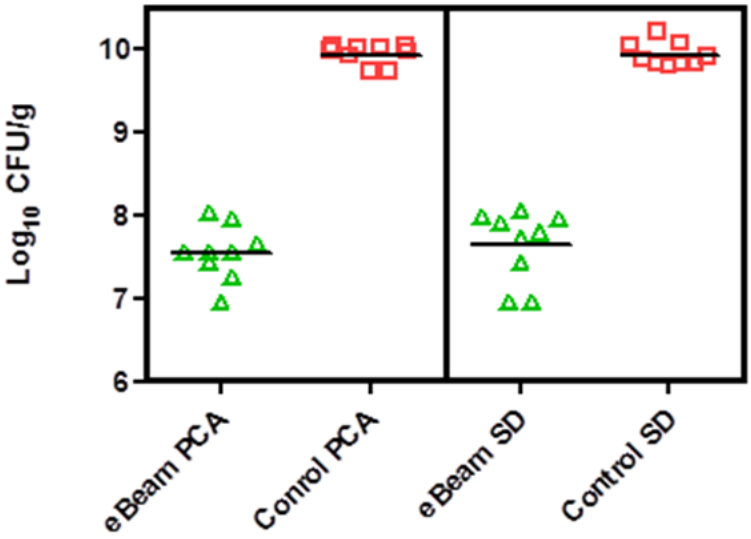


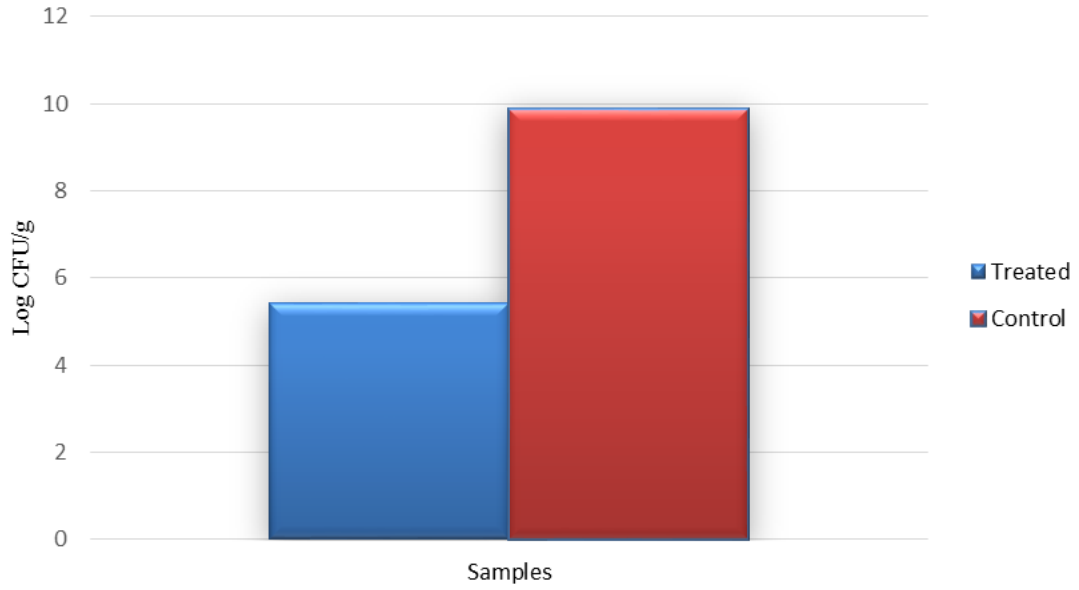
Figure 4.5 Effects of eBeam on Alfalfa sprouts at day 0 following treatment



4.2. Pathogen Reduction

Electron Beam treatment at 1 kGy was able to achieve a 6 Log, or 99.99%, reduction in a prepared inoculum of the big 6 strain cocktail of E. *coli* that was applied to the sprout samples.

Figure 4.6 Pathogen reduction of E *coli* cocktail following eBeam treatment



4.3. Sensory Analysis

Color

The eBeam treatment did not result in any statistical difference ($p<0.05$) in the lightness (L-Value), blue-yellow (B-Value), or red-green (A-Value) of the sprout samples when comparing treated and untreated samples.

Figure 4.7 L-value color from treated and control samples over 21 days

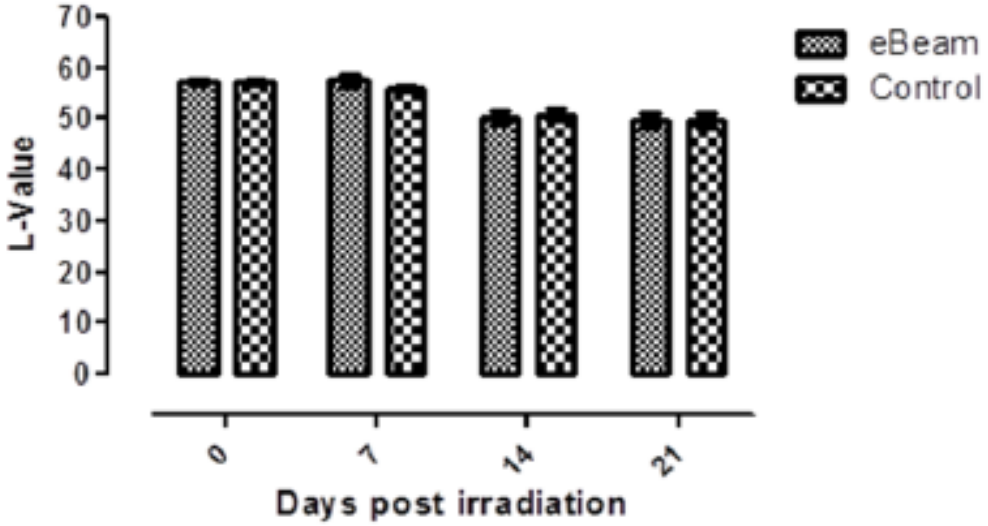


Figure 4.8 A-value color from treated and control samples over 21 days

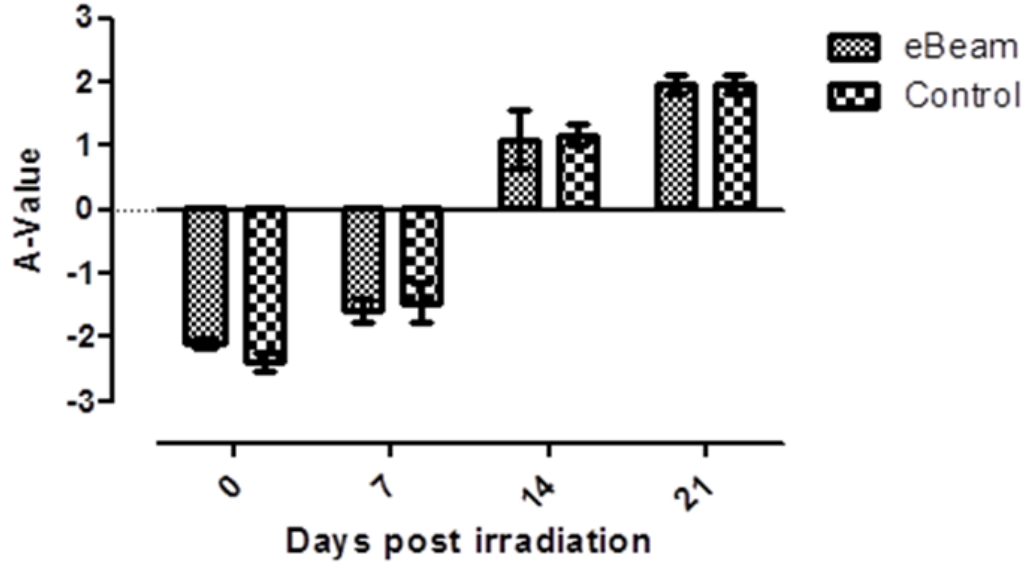
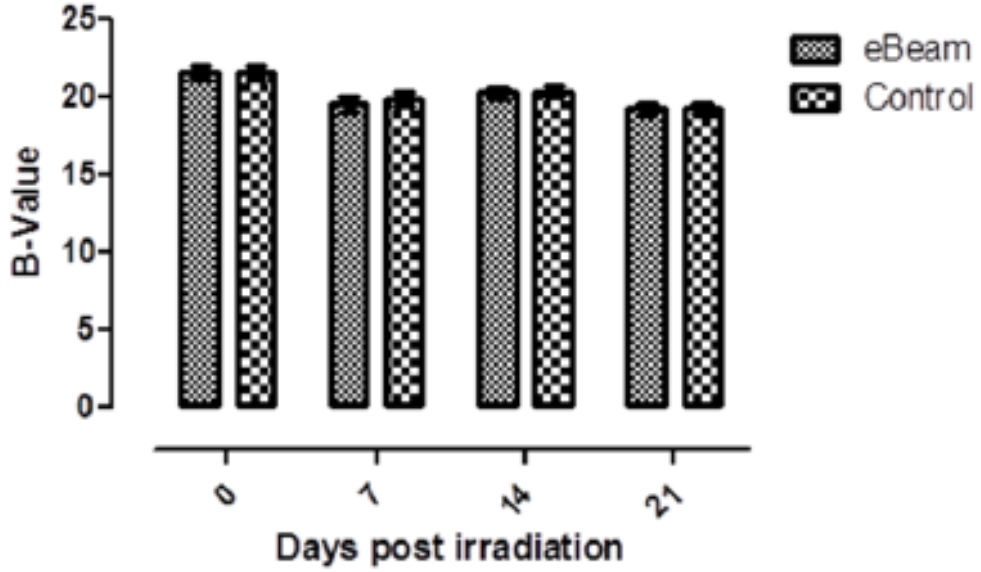


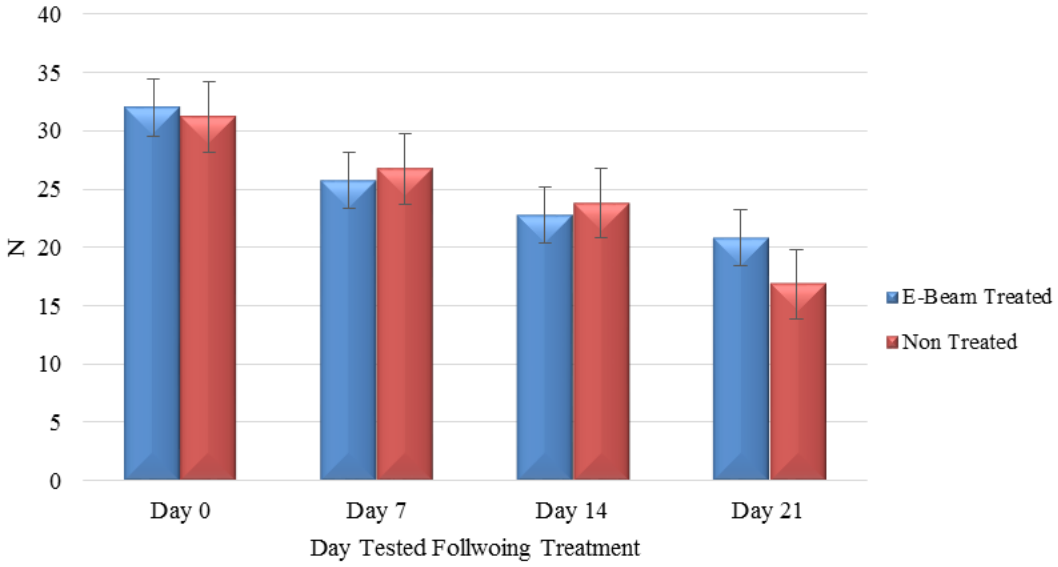
Figure 4.9 B-value color from treated and control samples over 21 days



Texture

Following the low dose eBeam treatment texture data taken on days 0, 1, 14, and 21 showed that there was no statistical difference between treated and control samples when observing the maximum force applied to the sprouts. This shows that the hardness or crispness of the sprouts, a desired quality by consumers, is not adversely affected with the treatment of eBeam.

Figure 4.10 Hardness of Alfalfa sprouts from treated and control samples over 21 days



4.4. Quantitative Microbial Risk Assessment

Using the reduction value found with the Big 6 strains of E. coli at a dose of 1 kGy the Monte Carlo simulation has shown that a reduction of illnesses from 24,000/100,000 to 2/100,000 is achieved when a starting concentration of 103 CFU/g is applied.

Figure 4.11 Monte Carlo simulation of infection risk before eBeam treatment

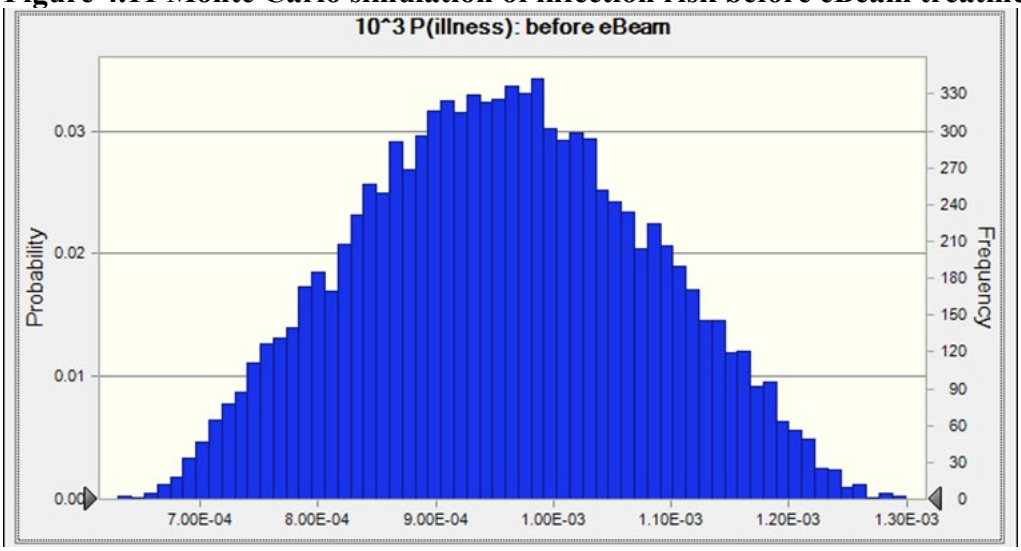
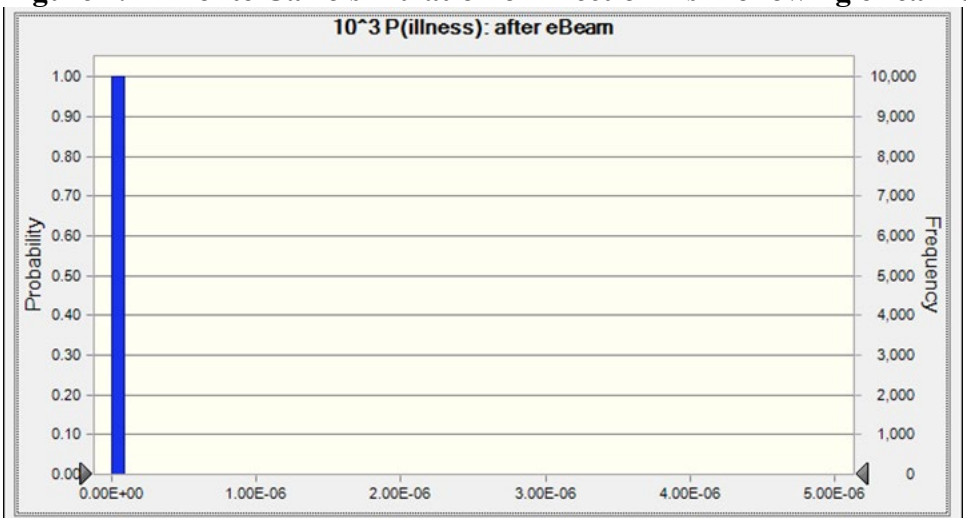


Figure 4.12 Monte Carlo simulation of infection risk following eBeam treatment



5. CONCLUSIONS AND FUTURE STUDIES

The studies presented in this thesis were conducted with the objective of determining the effectiveness of eBeam technology at current FDA approved doses on the reduction of selected pathogens and other indigenous microflora in raw alfalfa sprouts, and to conduct QMRA analysis of the reduction to quantify the real-world implications this technology would have to the industry. In addition, the sensory attributes of color and texture where analyzed following treatment to discover if any detrimental effects of the alfalfa sprouts occurred during treatment.

The microbial analysis looking at the natural bioburden concentrations showed a 2.03 and 2.11 log10 reduction in the aerobic bacterial and fungal populations when a 1kGy eBeam dose was administered. In addition to the natural bioburden reduction key pathogens were also examined. A cocktail of 6 serotypes of toxigenic non O157 E. *coli* were used to inoculate the alfalfa sprouts and determine the effectiveness key pathogen reductions. Following a 1kGy eBeam treatment a 4.44 log10 reduction of the inoculum was achieved. These reductions were significant, and higher than the reduction achievable with chlorine (Hypochlorite) washes (1-2 log10). The use of eBeam technology has the additional benefit of being a "clean" technology. As where the use of chemical based treatments is associated with negative health and environmental issues, eBeam is an on/off technology with no residuals left on the product and no hazardous waste to be dealt with. The penetrating ability of eBeam allows for products to be treated following packaging. This enables producers to lower the risk of cross contamination of

the product following treatment, helping to ensure the safety and quality of the products. It is important to note that this technology is not a “cleanup” technology and should be considered an additional asset in an already quality production. By coupling this eBeam with GMP’s and other hurdle technologies, such as modified atmosphere packaging, producers can help to ensure a safe and quality product.

Following the pathogen reduction testing an analysis of the reduction achieved by eBeam treatment was conducted using QMRA. This statistical analysis is used to demonstrate the theoretical reduction in the chances of contracting a foodborne illness from contaminated alfalfa sprouts when the eBeam technology is implemented. The results showed that a reduction in illnesses from 24,000/100,000 down to 2/100,000 if a consumer ate the average serving size of sprouts, 33g, that had a pathogen concentration of 103 CFU/g.

The sensory qualities of alfalfa sprouts were not affected by eBeam treatment in respects to their texture and color. Texture analysis was conducted using a texture analyzer and by an electrolyte leakage assay. In both studies it was shown that alfalfa sprouts treated by eBeam had no statistical difference from untreated sprouts. Color analysis also showed that treated sprouts had no statistical difference when compared to untreated sprouts throughout the 21 days of observation following treatment.

These studies have shown the promise of eBeam technology can have in the development of producing safe quality foods. If implemented properly this technology could help to reduce the risk of food borne illness outbreaks while maintaining the

sensory and nutritional qualities desired by costumers.

Additional studies are suggested to help in gaining further insight on the effects of eBeam processing in alfalfa sprouts. The first suggested is the conduction of a sensory panel study to determine if consumers are accepting of eBeam processed produce. To help determine if a difference between untreated alfalfa sprouts and eBeam treated can be detected by the consumer. Additionally, it would be suggested to carryout additional pathogen reduction test on other key microorganisms that are known to be prevalent in alfalfa sprouts.

The addition of other processing techniques should be tested in conjunction with eBeam. The addition of modified atmosphere packaging has shown to help in increasing the shelf-life of fresh produce. The combining of the two technologies could help to reduce the natural microflora and extend the products life. This study would need to be carried out use varying concentration of gases and packaging material to determine the optimum conditions in which pathogen and spoilage organism are reduced. In addition, the packaging material will need to be studied to determine its compatibility with eBeam treatment.

Alfalfa sprouts are regarded as a good source of vitamins and minerals. They are high in concentrations of vitamin K, Vitamin C, and calcium. It is suggested that studies be conducted to determine if the concentration and or quality of these nutrients are affected by the implementation of eBeam treatment as it could affect the consumers outlook on the product.

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