

ELECTRON BEAM IRRADIATION OF SLICED FRESH

CUCUMBER (*Cucumis sativus*)

A Thesis

by

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ABSTRACT

A healthy and balance diet includes consumption of fresh fruits and vegetables. Cucumber (*Cucumis sativus*) is one of the most cultivated vegetable in the world and is mostly consumed raw. Raw products are recognized as important vehicles for the transmission of human pathogens which causes foodborne illness. According to the Center of Disease Control and Prevention (CDC) Foodborne Outbreak Online Database, there were nine outbreaks due to *Salmonella* Poona associated with consumption of contaminated food between 1998 and 2008. From July 2015 until February 2016, multistate *Salmonella* Poona outbreak in the USA was reported due to the consumption of contaminated and imported Californian cucumbers. Hence, measures to decontaminate fresh produce are necessary. Electron-beam irradiation is an effective nonthermal method of decontamination against pathogens such as *Salmonella* Poona. However, irradiation of fresh produce such as cucumbers could produce negative quality effects such as loss of texture, flavor, and nutrients.

Therefore, the process must be characterized. The main objective of this study was to (1) determine the radiation D₁₀-value of *Salmonella* Poona on sliced cucumber; (2) quantify the effect of electron-beam irradiation on the product quality attributes (texture and color) throughout storage at refrigeration temperature (4-5°C); and (3) optimize irradiation treatment of sliced cucumbers to ensure proper decontamination (5D) while maintaining produce quality.

Five (± 2) grams of fresh cucumber was inoculated with 0.5 ml of a 10^8 CFU/mL of the bacterial culture of *Salmonella* Poona in sterile bags (18-oz). Samples were irradiated at room temperature with up to 1 kGy with an increment of 0.2 kGy using a low energy electron beam to find the D_{10} -value of the pathogens. Samples were also be irradiated at a 5D dose for decontamination of the pathogen. Irradiated samples were stored at 4-5°C and analyzed for product quality in terms of texture, color, water activity, moisture content, pH, and sensory characteristics for 3 days. All tests were performed in three replications. Non-irradiated samples served as controls.

The D_{10} -value of the *Salmonella* Poona strain used in this study was found 0.38 ± 0.03 kGy. Firmness (texture) of irradiated samples was significantly ($P < 0.05$) lower than the group of control samples. There was no negative effect ($P > 0.05$) in the other quality parameters of sliced cucumbers. Therefore, application of an e-beam irradiation decontamination step can significantly improve the microbiological safety of fresh sliced cucumbers.

DEDICATION

To my father, Abhay Joshi, my mother, Ansuya Joshi, for teaching me to believe in god, myself and believe in hard work and for earning an honest living for us.

To my fiancé, for his support, motivation, patience, and love.

To my sister, Hani Vyas and brother-in-law, Manav Vyas for supporting and encouraging me.

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Contributors

Part 1, faculty committee recognition

This work was supervised by a thesis committee consisting of Professor Elena Castell-Perez and Dr. Rosana G. Moreira of the Department of Biological and Agricultural Engineering and Professor Stephen Talcott of the Department of Nutrition and Food Science.

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The bacterial Species used in this study was provided by Professor Alejandro Castillo of the Department of Animal Science. All microbiological study in this research has been done in the laboratory of Professor Carmen Gomes of the Department of Biological and Agricultural Engineering.

Electron beam irradiation was carried out by Graduate student, Basri Omac of the Department of Biological and Agricultural Engineering.

All other work conducted for the thesis (or) dissertation was completed by the student independently.

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NOMENCLATURE

ANOVA	Analysis of variance
a_w	Water activity
CDC	Center of Disease Control and Prevention
DUR	Dose Uniformity Ratio
<i>E. coli</i>	<i>Escherichia coli</i>
e-beam	Electron-beam
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FDA	U S Food and Drug Administration
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis and Critical Control Point
IAEA	International Atomic Energy Agency
IFPA	Fresh-Cut Produce Association
kGy	kilogray
SCF	Scientific Committee on Food
USDA	US Department of Agriculture
WHO	World Health Organization

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1. INTRODUCTION

Food (including water), shelter and clothing are the traditional immediate basic human needs which human beings need for survival. Many modern lists draw attention to other basic needs such as sanitation, education, and healthcare. The basic needs approach aims to define the absolute minimum resources necessary for the long-term physical well-being of any human being. Man can live without basic needs other than traditional basic needs which are defined in modern lists. Similarly, he can live without clothing and shelter. However, he faces certain discomforts and inconveniences in life without them, and also he has to pass through multitudinous physical suffering and trauma without shelter. But “Food” is an essential commodity without which man cannot survive after a few days or weeks.

In the past, human beings had to rely on the food which was available locally. But now due to advance technology, fast transportation and modernization over the decades, dietary habits and health trends have been changed and food availability has increased. As a result, fruits and vegetable consumption has increased markedly as they are considered important components of the human diet (Yuk and others 2006). Several foodborne outbreaks are associated with the consumption of fresh fruits and vegetables. Because of foodborne outbreaks, consumption of fresh fruits and vegetables has led to an increased incidents of illnesses and an increased interest for the safety of these products (Tzortzakis and Chrysargyris 2017).

Fresh fruits and vegetable consumption are associated with a healthy lifestyle. The importance of fresh fruits and vegetables as a source of multiple nutrients has stimulated increased demand and consumption in recent years (Fabbri and Crosby 2016). Furthermore, the World Health Organization (WHO) encourages daily intake of at least 400 g of fruits and vegetables (excluding potatoes and other starchy tubers) for prevention of chronic diseases (Callejón and others 2015). According to evidence presented in World Health Report 2003, eating a mixture of various fruits and vegetables ensures an adequate intake of most micronutrients, dietary fibers and a host of essential non-nutrient substances. Therefore, food is not only consumed to satisfy the hunger and get necessary nutrients but also to improve physical and mental well-being by preventing nutrient-related diseases (Betoret and others 2011).

Insufficient consumption of fruits and vegetables increases the risk of obesity, coronary heart disease and stroke, type 2 diabetes, diverticulosis, hypertension, and epithelial cancers (for example, cancer of the lung, esophagus, mouth, stomach, colon, and pancreas) (Aldoori and others 1998; McCrory and others 1999; Bes-Rastrollo and others 2006; Hall and others 2009). There is evidence that can explain the consumption of high levels of high-energy foods, such as processed foods that are high in fats and sugars, promotes obesity compared to low-energy food such as fresh fruits and vegetables (Popkin 2001). Approximately 1.7 million of deaths and 16.0 million disability-adjusted life years (DALYs, the measure of the potential life lost due to premature mortality and the years of productive life lost due to disability) worldwide are reasoned to low consumption of fruits and vegetables (Vasileska and Rechkoska 2012;

World Health Organization). In addition, insufficient intake of fruits and vegetables is estimated to cause around 14% of gastrointestinal cancer deaths, 11% of ischemic heart disease deaths and 9% of stroke deaths globally (World Health Organization).

Cucumber (*Cucumis sativus*) is the fourth most cultivated vegetable in the world, and more than 60% of the production is done in China with the United States in fifth place. Consumption of fresh cucumber has increased worldwide. The US annual production in 2015 of fresh cucumbers was 1,066.9 million pounds and the consumption per capita was 7.5 pounds (USDA, 2016). The USA is a large importer of cucumbers.

As in the case of cucumbers, a large portion of fresh produce is consumed raw and foodborne disease outbreaks linked to these products are increasing (Olsen and others 2000; Sivapalasingam and others 2004). Raw products are recognized as important vehicles for the transmission of human pathogens which causes foodborne illness (Berger and others 2010). Globalization can also increase the risk of foodborne outbreaks, especially when produce comes from countries without maintaining safety standards. In the food chain, food can be contaminated at any point of processing which includes harvesting, transportation, processing and handling (Lynch and others 2009; Kozak and others 2013). The occurrence of foodborne infections related to fresh produce can be improved by better control interventions and improved prevention strategies worldwide. The understanding of the key contributing factors causing foodborne infection and the maintenance of the best practices can reduce and eliminate the problem of contamination in fresh produces (Kozak and others 2013).

The Center for Disease Control and Prevention (CDC) reported more than one million foodborne illnesses with 19,000 hospitalizations and 380 deaths because of *Salmonella* strains in the United States every year (Crowe and others 2015). *Salmonella* Poona is a rare serotype isolated in patients. According to the CDC Foodborne Outbreak Online Database, there were nine outbreaks due to *Salmonella* Poona associated with consumption of contaminated food between 1998 and 2008. From July 3, 2015- until February 29, 2016, multistate outbreak of *Salmonella* Poona infection in the United States was reported due to the consumption of contaminated and imported Californian cucumbers (CDC, 2016). According to the FDA report, 907 people were infected with the outbreak strains of *Salmonella* Poona across 40 states in the United States (Crowe and others 2015). 204 ill people were hospitalized, and 6 deaths were reported due to this foodborne outbreak.

Cucumbers imported from Mexico and distributed by Andrew & Williamson Fresh Produce (San Diego, CA) were the source of the infections in this multistate outbreak of *Salmonella* Poona as identified by the epidemiologic, laboratory, traceback and regulatory investigations. The investigation revealed that *Salmonella* Poona isolated from ill people and from contaminated cucumbers distributed by Andrew & Williamson Fresh Produce are closely related genetically. Two recalls of garden variety cucumbers distributed by the same distributor were announced due to the cucumbers were likely contaminated during the outbreak. According to the investigation on *Salmonella* Poona outbreak, the source of contamination of *Salmonella* Poona for cucumbers distributed by Andrew & Williamson Fresh Produce has not been identified. A

foodborne outbreak can happen at any step of distribution chain from farm to market or cross-contamination within the distribution chain.

A common practice to reduce the risk of contamination from fresh produces is washing them with chemicals such as hypochlorite, lactic acid, or other permitted antimicrobials or fumigation with ethylene oxide or methyl bromide (Rahman and others 2015); however, these treatments do not decontaminate internalized pathogens. A research study indicates ozone is a potent antimicrobial agent. Bactericidal action of ozone varies with the type of microorganism and medium. The issue to use ozone is, it is a less powerful against microorganisms in food than in pure cell suspensions (Kim 1998). Researchers have proposed irradiation treatment as a possible solution for pathogen decontamination (Lynch and others 2009; Chimbombi and others 2010, 2013; Borsa 2016).

Irradiation is a nonthermal technology (named cold sterilization) known to penetrate food tissues and eliminate pathogens from produce (Moreno and others 2007). Along with the reduction in microbial load, irradiation process allows for disinfestation and shelf life improvement in fresh produces. Many researchers have reported electron-beam irradiation and gamma irradiation as a potential method for reducing microbial growth and extending the shelf life of fresh fruits and vegetables (Sommer and others 2010). The joint committee on the wholesomeness of irradiated food reported that there is no toxicological hazard and no special nutritional or microbiological problems in any food commodity irradiated to an overall average dose of 10 kilogray (kGy) (World

Health Organization 1999). In the case of fresh fruits and vegetables, the maximum allowable dose is 1.0 kGy (FDA, 2016).

Few studies have evaluated the effectiveness of irradiation on decontamination of cucumbers and its effect on produce quality. There are contradictory reports on the effect of irradiation on the quality of cucumbers. Khattak and others (2005) observed a detrimental effect of gamma irradiation treatment on the firmness of cucumbers. No other relevant studies on electron-beam irradiation of cucumbers are currently available.

In this study, Potential effectiveness of electron beam irradiation in reduction of *Salmonella* Poona and extension of shelf-life of sliced cucumbers was evaluated. The reason behind selecting this research topic was the recent multistate foodborne outbreak of *Salmonella* Poona on cucumber in 2015. The effect of e-beam irradiation on quality attributes including texture, color, moisture content, water activity, pH, and consumer acceptance of fresh sliced cucumber was studied. This study will provide the basis for application of e-beam irradiation to other produce including whole cucumbers.

2. OBJECTIVES

The main goal of the study was to achieve optimize the irradiation treatment of fresh sliced cucumbers using electron beams. This goal was achieved after accomplishing the following objectives.

- 1) Determine the radiation D_{10} -value of *Salmonella* Poona on sliced cucumber.
- 2) Quantify the effect of electron-beam irradiation on the product quality attributes (texture and color) throughout storage at refrigeration temperature (4-5°C).
- 3) Optimize irradiation treatment of sliced cucumbers to ensure proper decontamination (5D) while maintaining produce quality.

3. LITERATURE REVIEW

3.1 Cucumber (*Cucumis sativus*)

Cucumbers are one of the most consumed food in the world due to low calorie value and high water content. It has been cultivated for least 3,000 years in Western Asia, and was probably introduced to other parts of Europe by the Romans. They are normally consumed as a vegetable but they are scientifically considered as a fruits as they develop from a flower and contain seeds. The cucumber is a creeping vine that grows up trellises or other supporting frames and their roots are in the ground. The cucumber plants have large leaves that form a canopy over the fruits. They usually grow in all tropical and subtropical countries. China is the largest cucumber producing country in the world. According to FAOSTAT, China has produced 56,904,098 tons of cucumber and gherkins in 2014. Average of 83% production share of cucumber and gherkins by Asia has been recorded between 1994 and 2014 (Figure 1). According to USDA, vegetable 2015 summery, Principle fresh market cucumber production was 305,040 metric tons in 2015 (USDA, 2016). Figure 2 and 3 illustrate the production trend of cucumbers and gherkins in the world and USA from 1994 to 2014 respectively.

Commercial production of cucumbers is usually classified into three types, Slicing cucumbers, Pickling cucumbers and Burpless cucumbers. Slicing cucumbers are the cucumbers which are grown to eat fresh. They are normally eaten in the unripe form as they become bitter and sour upon ripening.

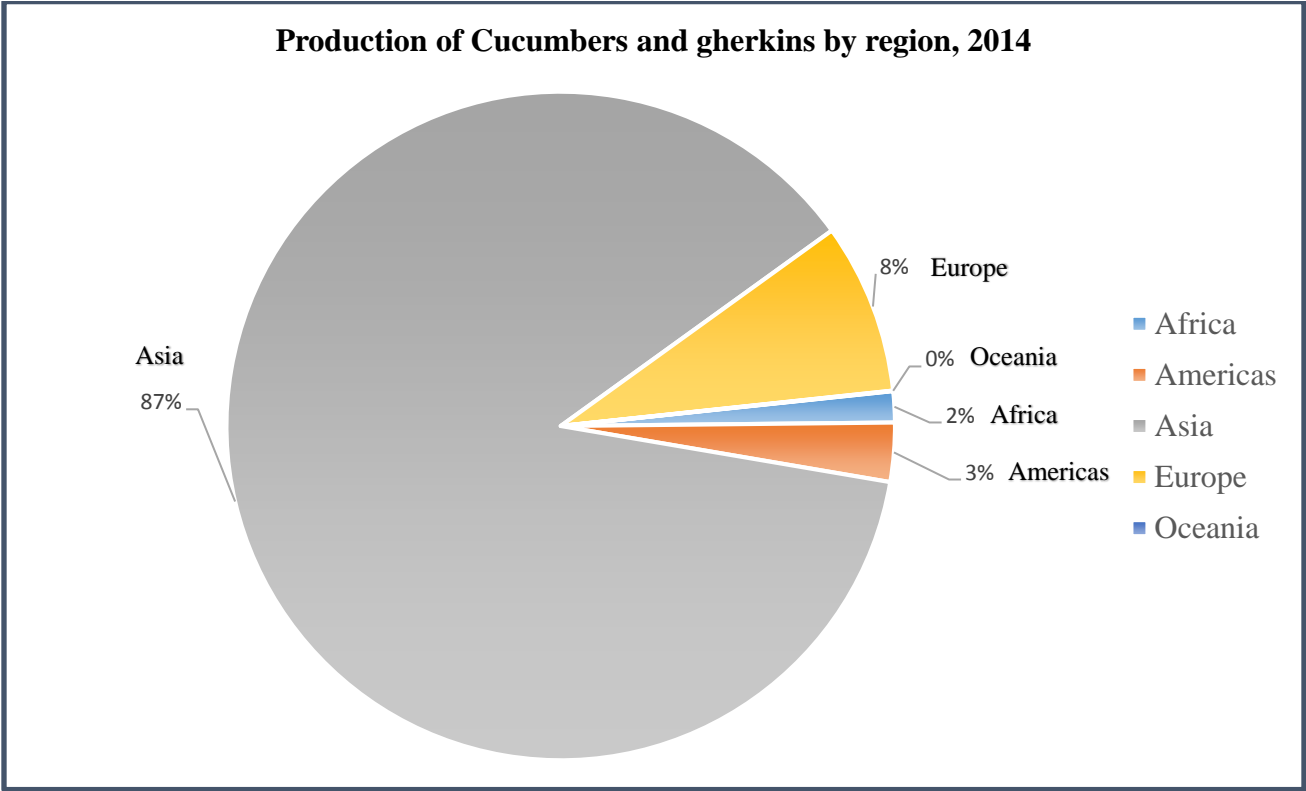


Figure 1. Production share of cucumbers and gherkins by region between 1994 and 2014 (Adapted from FAOSTAT, 2017).

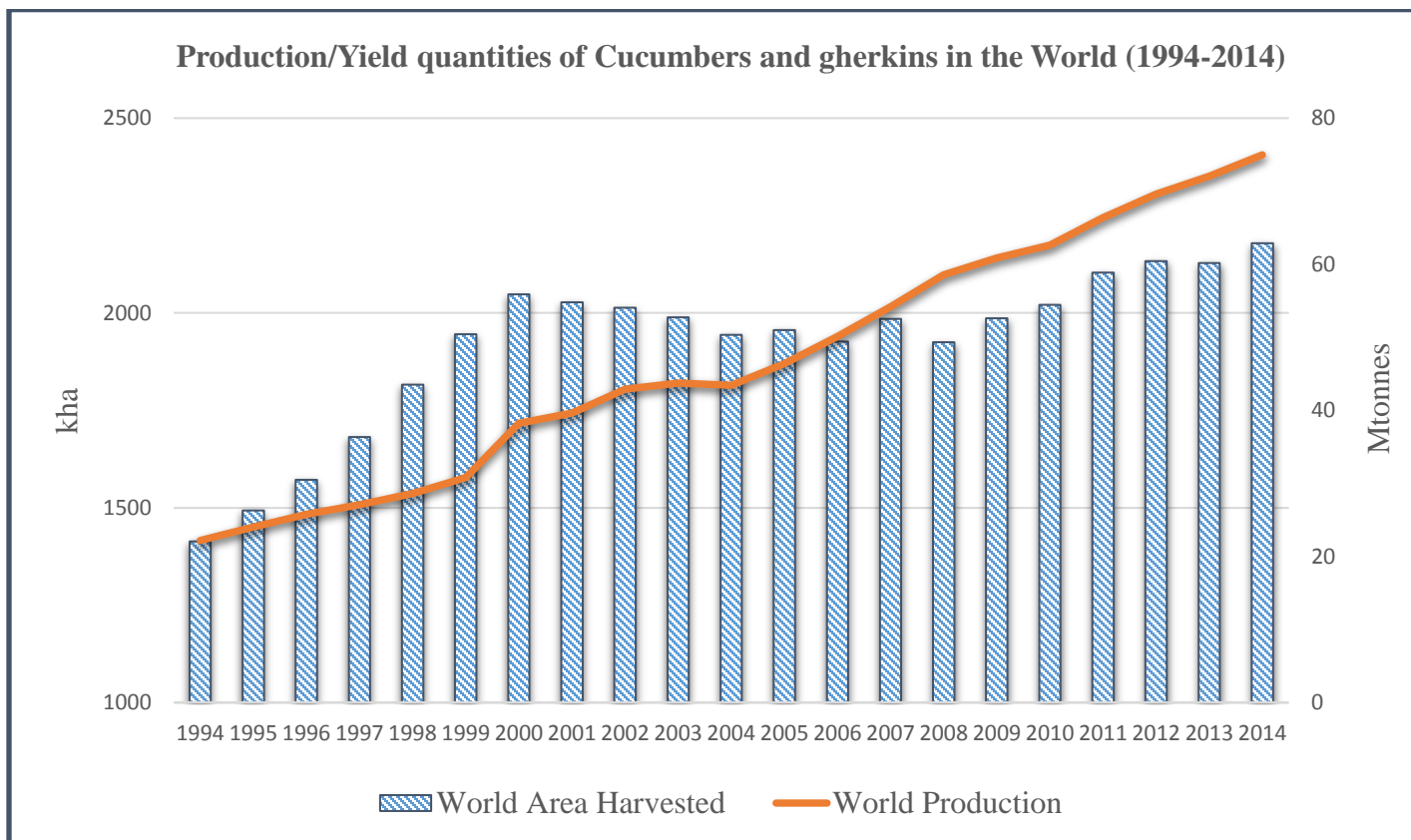


Figure 2. Production of cucumber and gherkins in the world between 1994 and 2014 (Adapted from FAOSTAT, 2017).

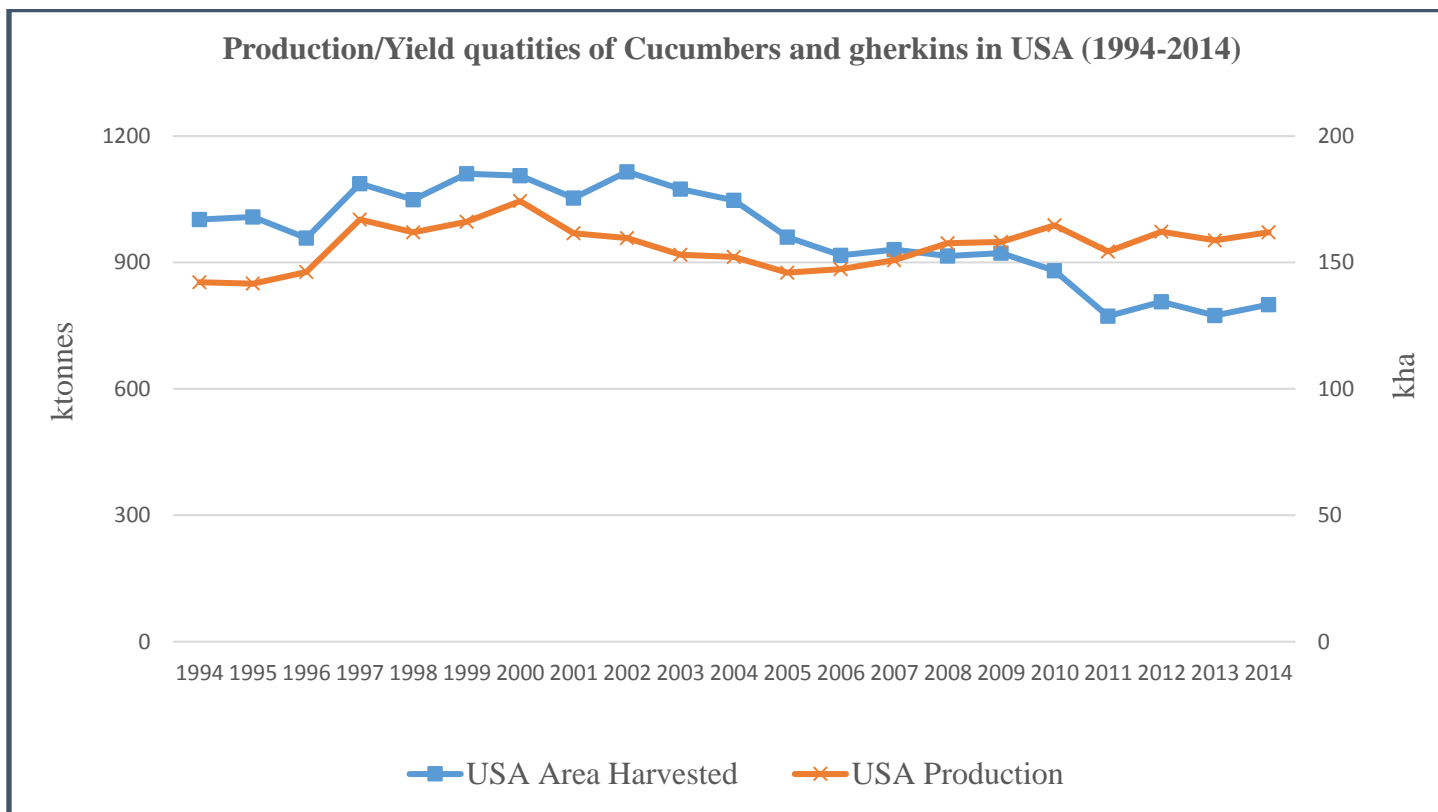


Figure 3. Production of cucumbers and gherkins in USA between 1994 and 2014 (Adapted from FAOSTAT, 2017).

Pickling cucumbers are (pickled) processed cucumber to develop flavor and extent shelf-life. Pickled cucumbers are soaked in brine or combination of vinegar and brine solution with various spices. Pickled cucumbers are smaller and thicker compared to slicing cucumbers. Burpless cucumbers are sweeter in taste. They are seedless, and have a thinner skin than other two types. They are normally grown in greenhouse.

Cucumbers are scientifically known as *Cucumis sativus*. They are widely cultivated plant throughout the world in the *Cucurbitaceae* family. According to USDA database, cucumbers are naturally low in calories, carbohydrates, sodium, and fat. Cucumbers contain many nutritional benefits, including hydrating properties (due to 95% water) and valuable nutrients. They are good source of phytonutrients such as flavonoids, lignans and triterpenes, which gives anti-cancer, antioxidant and anti-inflammatory benefits but notable only for vitamin K (16%) of the daily value. Table 1 summarize the nutrient values and weights for edible portion of raw cucumber (with peel).

Table 1. Chemical composition of cucumber (USDA, Food Composition Database)

Nutrient	Unit	Value / 100 g cucumber
Water	g	95.23
Energy	kcal	15
Protein	g	0.65
Total lipid (fat)	g	0.11
Ash	g	0.38
Carbohydrate	g	3.63
Fiber, total dietary	g	0.5
Sugar, total	g	1.67

3.2 Fresh-Cut Produce

Healthy and balance diet includes consumption of fresh fruits and vegetables. Therefore, fresh produce, fresh-cut products, and minimally processed food products are one of the major growing sectors of the food industry. According to the International Fresh-Cut Produce Association (IFPA), *“Fresh-cut produce is defined as any fresh fruit and vegetable or any combination of that has been physically altered from its original form, but remains in a fresh state. These fruits and vegetables have been trimmed, peeled, washed, and cut into a 100% usable product that is largely bagged or prepackaged to offer consumers high nutrition, convenience, and value while maintaining freshness”* (Bui and others 2010).

Fresh-cut products are rapidly expanding food category for the produce industry, food processors, retailers, and food service operators (Cantwell and Stockdale 2007). Initially, the food service industries were using fresh-cuts to reduce manpower and fresh produce waste. However, the importance of fresh-cut products in the retail groceries has increased due to fast pace life in developed countries. The U.S. fresh-cut fruits and vegetables is an estimated \$27 Billion market in 2014, and volume sales are increasing (Cook 2014). Fresh-cut products take less time in preparation and consumption. They are convenient for grab & go for healthy living busy families. Consumers know fresh-cut products are ripe and ready-to eat vs. figuring it out with the whole produce. They are different than traditional, whole fruits and vegetables in terms of their physiology, handling and storage requirements (Lamikanra 2002).

Fresh-cut products are highly perishable as a large proportion of their surface area is without epidermis which is the outer protective layer of tissues. Processing of fresh-cut produce including cutting which damage the integrity of the cells and cause tissue to suffer from wounding stress (Li and others 2017). The wounding stress accelerate deterioration of produce because of increase in respiration rate, water loss, tissue softening, color loss, oxidative browning, development of off-flavors, production of ethylene, and degradation of membrane lipids which reduce the shelf-life of product (Rolle and CHISM 1987; Soliva-Fortuny and Martín-Belloso 2003; Gil and others 2006; Hodges and Toivonen 2008). Fresh-cut produces raise food safety concerns as pathogens can be easily colonized on fresh-cut produces than intact produce because of higher availability of nutrients on cut surface (Leverentz and others 2003). Temperature, relative humidity, sanitation, atmosphere, and proper handling must be regulated to maintain good quality of fresh products (Watada and others 1996).

Cucumbers commonly harvested when they are green before full maturity. Harvesting delay can lead to lower quality of fruit and faster deterioration after harvest (Lamikanra 2002). Texture is an important quality attribute of cucumber. Consumer prefers a firm and crispy cucumber. Mold and other microorganisms grow rapidly on fresh cucumber at ambient temperature and high humidity due to higher water content and available nutrients for microorganisms. Cucumber contaminated with mold stored at ambient temperature can become soft in 12-18 hours (Costilow and others 1984). Food industry replaces fresh cucumbers with pickled cucumber because of less shelf life, quality and stability of fresh cucumbers (Dermesonlouoglou and others 2008). The

whole fresh cucumber can be stored for 7-10 days in refrigerator and shelf life for sliced cucumber is 1-2 days.

3.3 Fresh-Cut Produce Quality Attributes

Quality of fresh-cut produces is a combination of attributes and characteristics that dictate their value to the consumer. Quality attributes include appearance, texture, color, smell, flavor, and nutritional value. The importance of each quality attribute depends on the products and their use. At the time of purchase, appearance and freshness are important for consumer but subsequent purchase are also rely on texture and flavor of product upon eating. They also have concern regarding safety and nutritional value of product.

Quality of the whole fruit or vegetable depends on the cultivar, pre-harvest cultural practice, climate condition, maturity at harvest, harvesting method, handling procedures (Lamikanra 2002). Whereas, quality of whole fruit or vegetable, method of preparation, handling condition, and storage (temperature, relative humidity, packaging) affects the quality of fresh-cut produces.

Appearance of product is the most important parameter at the time of purchase. This may include size, shape, color, gloss, and defects (wound related effect, microbial colonization, chemical injuries, and various blemishes which results in unattractive product). Cucumbers are judged on the physical defects which include shriveling, wilting, internal drying of fruit, mechanical damage due to punctures, cuts, scratches, splits, skin abrasions, deformation, and bruising.

Texture of product is important factor for eating and cooking. Textural behavior of food is basically related to structure of food (Szczesniak 1963). Texture is a sensory property and is a response of tactile senses to physical stimuli that result from contact between some part of the body and the food material. This includes firmness, crispiness, juiciness, and toughness depending on the product. Understanding of food texture is related to study of the relationship between textural characteristics and chemical composition (Curwen and others 1966). Texture is also important for their transportation as soft and delicate fruits and vegetable cannot be shipped long distances without physical damage (Lamikanra 2002). Unfortunately, most of the processing techniques (such as cutting, freezing, blanching, sterilization) damages the cellular tissues of food and affect the textural properties of foods.

Flavor of product includes perception of aromas and tastes of many compounds. This includes sweetness, sourness, bitterness, astringency, and off-flavors. Flavor of most fruits and vegetables is influenced by sugar content, organic acids, phenolic compounds, and other volatile compounds. Objective analytical determination of critical components must be combined with subjective evaluation by a taste panel to produce useful and meaningful information about flavor of food product (Lamikanra 2002). However, consumer acceptability can only tested by large-scale testing by a representative sample of the consumers.

Fresh fruits and vegetables are considered as a source of important nutrition in human diet. They serve as source of vitamins, minerals, and dietary fibers. Some of the

nutrition helps to prevent health issues related to heart disease, cancer, and other disease. Nutritional value varies depending of commodity and cultivars.

3.4 Foodborne Disease Outbreaks

According to CDC, Foodborne disease outbreak can be defined as an incident in which two or more persons experience a similar illness after ingestion of a common food, and epidemiologic analysis implicates the food as the source of the illness. The reporting of foodborne and waterborne diseases started more than 80 years ago in the United States when the health officials were concerned about the high morbidity and mortality caused by typhoid fever and infantile diarrhea attributed to milk. The purpose of investigation was to obtain information about the role of food and water in outbreak diseases (Lynch and others 2006). Foodborne diseases are estimated to cause 9.4 million illness caused by 31 major pathogens every year (Scallan and others 2011). There are 31 known pathogens account for 20% of food poisoning every year and 80% of food poisoning are caused by unspecified agents (Sadilek and others 2016). According to the surveillance data from the US Center for Diseases Control and Prevention (CDC, 2016), 864 foodborne disease outbreaks with 13,246 illness, 712 hospitalizations, 21 deaths, and 21 food recall were reported in 2014. Out of these bacteria caused 247 outbreaks (53%), followed by 161 by viruses (35%), 46 by chemicals (10%), and 7 by parasites (2%). Figure 4 illustrate the number of foodborne disease outbreaks in the United States between 2000 and 2015.

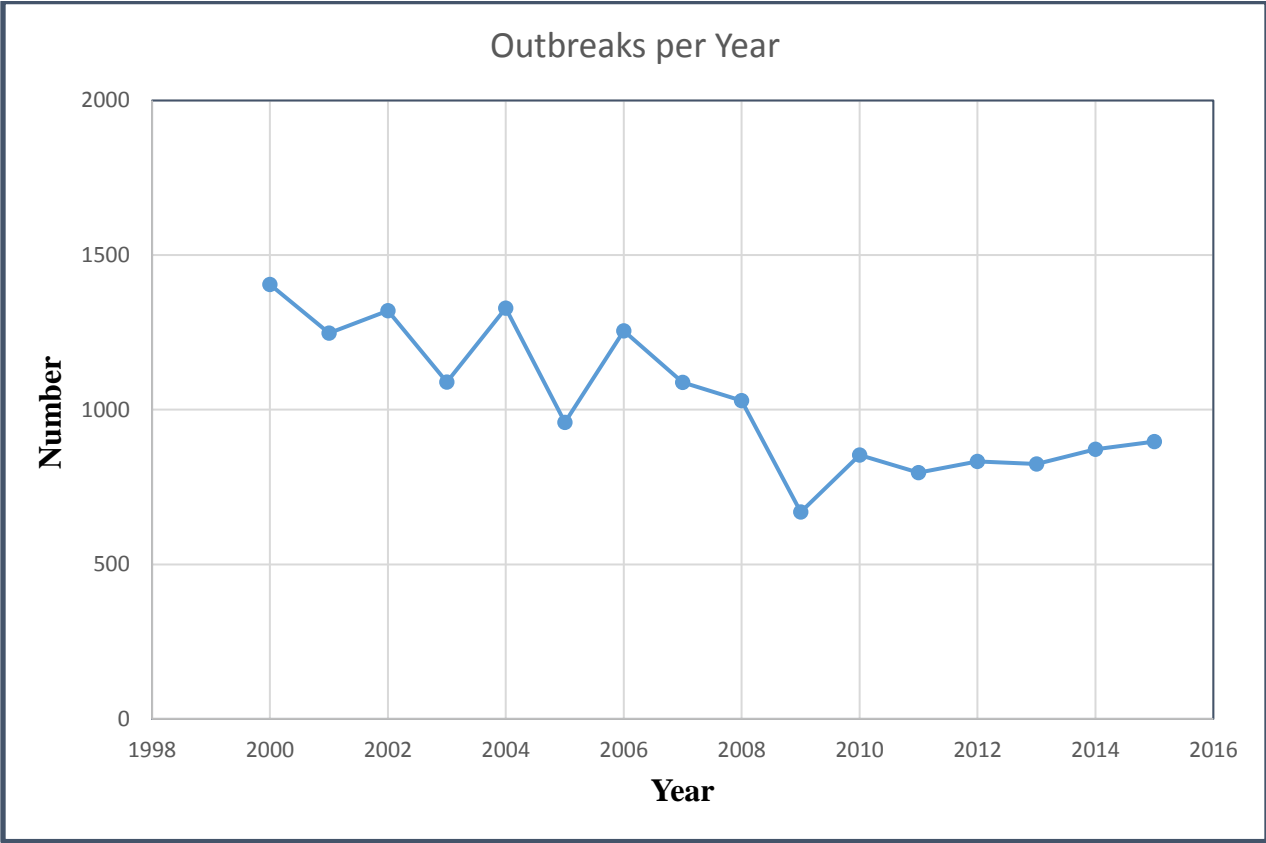


Figure 4. Foodborne disease outbreaks surveillance system data from 2000 to 2015 (Adapted from CDC 2016).

Pathogens that are the main concern today are Norovirus, *Salmonella* (Enteritidis, Thyphimurium, Javiana, and Newport), Shiga toxin-producing *Escherichia coli* (O157, O111, O26, O121, O103, O145, and O186), *Listeria monocytogenes*, *Campylobacter*, and *Clostridium Botulinum* (CDC 2016). Among them, Norovirus was accounting for 43% of illness, *Salmonella* for 27% illness. Food associated with outbreak illness were seeded vegetables (357), chicken (227), turkey (184), dairy products (144), and sprouts (155). The most common symptoms for food poisoning are vomiting, diarrhea, abdominal pain, fever, and chills but some pathogens can also cause symptoms of the nervous system (Sadilek and others 2016).

The United States Department of Agriculture (USDA), Food and Drug Administration (FDA), World Health Organization (WHO), industries, and retailers has a strong interest on the evaluation of alternatives to minimize fresh food contamination problem. The investigation of foodborne outbreaks leads to prevention and control measures in the food industries. Foodborne disease surveillance provides a basis for detecting disease and identify cause of outbreaks (Altekruse and others 1997). Hazard analysis and critical control point (HACCP) is a systematic approach to prevent foodborne outbreaks by identifying critical control points during processing.

3.5 Foodborne Disease Outbreaks Associated with Fruits and Vegetables

Foodborne outbreaks are related to raw produces and with the increase in raw product consumption for healthy lifestyle, risk of foodborne disease has been increasing (Goodwin and Brodwick 1995; Parish 1997; Gomes and others 2009; Callejón and

others 2015). Changes in dietary habits, methods of fruit and vegetable production and processing, packaging, distribution, global marketing technology, source of produce, and the emergence of pathogens previously not recognized for their association with raw produce have enhanced the possibility for foodborne outbreaks (Hedberg and others 1994; Beuchat 2002). According to US Department of Agriculture (USDA) food pyramid, an adult should include 3-5 servings of vegetables and 2-4 servings of fruits in diet. Fruits and vegetables are good source of vitamins, minerals, and fiber and are also associated with protection against various diseases, including cancers, and cardiovascular diseases (Lintas 1992; du Toit and others 2001).

Fresh fruits and vegetables are the known vehicles for the pathogens (Lynch and others 2009). Magnificent difference in surface morphology, internal tissue composition, and metabolic activities of leaves, stems, florets, fruits, roots, and tubers provide an extensive range of diverse ecological selection for specific groups of microorganisms (Beuchat 2002). Fruits and vegetables have opportunities and obstacles for pathogen contamination. They contain the nutrients which support the rapid and progressive growth of pathogens and they also have natural outer cover which protects interior of fruit or vegetable by preventing microbes to enter (Madden 1992). Fruits and vegetables can be contaminated with pathogens while growing in fields, during harvesting, post-harvest handling, distribution, or processing.

Table 2. Outbreaks associated with fresh raw fruits and vegetables between 2009 and 2014 (Adapted from CDC surveillance data 2009-2014).

Microorganism	Year	Produce
Shiga toxin-producing <i>E. coli</i> O157:H7	2014	Spinach, Pre-packaged salad, Leaf lettuce
	2013	Pre-packaged leafy greens, Romaine lettuce, Tomatoes, Cucumber
	2010	Romaine lettuce
	2012	Apple, Lettuce, Leafy green
	2014	Cabbage
Shiga toxin-producing <i>E. coli</i> O111	2014	Cabbage
<i>Listeria monocytogenes</i>	2014	Apple, Stone fruit, peaches
	2011	Cantaloupe
<i>Clostridium botulinum</i>	2012	Root and other underground vegetables, Beets
<i>Staphylococcus aureus</i>	2014	Cabbage, Carrots
<i>Salmonella enterica</i>		
Baildon	2014	Cantaloupe
Minnesota	2014	mango
Thompson	2013	Papaya
Typhimurium	2009	Lettuce
Javiana	2010	Yellow onion
Muenchen	2009	Blueberries
Saintpaul	2011	Cucumber, Tomato
Newport	2013	Tomato
Miami	2014	Romaine lettuce
Norovirus	2014	Lettuce
Norovirus Genogroup II	2014	Blueberries, Cantaloupe, Honeydew melon, Strawberries, mushrooms
Hepatitis A	2013	Strawberries

Microorganisms of concerns for fresh produces are *Salmonella* spp, *Escherichia coli*, *Shigella* spp, *Yersinia* spp, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium* spp (Yeni and others 2016). A multistate frozen strawberry outbreak of Hepatitis was reported in 1997 (Hutin and others 1999) and multistate outbreak of *Escherichia coli* O157:H7 in fresh spinach was reported in 2006 in United States (Sharapov and others 2016). The CDC surveillance data has reported 55 outbreaks related to plants in 2014 including *Salmonella* Minnesota outbreak in mangos (May), Shiga toxin-producing *E. Coli* O157:H7 outbreak in spinach (April) (CDC 2014). Outbreaks associated with fresh produces between 2009 and 2014 are listed in Table 2.

3.6 Attachment of Microorganisms to the Surface of Fruits and Vegetables

Fresh fruits and vegetables can harbor wide range of microorganisms. It is important to study presence and cause of contamination related to fresh produce to reduce the contamination. Fresh produces are usually in contact with soil, insects, animals, human, and water during growing, harvesting and processing. Pathogens such as *Listeria monocytogenes*, *Clostridium botulinum*, and *Bacillus cereus* are naturally present in some soil, and these are likely to contaminate fresh produce. Whereas, *Salmonella*, *E. coli* O157:H7, *Campylobacter jejuni*, *Vibrio cholera*, parasites, and viruses are more likely to contaminate fresh produce through vehicles such as contaminated water, improperly handled animal waste, and contact with insects, reptiles, mammals, and unpasteurized products from animal origin (Beuchat and Ryu 1997). Another post-harvest source of contamination includes feces, human handling,

harvesting equipment, transportation containers, transportation vehicle, wild and domestic animals, insects, rodents, dust, rinse water, ice, processing equipment, and packaging material (Janisiewicz and others 1999). Washing with water, chlorinated water, or some other disinfectant can reduce the population of microorganisms on fresh produce but they are not efficient to remove them completely as their inability to reach locations within structures and tissues that may harbor bacteria (Beuchat 2002).

The ability of pathogenic bacteria to adhere to fresh produce surface is a potential food safety concern to the fresh-cut produce industries. The attachment of bacteria on the surface of fresh fruits and vegetables is affected by a number of factors including the medium in which they are grown, motility, temperature and pH, length of contact time, water activity, and production of extracellular polysaccharides (Iturriaga and others 2003). The bacterial surface charge and hydrophobicity influence the bacterial attachment to fresh produce surface. Bacterial surface charge is influenced by carboxyl, amino, sulfate, and phosphate group within the cell envelop (Hassan and Frank 2004). The flagella, fimbriae, and outer membrane proteins may affect bacterial attachment to the surface (Ukuku and Fett 2002). Hassan and Frank (2004) studied effect of *E. coli* attachment on the surface of apple and lettuce related to cell hydrophobicity, surface charge, and capsule production. They did not found any significant effect of those factor except for capsule production which enhanced attachment.

A better understanding of microbial ecosystem on the surface of fresh produces would be useful to develop interventions to minimize contamination, prevent the growth and kill pathogens at various level of production from farm to fork (Beuchat 2002).

Bacteria also attach to cut surfaces, which directly expose produce cell walls to the environment and more susceptible to contamination by pathogens (Sze-Fan Tan and others 2016). Fresh fruits and vegetables often have punctures, cuts or splits due to improper handling during processing that influence the bacterial attachment. According to research study by Burnett and others (2000), punctured wounds harbored greater numbers of the pathogen at greater depths than the once without any injury.

3.7 Survival of Pathogens on the Surface of Fruits and Vegetables

The survival and growth of pathogens on fresh fruits and vegetables is depends on the microorganism, produce variety, and environmental condition of product storage (Stine and others 2005; Tian and others 2012). Survival and growth of pathogens depend on many factors including storage temperature, initial pH, water activity, relative humidity, sodium chloride content, atmosphere, and sodium nitrate concentration (Buchanan and Phillips 1990; Stine and others 2005). According to study by Watkins and SLEATH (1981), *Listeria monocytogenes* can survive in soil more than 8 weeks without any population reduction whereas, *Salmonella* population decreased to below detectable level after 6 weeks.

pH is an important factor as most of the microorganisms are inactive at lower pH values. Many fruits (pomegranate, oranges) are more acidic and do not support bacterial growth whereas fruits with higher pH values (> 5.0 pH value) (papayas, mangos, melons) are more susceptible to support bacterial growth (De Roever 1998). Tomatoes are of great interest as in spite of having low pH value (3.5-4.7), multiple outbreaks

associated with tomatoes have been reported. Multistate outbreak of *Salmonella* Nueport (2005), *Salmonella enterica* Baildon (1999), Hepatitis A (2009) were reported in fresh tomatoes (Cummings and others 2001; Greene and others 2008; Donnan and others 2012). The growth of acid tolerant yeast and mold populations in low acid products decreases product pH which makes the environment more suitable for pathogens (Francis and others 1999). *Listeria monocytogenes* has the ability to survive at extreme condition of low pH and temperature (Conway and others 2000). The growth and metabolism of *Listeria monocytogenes* reduce at low pH and temperature but they can survive longer under low temperature (5°C) than 30°C temperature (Cole and others 1990). Storage temperature becomes a critical factor for the low pH produce which allows the survival and growth of pathogens.

Survival of pathogens on the surface of fruits and vegetables is influenced by storage temperature. Temperature can influence the growth of bacteria because storage temperature determines the respiration rate of the produce which changes the gaseous atmosphere in the package, and, therefore influence the behavior of microorganisms (Nguyen-the and Carlin 1994). Tian and others (2012) studied survival and growth of four pathogens (*Salmonella enterica* Serovar typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, and *E. coli* O157:H7) on fresh vegetables (romaine lettuce, iceberg lettuce, perilla leaves, and sprouts) at different temperatures and conclude that population of all pathogens increased on all vegetables stored at 15°C and there was no significant ($P > 0.05$) difference was found at 4°C. Refrigerated temperatures cannot be relied upon to prevent growth of pathogens on produce, some pathogens remained

constant or grew on a variety of whole and cut produce store under refrigeration condition (Parish and others 2003). Growth of some pathogens may be inhibited by chilled temperatures but survival can be enhanced under certain condition. According to Knudsen and others (2001), *E. coli* O157:H7 and *Salmonella* are capable of survival without growth on fresh strawberries and both pathogens can survive better on cut strawberries at refrigerated temperature (5°C) than on whole fruits.

3.8 *Salmonella*

Salmonella is a rod-shaped (diameters between 0.7 and 1.5 µm, and length between 2 and 5 µm) gram-negative bacterium which belongs to *enterobacteriaceae* family. *Salmonella* species are non-spore-forming bacteria. They are facultative anaerobic intracellular pathogens (Li and others 2013). *Salmonella* infections are due to ingestion of contaminated food including animal-products, fruits, vegetables, and processed food. Salmonellae are chemoorganotrophic, with the ability to metabolize nutrients by both respiratory and fermentative pathways. They grow optimally at 37°C and catabolize D-glucose and other carbohydrates with the production of acid and gas (D'Aoust and Maurer 2007). Salmonellae cause typhoid fever, paratyphoid fever and food poisoning. *Salmonella* infected person develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection, And illness usually lasts 4 to 7 days. *Salmonella enterica* is estimated to cause 1.2 million illness every year including hospitalizations and death in the United States (Jackson 2013).

According to FDA, the genus *Salmonella* has over 2700 serotypes. *Salmonella enterica* is the type of species and is further divided in several subspecies. Multistate foodborne outbreak of *Salmonella* Mbandaka infection associated with alfalfa seed was reported in 1999 (Gill and others 2003). Over a six-week period, 25 episodes of *Salmonella* Eimsbuettel infection reported due to contaminated rectal thermometers (McAllister and others 1986). The optimum pH for the growth of salmonellae is between 6.5 and 7.5 but study have shown survival of *Salmonella* in acidic environment. Outbreak of *Salmonella* Braenderup infection associated with roma tomatoes was reported in 2004 in the United States (Gupta 2007). Although *Salmonella* normally grow at 37°C, they can survive and even grow at lower temperature (Matches and Liston 1968).

D₁₀-value for *Salmonella* are specific to each species and also specific to strains within the same species in some cases. Research initiatives in the past have been focused on determination of D₁₀-value for pathogens in various foods using gamma radiation with little work done on electron-beam irradiation. A number of factors play role in the determination of D₁₀-value. Several studies have been conducted in the past on Gamma radiation or e-beam radiation of meats, sprouts, however, not much has been reported on fresh produces.

One of the factor which significantly affects the D₁₀-value is a composition of the medium. According to the research study by Thayer and others (1990), D₁₀-value for *Salmonella spp.* are lower when they are suspended in phosphate buffer and microorganisms are more resistant to the effect of irradiation in presence of air or

vacuum. Variations in sample preparation, sample composition, inoculation method, headspace composition (if samples are irradiated in sealed bags), microbiological method, and irradiation type and set-up can affect the D_{10} -value for different pathogens on food (Moreira and others 2012). When irradiation is done on sample sealed in a bag, headspace composition can affect the pathogen's response to irradiation as respiration rate of fresh produce will change the headspace composition. Other parameters that could possibly effect the D_{10} -value are the temperature of the product and moisture content.

Salmonella Poona is a rare serotype responsible for national and international foodborne outbreaks. CDC has reported multiple multistate outbreaks of *Salmonella* Poona in infections associated with cantaloupe in 1991 and in the spring of consecutive years during 2000-2002 (Centers for Disease Control (CDC) 1991; Centers for Disease Control and Prevention (CDC) 2002). Many researchers have studied action of *Salmonella* Poona in food and inactivation of pathogens with various treatments. Mahmoud and others (2008) achieved more than a 5 log CFU/cm² reduction of *Salmonella* Poona after treatment with 3.0 and 5.0 mg/L chlorine dioxide gas for 10 minutes. According to study of Annous and others (2004), surface pasteurization of whole cantaloupes at 76°C can reduce 5 log CFU/cm² of *Salmonella* Poona.

3.9 Incidence of *Salmonella* in Cucumbers

Cucumbers are normally grow on the ground, and can be contaminated with a human pathogen such as *Salmonella* anytime during production including harvesting,

processing, transportation, packaging, and storage. Cucumber is considered a potentially hazardous food because it is capable of supporting the growth of pathogens due to low acidity (pH 5.1 to 5.7), high water content (95%) and water activity (~96%). The US Food and Drug Administration (1997) has issued safety guidelines for fresh-cut products to prevent foodborne outbreaks. This guideline includes possible microbial hazards throughout the processing and appropriate control measures for such hazards to occur.

As discussed earlier, multistate outbreak of *Salmonella* Poona infection in the United States was reported due to the consumption of contaminated and imported Californian cucumbers (CDC, 2016). This led to 907 confirmed cases of outbreak strains of *Salmonella* Poona across 40 states with 204 ill people hospitalized, and six deaths.

3.10 Decontamination Methods for Fresh Produce

As we have discussed earlier, fresh fruits and vegetables are associated with foodborne disease outbreaks due to presence or contamination of pathogenic microorganisms. Quality and safety of fresh produce depend on their microbiological flora. Hence, sanitation and attention to microbiology are very important in maintaining shelf stability and safety of fresh produce (Brackett 1992). There are three main reasons for industries to concern with sanitation and disinfection of fresh fruits and vegetables: Necessity, quality, and safety.

There are variety of methods used to eliminate or reduce microbial population on whole and fresh-cut produces. Each method has specific advantages and disadvantages depending upon the type and use of product. The best method to eliminate pathogens

from the fresh produce is to prevent contamination in the first place. To minimize the risk of contamination with fresh produce, potential source of contamination throughout the production to consumption should be identified and eliminated. However, this is not always possible, the need to wash and sanitize the produce is considered as an important tool for decontamination to prevent foodborne outbreaks related to fresh produces.

Microbiological safety of fresh fruits and vegetables are affected by initial quality and handling of produces.

Decontamination methods for fresh fruits and vegetables can be broadly classified in chemical and physical treatment. The efficiency of any decontamination treatment used for pathogen reduction will depends on several factors including type of treatment selected for the produce of interest, characteristics of produce surface such as cracks, cuts, texture and hydrophobic tendency, the type and physiology of target pathogen, treatment exposer time, concentration of the sanitizer, pH and temperature of produce and sanitizing agent (Parish and others 2003).Storage of produces at refrigerated condition is not enough to prevent growth and survival of all microorganisms. Several studies has demonstrated the ability of *Listeria monocytogenes* and, *Salmonella* spp to grow and survive on whole and fresh-cut produces stored at refrigerated temperature (Gandhi and Chikindas 2007; Pasquali and others 2016). Proper handling and sanitization at all levels in the fresh produce chain is crucial to prevent foodborne outbreaks.

According to FDA, sanitizer is defined as an agent that reduces contaminants in the inanimate environment to levels considered safe as determined by Public Health

Ordinance, or that reduces the bacterial population by significant numbers where public health requirements have not been established. FDA had approved seven general classes of food contact sanitizers which includes acid-anionic surfactants, carboxylic acids, chlorine and chlorine compounds, iodine complexes, peroxide and peroxyacid mixtures, phenolic, and quaternary ammonium compounds (Block 2001). Food contact sanitizers must receive additional approval from the Environmental Protection Agency (EPA).

Chlorine and chlorinated agents are most widely used as a chemical disinfectant in the fresh fruits and vegetables (Selma and others 2008; López-Gálvez and others 2010). The most common forms of free chlorine are liquid chlorine and hypochlorite. They are commonly used to sanitize produce at a concentration of 50-200 ppm with the contact time of 1- 2 min (Abadias and others 2011). The antimicrobial efficiency of chlorine is pH dependent and effectiveness of chlorine solution was observed at pH range of 6.5-7.5 (Ölmez and Kretzschmar 2009). The ability of chlorine to destroy microorganisms depends on the amount of free residual chlorine (chlorine remaining after the reaction with organic matter) in water (Vadlamudi 2005). Brackett (1987) reported 2 log unit reduction of *Listeria monocytogenes* on Brussels sprouts when treated with 200 ppm chlorine solution.

Organic acids (citric acid, acetic acid, lactic acid) are GRAS (Generally Recognized as Safe) and known to have antimicrobial properties. The antimicrobial activity of organic acids is a function of reduction of pH in the microbial environment leading to disruption of membrane transport and permeability, accumulation of anions and lowering of intracellular pH within the cell by dissociation of hydrogen ions from

the acid. Organic acids act rapidly and kill a broad spectrum of bacteria. Advantage of using organic acids is they are effective within a wide range of temperature and cannot be affected by hard water. However, they also have disadvantages such as high cost, odor, and corrosiveness (Ölmez and Kretzschmar 2009). According to research study by Sagong and others (2011), Treatment of organic fresh lettuce with three organic acids (2%) (malic acid, lactic acid, and citric acid) reduced 1-2 log units of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*.

Ozone is a strong antimicrobial agent with potential applications in food industries. It has been used widely in water applications for many years. After it gained GRAS (Generally Recognized as Safe) status in 1997, use of ozone as disinfectant has been approved in Europe and in the United States. It is effective to reduce many bacteria, mold, and yeast at low concentrations (1-5 ppm) with the contact time of 1-5 min (Ölmez and Kretzschmar 2009). Hirneisen and others (2011) concluded that 3 log unit reduction of Norovirus can be achieved with the application of ozone. However, ozone inactivation of norovirus is dependent upon the presence of a food matrix and the time of treatment.

Physical removal of microorganism is removal of soil and microorganisms using brushes to scrub surfaces of whole fruits and vegetables. This is often done in conjunction with a detergent followed by a portable water rinse. Problem with treatment is brushing also removes a portion of the natural waxy layer from the surface that helps to prevent microorganisms. Hence, produce with delicate skin cannot be treated physically.

Many treatments are known to be partially effective in reducing microorganisms from the surface of whole and fresh-cut produce, none of them are effective enough to completely eliminate microorganisms. Treatment of fruits and vegetables with sanitizers often results in 2-3 log units of reduction in pathogen population on fresh produce but cannot be relied upon to eliminate safety risk (Sy and others 2005). Another challenge for the food industries is the minimization of water consumption and wastewater discharge rates. Due to the above mentioned problems, new intervention strategies such as irradiation are worth their evaluation.

3.11 Irradiation as an Intervention Strategy

3.11.1 History

The early history of food irradiation (1890s-1940s) is related to radiation physics and to the development of the systems and sources to be used in food irradiation. Irradiation on meat, poultry, and minimally processed foods has attracted more attention in the 1990s than insect disinfestation of stored dried foods, which was studied mainly between the 1960s and 1970s (Molins 2001). During the 1950s-1960s, the US army conducted research into irradiation of military rations using various levels of doses. These experiments prompted similar studies in Belgium, Canada, France, The Federal Republic of Germany, Netherlands, Poland, the Soviet Union, and the United Kingdom during mid- or late 1950s (Diehl 2002). Health authorities in these countries were hesitant to grant permissions for marketing irradiated food because of unanswered questions about the safety of irradiated food for human consumption. After long-term animal feeding

studies, short-term screening tests, and the study of chemical changes in food products, the joint committee including Food and Agricultural Organization (FAO), International Atomic Energy Agency (IAEA), and World Health Organization (WHO), on the wholesomeness of irradiated food reported that there is no toxicological hazard and no special nutritional or microbiological problems in any food commodity irradiated to an overall average dose of 10 kilogray (kGy) (Diehl 1999; World Health Organization 1999).

Irradiation is an effective method of preservation by reducing food spoilage organisms and for increasing shelf-life of food product (IFT 1983; World Health Organization 1988). In 1986, 1992, and 1998, the Scientific Committee on Food (SCF) expressed favorable opinions on irradiation of fruit, vegetable, camembert from raw milk, frog legs, gum Arabic, casein/caseinates, egg white, cereal flakes, rice flour, and blood products (Arvanitoyannis 2010). The first half of the 1900s could be called as the age of inventors as radiation facilities were not of suitable capacities for practical applications during that period (Diehl 2002). Selected historical milestones of irradiation of food research and developments are summarized in Table 3.

Table 3. Historic milestones of food irradiation (Adapted from Farkas and Mohácsi-Farkas 2011).

Age of inventors
1905: J. Appleby & A.J. Banks: British patent: “to bring about an improvement in the conditions of foodstuffs” and in “their general keeping quality by radiation of radioactive substances”
1921: B. Schwartz (US): published use of X-rays for inactivating trichinae in raw pork
1930: O. Wüst: French patent: kill bacteria in packaged food with X-rays Radiation facilities not yet suitable for practical application
1957: First commercial application: electron beam irradiation of spices in Germany
1966: First International Symposium on Food Irradiation, Karlsruhe, Germany
1970–1982: International Project in the Field of Food Irradiation (IFIP) (19→24 countries + FAO/IAEA, OECD→WHO)
1980 (1964, 1969, 1976): Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI). Landmark decision, doses up to 10 kGy
1983–1984: Codex Alimentarius “General Standard for Irradiated Foods” and “Recommended International Code of Practice for the Operation of Radiation Facilities”
1979–1990: Assisting developing countries by training and demonstration: FAO/IAEA International Facility for Food Irradiation Technology (IFFIT), Wageningen, The Netherlands
1983–2004: International Consultative Group on Food Irradiation (ICGFI)

3.11.2 Ionizing Radiation and its Sources

Ionizing radiation produces electrically charged particles or ions by removal of electrons from an atom. It has a higher energy than non-ionizing radiation like radio waves, microwaves, infrared, and light that has different frequencies and energy associated with different radiations in the electromagnetic spectrum. When ionizing radiation strikes bacteria and other microbes, its high energy breaks chemical bonds in molecules that are vital for cell growth and integrity. As a result of this radiolysis, the microbes will die, or can no longer multiply causing spoilage or illness. Food is commonly irradiated through the application of gamma rays (with ^{60}Co or ^{137}Cs radioisotopes), X-rays (high energy of up to 7.5 Mev), or electron beam (high energy of up to 10 Mev). None of these sources induce radioactivity in the food or its packaging, and the treatment has many feasible applications including significantly improving microbial safety and/or storage stability of foods (Farkas and Mohácsi-Farkas 2011).

Gamma rays are produced by neutron bombardment in a nuclear reactor of the metal Co-60 or Cs-137 and contains energy of about 1 to 2 Mev. Co-60 yields uniformity of dose in the food product and 95% of its emitted energy is available for use. It can penetrate up to 70 cm of depth of the product. Co-60 is the most widely used radioisotope source in radiation facilities for use in the irradiation of food, sterilization of medical product, and radiotherapy (Farkas 1989; Lee and Chu 1996; Xia and others 2006; Haji-Saeid and others 2007). It also has disadvantage of low dose rate and long treatment time.

X-rays were discovered by Roentgen in 1895 (Diehl 2002). They can be produced by bombarding a metal with high-energy electrons. Although some energy is absorbed, the rest is converted to X-rays. X-ray tube or vacuum tube can be used to produce X-rays which uses a high voltage to accelerate the electrons released by a hot cathode to a high velocity. Though X-rays have high penetrating power (up to 100cm depth) and dose rate, they are not used in food irradiation due to poor conversion of accelerated electrons to X-rays (Thakur and Singh 1994).

Electron beam facilities generate e-beams with a linear accelerator powered by electricity. The electrons are concentrated and accelerated to 99% of the speed of light and energies of up to 10 MeV. The best application of e-beam is surface and subsurface irradiation of foods because of their low penetration potential. E-beams must be converted to x-rays to penetrate large items. The advantage of a linear accelerator over using gamma rays is the speed with which product can run over a conveyor through the beam.

3.11.3 Role of Food in Deciding Irradiation Dose

The irradiation dose is defined as the quantity of radiation energy absorbed by the food and it is the most critical factor in food irradiation (World Health Organization 1988). The absorbed dose is measured by the unit gray (Gy). The intended effect or irradiation may not be achieved without proper amount of absorbed radiation dose. The irradiation used for food is depends on several factors including physical and chemical composition of the food being irradiated and desired effect (World Health Organization 1988).

Composition of food plays major role in determining the radiation dose as radiation often induces undesirable changes in taste and/or appearance in foods. Food high in lipids (meat, nuts) tends to develop rancidity at high dose which develops bitter, metallic, or burnt flavors in food. Radiation of fat in presence of oxygen and water tends to accelerate lipid peroxidation and rapid onset of rancidity (Molins 2001). This can be minimized by irradiating high fat product in the frozen state and/or by vacuum packaging where oxygen is removed before treatment (Matsuyama and others 1964; Mbarki and others 2009). Irradiation of food high in carbohydrates results in depolymerization and oxidation of polysaccharides which leads to softening of the food. Irradiation also adversely affects the vitamins particularly vitamin C and E (Thakur and Singh 1994). Fruits are very prone to spoilage by radiation because of tissue softening. The absorptive irradiation dose and exposure time must be adequate to achieve the required biological effects on food product without any alteration of the quality (Mustapha and others 2014). The FDA has found irradiation of food to be safe under several conditions (Table 4).

Microbial decimal reduction due to irradiation (D_{10} -value) is defined as the irradiation dose (kGy) required for one-log-reduction of the initial microorganism population and is calculated from the negative inverse slope of the logarithm of viable population (CFU/mL) versus dose (kGy). Rajkowski and others (2003) found the D_{10} -value of 0.74 and 1.10 kGy for the nonvegetable and vegetable isolated strains of *Salmonella* spp and 1.43 and 1.11 kGy for the nonvegetable and vegetable isolated

Table 4. Food permitted to be irradiated under FDA's regulations (Adapted from Morehouse 2002).

Food	Purpose	Dose [kGy]
Fresh pork	Control <i>Trichinella spiralis</i>	0.3 min. – 1 max.
Fresh foods	Growth and maturation inhibition	1 max.
Foods	Arthropod disinfection	1 max.
Dry enzyme preparation	Microbial disinfection	10 max.
Dry spices/seasonings	Microbial disinfection	30 max.
Poultry	Pathogen control	3 max.
Frozen meats (NASA)	Sterilization	44 min.
Refrigerated meat	Pathogen control	4.5 max.
Frozen meat	Pathogen control	7 max.
Shell eggs	Pathogen control	3 max.
Seeds for sprouting	Pathogen control	8

strains of *E. coli* O157:H7 on broccoli sprouts, respectively. On the other side, D₁₀-value for *Salmonella* spp. were between 0.29-0.43 kGy on minimally processed watercress (Martins and others 2004) and D₁₀-value for *Salmonella* Typhimurium were 0.164 kGy in carrots and 0.178 kGy in cucumbers (Dhokane and others 2006). This is due to D₁₀-value of microorganisms varies with water content of food. Product with high moisture

content require less amount of irradiation for disinfestation. Food with low water content and water activity contribute to greater resistance of microorganisms against irradiation.

3.11.4 Irradiation of Fresh Cut Produce

Irradiation process on food prevents the growth of microorganisms and inhibits biochemical reaction in the process of maturation which slows down the process of maturation of many fruits and vegetables (Rahman and others 2015). It is important to control toxicological side effects of irradiation process such as degradation of nutrients, organoleptic properties or high level of the radioactivity. According to Thomas (2001), the objective of radiation of fruits and vegetables includes

- I. Extension of shelf life by delaying maturation and ripening;
- II. Control of fungal pathogens causing post-harvest rot;
- III. Inactivation of human pathogens to maintain the microbiological safety and quality;
- IV. As a quarantine treatment for commodities subject to infestation by insect pests of quarantine importance; and
- V. To increase juice recovery from berry fruits

Different dose levels for fresh fruits and vegetables can be divided into three levels based on dosage: (1) Low-dose irradiation (<1.0 kGy) can be used for inhibition of infection and germination, delay of ripening; (2) Intermediate-dose irradiation (1.0 - 3.0 kGy) is recommended for delaying the maturity of fresh produces and for eliminating microbial contamination; and (3) High-dose irradiation (>3.0 kGy) can be

used for extraction of bioactive compounds, reduction in number of microorganisms to the point of sterility (Lung and others 2015).

Studies completed on irradiated fruits and vegetables provide information on the tolerance of these products to ionizing radiation. Yu and others (1995) found that irradiation on strawberries with dose of up to 2 kGy and Moreno and others (2007) found that irradiation on blueberries up to 1.6 kGy is a feasible decontamination treatment that maintains the overall quality of fruit. Radiation beyond 0.34 kGy resulted in quality (firmness) degradation in fresh-cut apples, but treatment with calcium with irradiation prevented softening of apple slices (Gunes and others 2001). Lu and others (2005) found that irradiation dose of 1.0 kGy can reduce population of bacteria, fungi, and *E. coli* on fresh-cut celery with better retention of vitamin C, soluble solids, total sugars, and sensory attributes than those of non-irradiated celery. The fresh-cut packaged cantaloupe irradiated with dose between 1.0-1.5 kGy had better quality attributes with slightly increased in carotene content (Castell-Perez and others 2004), with modified-atmosphere packaging offers extension in shelf-life (Boynton and others 2006). Pinela and others (2016) studied the suitability of irradiation dose of up to 5 kGy for preserving fresh-cut watercress and found that the overall quality was better preserved with the 2 kGy dose, however antioxidant activity and total flavonoids preserved with irradiation dose of 5 kGy. Bari (2005) observed 4.88, 4.57, 5.25, and 4.14 log CFU/g reduction of *Listeria monocytogenes* on broccoli, mung bean sprouts, cabbage, and tomato respectively up on 1.0 kGy ionizing irradiation.

4. MATERIALS AND METHODS

4.1 Reduction of *Salmonella* Poona on Irradiated Cucumber Slices

4.1.1 Media Preparation

Enumeration of salmonellae was carried out on MacConkey agar (Becton, Dickinson and Company, Sparks, MD, USA) supplemented with 80 µg/mL of rifampicin (MCAR; Sigma, St. Louis, MO, USA). Rifampicin was added as the selective agent for the rifampicin-resistant (Rif⁺) strain of *Salmonella* Poona, which was used for inoculation on the sliced cucumber in this study. Rifampicin (80 µg) was dissolved in 5 ml methanol and then added to 1 L of sterile MacConkey agar at 45°-50°C; plates were then poured at 45°-50°C and stored at 5°C in the refrigerator until use.

4.1.2 Bacterial Cultures

A rifampicin-resistant (Rif⁺) strain of *Salmonella Enterica* serotype Poona for this study was provided by Dr. Alex Castillo of the Texas A&M University at College Station (College Station, TX, USA). The *Salmonella* Poona organism was preserved in CryoCare Bacteria Preserves (Key Scientific Products, Inc, Stamford, TX, USA) at -80°C in the laboratory freezer (Sanyo North America Corporation, San Diego, CA, USA). When needed, the frozen culture was revived by transferring one loop to 9 mL of tryptic soy broth (TSB; Becton, Dickinson, and Co., Sparks, MD, USA) and incubating for 24 h at 37 °C. One loop of the broth culture was streaked on the plate of tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD, USA) and incubated at 37 °C for

24 h. A single colony was picked up from the plate, transferred to a TSA slant, incubated at 37 °C for 24 h and was used as working culture.

4.1.3 Inoculum Preparation

The rifampicin-resistant (Rif⁺) *Salmonella* Poona maintained on TSA slant was transferred to a tube containing 9 ml of TSB with a loop and grown by incubating at 37 °C for 24 h. After incubation, the culture was centrifuged (Centrifuge B4i, Jouan, Thermo-Fisher Scientific, Waltham, MA, USA), washed for three times with equal volumes of sterile buffer peptone water (3000 × g for 15 min) in sterile tubes and again washed for three times. An estimated initial concentration of 10⁸ CFU/ml was prepared using 0.5% of absorbance (Milton Roy Spectronic 20D turbidity meter, OD 600 nm, Milton Roy Co, CA, USA) as a reference. The initial concentration was confirmed by making serial dilutions of the inoculum suspension in 9 mL culture tubes of 0.1% the Peptone water and plated on MacConkey Agar supplemented with 80 µg/mL of rifampicin. *Salmonella* Poona grows as colorless colonies on MacConkey plates. The plates were incubated at 37°C until visible colonies can be count.

4.1.4 Sample Preparation and Inoculation

Cucumbers (free from any visual defects such as bruises, cuts or abrasions) were purchased from a local supermarket (College Station, Texas, USA) and stored at 4-5°C temperature and 95% relative humidity in a laboratory refrigerator. Cucumbers with an average diameter of 4-5 cm were selected and brought to ambient temperature prior to

inoculation. In the laboratory, the samples were washed thoroughly with sterile distilled water and sliced to a thickness of 4 ± 0.2 mm using a stainless steel adjustable slicer (Farberware Slicer, Walmart, College Station, TX). All handling materials were continuously sanitized by using 70% ethanol to prevent cross contamination. Each slice (Approximate 5g) was placed in 7.6 cm \times 18.4 cm Whirl-Pak® bags (Nasco, Fort Atkinson, WI) (Figure 5) and 0.5 ml of the 10^8 CFU/mL of bacterial culture was inoculated right onto the center of the slice with the help of a sterile pipette. The inoculated sample bags were then sealed in a LABCONCO purifier Biological Safety Cabinet (Labconco Corporation, Kansas City, MO 64132-2696) to ensure hermetic conditions. The bags were left to dry at room temperature for 2 hours in a biosafety cabinet.



Figure 5. Packaging of cucumber slice in a Whirl-Pak® bags.

4.1.5 Microbiological Analysis

Enumeration of Rif⁺ *S. Poona* was carried out on MacConkey agar plates supplemented with rifampicin (80 µg/L; Sigma Aldrich Co., St. Louis, MO, USA). A 5 g cucumber sample was smashed by using a small meat hammer to break all the tissues of the cucumber and placed with 45 ml of buffered peptone water in 7.6 cm × 18.4 cm Whirl-Pak® bags. The mixture was mixed thoroughly for 5 minutes. Then appropriate serial dilution (using 9 mL of 0.1% peptone water) were made from this homogenate mixture and spread onto MacConkey-RC (limit of detection by plating: 10 log CFU/g). Plates were then incubated at 37°C until visible colonies can be counted with the use of a magnifier counter. All *salmonella* counts (CFU/g) were transformed into log CFU/g. This measurement was conducted in four replications.

4.2 Electron-beam Irradiation Procedure

The inoculated sample bags (5 ± 3 grams) were taken inside a cooler to the 1.35 MeV Van de Graaff accelerator facility located at the Texas Food Safety Engineering laboratory of Texas A&M University. Since the irradiation process took 5-6 hours, all sample bags were kept in a refrigerator at 5°C to prevent microbial outgrowth during processing. For D₁₀-value determination, four independent samples (Whirl-Pak® bags) were irradiated up to a dose of 1.0 kGy with an increment of 0.2 kGy at room temperature (Figure 6, 7). Non-irradiated samples were considered as control samples. After irradiation, samples were stored at 4-5°C for microbial analysis.

Samples were also be irradiated at a 5D dose for decontamination of the pathogen. After this irradiation treatment, samples were stored at 4-C°C for microbial quality analysis up to 3 days. Quality attributes were measured every day.

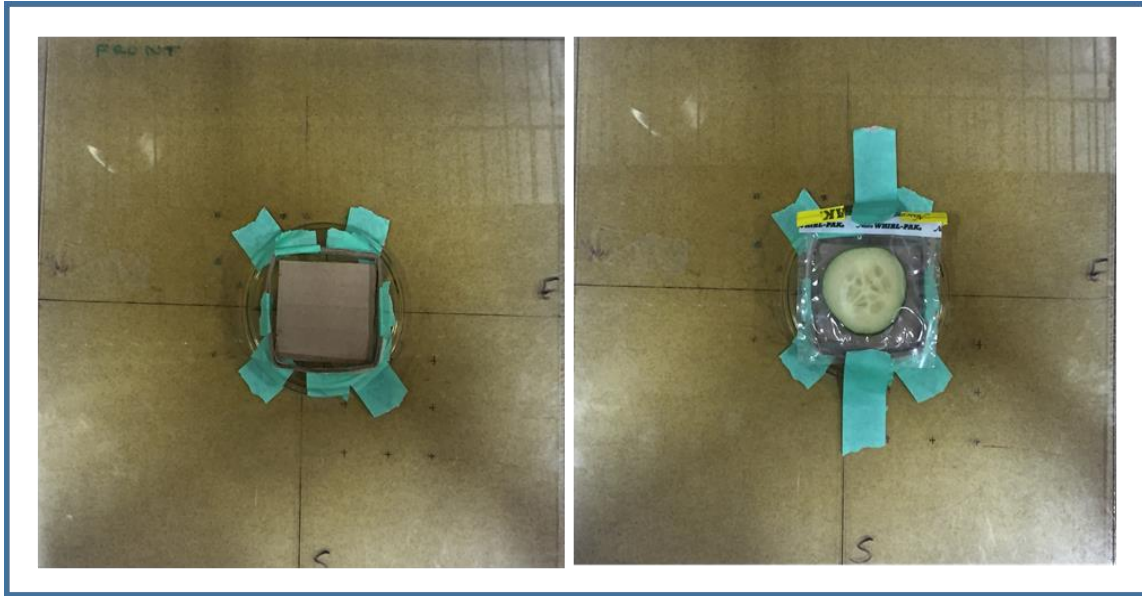


Figure 6. A sample-place board with sample for e-beam irradiation.

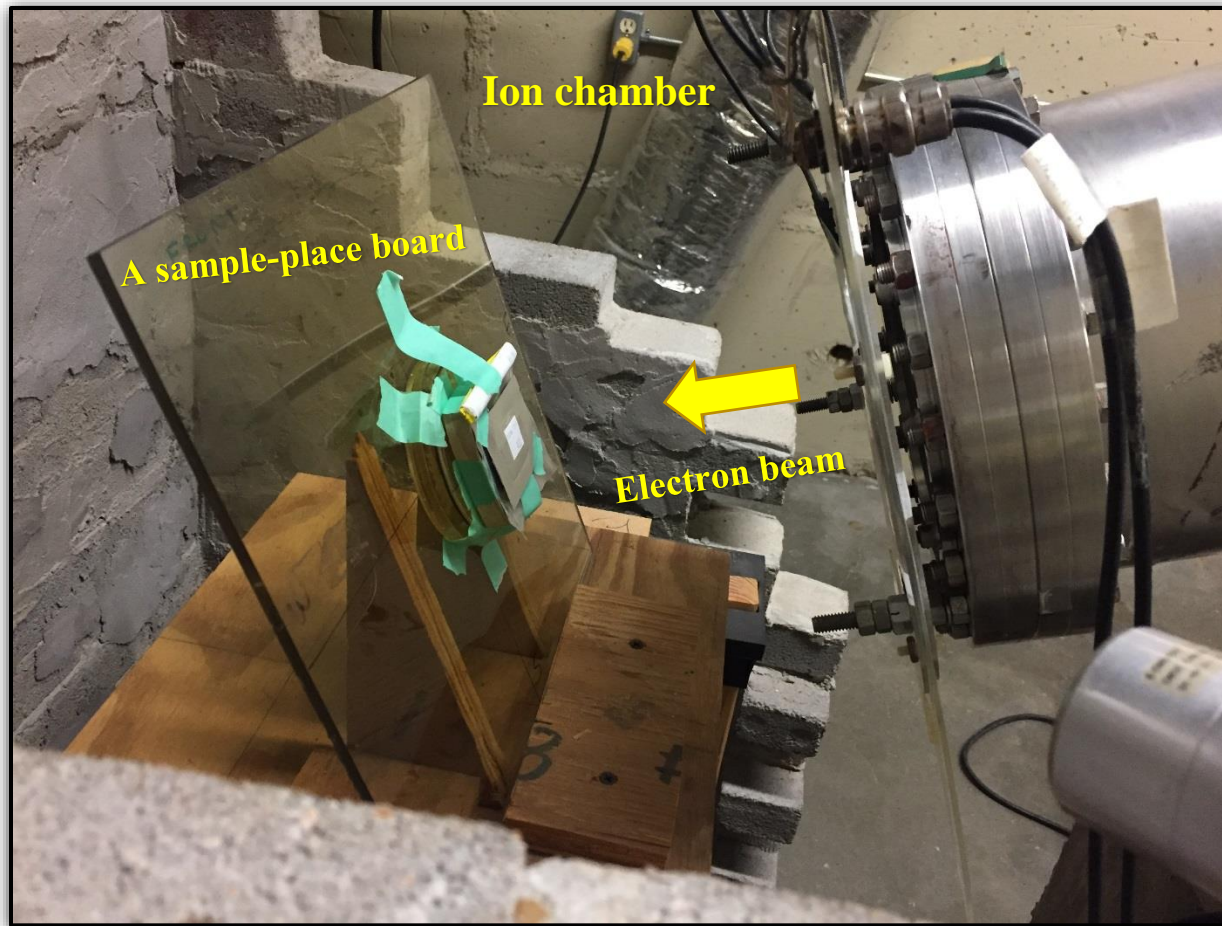


Figure 7. The 1.35 MeV Van de Graaff accelerator, with ion chamber and a sample-place board.

4.2.1 Dose Mapping Study

A dose mapping study was conducted to determine the uniformity of electron beam irradiation using an Ion farm chamber (Markus chamber, Type 23343). Irradiation dosage was measured by placing radiochromic film dosimeters (B3WIN Radiochromic films, Gex Corporation, centennial, CO) at the front and backside of the sample, for a total of 2 dosimeters. The radiochromic films were read after stabilization using a radiochromic reader model 92 (Far West Technology Inc., Goleta, CA, USA).

The Dose Uniformity Ratio (DUR) is defined as the ratio of maximum to minimum absorbed dose,

$$DUR = \frac{D_{max}}{D_{min}} \quad (1)$$

Where, D_{max} = maximum absorbed dose

D_{min} = minimum absorbed dose

It should be close to the value of 1.0 (IAEE 2002). The cucumbers were sliced at a thickness of 4 mm and packed in individual Whirl-Pak® sample bags. The distribution of irradiation dosage was measured for cucumber slice when irradiated at 1.0 kGy by placing two Radiochromic film dosimeters each at the front, center, and backside of the cucumber slice, for a total of six dosimeters (one at middle and one on the corner of the sample) (Figure 8). Radiochromic film was read after stabilization using a Radiochromic reader model 92.

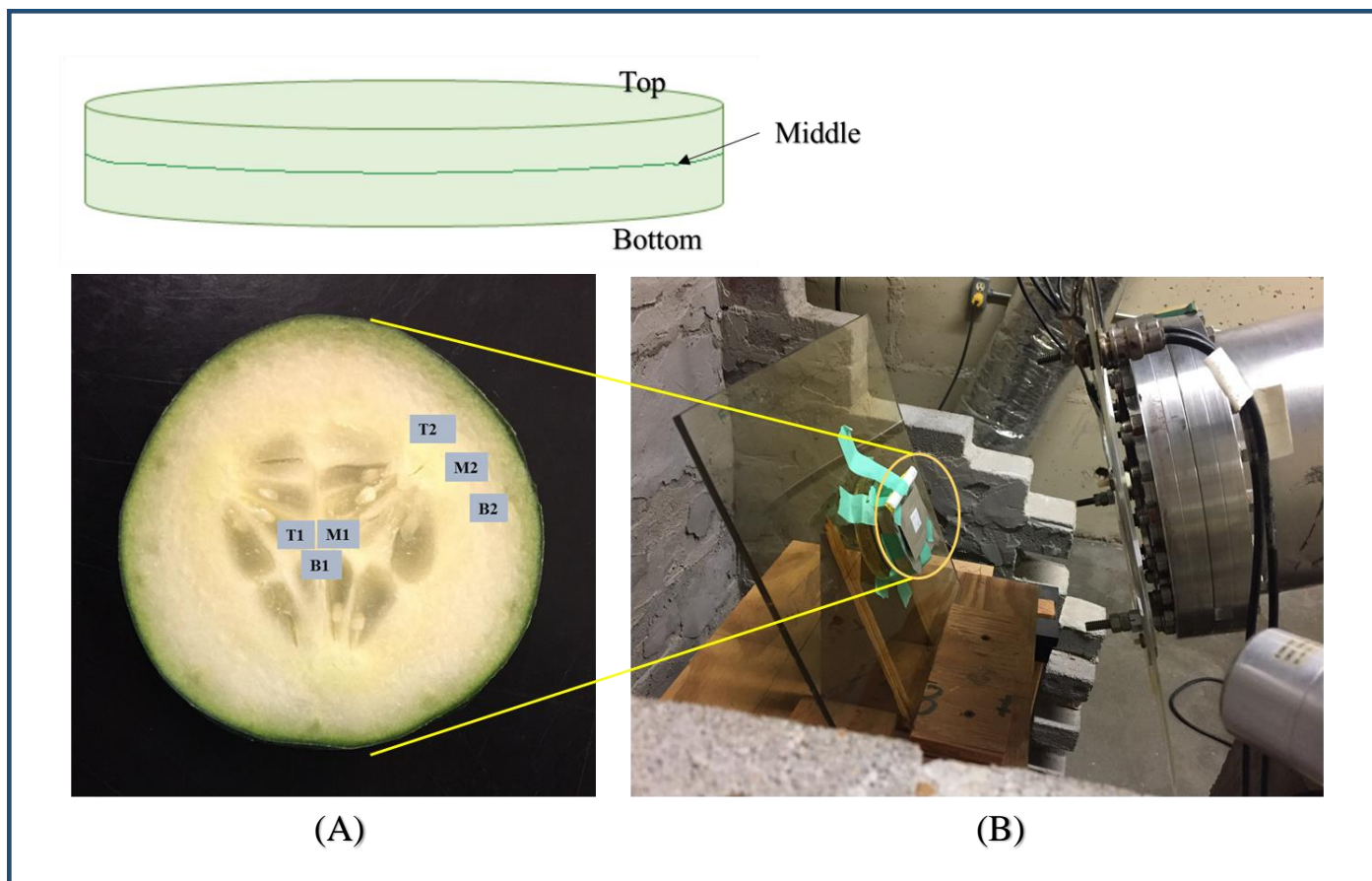


Figure 8. Experimental set-up to irradiate cucumber slice for dose mapping study. A: Location of dosimeters (T=top; M=middle; and B=bottom). B: Product irradiation.

4.2.2 D₁₀-value Determination

The D₁₀-value for *Salmonella Poona* in cucumber was estimated from the negative inverse slope of the logarithm of viable population (CFU/ml) versus dose (kGy) as discussed before and,

$$D_{10}Value = \frac{1}{slope} \quad (2)$$

Tests were performed in four replications at each dose level.

4.3 Quality Attributes of Irradiated Cucumber Slices

4.3.1 Sample Preparation and Packaging

Cucumbers were purchased from a local supermarket (College Station, Texas) and stored at 4-5°C temperature and 95% relative humidity in a laboratory refrigerator. Cucumbers with an average diameter of 4-5 cm was selected and washed thoroughly with sterile distilled water. The washed cucumbers were sliced to a thickness of 4 ± 0.3 mm using a stainless steel adjustable slicer. Each slice was sealed packed in 7.6 cm × 18.4 cm Whirl-Pak® bags (Figure 5).

4.3.2 Measurement of Texture (Firmness)

The texture of cucumber slices was measured using a TA-CT3 Texture analyzer (Brookfield AMETEK, MA, USA), which was equipped with a stainless still probe with the diameter of 4-mm (TA 39) for penetration and a 10 kg load cell. In cucumber, the firmness of the tissues varies from center to the periphery due to a structural difference. The peripheral part of the sample was considered to measure firmness as it is the section

that gives crunchiness to cucumber (Figure 9). The texture (firmness) was determined as the maximum force to penetrate the probe into the sample to a 3 mm depth from the surface of the sample. The non-irradiated and irradiated samples were tested every day of storage up to 3 days. The instrument was calibrated before each use. This measurement was conducted in triplicate.



Figure 9. Brookfield CT3 with sample under compression test.

4.3.3 Measurement of Color

The color of the cucumber samples (control and irradiated samples) was determined at room temperature by using a LAB Scan XE colorimeter (Hunter Lab, Inc, VA, USA) with D₆₅ illuminant and 10 degree standard observer. Readings were recorded as L^* (lightness), a^* (Red-green), b^* (Yellow-blue) for each sample. The L^* , a^* , b^* values were calculated from following equations,

$$L^* = 100 \sqrt{\frac{Y}{Y_n}} \quad (3)$$

$$a^* = K_a \left(\frac{X/X_n - Y/Y_n}{\sqrt{Y/Y_n}} \right) \quad (4)$$

$$b^* = K_b \left(\frac{X/X_n - Z/Z_n}{\sqrt{Y/Y_n}} \right) \quad (5)$$

Where,

X , Y , and Z are the CIE tristimulus values;

X_n , Y_n , and Z_n are the tristimulus values for the illuminant,

$X_n = 94.83$, $Y_n = 100.00$, and $Z_n = 107.38$;

K_a and K_b are chromaticity coefficients for the illuminant,

$K_a = 172.10$ and $K_b = 66.70$.

Hue and Chroma values were calculated using following equations,

$$\text{Hue} = \tan^{-1} \frac{b^*}{a^*} \quad (6)$$

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad (7)$$

LAB Scan XE colorimeter was calibrated with a standard plate ($Y=94.00$, $x=0.3578$, $y=0.4567$) each time before use. This measurement was conducted in triplicate.

4.3.4 Measurement of Water Activity

Samples from each group (irradiated and control sliced cucumbers) were selected and the Rotronic Hygroskop DT (Rotronic Instruments Corp, NY, USA) used to measure relative humidity and temperature. The equipment was calibrated with humidity standards before use. The Water Activity (a_w) was determined using this equation,

$$a_w = \frac{\text{Relative humidity (\%)}}{100} \quad (8)$$

This measurement was conducted in triplicate at room temperature.

4.3.5 Measurement of Moisture Content

Moisture content was determined by the direct method using vacuum oven (Lab-Line Instruments Inc., Melrose Park, IL, USA) which measures weight loss of sample after drying in a vacuum oven at 70°C for 9 hours. Each sample's weight, approximately 4g, were recorded before and after drying. The samples were first chopped into small pieces and placed in aluminum canisters prior starting the drying process. The weight of canisters was also being recorded for more accurate measurements. After removing the samples from the vacuum oven the samples were placed in a desiccator to cool down before recording the final weight. The wet-basis moisture content was determined using this equation,

$$m = \frac{W_m}{W_m + W_d} = \frac{W_m}{W_{Total}} \quad (9)$$

Where,

m = wet-basis moisture content expressed as a decimal fraction

W_m = mass of water;

W_d = mass of dry matter;

W_{total} = mass of the original (wet) sample

This measurement was conducted in triplicate.

4.3.6 Measurement of pH

Samples from each group (irradiated and control sliced cucumbers) were selected randomly and the pH of sliced cucumbers was measured using a portable digital pH meter (Cole Parmer, pH 500 series, Model # 59003-20, Singapore). The pH meter was properly calibrated with standard solutions, pH 4, 7, and 10 and sanitized before the each experiment. The experiments were carried out at room temperature. This measurement was conducted in triplicate.

4.3.7 Sensory Evaluation

Sensory evaluation of irradiated and control samples was carried out under specific conditions on days 0, 1, 2, and 3 of refrigerated storage. Two samples were presented to panelists inside plastic containers with labeled 2 random digits. Panelists (50) were asked to evaluate the samples by visual inspection of color, texture, smell and overall quality. A consumer test was carried out among (50 panelists) students, staff, and faculty at Texas A&M University. Panelists scored the samples using a 9-point hedonic scale where a score of 9 represents “like extremely” and a score of 1 represents “dislike extremely”. Scores higher or equal to 5 were considered as acceptable (Amerin and others 2013).

4.3.8 Analysis of Data

All experimental data recorded for each parameter tested in replicated three times. Data recorded for each parameter were analyzed by one-way analysis of variance (ANOVA) with <0.05 significance level using Statistical Analysis Software JMP. (SAS Institute, Cary, NC, USA). Mean separation was carried out using Tukey's test when ANOVA indicated a significant ($P < 0.05$) difference.

All microbial count data were converted into \log_{10} CFU/g before analysis. For the pathogen reduction study, Average microbial count (\log CFU/g) was plotted against irradiation dose to reveal the pathogen reduction achieved due to irradiation and the effect of storage at 4°C over 3 days.

5. RESULTS AND DISCUSSION

5.1 Dose Mapping Study

For an electron beam irradiation treatment, a dose mapping study of sliced cucumber was conducted using the Ion farm Chamber to determine the Dose Uniformity Ratio (DUR). Figure 10 show a distribution of the dose over the cucumber slice. Dosimeters located at the middle of the slice (Penetration depth = 2mm) absorbed the highest amount of dose by 28% and 56% compared to dosimeters located at front and back respectively. The explanation for the highest absorbed dose on dosimeter at the center is the effect of scattered electrons that cause the additional absorption of dose in addition to the primary incident electrons from the e-beam. Table 5 illustrates absorbed doses at different depths of cucumber slice. The Dose Uniformity Ratio for the experiment was 1.56.

The DUR value for the research should be as close to 1.0 as possible to get uniform dose distribution in small research samples. The experimental results of DUR can demonstrate the dose-effect relationship, while for irradiation of large industrial production, wider dose variation is unavoidable (IAEA 2002). It is useful for irradiator designers and food technologist to design large production industrial applications. Various techniques are used in industries to minimize the DUR to optimize product quality and to maximize throughput of an irradiation facility.

Table 5. Dose distribution on sliced cucumber irradiated at 1.0 kGy.

Dose Distribution (kGy)		
Penetration depth (mm)	Absorbed dose at center of the slice (kGy)	Absorbed dose at corner of the slice (kGy)
0	^y 1.28 ^a ^l (0.13)	^y 1.28 ^a (0.08)
2	^z 1.61 ^a (0.22)	^z 1.66 ^a (0.21)
4	^x 1.14 ^b (0.14)	^x 0.96 ^a (0.18)

Values are means of four replications

^l Standard deviation Values are means of three replications

^{x, y, z} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

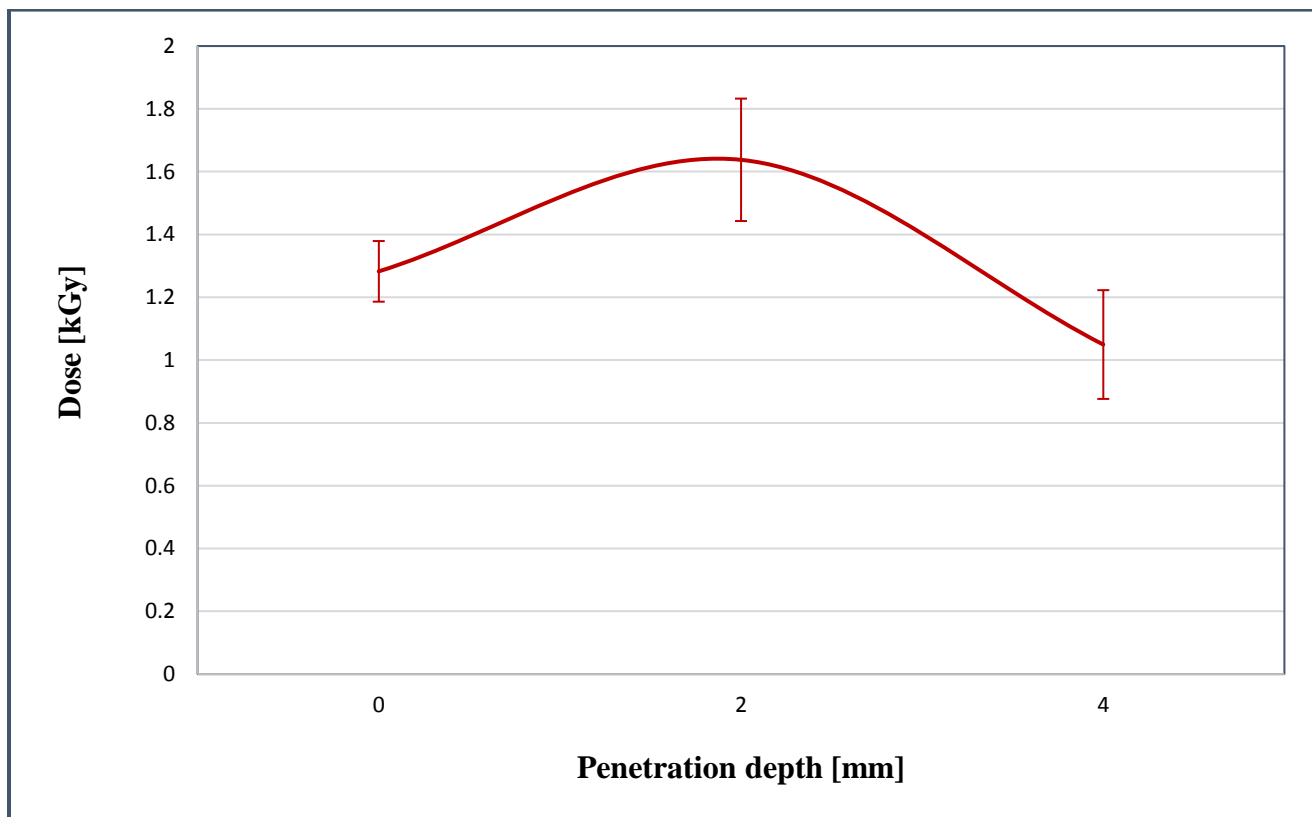


Figure 10. Dose penetration depth for 4mm thick cucumber slice irradiated with a 1.35 MeV e-beam accelerator. Values are means of four replications.

5.2 D₁₀-value for *Salmonella* Poona on Cucumber Slices

Figure 11 shows the death curve for *Salmonella* Poona on a cucumber slices when irradiated up to a dose of 1.0 kGy with an increment of 0.2 kGy. Each of the data points is an average count of *Salmonella* Poona from four cucumber samples (Table 6). There was a reduction of 2.6 log CFU/g due to irradiation at a dose of 1.0 kGy compared to non-irradiated samples. The slope of the line, obtained from the linear equation $y = mx + c$ where m stands for the slope of the line, was -2.6233 (Figure 11). The negative value of the slope is due to the downward trend of the line. The D₁₀-value of 0.38 ± 0.03 kGy for *Salmonella* Poona in cucumber was established in this study.

Rajkowski and Thayer (2000) indicated the D₁₀-value of 0.54 and 0.46 kGy for the *Salmonella* spp. cocktails made with meat and vegetable isolates respectively on sprouts. Thayer and others (1995) found the D₁₀-value of 0.7 for *Salmonella* spp. on all the meat at 5°C. In a study conducted by Thayer and others (1990), D₁₀-value were different for different species ranging from 0.77 for *Salmonella* Enteritidis to 0.38 *Salmonella* Typhimurium in mechanically deboned chicken. In another study on orange juice conducted by Niemira and others (2001), D₁₀-value were different for different spices ranging from 0.71 kGy for *Salmonella* Anatum to 0.35 kGy for *Salmonella* Infantis. These studies indicate that all *Salmonella* species have different D₁₀-value and it also differs with type and composition of food. All possible physical and environmental parameters besides the characteristics of the target microorganism should be considered to determine D₁₀-value of any food product.

Table 6. Effect of e-beam irradiation at various level of absorbed doses (0 to 1.0 kGy) on *Salmonella* Poona population (log CFU/g) stored at 4°C.

Dose [kGy]	Population of <i>Salmonella</i> Poona (log CFU/g)
0	^z 6.75 ¹ (0.26)
0.23	^y 5.77 (0.19)
0.41	^{x, y} 5.41 (0.46)
0.66	^{w, x} 4.79 (0.57)
0.84	^w 4.26 (0.24)
1.0	^w 4.10 (0.26)

Values are means of four replications

¹ Standard deviation Values are means of three replications

^{w, x, y, z} Means within a column, which are not followed by a common superscript letter are significantly different (P < 0.05)

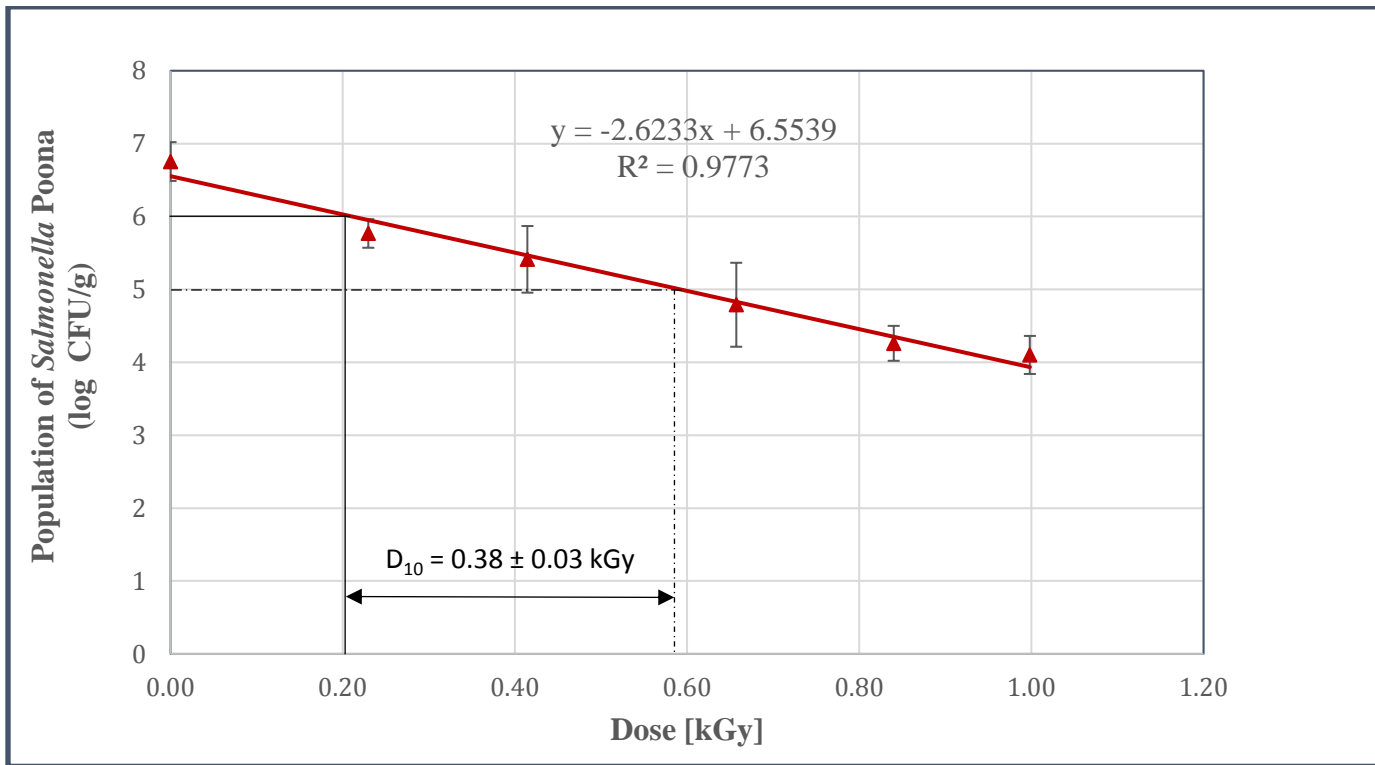


Figure 11. Death curve of *Salmonella* Poona for sliced cucumber at room temperature ($R^2 = 0.98$). Values are means of four replications.

5.3 Effect of E-beam Irradiation at 1.9 kGy (5D log *Salmonella* Poona reduction) on the Microbial Population of *Salmonella* Poona on Cucumber Slices

Salmonella Poona counts in the non-irradiated and irradiated cucumber samples are presented in Table 7. Electron beam irradiation at the dose of 1.9 kGy reduced *Salmonella* Poona by 4.96 log (CFU/g) (~ 5.0 Log CFU/g) (Figure 12), compared with the non-irradiated control samples on the day 0. The difference in *Salmonella* counts on the day zero indicates the immediate effect of irradiation treatment on the microorganism. At the end of day 3, the non-irradiated samples had 8.61 log CFU/g while samples irradiated at 1.9 kGy had 3.10 log CFU/g (difference 5.5 log CFU/g) *Salmonella* counts due to subsequent refrigerated storage. Approximately 0.6 Log CFU/g (17%) reduction in *Salmonella* counts with time was observed during the storage of the irradiated sliced cucumbers at 4°C. There was no significant ($P > 0.05$) reduction in *Salmonella* counts for the group of non-irradiated samples. The effect of electron beam irradiation on quality and safety of sliced cantaloupe was studied by (Palekar and others 2015). They found that irradiation reduces *Salmonella* concentration by 1.1 log CFU/g at 0.7 kGy and 3.6 log CFU/g at 1.5 kGy with the gradual reduction in *Salmonella* counts with time during storage of 21 days.

The results confirm that electron-beam irradiation treatment of sliced cucumbers is effective in reducing a concentration of *Salmonella* and subsequent storage of treated cucumber samples at the temperature of 4°C prevents the reproduction of *Salmonella* cells. This finding emphasizes the importance of proper storage and handling followed by processing to minimize the risk of pathogen regrowth and recontamination. The

concentration of pathogens in fresh produce is usually low (~ 3 Log CFU/g) (Prakash and others 2000). Taken this into consideration, a 5 log reduction can completely eliminate the contamination of pathogens from the fresh produce.

(Huang and others 2015) studied the effect of chronic temperature abuse on the growth of *Salmonella* on fresh-cut cantaloupe. Chronic temperature abuse is a sustained storage of time and temperature controlled food products at a temperature exceeding that prescribed for safe storage. They found an increase in growth of *Salmonella* at a temperature of 8°C and 12°C through storage of 7 days. However, at 4°C, the population of *Salmonella* did not increase. Our data shows a slow reduction in *Salmonella* population during storage of 3 days at 4°C. On that account, it shows signs of being more factors other than the temperature of storage that contributed to the reduction in the population of *Salmonella* on irradiated cucumber samples. One of the factors might be the reproduction of yeasts and/or molds that would compete with the pathogen for survivor (Palekar and others 2015). Yeasts and molds have a competitive advantage over pathogens that they can grow at refrigeration temperature and low pH. They compete with the weaker survivors for space and nutrients. When surface tissue of fruits and vegetables are punctured or cut by insects or mechanical abuse, yeasts, and molds naturally present on the surface (Beuchat 2002). There has not been much work done to study the interaction of pathogens with yeasts and molds on irradiated fresh produces.

Table 7. Effect of e-beam irradiation at 1.9 kGy on the *Salmonella* Poona population (log CFU/g) on cucumber slices stored at 4°C for 3 days.

Population of <i>Salmonella</i> Poona (log CFU/g)		
Time (days)	Control	Irradiated (1.9 kGy)
0	$\bar{x}8.73^b$ ¹ (0.10)	$\bar{y}3.77^a$ (0.21)
1	$\bar{x}8.68^b$ (1.13)	$\bar{x}, \bar{y}3.56^a$ (0.55)
2	$\bar{x}8.66^b$ (0.69)	$\bar{x}, \bar{y}3.20^a$ (0.17)
3	$\bar{x}8.61^b$ (1.67)	$\bar{x}3.10^a$ (0.17)

Values are means of four replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

\bar{x}, \bar{y} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

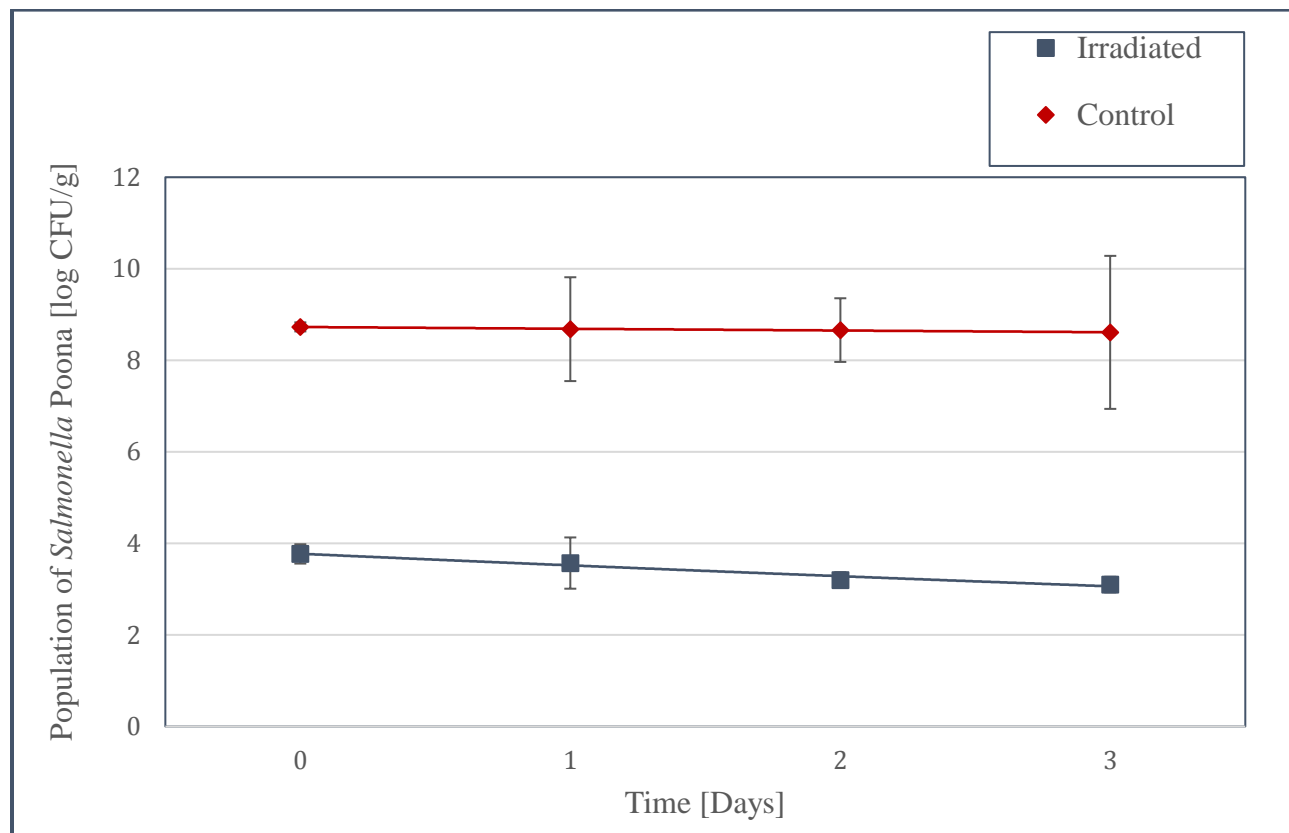


Figure 12. Effect of e-beam irradiation at 1.9 kGy on population of *Salmonella* Poona stored at 4°C for 3days.

Control means non-irradiated samples. Values are means of four replications.

5.4 Effect of E-beam Irradiation at 1.9 kGy on the Quality Attributes of Cucumber Slices

5.4.1 Texture

Figure 2 (Appendix B) represents the values of maximum force required to shear the sliced cucumber samples during storage time. During the analysis, the control group started to lose surface moisture and the dry surface was observed. Samples from control groups were observed brittle up on breaking them using hands. However, no significant difference ($P > 0.05$) in firmness was found up to second day of storage. Samples on the third day showed significantly ($P < 0.05$) higher firmness.

The firmness of irradiated samples was softer ($P < 0.05$) compared to control samples. The firmness of sliced cucumbers was reduced by 50% because of irradiation treatment. The main problem with irradiation treatment of fruits and vegetables is the change (reduction in firmness) in texture. Radiation of fruits and vegetables induces depolymerization of cellulose, hemicelluloses, starch, and pectin, which results in softening of tissue. During storage, the group of irradiated samples did not lose surface moisture like control samples. Visually, irradiated samples seemed fresher on the second and third day of storage than the control samples. No significant ($P > 0.05$) differences were found among the irradiated samples during storage time.

There was a significant ($P < 0.05$) difference between maximum force values for irradiated and control sliced cucumber samples (Table 8). Firmness of irradiated samples were markedly (~50%) lower than the control samples.

Table 8. Effect of e-beam irradiation at 1.9 kGy on the maximum force (N) required to shear the cucumber slices stored at 4°C for 3 days.

Texture, [N]		
Time (days)	Control	Irradiated (1.9 kGy)
0	^x 13.86 ^b ¹ (1.62)	^x 6.02 ^a (1.11)
1	^x 14.34 ^b (0.56)	^x 6.31 ^a (0.47)
2	^x 13.67 ^b (1.06)	^x 6.90 ^a (0.96)
3	^y 15.02 ^b (0.96)	^x 6.46 ^a (1.42)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

5.4.2 Color

The effect of irradiation treatment on the color of the sliced cucumber samples is presented in Figures 3 to 5 (Appendix B). The lightness (L^*) values were used to estimate changes in the white color of sliced cucumbers during storage. L^* is perceived lightness approximately ranging from 0.0 for black to 100.0 for white color. Values for lightness varied significantly ($P < 0.05$) with a trend towards higher values through storage time for control samples. No significant ($P > 0.05$) differences were found in all irradiated samples through storage (Table 9). The Lightness of the irradiated samples was higher than control samples for zero and first day but after that on second and third day, irradiated samples were darker than the non-irradiated samples (Figure 3, Appendix B). However, there were no significant differences ($P > 0.05$) between all irradiated and non-irradiated samples (Table 9).

The a^* values estimate redness-greenness of the sliced cucumbers during storage. Here, All the values for a^* were in negative values as cucumbers exhibits shades of green color (Figure 4, Appendix B). Values for control samples varied significantly ($p < 0.05$) with a trend towards higher values through storage time. For irradiated samples, a^* values on day 1, day 2, and day 3 were significantly ($P < 0.05$) lower than the a^* value on the zero day. There were significant ($P < 0.05$) difference between samples on comparing irradiated samples with control samples (Table 9).

The b^* values estimate yellowness-blueness of the sliced cucumbers during storage. All the positive values for b^* were displaying shades of yellowness of cucumber slice. The b^* values of irradiated and control sliced cucumbers decreased significantly (P

< 0.05) with storage time. No significant ($P > 0.05$) difference was observed among the irradiated and control samples (Figure 5, Appendix B) (Table 6).

Hue describes an angle to the color wheel which distinguish the color position around the color wheel. Table 10 indicates that there was no significant ($P > 0.05$) difference in hue value in all of the samples. All samples (control and irradiated) were on same angle throughout the storage.

Chroma is the quality of a color's purity, intensity or saturation. It is a magnitude of color. There was no significant ($P > 0.05$) difference among group of control and irradiated samples. However, Intensity of color varied significantly ($P < 0.05$) with a trend towards lower values through storage time (Table 10).

Table 9. Effect of e-beam irradiation at 1.9 kGy on color L^* , color a^* , and color b^* values for cucumber slices stored at 4°C for 3 days.

	Color L^*		Color a^*		Color b^*	
Time (days)	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)
0	$^x67.27^a$ $^1(0.69)$	$^x68.95^b$ (3.42)	$^x-3.67^b$ $^1(0.16)$	$^x-3.39^a$ (0.15)	$^z13.81^a$ $^1(0.61)$	$^y13.78^a$ (0.98)
1	$^x68.92^a$ (0.54)	$^x69.17^a$ (1.73)	$^x-3.55^b$ (0.13)	$^y-3.19^a$ (0.21)	$^z, ^y13.16^a$ (0.46)	$^x12.87^a$ (0.68)
2	$^y69.47^b$ (0.97)	$^x68.17^a$ (0.79)	$^y-3.04^a$ (0.09)	$^y-3.15^b$ (0.06)	$^y12.59^a$ (0.37)	$^x12.68^a$ (0.38)
3	$^y70.40^a$ (1.06)	$^x69.91^a$ (2.99)	$^z2.86^a$ (0.07)	$^y-3.13^b$ (0.14)	$^x12.47^a$ (0.30)	$^x12.55^a$ (0.56)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y, z} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

Table 10. Effect of e-beam irradiation at 1.9 kGy on Hue and Chroma values for the cucumber slices stored at 4°C for 3 days.

Time (days)	Hue		Chroma	
	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)
0	^x -0.99 ^a ¹ (0.00)	^x -0.99 ^a (0.00)	^z 14.28 ^a ¹ (0.62)	^y 14.19 ^a (0.99)
1	^x -0.99 ^a (0.00)	^x -0.99 ^a (0.00)	^z 13.62 ^b (0.48)	^y 13.26 ^a (0.71)
2	^x -0.99 ^a (0.00)	^x -0.99 ^a (0.00)	^y 12.95 ^a (0.38)	^y 13.06 ^a (0.38)
3	^x -0.99 ^a (0.00)	^x -0.99 ^a (0.00)	^x 12.80 ^a (0.30)	^x 12.94 ^a (0.53)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y, z} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

5.4.3 pH

pH is a rough measure of the microbiological activity in foods. According to FDA, pH of cucumber is between 5.1 and 5.7. Thus, it is capable of supporting the growth of pathogens due to low acidity. The pH values for the control and irradiated cucumber samples are presented in Figure 6 (Appendix B). All the samples showed a slight trend towards an increase of pH through storage. On the third day of storage, pH of both irradiated and control samples were significantly ($P < 0.05$) higher compared to day 0, 1 and 2 of storage. However, when comparing the irradiated samples with the control samples, the pH of the irradiated samples was greater than the control samples. The pH of irradiated samples on the second and third day of storage was significantly ($P < 0.05$) higher than the control samples on the same days of storage (Table 11).

5.4.4 Water Activity (a_w)

The effect of the irradiation treatment on the water activity (a_w) in the sliced cucumber samples during three days of storage is presented in Figure 7 (Appendix B). All samples have water activity values between 96.4 and 97.0 %. There was a slight difference between the irradiated and control samples. Values of Irradiated samples were slightly higher than control samples through storage. However, no significant ($P > 0.05$) difference was found among all the samples (Table 12).

Table 11. Effect of e-beam irradiation at 1.9 kGy on pH values for cucumber slices stored at 4°C for 3 days.

pH		
Time (days)	Control	Irradiated (1.9 kGy)
0	^x 5.5 ^a ¹ (0.10)	^x 5.6 ^a (0.06)
1	^x 5.5 ^a (0.08)	^x 5.6 ^a (0.05)
2	^x 5.6 ^a (0.16)	^x 5.7 ^{a, b} (0.09)
3	^y 5.9 ^{a, b} (0.02)	^x 6.0 ^b (0.10)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

Table 12. Effect of e-beam irradiation at 1.9 kGy on water activity values for cucumber slices stored at 4°C for 3 days.

Water Activity (a_w)		
Time (days)	Control	Irradiated (1.9 kGy)
0	^y 0.968 ^a ¹ (0.67)	^x 0.969 ^a (0.42)
1	^x 0.965 ^a (0.26)	^x 0.967 ^a (0.06)
2	^x 0.966 ^a (0.06)	^x 0.968 ^a (0.30)
3	^x 0.967 ^a (0.11)	^x 0.968 ^b (0.15)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

5.4.5 Moisture Content

The effect of the irradiation treatment on the moisture content of sliced cucumber during storage is presented in figure 8 (Appendix B). The moisture content of cucumber is important to consider as it affects physical, chemical aspects of fresh cucumber which relates with the freshness and stability of cucumber during the storage period. Moisture content relates with the texture, taste, and appearance of the slice of fresh cucumber and also microbial activity has a direct relation with moisture availability in the cucumber. There was not specific trend observed in moisture content during the storage however, the surface of sliced cucumber became dry during storage due to evaporation of surface moisture. All samples have moisture content values between 96.6 and 97.0. No significant ($P > 0.05$) difference was found among all the samples (Table 13).

5.4.6 Sensory Attributes

Results of sensory evaluation are presented in Figures 9 to 12 (Appendix B). A score of 1 represented dislike extremely, 5 represented neither like nor dislike, and 9 represented like extremely. A value of 5 or above was considered acceptable in this study (Appendix A).

Table 13. Effect of e-beam irradiation at 1.9 kGy on moisture content (wet basis) values for cucumber slices stored at 4°C for 3 days.

Moisture Content (wet basis), [%]		
Time (days)	Control	Irradiated (1.9 kGy)
0	^x 96.8 ^a ¹ (0.06)	^x 96.7 ^a (0.11)
1	^x 96.7 ^a (0.08)	^x 96.8 ^a (0.08)
2	^x 96.9 ^a (0.15)	^x 96.8 ^a (0.02)
3	^x 96.8 ^a (0.04)	^x 96.9 ^a (0.27)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation Values are means of three replications

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

^{x, y} Means within a column, which are not followed by a common superscript letter are significantly different ($P < 0.05$)

Color acceptability scores of the control and irradiated samples were not significantly ($p > 0.05$) different during storage (Figure 9, Appendix B). On the zero and first day of evaluation, there was a significant ($P < 0.05$) difference among the color acceptability scores of the both groups. Controls were more acceptable ($P < 0.05$) to the consumer than irradiated samples. Acceptability was similar ($P > 0.05$) for both group of samples for day 2, and 3 of storage. The group of control samples showed a decreasing trend by storage time but they were acceptable by day 3 of storage. The group of irradiated samples was also acceptable by the panelists. These results are supported by the objective color measurements.

Odor score values for consumer acceptance are significantly ($P < 0.05$) different for control and irradiated samples (Table 14). Acceptance score for irradiated samples was higher than control samples after the first day of analysis. Cucumber slices lost their original smell with storage time, but irradiated samples did not exhibit unfavorable odor changes. This is because irradiation controls the microbial growth which prevents the development of off odor in cucumber samples. Both groups of samples was acceptable by the panelists.

Texture values for control and irradiated samples were significantly ($p < 0.05$) different on the 0 and 3 days of storage (Table 14). On the zero day, texture acceptability value for control samples was higher than irradiated samples. Panelist found that irradiated samples were losing water on the surface and softer in texture. However, on the day 3, irradiated samples were more acceptable than control. According to comments, a surface of the control sample was dry because of loss of surface moisture

and irradiated samples looked fresher compared to the controls. Objective texture analysis did not observe any difference ($P > 0.05$) throughout the storage period of 3 days for both groups but the texture (firmness) of irradiated samples were significantly ($P < 0.05$) lower than control samples (Figure 11, Appendix B). Relationship between objective texture analysis and sensory texture analysis was studied using Pearson correlation using equation,

$$r_{xy} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 \sum_{i=1}^n (y_i - \bar{y})^2}} \quad (10)$$

The Pearson's correlation coefficient for non-irradiated samples was -0.20 and for irradiated samples was -0.03, which means there was no correlation between objective and sensory texture analysis.

The overall quality scores are shown in Figure 12 (Appendix B). There were no significant ($P > 0.05$) differences observed in overall acceptability of irradiated and control samples during storage (Table 14). On day zero, overall acceptability of control samples was higher than irradiated samples but there was no difference in acceptability values during storage. The overall acceptability of cucumber samples was highly affected by the appearance and odor of the samples. Both sample groups were equally accepted by panelists.

Table 14. Effect of e-beam irradiation at 1.9 kGy on sensory attribute values for cucumber slices stored at 4°C for 3 days.

Time (days)	Color		Odor		Texture		Overall acceptability	
	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)	Control	Irradiated (1.9 kGy)
0	\bar{x} 7.90 ^b (1.29)	\bar{x} 7.10 ^a (1.71)	\bar{y} 7.85 ^b (1.35)	\bar{x} 7.00 ^a (1.68)	\bar{y} 7.60 ^b (1.53)	\bar{x}, \bar{y} 7.00 ^a (2.00)	\bar{x} 7.80 ^b (1.24)	\bar{x} 7.15 ^a (1.63)
1	\bar{x} 7.38 ^b (1.33)	\bar{x} 7.12 ^a (1.45)	\bar{x} 6.66 ^b (1.38)	\bar{x} 7.41 ^a (1.43)	\bar{x} 6.51 ^a (1.67)	\bar{x} 6.56 ^a (1.68)	\bar{x} 7.51 ^a (1.25)	\bar{x} 7.31 ^a (1.51)
2	\bar{x} 7.33 ^a (1.17)	\bar{x} 7.37 ^a (1.30)	\bar{x} 6.37 ^a (1.76)	\bar{x} 7.57 ^b (1.23)	\bar{x} 6.50 ^a (1.81)	\bar{x}, \bar{y} 6.81 ^b (1.79)	\bar{x} 7.33 ^a (1.33)	\bar{x} 7.48 ^a (1.22)
3	\bar{x} 7.37 ^a (1.19)	\bar{x} 7.40 ^a (1.27)	\bar{x}, \bar{y} 6.82 ^b (1.72)	\bar{x} 7.62 ^a (1.27)	\bar{x}, \bar{y} 6.74 ^a (1.99)	\bar{y} 7.62 ^a (1.27)	\bar{x} 7.05 ^a (1.65)	\bar{x} 7.17 ^a (1.28)

Values are means of three replications

Control means non-irradiated cucumber slices

¹ Standard deviation

^{a, b} Means within a row, which are not followed by a common superscript letter are significantly different (P < 0.05)

\bar{x}, \bar{y} Means within a column, which are not followed by a common superscript letter are significantly different (P < 0.05)

6. RECOMMENDATIONS FOR FUTURE STUDY

Recommendation for future research on electron beam irradiation on fresh produce include:

- Effect of e-beam irradiation on growth and survival of other pathogens.
- Application of other treatments in combination of irradiation to reduce required dose of irradiation for decontamination.

7. CONCLUSION

The effect of electron beam irradiation treatment on the microbiological safety and quality attributes (texture, color, pH, water activity, moisture content, and sensory attributes) of fresh sliced cucumber was evaluated. In addition, D₁₀-value of *Salmonella* Poona was determined using various dose of e-beam irradiation. Electron beam irradiation was found to be an efficient tool for decontamination of *Salmonella* Poona as well as extension of shelf-life of sliced cucumbers due to following reasons:

- The D₁₀-value for *Salmonella* Poona on electron beam irradiated sliced cucumber was found to be 0.38 ± 0.03 kGy.
- This study showed that a 5 log CFU/g reduction of *Salmonella* Poona was achieved when irradiating the slices with 1.9 kGy. Irradiation followed by refrigerated storage was efficient at pathogen reduction and maintaining quality of sliced cucumber.
- The e-beam irradiation of fresh sliced cucumber did not affect its quality parameters including color, pH, water activity, and moisture content. Only a slight increase in pH values were observed during storage in both groups of irradiated and non-irradiated cucumber slices.
- The firmness of sliced cucumbers was adversely affected by e-beam irradiation. The firmness of irradiated sliced cucumbers was decreased by 50% during objective measurement compared to non-irradiated group. No particular trend was observed in firmness values of all samples during storage of 3 days.

- Although firmness of sliced cucumber decreased significantly ($p < 0.05$) with the application of irradiation treatment, the irradiated samples were found acceptable during the consumer sensory test.

In summary, the results from this study indicate that electron beam irradiation can be an effective intervention strategy in the processing of sliced cucumber and it provides basis for application of e-beam irradiation on whole cucumber.

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APPENDIX A

Sensory Evaluation Form

Product Name: **Slice of Cucumber**

Date:

Instruction: You are presented with two food samples. Please evaluate both samples for each quality parameter and use the number scale below to mark which number that you liked or disliked about each sample.

Note: You can touch samples to check texture (hardness). Please do not eat the sample.

- 9 - Like extremely
- 8 - Like very much
- 7 - Like moderately
- 6 - Like slightly
- 5 - Neither like nor dislike
- 4 - Dislike slightly
- 3 - Dislike moderately
- 2 - Dislike very much
- 1 - Dislike extremely

	Sample 1	Sample 2
General appearance		
Color		
Smell		
Texture		

Comments:

APPENDIX B

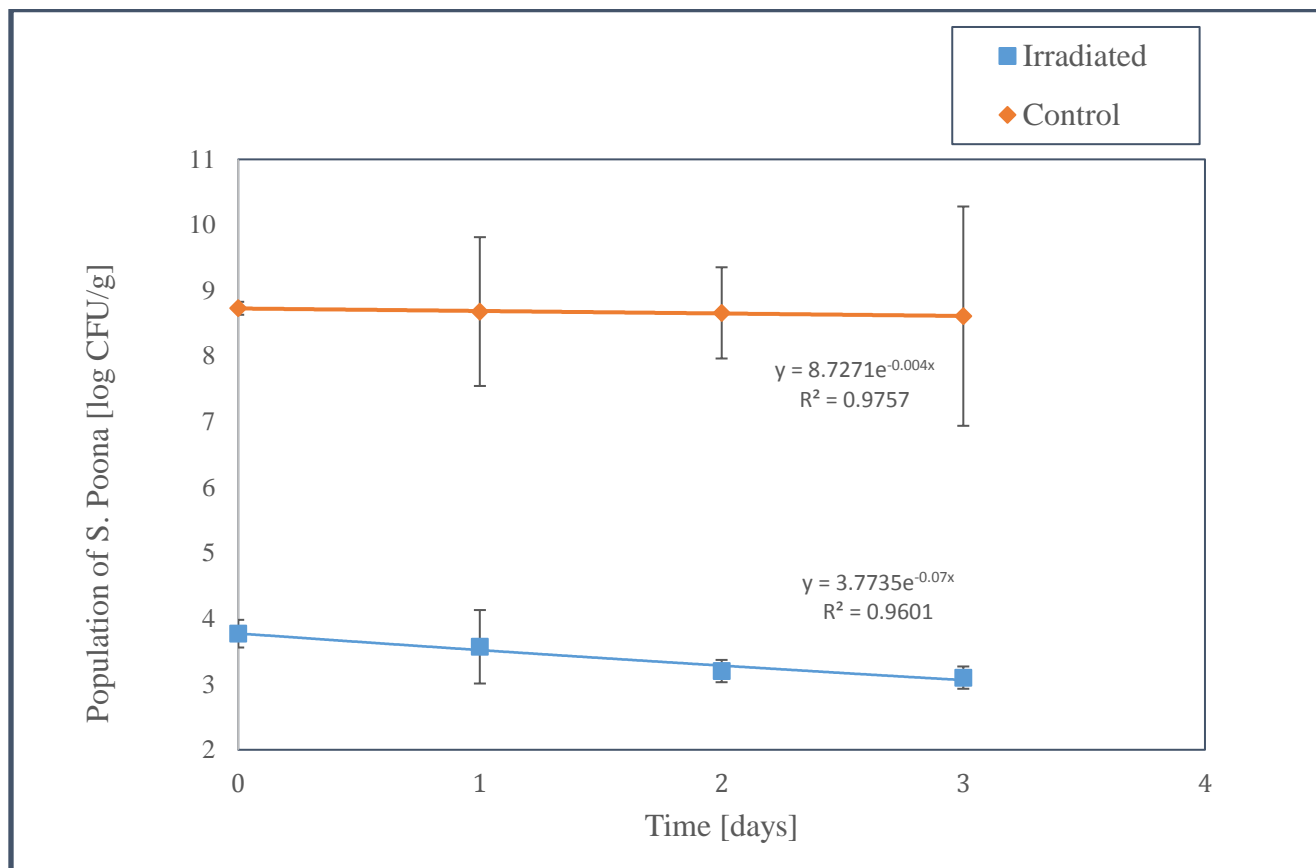


Figure 1. Inactivation kinetics of *Salmonella Poona* in cucumber slices irradiated at 1.9 kGy stored at 4°C for 3 days. Control means non-irradiated samples. Data points are means of four replications.

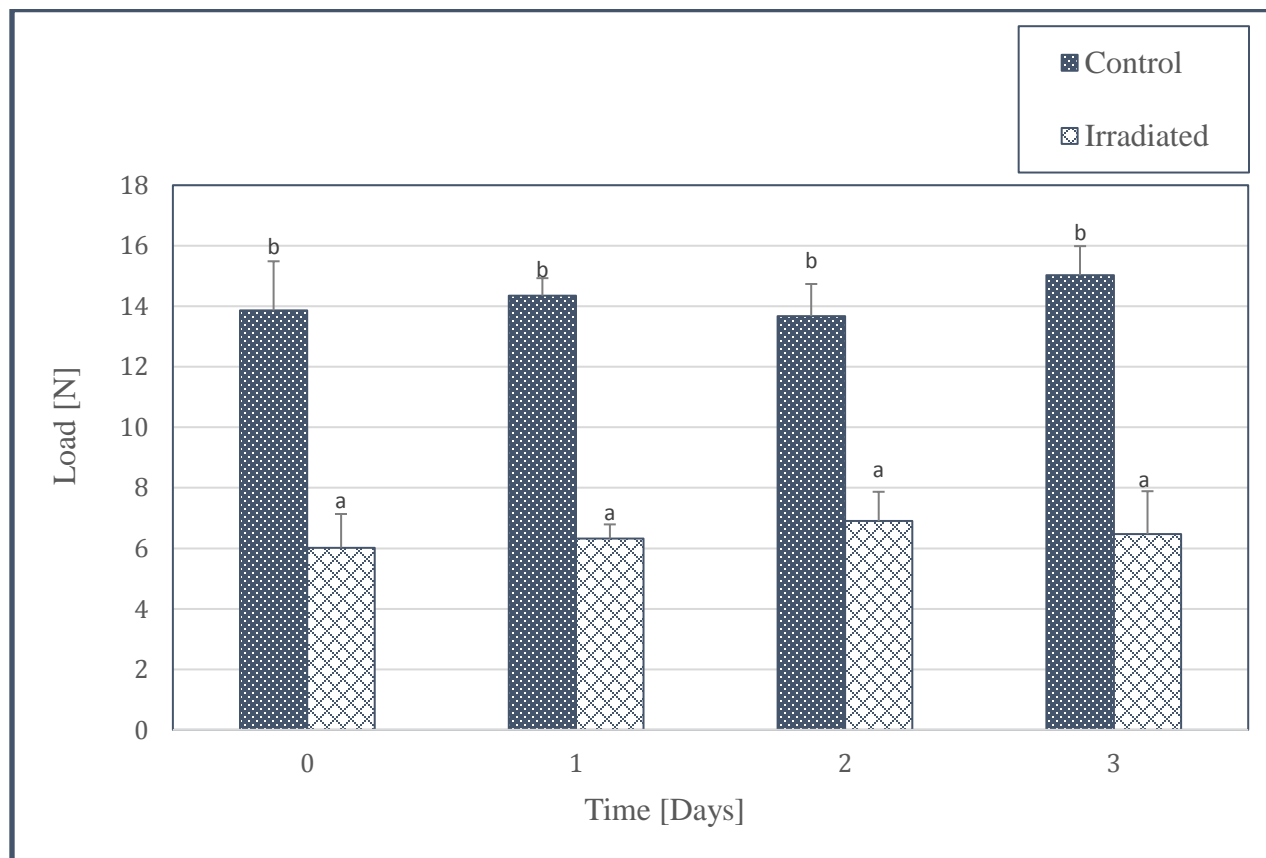


Figure 2. Effect of e-beam irradiation at 1.9 kGy on maximum force (N) required to shear cucumber slices stored at 4°C for 3 days. Control means non-irradiated samples. Values are means of three replications.

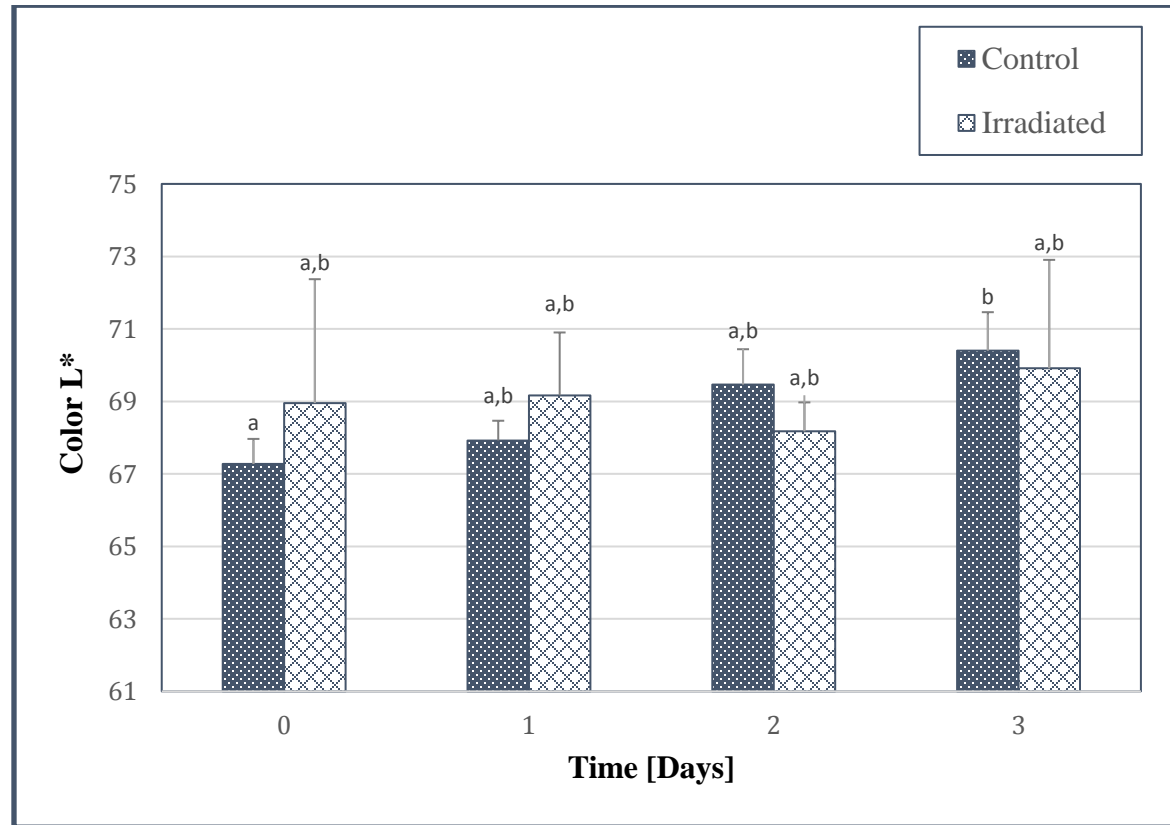


Figure 3. Effect of e-beam irradiation at 1.9 kGy on color L^* values (Equation 3) for cucumber samples stored at 4°C for 3 days.

Control means non-irradiated samples. Values are means of three replications.

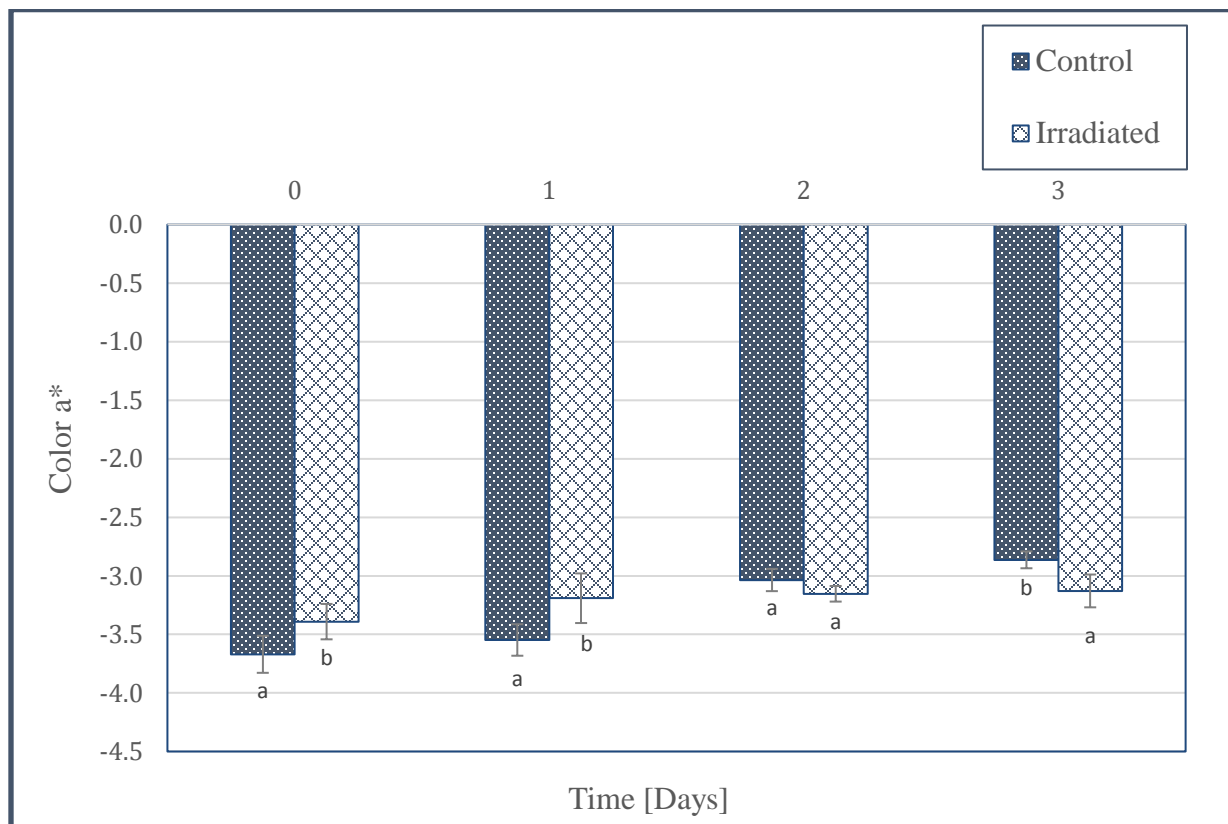


Figure 4. Effect of e-beam irradiation at 1.9 kGy on color a^* values (Equation 4) for cucumber slices stored at 4°C for 3 days.

Control means non-irradiated samples. Values are means of three replications.

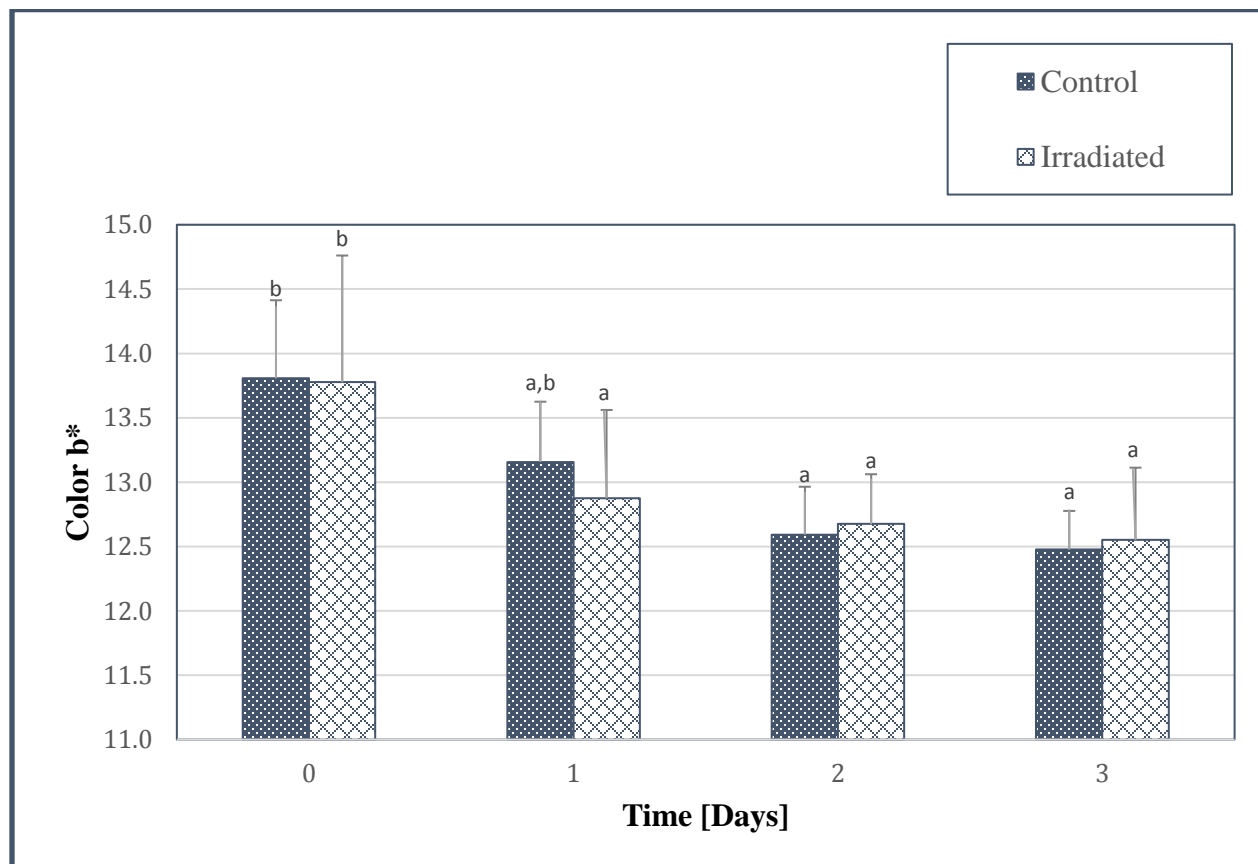


Figure 5. Effect of e-beam irradiation at 1.9 kGy on color b^* values (Equation 5) for cucumber slices stored at 4°C for 3 days.

Control means non-irradiated samples. Values are means of three replications.

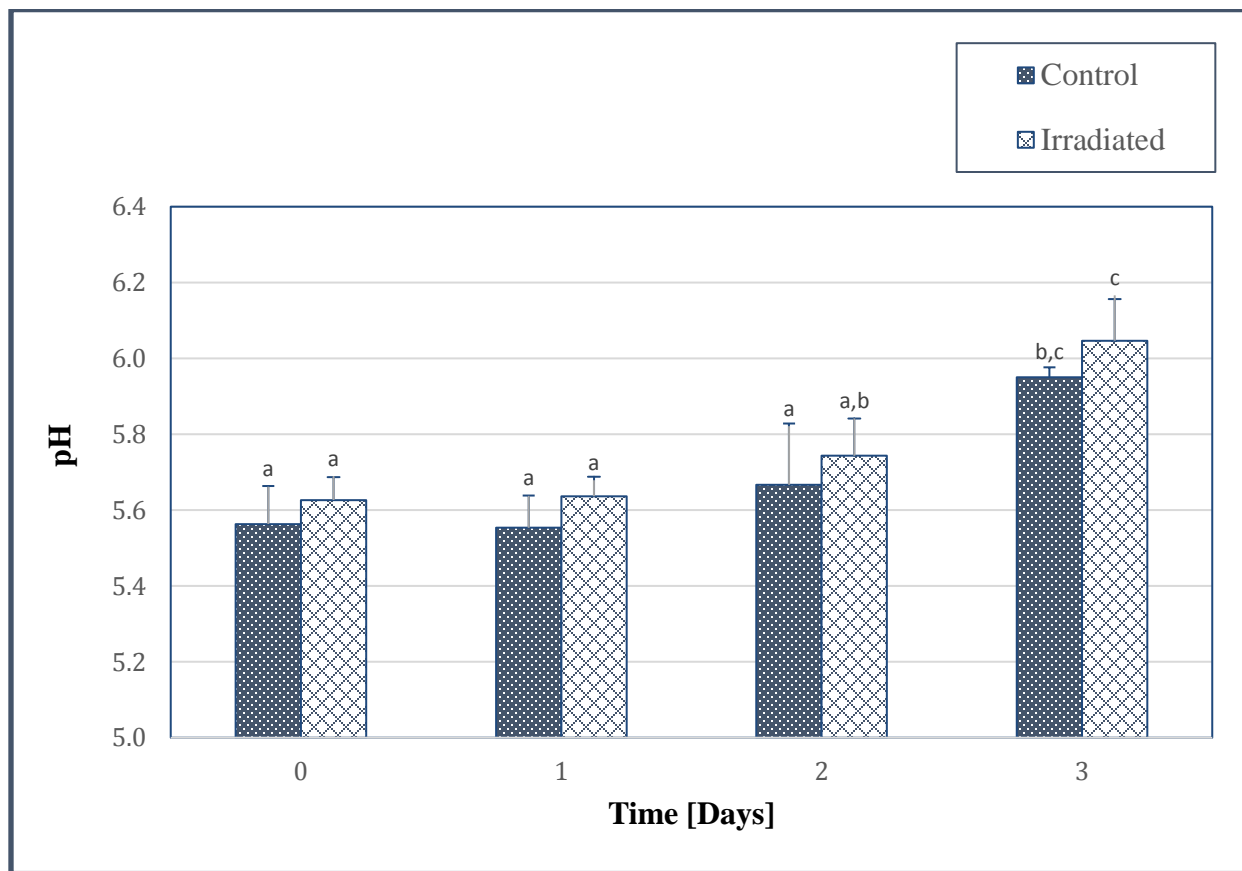


Figure 6. Effect of e-beam irradiation at 1.9 kGy on pH values for cucumber slices stored at 4°C for 3 days.

Control means non-irradiated samples. Values are means of three replications.

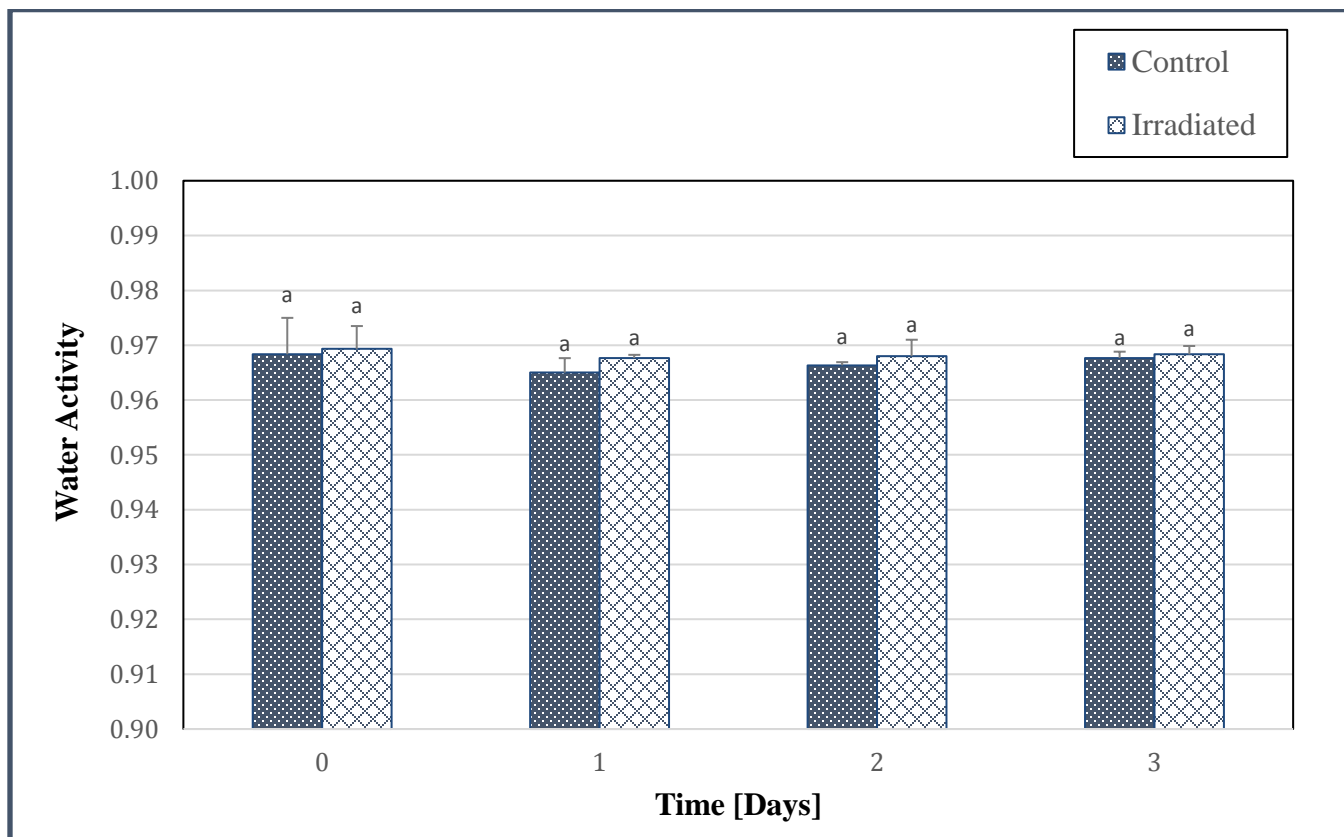


Figure 7. Effect of e-beam irradiation at 1.9 kGy on water activity values for cucumber slices stored at 4°C for 3 days.

Control means non-irradiated samples. Values are means of three replications.

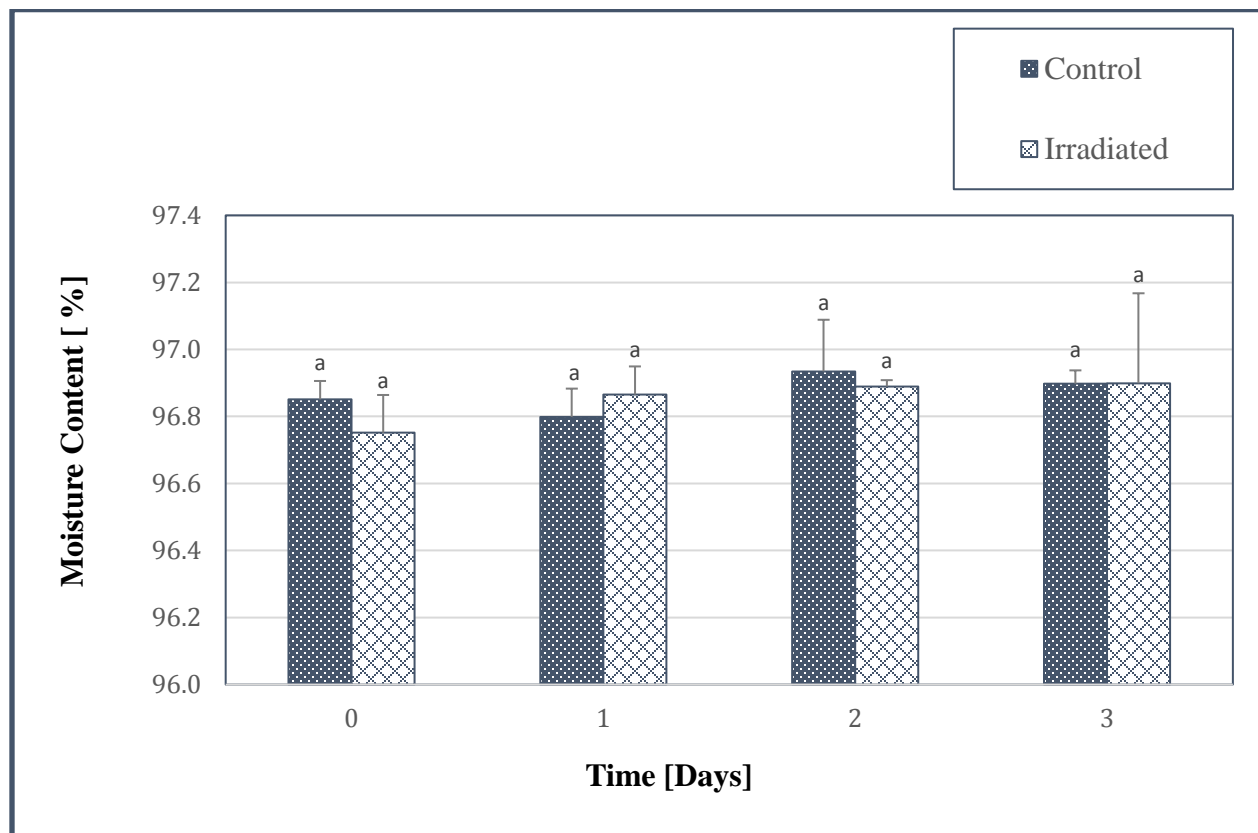


Figure 8. Effect of e-beam irradiation at 1.9 kGy on moisture content (wet basis) values for cucumber slices stored at 4°C for 3 days. Control means non-irradiated samples. Values are means of three replications.

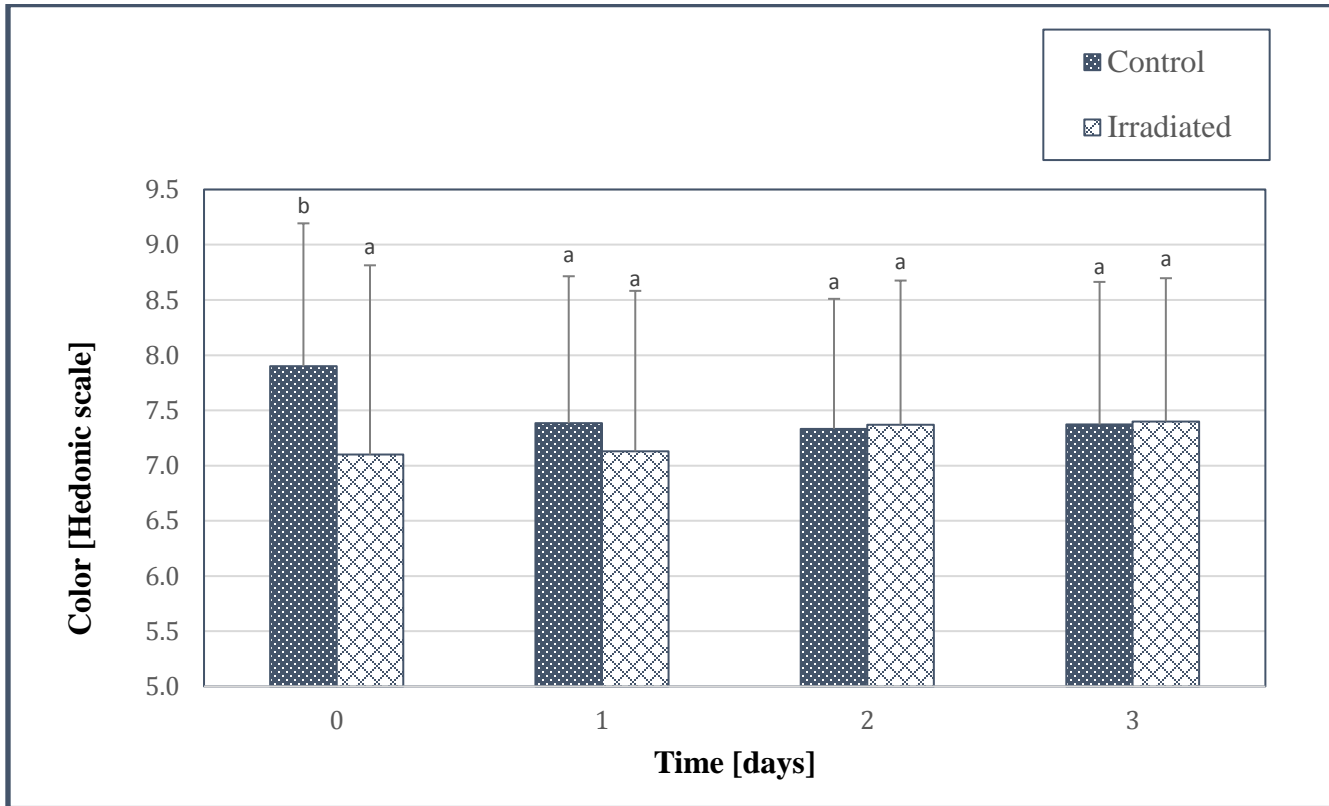


Figure 9. Effect of e-beam irradiation at 1.9 kGy on sensory color scores for cucumber slices stored at 4°C for 3 days. A score of 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Control means non-irradiated samples. Values are means of fifty replications.

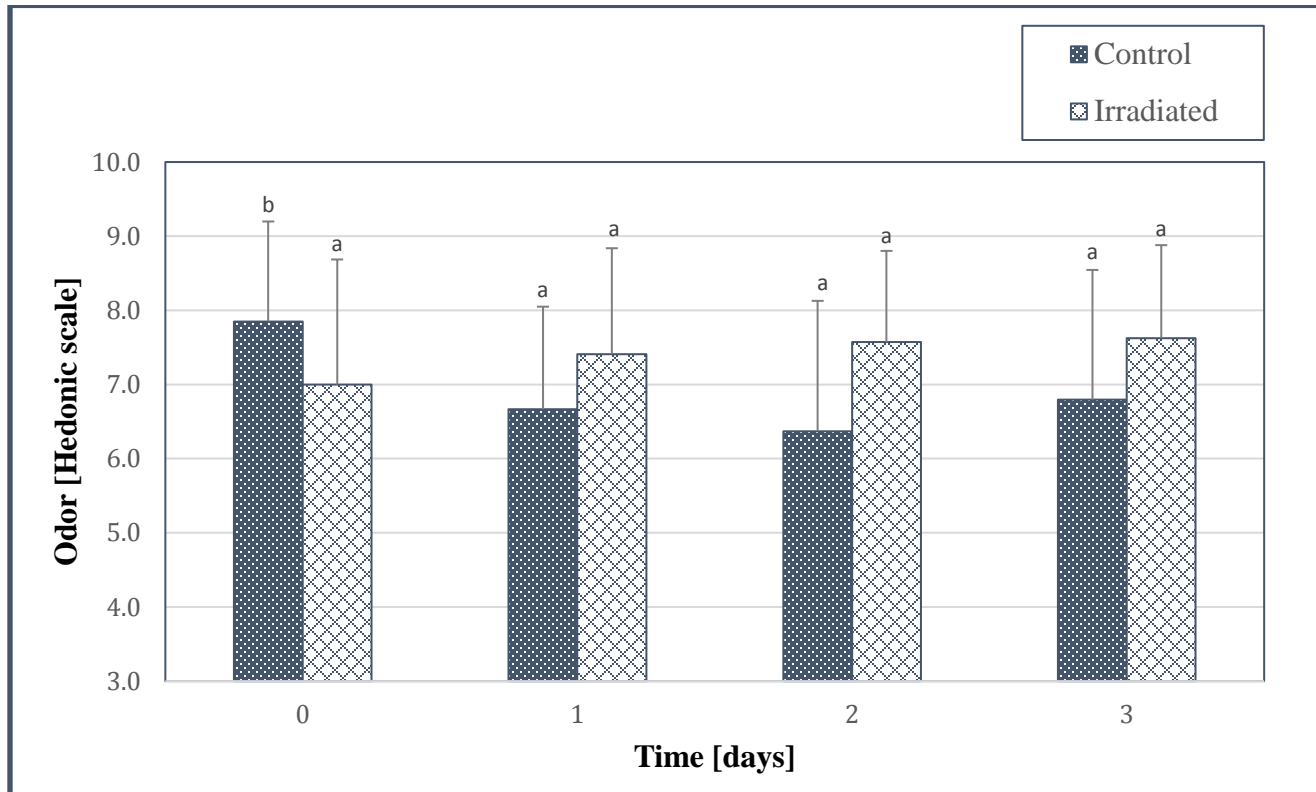


Figure 10. Effect of e-beam irradiation at 1.9 kGy on sensory odor scores for cucumber slices stored at 4°C for 3 days. A score of 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Control means non-irradiated samples. Values are means of fifty replications.

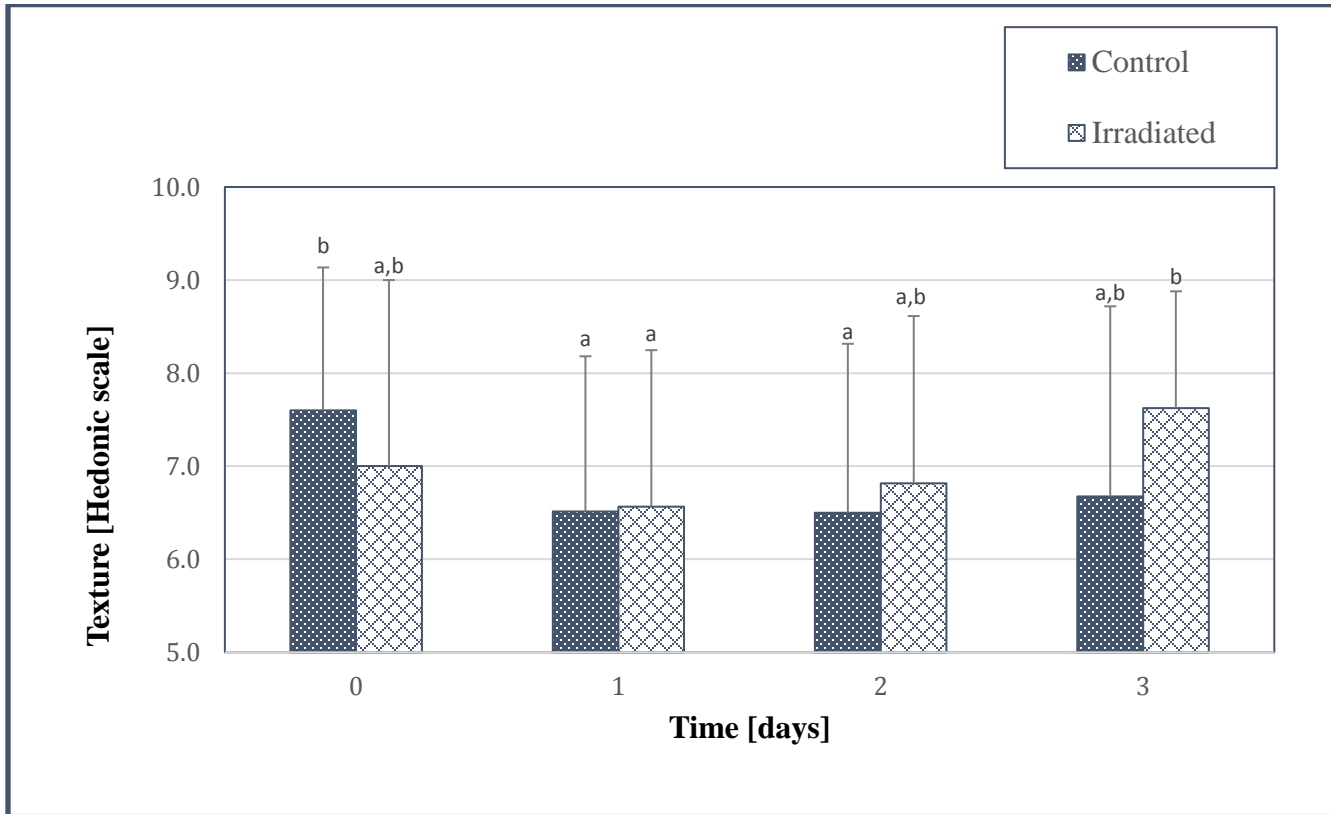


Figure 11. Effect of e-beam irradiation on sensory texture scores for cucumber slices stored at 4°C for 3 days. A score of 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Control means non-irradiated samples. Values are means of fifty replications.

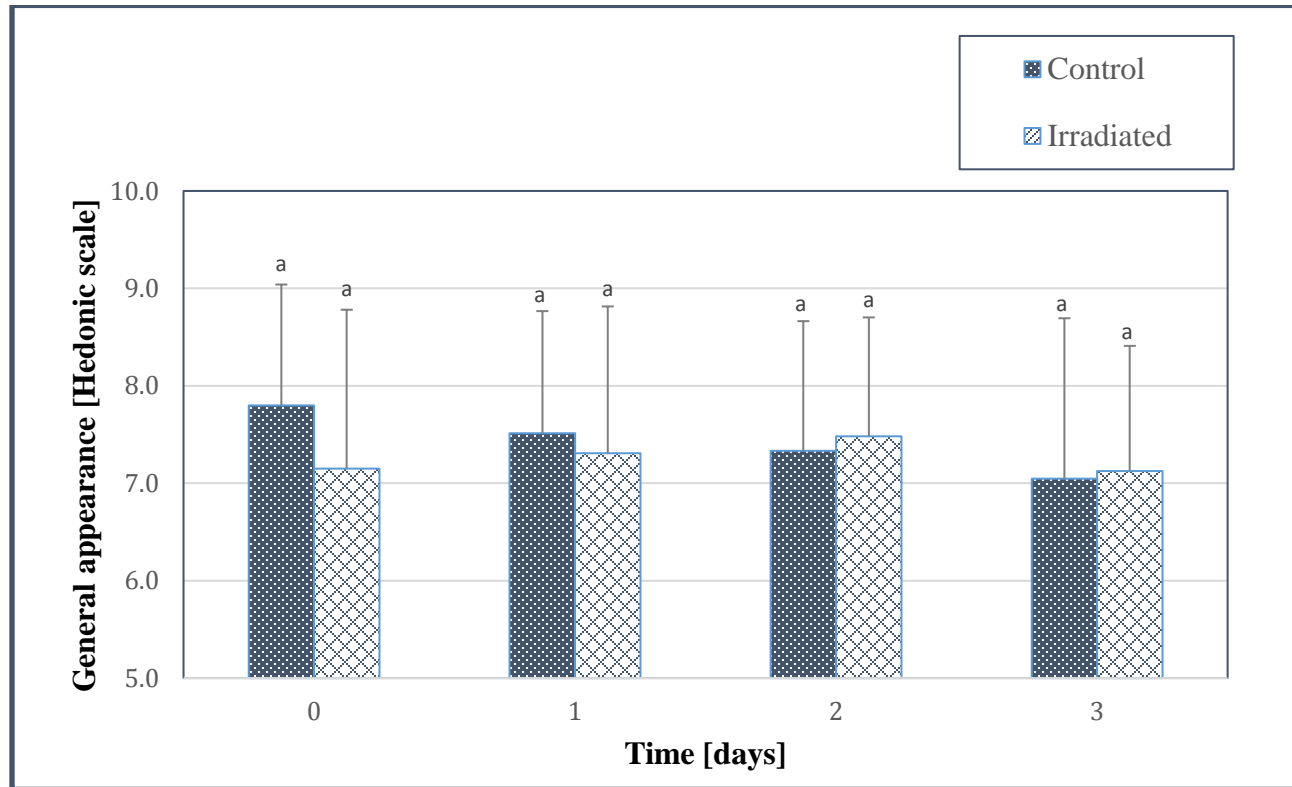


Figure 12. Effect of e-beam irradiation on sensory general appearance scores for cucumber slices stored at 4°C for 3 days. A score of 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Control means non-irradiated samples. Values are means of fifty replications.