ENHANCING MICROBIOLOGICAL SAFETY OF CUCUMBERS AND REDUCING SPOILAGE OF WHITE ONIONS USING ELECTRON BEAM PROCESSING

An Undergraduate Research Scholar Thesis

by

LUIS VALENCIA

Submitted to the Undergraduate Research Scholars program
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Suresh Pillai

May 2016

Major: Food Science & Technology

TABLE OF CONTENTS

ABSTRACT

Enhancing Microbiological Safety of White Onions and Cucumbers using Electron Beam processing

Luis Valencia
Department of Nutrition & Food Science
Texas A&M University

Research Advisor: Dr. Suresh Pillai
Departments of Poultry Science & Nutrition and Food Science

Microbial contamination is of significant importance to the economic value and microbiological safety of fresh produce. Two fresh produce commodities that are of significant consumer demand are cucumbers (*Cucumis sati*vus) and white onions (*Allium cepa* L.). Product quality of onions in terms of extended shelf life is of economic importance to growers, distributors, and retailers. Similarly, elimination of the bacterial pathogen, *Salmonella* spp., on cucumbers is of significant importance to public health. Electron beam (eBeam) is a non-thermal food processing technology that is approved by the US FDA but has not been widely adopted by the fresh produce industry. My hypothesis was that there will be a reduction in the overall bioburden (which could result in extending the shelf life as well as elimination of potential targeted organism when white onions and cucumbers are treated with low (≤ 1 kGy) eBeam dose. To test the hypothesis, we will inoculate white onions and cucumbers with laboratory grown strains of *Enterobacter cloacae* and *Salmonella* Typhimurium and expose them to eBeam doses of ≤ 1kGy, which is currently approved by the FDA for fresh produce. The FDA approved microbiological methods will be used determine the natural bioburden and pathogen reduction after eBeam processing at doses under 1kGy.

SECTION I

INTRODUCTION

Background

Fresh produce consumption has become the trend for a healthier lifestyle due to an increase of health problems across the United States. Consumer demand of fresh produce has been the reason for a significant increase in harvesting, processing, and packaging, and as a result it has increased the vulnerability of produce to microbial pathogens and spoilage organism [1]. Fresh produce has become one of the predominant vehicles of pathogen transmission in foodborne outbreaks [2]. One of the main foodborne pathogens linked to many gastrointestinal illnesses in fresh produce is *Salmonella* [3]. *Salmonella* is a gram-negative bacterium that is capable of being transmitted via many food commodities, but of late has been linked to fresh produce [4]. Cucumbers are of great concern because of the risk of microbial pathogen contamination and them serving as potential vehicles for pathogens such as *salmonella*. According to the CDC and FDA, in the last couple of years there have been numerous outbreaks and casualties linked to cucumbers contaminated with *salmonella*. Andrew and Williamson Fresh Produce out of San Diego, California was one of the most recent companies to have had a multistate recall due to 285 confirmed cases for consumption of cucumbers contaminated with *salmonella* [5]. The other food commodity of interest that is significantly consumed throughout the USA because of its ability to enhance flavor are white onions. Due to its high consumer demand, the United States harvests an estimated 6.2 billion pounds per year to be distributed both domestically and internationally [6]. The spoilage organism of concern to onions is *Enterobacter cloacae* [7]. The spoilage organism causes a disease known as "Enterobacter Bulb Decay"; the exterior region of the white onions seems white and without decay, but the inner region of the bulb shows discoloration and decay [7]. This bacterium possesses the capability to influence supplies and market price.

Electron beam irradiation

To enhance product quality and shelf life of fresh produce, Electron Beam (eBeam) processing has been approved by the US Food and Drug Administration (FDA). However, the produce has to be labeled since the FDA considers this food processing technology as a "food additive" [8]. eBeam processing has the capability to revolutionize the food industry. This technology involves the use of ionizing radiation to enhance food safety and quality, and prolong shelf life [9, 10]. eBeam technology converts commercial electricity into high energy electrons which carry enough energy to break chemical bonds indiscriminately, hence eliminating insects, pests, and microbes, including foodborne pathogens [9]. This technology has shown to be effective at reducing microbial pathogens with a low dose without depriving the fruits and vegetables of nutrient content. Its application will create a higher quality and safety standard for a wide variety of fresh fruits and vegetables.

Objectives

My research hypotheses are 1. There will be a reduction in the overall bioburden resulting in elimination of *Enterobacter cloacae* and thereby an extension of shelf life white onions when treated with low (≤ 1 kGy) eBeam doses, 2. eBeam doses (≤ 1 kGy) will eliminate microbial bioburden and low levels of *Salmonella* Typhimurium on cucumbers.

CHAPTER II

METHODS

Microbiological culture

The FDA Bacteriological Analytical Manual's (BAM) microbiology procedures were employed to detect and enumerate the microbial bioburden and target organisms. A Biosafety Level 2 lab with access to the National Center for Electron Beam Research (NCEBR) was used in this study [9]. Both *Salmonella* Typhimurium 14028 and *Enterobacter cloacae 13047* were gathered from the American Type Culture Collection (ATCC). The bacteria cultures were both cultured utilizing BD Tryptic Soy Broth (TSB). Overnight cultures were grown in TSB prior to each individual trial to grow subsequent log phase cultures to be utilized in the experiments. Log phase cultures were centrifuged at 4000xg for 10 minutes and bacterial pellets were re-suspended in phosphate-buffered saline (PBS). The selective agar utilized to identify *salmonella* was Difco Xylose Lysine Deoxycholate (XLD). XLD was utilized because it contains sodium desoxycholate to inhibit all gram-positive organism and lysine to enable differentiation between *salmonella* spp. and other gram-negative organism. To identify and enumerate *enterobacter* spp., Eosin Methylene Blue (EMB) Agar was utilized.

eBeam Processing Sample Preparation

The samples were prepared as follows: fresh cucumbers and onions were cut into equal size slices and weighed to a total volume of 25g. The weighed samples were individually placed in Whirl-Pak™ bags and each individual produce sample was spiked with approximate ($1x10^8$ CFU/25g) of the appropriate bacterium. All bacterium-spiked sample bags were left inside the hood at room temperature for 45 minutes to allow complete drying. Once the bacteria seem to have dried on the produce they were ready to be packaged for eBeam processing.

Packaging samples for eBeam processing

Bacteria-spiked sample bags were triple heat-sealed utilizing Whirl-Pak™ bags to be within the University biosafety guidelines to transport opportunistic food borne pathogens. The first Whirl-Pak™ bag-containing 25g of spiked sample was heat-sealed and then placed inside a larger Whirl-Pak™ bag and heat-sealed again. Once the specimens were heat-sealed and re-inspected for tears they were placed on transport containers, and were shipped for eBeam processing.

eBeam Processing

The bacteria-spiked samples were then treated with four different target eBeam doses of 0, 400, 600, and 1kGy at the NCEBR. Once the samples were successfully treated they were shipped back to the lab for further processing. The treated samples were diluted to a 1:10 ratio utilizing an FDA recommended diluent (Buffered peptone water) to help dislodge microorganism from inner and outer surfaces of the produce. Samples were than placed in stomacher for a period of 2 minutes to allow total homogenization of the sample. Once samples were totally homogenized, 1mL from each individual dose treated bags was extracted and dispensed into 2mL centrifuge tubes. To acquire a countable colony forming unit (CFU) 10-fold serial dilution were performed using phosphate-buffered saline (PBS). 100 µL aliquots were spread plated onto selective media and incubated at 37^{o}C for 24 hours.

CHAPTER III

RESULTS

Bacterial inactivation during eBeam processing

The inactivation of the inoculated *Salmonella* Typhimurium 14028 in cucumber samples is shown in **Fig. 1.** e-Beam processing showed that when exposed to nearly 1.0 kGy, *S.*Typhimurium numbers decreased by approximately 4.0 log units. The inactivation of inoculated *Enterobacter cloacae* 13047 in white onions samples is shown in **Fig. 2** e-Beam processing showed that when exposed to nearly 1.0 kGy, *E. cloacae* numbers decreased by approximately 4.5 log units. Based on the inactivation kinetics, *S.*Typhimurium was calculated to have a D_{10} value of 270 ± 30.16 Gy in sliced cucumbers (**Table 1)** while *E. cloacae* was calculated to have a D_{10} value 208.42 ± 6.14 Gy in treated sliced white onions (**Table 2)**.

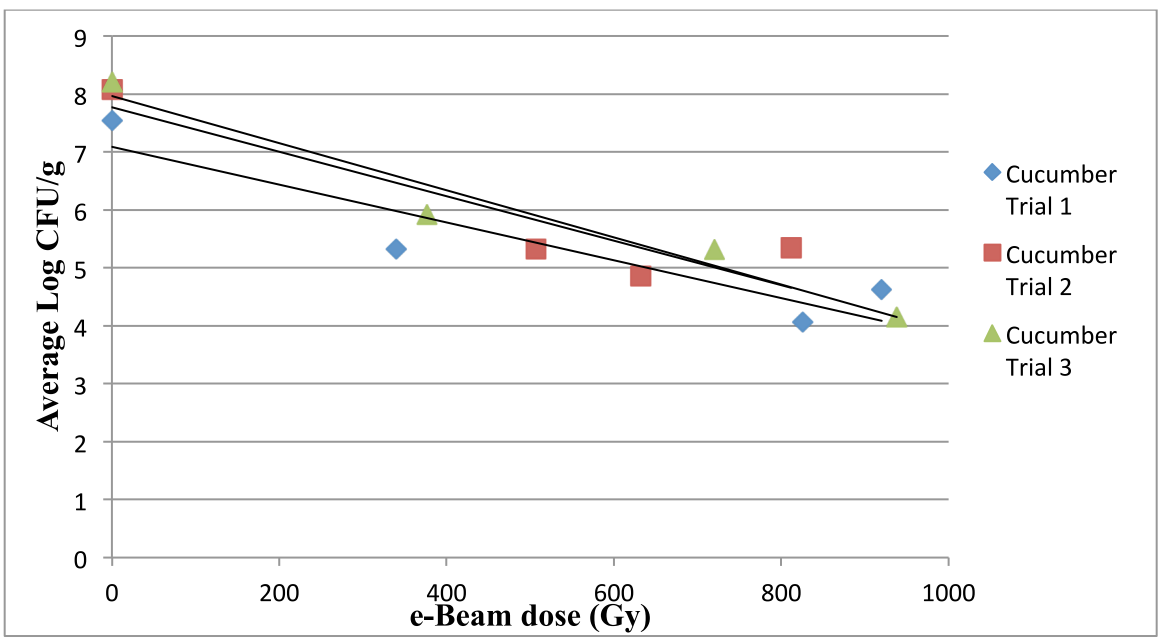

Fig. 1. Inactivation of *Salmonella* Typhimurium 14028 spiked in treated cucumber samples (n=3) after subjecting to e-Beam processing.

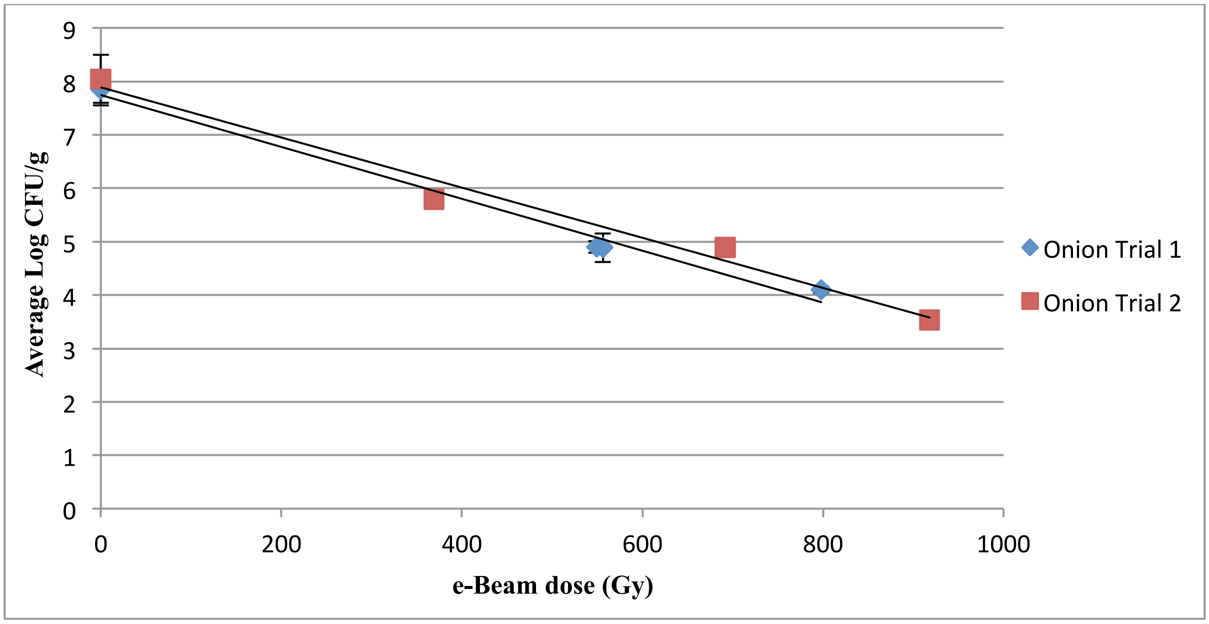

Fig. 2. Inactivation of *Enterobacter cloacae 13047* spiked in treated white onion samples (n=2) after subjecting to e-Beam processing.

Table 1
Mean D_{10} values of target organism in cucumber samples upon eBeam processing.

Target Organism	Mean D_{10} value (Gy)			
	Trial 1	Trial 2	Trial 3	Average
Salmonella Typhimurium 14028	263.16	303.03	243.9	270 ± 30.16

Table 2
Mean D_{10} values of target organism in white onion samples upon eBeam processing.

Target Organism	Mean D_{10} value (Gy)		
	Trial 1	Trial 2	Average
Enterobacter cloacae 13047	204.08	212.77	208.42 ± 6.14

CHAPTER IV

CONCLUSION

This study has shown that e-Beam processing has the potential to significantly reduce the amount of a specific target organism with a uniform dose under 1kGy. Its ability to maintain dose uniformity throughout various samples makes it ideal for not just research purposes, but for commercial utilization. The FDA recommends utilizing this source of processing when compared to other sources of irradiation because of its precision in deliberating a uniform dose. The bacterial organisms that were tested seem to have been both susceptible to the process regardless of the product that they were inoculated into. This study illustrates the potential and impact that e-Beam processing could have in the food industry because it doesn't discriminate on what products it can and can't be used. This source of high-energy Electron-Beam irradiation could perhaps increase the safety and quality standards that have been set for food commodities to potentially prevent future food borne illnesses. Its ability to enhance and prolonged the shelf life of food commodities could in fact revolutionize the market for perishable food commodities.

REFERENCES

[1] Beuchat LR and Ryu J-H. Produce handling and processing practices. Emerg Infect Dis 3 (1997):459–465.

[2] Falomir, M. P., Gozalbo, D., and Rico, H. Coliform bacteria in fresh vegetables: from cultivated lands to consumers, In A. Mendez (ed.), Current research, technology and educationaltopics in applied microbiology and microbial biotechnology. FormatexResearch Center, Badajoz, Spain, (2010): p. 1175–1181.

[3] Heaton, J. C., and Jones, K. Microbial contamination of fruit and vegetables and the behavior of enteropathogens in the phyllosphere: a review. J. Appl. Microbiol. 104 (2008):613–626.

[4] Jay, James M., Loessner, Martin J., and Golden, David A. Modern Food Microbiology, Seventh Edition. Springer, New York, NY, (2005): 619-634.

[5] U.S Food and Drug Administration. Andrew and Williamson Fresh Produce Recalls Limited Edition Brand Cucumbers Because of Possible Health Risk. U.S. Food and Drug Administration, Silver Spring, MD, (2015)

[6] National Onion Association. How and where Onions are grown. National Onion Association, Greeley, CO, (2011).

[7] Conn, Kevin E., Lutton, Jeffrey S., and Rosenberger, Staci A. Onion Disease Guide.

[8] U.S Food and Drug Administration. Regulatory Report: Irradiation of Food Packaging Materials. U.S. Food and Drug Administration, Silver Spring, MD, (2008)

[9] Pillai, S. D., Shayanfar, S. Electron Beam Pasteurization and Complementary Food Processing Technologies, USA, Elsevier, (2015): p.352

[10] Beier, Ross C., Pillai, Suresh D., Phillips, Timothy D. Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions. Blackwell Publishing, Ames, IA, (2004): ch28.

[11] U.S Food and Drug Administration. Bacteriological Analytical Manual, 8th Edition, Revision A. U.S. Food and Drug Administration, Silver Spring, MD, (1998): ch1, 3, 5, 18.