ELECTRON BEAM IRRADIATION FOR IMPROVING SAFETY OF FRUITS AND VEGETABLES

A Thesis

by

MEGHA SARTHAK ADAVI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Food Science and Technology

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ABSTRACT

Electron Beam Irradiation for Improving Safety of Fruits and Vegetables. (May 2011)

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Increase in consumption of fresh cut produce over the past decade has resulted in a rise in incidents of food borne outbreaks due to pathogens. Conventional techniques of sanitizing washes may not be effective since the organic matter released from the fresh produce use up the free chlorine thus reducing the sanitizing potential of wash water just when it is needed most and a heat treatment step to kill pathogens cannot be applied if the purpose is to consume fresh produce. Electron beam (e-beam) irradiation was used to treat cut cantaloupe, cut roma tomatoes, baby spinach, romaine lettuce which were surface inoculated with a cocktail of *Salmonella* and *E. coli* O157:H7. Results showed that irradiation reduced *Salmonella* and *E. coli* O157:H7 significantly with increasing doses at 0.2, 0.4, 0.6, 0.8, and 1.0 kGy. The D_{10}-value for *Salmonella* on irradiated cut cantaloupe, cut roma tomatoes, baby spinach, and romaine lettuce was found to be 0.71 kGy, 0.64 kGy, 0.19 kGy, and 0.23 kGy respectively. The D_{10}-value for *E. coli* O157:H7 on the produce listed above was found to be 0.73 kGy, 0.54 kGy, 0.18 kGy, and 0.20 kGy respectively.

Low dose e-beam irradiation was found to be an excellent tool for ensuring the reduction of spoilage organisms and extending shelf life in cut cantaloupe, cut roma tomatoes, baby spinach, romaine lettuce, strawberries, and green onion. The produce were tested for 12 days of storage for aerobic plate count, yeast and mold, lactic bacteria, color, texture, and respiration rate as a function of irradiation doses 0, 1, 3, and 5 kGy. Aerobic plate counts, yeast counts, and lactic acid bacteria were reduced appreciably at all doses tested on all commodities. Molds did not grow on any samples including control for cut cantaloupe, cut tomatoes, and green onion but for the other commodities, mold was reduced at the same rate as yeasts and vegetative bacteria. Lactic acid bacteria were reduced at all doses while the reduction was highest with 5 kGy in all commodities. When irradiated with 5 kGy, during storage, strawberries, spinach, and green onion displayed wet, soggy and mushy appearance, romaine lettuce leaves were wilted, had a translucent midrib and brown pigmentation. E-beam irradiation increased respiration rate for all samples on day 0 compared to non-irradiated control irrespective of the commodity type and the effect was dose dependent. Firmness reduced appreciably for cut roma tomatoes, baby spinach, strawberries, romaine lettuce, and green onion with increasing doses. Cut cantaloupe was low in firmness but the effect was not dose dependent.

Irradiation at low doses is a promising tool to reduce pathogens and enhance keeping quality of cut cantaloupe, cut tomatoes, baby spinach, romaine lettuce, strawberries, and green onion. Irradiation is to be implemented as part of an overall HACCP plan and is not meant to replace existing control measures.

DEDICATION

To my family for their sacrifice, support and prayers that helped me realize this dream.

Thank you for the patience, understanding, encouragement, and love you have bestowed on me throughout these past years.

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INTRODUCTION

The food supply in the United States remains one of the safest in the world according to Food and Drug Administration (FDA) and yet there have been 72 food-borne illness outbreaks associated with fresh produce from 1996 to 2006, and a quarter of those outbreaks were tied to fresh-cut produce *(43)*. Fresh produce is usually consumed raw, and there is no kill step to eliminate pathogens. Conventional techniques of decontamination used in the industry include sanitizing washes containing chlorine to kill microorganisms which have not proven effective against internalized or embedded pathogens. It is a well-known fact that the best method to eliminate pathogens from produce is to prevent contamination, as washing, even with disinfectants, can only reduce but not eliminate pathogens. Hence, the fresh produce industry is in need of a kill step to ensure the safety of produce. An alternative may be irradiation which is emerging as a promising tool to enhance safety and extend shelf life of fresh and fresh cut produce.

Gamma rays have been the most extensively studied form of irradiation and have been successfully applied to spices, tubers, grains and meat products for the space program. However, consumer reluctance has limited its application over a broad range of food stuffs. As a result, alternate irradiation technologies such as e-beam and X-rays are attracting attention as possible decontamination tools. The FDA recently has amended food additive regulations to permit the irradiation of fresh iceberg lettuce and spinach in

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This thesis follows the style of *Journal of Food Protection.*

order to kill microbial pathogens and enhance shelf life. The potential for application of this novel irradiation technology needs to be studied more extensively so that the treatment can be applied to a variety of fresh cut produce. The present study was conducted to evaluate the effectiveness of e-beam irradiation in pathogen reduction in cut roma tomatoes, cut cantaloupe, baby spinach, and romaine lettuce. Based on the results, D_{10}-values for reduction of *Salmonella* and *E. coli* O157:H7 were computed. These commodities have been associated with outbreaks linked to pathogens. Also, the effect of e-beam irradiation on the shelf- life of cut roma tomato, cut cantaloupe, baby spinach, romaine lettuce, strawberries, and green onion was evaluated. Effect of irradiation on quality parameters such as color, texture, respiration rate, and microbial counts was studied. Recommendations on keeping quality of the packed commodities based on microbial safety and acceptable quality was proposed.

REVIEW OF LITERATURE

Produce borne illness

Outbreaks linked to fresh produce

During the last three decades, consumption of fresh fruits and vegetables in the US has increased due to awareness of the health benefits from eating fresh produce. Fresh produce is an important part of a healthy diet, but concerns about its safety have been raised as they represent the second leading cause of food borne illnesses in the US *(41, 93)*. An increasing number of outbreaks caused by food borne pathogens that were reported by the Centers for Disease Control and Prevention (CDC) *(18)* have been associated with fresh produce consumption. The proportion of outbreaks linked to fresh produce increased from <1% of all reported outbreaks with a known food vehicle in the 1970s to 6% in the 1990s *(93)*. Since the mid-1990s, outbreaks linked to raspberries, green onions, and strawberries along with other commodities have also been reported. In 2008, a large outbreak of salmonellosis caused by *Salmonella* Saintpaul was traced to hot peppers and possibly tomatoes *(19). Salmonella* Poona has been repeatedly linked to imported cantaloupes *(16)* which underline the challenges related to fresh produce.

Outbreaks of foodborne disease associated with cantaloupe

Outbreaks linked to *Salmonella* have been reported throughout the US and Canada and cantaloupe has been the commodity implicated *(7, 16, 61)*. Three multistate outbreaks of *S.* Poona infections associated with eating cantaloupe imported from

Mexico occurred in the spring of 2000, 2001 and 2002 and *S*. Poona was implicated in these *(16)*. In 1997, an outbreak (24 cases) of *Salmonella* serogroup Saphra infection in California was associated with imported Mexican cantaloupe *(61)*. As a result, in October 2002, the FDA banned import of cantaloupes grown in Mexico citing unsanitary conditions and the outbreaks as prime reasons.

DelRosario et al. *(23)* reported an outbreak of foodborne illness caused by *E. coli* O157:H7 which was apparently caused by the consumption of cantaloupe in a restaurant in Oregon *(23)*.The consumption of cantaloupe or other items in the salad bar were most likely cross-contaminated by meat products during preparation *(33)*.

Outbreaks of foodborne disease associated with tomatoes

Four separate outbreaks of foodborne illness associated with the consumption of fresh produce were reported in 2006 in the US and the commodities identified to be the vehicles were spinach, lettuce, and tomatoes *(25)*. Tomatoes are more commonly associated with outbreaks of *Salmonella* infection, although *Shigella* also has been associated with consumption of tomatoes (*88)*. In 2004, the CDC reported three outbreaks of *Salmonella* infection associated with eating roma tomatoes. In one of these outbreaks, which was linked to eating roma tomatoes at various sites of a delicatessen chain, multiple serotypes were isolated. A field packing operation, and 3 packing house operations were identified as possible sources of the *Salmonella*. However, a specific source of contamination during packing could not be identified. A number of *Salmonella*

serovars (Javiana, Montevideo, Anatum, and Newport) have been repeatedly implicated in outbreaks linked to whole or chopped tomatoes *(18).*

Outbreaks of foodborne disease associated with leafy greens

In 2006, pre-bagged baby spinach contaminated with *E. coli* O157:H7 caused 205 illnesses and 3 deaths across 26 US states and one Canadian province *(98)*. *E. coli* O157:H7 is clearly a public health concern since it is the second most important causal agent of outbreaks linked to fresh produce *(93)*. The impact of the 2006 bagged spinach outbreak was recognized with a marked decrease in consumption of spinach and related products. The consumption of bagged spinach decreased by 43%, bagged salad containing spinach decreased by 42%, and bagged salad not containing spinach decreased by 8% in a span of 24 weeks *(34)*.

According to Rangel et al. *(86)* and Delaquis et al. *(22)*, lettuce was the single most frequently implicated commodity among the reported outbreaks in fall 2006. The US CDC reported 634 cases of illness due to 20 outbreaks linked to the consumption of lettuce contaminated with *E. coli* O157:H7 during 1998 to 2005 *(56)*. Lettuce appears to be more susceptible to bacterial contamination. A number of outbreaks caused by *E. coli* O157:H7 have been linked to the consumption of lettuce *(95). E. coli* O157:H7 is the most commonly implicated pathogen causing outbreaks associated with spinach and other leafy greens *(10, 86, 93)* and Solomon et al. *(94)* reported that the pathogen is transmitted through manure, irrigation water and can be eventually be internalised in the lettuce leaves.

Outbreaks of foodborne disease associated with berries

Outbreaks caused by Hepatitis A Virus (HAV) have been associated with fruits including frozen raspberries and strawberries and have been traced to poor handling issues *(85, 87)*. In these outbreaks, the fruits used were frozen and the foods had not been cooked, thus the HAV was not destroyed. A multistate outbreak of HAV was traced to frozen strawberries processed at a single plant and Niu et al. *(74)* found that among 827 students and 60 teachers at an elementary school in Georgia during a 2-week period, 15 developed HAV. An outbreak of cyclosporiasis was reported in North America in 1996 associated with the consumption of Guatemalan raspberries *(42)*. Although various berries, including strawberries which were served at various events, were thought to be possible means of infection, Guatemalan raspberries were ultimately implicated *(42)*. Guyader et al. *(52)* reported a gastroenteritis outbreak in Sweden caused by norovirus traced back to consumption of raspberry cakes as source of outbreak. Raspberries have been implicated in outbreak of gastroenteritis caused by calcivirus linked to consumption of frozen raspberries in Finland *(78)*.

Outbreaks of foodborne disease associated with green onions

Several recent outbreaks of foodborne illness have been associated with consumption of green onions contaminated with HAV *(24)*. The role of food in the transmission of the norovirus *(20)* and (HAV) has been reported *(60)*, with fresh produce being one of the most commonly implicated foods *(7)*. HAV can contaminate foods through infected workers and food handlers in processing plants and restaurants; these

were the likely causes of numerous outbreaks involving fresh produce like green onions *(17)*. The largest documented food borne outbreak of HAV in the US occurred in 2003 in Pennsylvania and resulted in more than 600 cases and 3 deaths *(107)*. This outbreak was traced to green onions that came from two farms in Mexico after ruling out the food handlers as source of contamination.

The likely source of the HAV appeared to be the raw onions themselves, which had been contaminated either before or during packing into boxes at the farms. Because onions are used as an ingredient in several dishes served at the restaurant, the contaminated green onions were chopped and stored in bulk, providing the opportunity for cross-contamination to other onions and ingredients, which may have contributed to the size of this outbreak *(107)*.

Decontamination strategies for fresh produce

Risk management tools used currently for controlling contamination of fresh green leafy vegetables are based on prevention and are mostly centered on good agricultural practices (GAPs) and good manufacturing practices (GMPs), which provide guidelines to prevent and reduce biological contamination of fresh vegetables from known sources. In addition, methods of decontamination are commonly used, and include washed with chlorinated water or other approved sanitizers. According to FDA's *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables,* to sanitize is to treat clean produce by a process that is effective in destroying or substantially reducing the numbers of microorganisms of public health concern as well

as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer *(99)*.

However, the effectiveness of these applications may be limited by a variety of factors. Chlorine, which is the most widely used sanitizer for disinfecting fruits and vegetables in the food industry depletes rapidly in presence of high organic material. It can also form potentially carcinogenic organochlorine compounds by reacting with trace amounts of organic material *(28)*. While microbial reduction occurs with use of sanitizing solutions, the effect is short lived as the microorganisms soon grow back to similar numbers as the control (water wash) *(35)*. There is a real need to find alternatives for reducing pathogens in fresh cut fruit and vegetables in order to improve the efficacy of washing treatments.

Chlorine

Chlorine is the most commonly used sanitizer by the fresh produce industry. A wash with 1% H_2O_2 results in pathogen reduction at the same level as chlorine according to Sapers et al. *(91)*. Aqueous chlorine dioxide (ClO_2) has been proposed as an alternative to sodium hypochlorite (NaClO) for fresh-cut produce sanitization by Lopez –Galvez et al. *(55)*. According to these authors, the advantage of using aqueous chlorine dioxide is eliminating risks associated with chlorination byproducts, and they saw that trihalomethanes (THMs) were detected only in wash water containing sodium hypochlorite used to wash lettuce. Lopez-Galvez et al. *(55)* have suggested that aqueous chlorine dioxide is equally suitable as sodium hypochlorite for fresh-cut lettuce

sanitation with the advantage of preventing the formation of THMs. Casteel et al. *(13)* reported that coliphage MS2 (bacteriophage which is commonly used as a surrogate for some pathogenic viruses and indicator of fecal contamination) was reduced on inoculated strawberries by 68, 92 and 96% when treated with water added with sodium hypochlorite at concentrations of free chlorine of ≤ 2, 20 and 200 mg/l, respectively *(13)*.

Although use of chlorine by the produce industry has been reported for several decades, Wei et al. *(106)* reported that treatment with chlorine at 100 ppm for 2 min did not significantly reduce *Salmonella* both at the stem scar (3.98 log CFU/g) and the skin (3.25 log CFU/g). The fruit and vegetable structure and morphology like the hydrophobic cutin, uneven or damaged epidermis, as well as the ability of microorganisms to internalize in the product limit the efficacy of decontamination treatments *(11)*. Ukuku *(102)* reported that cantaloupes which are sanitized using sanitizers (200 ppm chlorine or 2.5% hydrogen peroxide for 2 min.) show reduction in *Salmonella* population but are more susceptible to recontamination with the bacterial pathogen compared to untreated controls during subsequent handling.

Chlorine solutions are widely used in the fresh and fresh-cut produce industry to disinfect the wash water used in washing/rinsing fresh produce. Chlorine water reduces microbial populations and prevents cross contamination during water re-use and re-circulation. However, the large amount of organic material present on the fresh produce takes up free chlorine thus reducing the sanitizing potential of wash water just when it is needed most *(108)*.

Organic acids

Several studies demonstrated the limited efficacy of chlorine in reducing microbial populations on the surface of fresh produce. Hence, alternatives such as use of organic acids have gained importance. The efficacy of organic acid washes (lactic, acetic, citric, peracetic, propionic) in reducing microbial populations on the surface of fruits and vegetables has been investigated *(67)*. Alvarado-Casillas et al. *(2)* have reported that 2% L lactic acid by spray was the treatment that resulted in the lowest bacterial counts on both cantaloupes and bell peppers without any changes in sensorial characteristics. Lactic acid was reported to be superior compared to chlorine in effectively reducing internalized salmonellae in tomatoes *(45)*.

Treatment with organic acids combined with ozone has been investigated by Yuk et al. *(111)* who demonstrated that the combination did not have a residual antimicrobial effect during storage and the pathogen counts increased up to 9 log CFU/g. The combination of ozone-organic acid treatment was found to be more effective in reducing the pathogen levels on lettuce than individual treatments *(111)*. When lettuce was treated with a combination of lactic acid and hydrogen peroxide, a 4-log reduction for *E. coli* O157:H7 and *Salmonella* and a 3-log reduction for *Listeria monocytogenes* were obtained *(54)*.

Irradiation as an alternative treatment

History

Food irradiation has been in development since the early decades of the twentieth century. In 1963, the US saw its first approval of food irradiation when FDA approved its use to control insects in wheat and wheat flour. In 1964, additional approval was given to inhibit the development of sprouts in white potatoes *(3)*. . From then on, through 2008, various foods have been approved for irradiation including various herbs, spices, vegetable seasonings, pork, fruits, vegetables, grains, frozen poultry, chilled red meat and meat products. Higher doses that sterilize frozen and packaged meats were approved in 1995 for use by National Aeronautics and Space Administration (NASA) only *(101)*. Irradiation has been approved by FDA to control food borne pathogens in raw poultry with a dose range of 1.5 – 3.0 kGy. In addition, irradiation of raw refrigerated and frozen meat has been approved recently with maximum doses of 4.7 and 7 kGy, respectively *(100)*. FDA has also recently approved use of irradiation dose up to 3 kGy in shell eggs with the intention of significantly reducing populations of *Salmonella* spp *(100)*. However, irradiation has not yet obtained a significant place in the US food industry.

Ionizing radiation

There are three types of ionizing energy that can be used for irradiation of food: X-rays, electron beam and gamma rays. Linear accelerators are used to generate a beam of electrons and the food can be treated by exposure to these electrons. X-rays are

produced when the electrons from the accelerator are stopped by a metal target (such as tungsten) and the food is therefore exposed to X-rays *(4)*. The amount of energy produced by electron beam can be adjusted. Gamma rays are produced by radioisotopes that continuously emit the high energy gamma rays. The approved sources of gamma rays for food irradiation are cobalt- 60 (the most common) and cesium-137 *(4)*.

Both, gamma and x-rays have no mass and hence have higher penetrating power whereas electrons have a small mass and hence get slowed down as they enter the target matrix. X-rays are slightly more penetrating than gamma rays *(4)*. Electron beams have a higher energy level (10 MeV) compared to gamma rays but low penetration potential (3.5 in, dual beam) and hence are best used for surface and subsurface irradiation of foods *(45)*. The advantage of electron beam over a radioisotope is high dose rate as opposed to the slower process of exposure of the product to gamma rays that originate from a source that has to be stored under water.

Dosimetry

The success of radiation processing of food depends to a large extent on the ability of the processor to measure the dose delivered to the food product. Radiation dosimetry is the calculation of the absorbed dose in matter and tissue resulting from the exposure to ionizing radiation indirectly and directly. Dosimetry is achieved using dosimeters which are made of radiation sensitive material like alanine or radiochromic films. It is paramount to have a reliable dosimetry since the decontamination processes for food industry and sterilization of health care products are highly regulated, especially

with respect to absorbed dose. Thus, accurate dosimetry is indispensable. According to Olson *(75)*, irradiation of foods causes release of free radicals that are unstable and react with other chemical compounds in the food system to form stable radiolytic compounds. The radiochromic films change color when irradiated and can be quantified using a spectrophotometer. The amino acid alanine, when exposed to irradiation forms free radicals that can be measured and correlated to the absorbed dose using electron spin resonance spectrometry *(75)*.

Irradiation in fresh cut produce

Food irradiation is a promising new food safety technology that can eliminate disease-causing microorganisms from foods. The application of ionizing irradiation to control spoilage microorganism increases shelf life of irradiated strawberries, lettuce, sweet onions, and carrots *(96)*. Zhang et al. *(113)* showed experimentally that the number of aerobic mesophilic bacteria on fresh cut lettuce irradiated with 1.0 kGy was reduced by 2.35 logs and also proved that the best treatment of maintaining quality of fresh-cut lettuce appeared to be 1.0kGy irradiation. The added advantage in this case was that the sensory quality was maintained for a period of 8 days at 4°C. Farkas et al. *(32)* showed that ionizing radiation at 1kGy reduced loads of bacteria, improved microbiological shelf life, and extended sensory quality of pre-cut bell peppers and carrots thus proving that irradiation are highly effective for inactivation of food borne pathogens and parasites in various vegetables.

Studies on irradiation were done in combination with other methods of sanitation as in case of Kim et al. *(48)*, who studied the effect of warm water dip in combination with irradiation on quality of fresh-cut green onions. Irradiation at all tested doses reduced APCs and the development of decay and off-odor, improved visual quality, and preserved green color. The warm water dip followed by irradiation showed reduction in APCs by 0.9 log CFU/g initially but the beneficial effect disappeared during storage. Boynton et al. *(8)* studied effects of low dose e-beam radiation on fresh cut cantaloupes and showed that a high dose of 1.5kGy resulted in initial APC reductions of 1.5 log compared to the non-irradiated controls, and also prevented the 2.5 to 3 log APC increases seen in controls after 10 – 11 days of storage.

D_{10}-values for Salmonella *and* E. coli *O157:H7 in fresh cut produce*

Studies have been conducted in the past to determine the D_{10}-value for pathogens in various foods using gamma irradiation however, not much has been reported for D_{10}-value using electron beam irradiation. Various factors play a role in determining the radiation sensitivity of organisms and hence their D_{10}-value is specific to certain parameters under which it is determined. Composition of the medium is the most significant factor along with temperature of the product and moisture content. Niemira *(68)* studied effect of gamma irradiation on spinach and lettuce with internalized pathogens using a cocktail of 3 isolates of *E. coli* O157:H7. A D_{10}-value of 0.39 kGy was reported in romaine lettuce. In spinach leaves, the pathogen had a biphasic response,

with a D_{10}-value of 0.27 kGy in the range of 0 to 0.75 kGy but only slight additional reductions from 0.75 to 1.5 kGy.

Treatment by gamma radiation on seasoned spinach gave a D_{10}-value of 0.37 kGy for *S.* Typhimurium according to Lee et al. *(53)*. Low energy X-ray was used to determine D_{10}-value for *E. coli* O157:H7 on iceberg lettuce by Sanghyup et al. *(90)*. A dip inoculation protocol yielded a D_{10}- value of 0.04 kGy whereas the spot inoculation method gave a D_{10}- value of 0.078 kGy *(90)*. In another study by Neal et al. *(66)* treatment by e-beam radiation at a dose of 0.40 kGy on baby spinach resulted in a reduction in populations of *E. coli* O157:H7 and *Salmonella* of 3.7 and 3.4 log cycles, respectively. However, at 0.70 kGy, both pathogens were reduced by 4 logs.

In one of the studies Prakash et al. *(81)* examined the efficacy of combined irradiation and a 1% calcium chloride dip to reduce the population of *Salmonella* enteric strains on diced tomatoes. D_{10}-value results ranged from 0.26 to 0.39 kGy, indicating that a 5 log CFU/g reduction in *Salmonella* spp. in diced tomatoes needs a dose of 1.3 – 1.95 kGy. Another study on cut tomatoes by Schmidt et al. *(92)* noticed different radiation responses for two serotypes of *Salmonella* which illustrates that the radiation sensitivity varies within the same pathogen species. An interesting finding by James et al. *(46)* who studied the e-beam effect on nalidixic acid-resistant *S.* Montevideo in cooked tomato puree of various pH values found that at pH 4.4 and 4.9, *S.* Montevideo had a D_{10}-value of 1.07 and 1.50 respectively.

Studies on pathogen reduction on cantaloupes using e-beam are very limited. Palekar *(77)* reported a D_{10}-value of 0.211 kGy for *S.* Poona on cut cantaloupes treated

with e-beam irradiation in one study and in another study reported a 1.1 log reduction at 0.7 kGy and 3.6 log reduction at 1.5 kGy on cut cantaloupes for *S*. Poona. This indicates that radiation sensitivity of a pathogen is dependent on various factors. Radiation sensitivity can be a function of product type, dose rate, sample preparation, and type of radiation. For example, the D_{10}-value of *E. coli* O157:H7 and *S.* Typhimurium inoculated on broccoli, red radish, broccoli sprout, and red radish sprout showed statistically significant differences (0.09, 0.86 kGy) between samples treated with E-beam (2.5 MeV) and those treated with gamma ray *(105)*.

To make efficient use of irradiation as a treatment to control pathogens, it is imperative to plan a study to obtain information on fresh produce. This study is expected to facilitate collection of useful data for further development of strategies for reducing pathogens in fresh and fresh-cut produce after GAPs and GMPs are applied.

Accordingly we hypothesize that the D_{10}-values for *Salmonella* and *E. coli* O157:H7 have a non-linear relationship with the e-beam dose applied and will depend on the type of produce used. Thus, higher D_{10}-values will be obtained at lower e-beam doses and lower D_{10}-values at higher e-beam doses.

Furthermore, we propose that the range of D_{10}-values that are useful for these microorganisms will be defined by the maximum e-beam dose that is tolerable to the type of produce used. Thus, a high dose of e-beam can be applied to produce in question without causing any perceivable change in quality parameters.

The purpose of this study was to determine D_{10}-value for *Salmonella* and *Escherichia coli* O157:H7 on romaine lettuce, spinach, fresh-cut cantaloupe, and fresh-

cut roma tomatoes irradiated with electron beam. In addition also evaluate efficacy of e-beam irradiation to determine the maximum dose of e-beam irradiation that can be irradiated onto fresh cut roma tomatoes, fresh cut cantaloupe, romaine lettuce, baby spinach, strawberries, and green onion without changes in firmness, color, respiration rate, and microbial counts.

MATERIALS AND METHODS

Phase 1

Determination of D_{10}-value for *Salmonella* and *E. coli* O157:H7 on lettuce, spinach, fresh-cut cantaloupes, and fresh-cut tomatoes.

Bacterial cultures

Rifampicin-resistant strains of *Salmonella enterica* serotypes Poona, Montevideo, Agona, Michigan, and Gaminara and rifampicin-resistant strains of *E.coli* O157:H7 (designated R1, R8, R18, R41, and R34) were obtained from the culture collection of the Texas A&M University's Center for Food Safety. All isolates were derived from parent strains obtained from beef cattle carcass sources. The strains (Rif+) were stored at -80°C until further use.

Inoculum preparation

The microorganisms were resuscitated by transferring to tryptic soy broth (TSB; Difco, Detroit, MI) and incubated at 35°C for 18-24 h. The cells were then streaked on tryptic soy agar (TSA; Difco, Detroit, MI) slants and stored at 4-5°C until they were needed for the experiment. Prior to use, resistance to rifampicin was confirmed by streaking each organism onto TSA plates supplemented with 0.1 g/L rifampicin (Sigma, St Louis, MO) and incubated at 35°C for 18-24 h. Confirmation of *Salmonella* cultures was done by biochemical tests on triple sugar iron agar (TSIA, BD Diagnostic Systems,

Sparks, MD) and lysine iron agar (LIA, BD Diagnostic Systems, Sparks, MD) slants. Serotypes of *E.coli* O157:H7 were streaked on sheep blood agar plates (BD Biosciences, Cockeysville, MD). Isolated colonies were then confirmed for O157 and H7 antibodies using the RIM *E.coli* O157:H7 kit (Remel, Lenexa, KS). Characteristic colonies were then maintained on TSA slants until the day of the experiment. The day before the experiment, each isolate was transferred into 20 ml TSB and incubated at 35°C for 18-24 h. The cells were then harvested by centrifugation at 2191 x g in a Jouan B4i centrifuge (Thermo-Fisher Scientific) for 15 min at 25 °C. Each of the cell pellets obtained were re-suspended in 25ml 0.1% sterile phosphate buffer saline (Calbiochem, San Diego, CA). A bacterial cocktail of all the 10 isolates (5 each of *Salmonella* and *E. coli* O157:H7) was prepared by mixing 25 ml each of the re-suspended pellet in a sterile Erlenmeyer flask prior to inoculating. The average final concentration of *E. coli* O157:H7 and *Salmonella* in the cocktail was 8.0 and 8.6 CFU/ml respectively.

Produce

Fresh romaine lettuce heads, baby spinach, cantaloupes and roma tomatoes were obtained from a local wholesaler (Scarmardo Produce Co., Bryan, TX) and stored at 5°C overnight. The inoculation was done on the day before the irradiation treatment. For each commodity, a total of 54 samples (9 samples x 6 doses) were packed. Two additional sets of 9 samples were packed for back- up and speed check.

Inoculation of produce

Romaine lettuce and baby spinach – Romaine lettuce heads were separated into individual leaves. For both lettuce and spinach, leaves that were damaged, bruised or dirty were discarded. For the treatment, 10 g leaves (typically a single leaf for romaine lettuce) were weighed out and placed inside a sterile Stomacher® bag (Seward Scientific, London, England). The leaf/leaves inside the Stomacher® bag was surface inoculated with 1 ml of the cocktail previously described with a micropipette inside a microbiological safety cabinet. The bag was lightly shaken just enough to ensure uniform distribution of the inoculum on the entire leaf surface. The leaves were then left to dry inside the cabinet for 1 h until the leaves were dry enough and the inoculum did not flow outside the leaf surface. The Stomacher® bags were then vacuum sealed to avoid air pockets in the sample. For each treatment dose, nine samples were used. Three Stomacher® bags were packed one beside the other inside one 95 kPa leak-proof specimen transport bag (Inmark, Inc., Atlanta, GA) with the inoculated surface facing up.

Roma tomato and cantaloupe - The tomatoes and cantaloupes (skin peeled with sterile knives) were cut into cylinders of uniform diameter using a sterile hollow stainless steel cylinder of 1.5 in diameter (area 1.77 in^2). Extra flesh was removed using a sterile knife to get a uniform thickness of approximately 5mm for tomatoes and 3.5 mm for cantaloupe. Each piece weighing about 10 g was placed in sterile petri dishes with flesh facing up. The surface was inoculated with 0.1 ml of the bacterial cocktail previously described with a micropipette, swirled lightly ensuring uniform distribution

and air dried inside a microbiological safety cabinet for 1 h. For each treatment dose, nine pieces/samples were arranged inside a sterile Stomacher® bag so as to have 3 pieces in a row and 3 such rows were arranged on top of another using sterile forceps. The inoculated surface was facing up and the packs were vacuum sealed. The Stomacher® bag was then packed inside a leak-proof specimen transport bag as described previously. In this case, one transport bag had all the nine samples.

Irradiation treatment

The irradiation treatment was carried out at the Texas AgriLife Research's National Center for Electron Beam Food Research located within the Texas A&M campus. A pit and tower system with a single beam 10-MeV and 15-kW linear accelerator was used for this study (LINAC, Varian, Palo Alto, CA). The inoculated samples contained within the specimen transport bags were transported to the E-beam center in an insulated cooler (Igloo Products Corp, Katy, TX) with refrigerant packs (UTEK 30°F, Polyfoam Packers Co. Wheeling, IL) and were subjected to e-beam irradiation at increasing doses between 0 (control) and 1kGy, with increments of 0.2 kGy. The electron beam was incident from the top and the samples were made to pass under the beam in a cardboard tray placed on a conveyor belt. The area of operation inside the e-beam center was maintained at 5°C.

Speed check bags for the 4 commodities were run with the inoculated surface facing up to check for the surface dose. A pack of 11 HDPE attenuation sheets (12 in x 12 in x 4.8 mm) and 1 sheet (12 in x 12 in x 1.6 mm) (King Plastic Corp Inc, North Fort,

FL) were used on the top and bottom of the bags and the conveyor belt speed was adjusted as needed to achieve the target dose.

Dose mapping

Dose mapping was achieved using alanine pellets (Bruker Instruments, Rheinstetten, Germany) placed on the bag between 2 pieces of the produce so that the leaf and the pellet are at the same level. Each pack was run with 2 dosimeters placed diagonally opposite on top. Absorbed dose on the dosimeters was analyzed using an electron paramagnetic resonance instrument (EMS 104 EPR analyzer, Bruker Instruments, Karlsruhe, Germany). The average final doses for all commodities are shown in Table 1.

TABLE 1. *Commodities with average dose delivered for D_{10}-value determination*

Commodity	Average Dose (kGy)
Cut cantaloupe	0, 0.20, 0.40, 0.66, 0.79, 1.0
Cut tomtato	0, 0.21, 0.40, 0.59, 0.80, 1.0
Baby spinach	0, 0.22, 0.38, 0.60, 0.83, 1.08
Romaine lettuce	0, 0.22, 0.40, 0.65, 0.84, 1.09

Sampling and microbiological analysis

Produce treated with irradiation were analyzed for presence of *E. coli* O157:H7 and *Salmonella*. For romaine lettuce and baby spinach, contents of each Stomacher® bag

were pummeled with 90 ml of sterile 0.1% peptone water (BD Diagnostic Systems, Sparks, MD) in a stomacher (Seward Scientific, London, England) for 1 min. For tomato and cantaloupe, each piece was transferred into a sterile Stomacher® bag and the contents were pummeled with 90 ml sterile 0.1% peptone water (BD Diagnostic Systems, Sparks, MD) in a stomacher (Seward Scientific, London, England) for 1 min. Serial dilutions were made and spread plated onto lactose sulfite phenol red rifampicin (LSPR), a selective and differential medium designed for simultaneous enumeration of rifampin-resistant *E. coli* and *Salmonella (15).* Plates were incubated for 24 - 28 h at 35°C. Rifampicin-resistant *E. coli* O157:H7 produced yellow colonies on the medium, whereas rifampin-resistant S*almonella* developed colonies with a black center surrounded by a pink halo. Counts of *E. coli* O157:H7 and *Salmonella* were made independently.

Statistical analysis

The microbiological data (plate counts) were transformed to log value for data analysis. Log (N/N_0), where N is survival population and N_0 is initial population for each commodity were plotted against the dose of e-beam energy applied. Linear regression analysis was conducted to establish the effect of the dose on the bacterial counts using Microsoft Excel 2007 (Microsoft Corp. Redmond, WA). From the regression line, the slope of the line was calculated and the D-value (dose necessary to reduce the bacterial population by 1 log cycle) was reported as the reciprocal of the slope of the regression line.

Phase 2

Determine the maximum dose of e-beam energy that can be irradiated onto romaine lettuce, baby spinach, roma tomatoes, cantaloupes, green onion, and Strawberries without changes in firmness, color, respiration rate, and microbial counts over a storage period of 12 days.

Produce

Fresh romaine lettuce heads, baby spinach, cantaloupes, green onions, roma tomatoes, and strawberries were obtained from a local wholesaler (Scarmardo Produce Co., Bryan, TX) and stored at 5°C overnight.

Packing of produce for treatment

Produce was packed in Ziploc® bags as described below for each commodity. To prevent atmosphere modification inside the bag, small perforations (~20) were made on each bag using sterile needles. Then, air was manually flushed out before bags were closed. Produce was packed the day before the treatment and stored at 5°C overnight. In all cases, the contents of the bag were spread evenly to make the bag as flat as possible. All handling and packing was done using sterile gloves and forceps.

For each treatment dose, separate packs for texture, respiration rate, and microbiological analysis were used and all packs were prepared in triplicate. For color and respiration rate, the same packs were used throughout the storage. For irradiation treatments, 1 bag for speed check and an additional bag for storage were prepared for

each treatment dose in all 6 commodities. The packed commodities were all stored at 5°C overnight until treated. Post treatment, packs were stored at 5°C for 12 days and samples were analyzed on 0, 3, 6, 9, and 12 days of storage for color, texture, respiration rate and microbiological counts. For microbiological counts and respiration rate analysis, 1 sample each from the three bags was used each sampling day. For color and texture, 3 samples from each of the three bags were analyzed. Produce that did not receive any treatment was used as control or dose 0.

Romaine lettuce - romaine lettuce heads were separated into individual leaves and outer leaves which were damaged, bruised or dirty were discarded. For color analysis, 3 leaves marked as 1, 2 and 3 were packed in a Ziploc® bag (10.75 in x 12 in). For texture, a total of 15 leaves were packed (5 leaves x 3 Ziploc® bags). For respiration rate, 6 leaves and for microbial analysis, 6 leaves each weighing 10 g was packed in a Ziploc® bag.

Baby spinach - For texture and microbial analysis, 60 g spinach was packed in a Ziploc® bag (10.75 in x 12 in). For respiration rate, 50 g spinach was packed in a Ziploc® bag (10.75 in x 12 in). After treatment, 3 leaves each from each replicate were removed from the bags marked for texture and were numbered 1, 2, and 3. These leaves were packed in a Ziploc® bag (6.75 in x 7 in) and used for color measurement throughout the study.

Roma tomatoes – Tomatoes were cut into 3.5 cm x 3.5 cm pieces and the core and seeds removed using sterile knives. For color, 3 pieces were packed in a Ziploc® bag (6.75 in x 4.25 in). For texture, 15 pieces were packed in a Ziploc® bag (6.75 in x 7 in).

For respiration rate, 8 pieces were packed, and for microbial analysis, 6 pieces were packed in a Ziploc® bag (6.75 in x 4.25 in).

Cantaloupe – Cantaloupes were washed with water, peeled and cut into pieces of size 3.5 cm x 3.5 cm x 1.5 cm using sterile knives. Separate knives were used for peeling and cutting to avoid cross contamination. For color, 3 pieces were packed in a Ziploc® bag (6.75 in x 4.25 in). For texture, 15 pieces were packed in a Ziploc® bag (6.75 in x 7 in). For respiration rate, 8 pieces were packed, and for microbial analysis, 6 pieces were packed in a Ziploc® bag (6.75 in x 4.25 in).

Strawberries - For color, 3 fruits were packed in a Ziploc® bag (6.75 in x 4.25 in). For texture, 15 fruits were packed in a Ziploc® bag (6.75 in x 7 in). For respiration rate, 4 pieces were packed and for microbial analysis, 6 small fruits weighing ~ 10 g were packed in a Ziploc® bag (6.75 in x 4.25 in).

Green onion – The leaves of green onion were cut by an inch and 10 pieces were packed in a Ziploc® bag (10.75 in x 12 in) fo texture, respiration rate and microbial counts. After treatment, 3 pieces each from each replicate were removed from the bags marked for texture and were numbered 1, 2, and 3. They were packed in another Ziploc® bag (10.75 in x 12 in) and used for measuring color.

Irradiation treatment

Samples were transported to the Texas AgriLife Research's National Center for Electron Beam Food Research located within the Texas A&M campus and the irradiation treatment was carried out as described previously. Irradiation was done at

target doses of 1, 3, and 5 kGy. The electron beam was incident from the top and the samples were made to pass under the beam in a cardboard tray placed on a conveyor belt. The area of operation inside the e-beam centre was maintained at 5°C. All the speed check bags were run to check for the entry and exit dose and the conveyor belt speed was adjusted accordingly to achieve the target dose.

Dose mapping

Dose mapping was achieved using alanine dosimeter film strips (BioMax, Eastman Kodak Co., Rochester, N.Y.). For running speed checks, two alanine strips were taped to the pack, one top and one at the bottom. The pack was secured on a cardboard tray in between 2 single layers of HDPE attenuation sheets (12 in x 12 in x 4.8 mm) with an adhesive tape. The produce packed for treatment were split between 2 cardboard trays and for each tray, 2 dosimeters were placed diagonally on top and 2 dosimeters were placed diagonally on the bottom of a pack. The packs were secured in the same way before placing them on the conveyor belt. Absorbed dose on the dosimeters was analyzed using an electron paramagnetic resonance instrument (EMS 104 EPR analyzer, Bruker Instruments, Karlsruhe, Germany). The average final doses for all commodities are shown in Table 2.

Color analysis

Color was measured using Minolta CR-300 handheld colorimeter (Minolta Camera Co., Osaka, Japan). The colorimeter was calibrated with a standard white plate

TABLE 2. *Commodities with average dose delivered for quality analysis*

Commodity	Average Dose (kGy)
Cut cantaloupe	1.09, 3.22, 5.20
Cut tomato	1.03, 3.15, 5.17
Baby spinach	1.01, 3.05, 5.16
Romaine lettuce	1.10, 3.07, 5.07
Strawberries	1.07, 2.92, 5.43
Green onion	1.03, 3.05, 5.11

(C, Y = 93.4, x = 0.3136, y = 0.3195) before use each sampling day. L, a, b values were measured under 'C' illumination. For measuring color of cantaloupe, tomato, and strawberries, calibration was done with a cling wrap placed on the white tile since the samples were also covered with the wrap for color measurement. For lettuce, spinach and green onions, no wrap was used. Nine measurements were done in L, a, b color space per commodity per dose. Results for color were reported as chroma values calculated as $(a^2+b^2)^{1/2}$. The chroma or saturation of a color is a measure of how intense or pure the color is. High chroma colors look rich and full. Low chroma colors look dull and grayish.

Texture (firmness) analysis

Texture (firmness) of the produce was measured using a texture analyzer TA-XT2i texture analyzer (Texture Technology Corp. - Stable Micro Systems Ltd., Surrey, UK) with a 50 kg load cell. The instrument was calibrated prior to use on each day of analysis. The probes used were different for different produce due to the nature of the produce. Individual tomato pieces were placed over a metal plate with the flesh facing up and punctured equatorially through the flesh using a puncture probe with a diameter of 3 mm, a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s to a penetration depth of 5 mm. Individual cantaloupe pieces were placed over a metal plate and punctured equatorially through the flesh using a puncture probe with a diameter of 3 mm, a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s to a penetration depth of 8 mm. Each strawberry was cut on either side along the length to make it flat and placed flat over a metal plate and punctured equatorially through the flesh closer to the skin and away from the centre hole using a puncture probe with a diameter of 3 mm, a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s to a penetration depth of 5 mm.

Kramer shear cell with a sample holder (internal dimension $82 \times 63 \times 89$ mm^3) and a five blade attachment was used in analyzing texture for spinach, lettuce, and green onion. The texture analyzer was calibrated each time a measuring probe was changed. Ten g of spinach was placed inside the sample holder and punctured with 5 parallel blades at a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s until all the leaves were crushed. Lettuce leaf weighing 10 g was cut across the

leaf into pieces of 8 cm length to fit inside the sample holder and punctured with 5 parallel blades at a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s until the entire leaf was crushed. Green onion weighing 10 g was cut into pieces of 8 cm length to fit inside the sample holder and punctured with 5 parallel blades at a pre-test speed of 10 mm/s, test speed of 1 mm/s and a post test speed of 5 mm/s until the entire leaf was crushed. Nine measurements were done per commodity per dose and the mean force in Newton was recorded.

Respiration rate analysis

Respiration rate was measured using the closed system methodology. The irradiated produce in each replicate bag was weighed and transferred to an airtight glass jars at 5°C and stored for a set period of time. Cut tomato and green onion were stored for 2 h, cut cantaloupe, strawberries, spinach, and lettuce were stored for 1 h. A rubber septum was attached in the center of a rubber lined lid for each glass jar for gas sampling. Gas samples in duplicate were taken at the designated time intervals, using a 1 ml syringe. The needle was inserted into a CO_2 gas analyzer Horiba PIR-2000 (Horiba, Irving, CA) to measure CO_2 composition. Each sampling day, 1 ml of 0.5% CO_2 was injected as a reference. After sampling of gas from the jars, the produce was put back in the same Ziploc® bag for use on the next sampling day. Produce samples were taken from 3 bags and placed in 3 glass jars per dose and 2 headspace samples were drawn from each jar.

Microbiological analysis

Each sampling day, 10 g were removed from the Ziploc® bag using sterile forceps, transferred to a Stomacher® bag containing 90ml sterile 0.1% peptone water and pummeled in a stomacher 400 for 1 min. From this homogenized sample, serial ten-fold dilutions were made and 1ml from each dilution was plated on Petrifilm™ (3M Corp., St Paul, MN) for Aerobic Plate Counts (APC) and Yeast and Mold counts (YNM). For Lactic Acid Bacteria counts (LAB), the pour plate technique was used in De Mann Rogosa Sharpe Agar media (MRS, Difco, Detroit, MI). After the agar solidified, an additional layer of the medium was poured before incubation. The APC Petrifilm™ were incubated at 25°C for 48 h, YNM at 25°C for 3-5 days and LAB plates at 35°C for 48h. Three samples were analyzed per commodity per dose.

Statistical analysis

Microbial counts were transformed logarithmically before statistical analysis. Difference in firmness and chroma values, as well as microbiological counts over storage time were determined as a function of the doses of energy applied using the Least Square Mean function in SAS. All the values are presented at a level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

D_{10}-value for *Salmonella* and *E. coli* O157:H7 on irradiated baby spinach, romaine lettuce, cut cantaloupe, and cut roma tomatoes

Baby spinach

Irradiation of baby spinach at increasing dose levels between 0 and 1.08 kGy yielded a linear dose-survival curve for *Salmonella* (R^2= 0.98) and for *E. coli* O157:H7 (R^2 = 0.97) (Fig. 1). There was a5.5 log CFU/g reduction from the non- irradiated control (7.6 log CFU/g) and samples irradiated at 1.08 kGy (2.1 log CFU/g) for *Salmonella* and a 5.7 log CFU/g reduction from the non- irradiated control (7.6 log CFU/g) and samples irradiated at 1.08 kGy (1.9 log CFU/g) for *E. coli* O157:H7.

A D_{10}-value of 0.19 kGy for *Salmonella* and 0.18 kGy for *E. coli* O157:H7 in baby spinach is reported in this study. This is in agreement with the D_{10}-value determined by Neal et al. *(61)* and Gomes et al. *(33)* for *E. coli* O157:H7. Both these studies determined D_{10}-value only for *E. coli* O157:H7 using e-beam but the dose range was higher in the study done by Gomes et al. (upto 2.5 kGy). In both studies, the inoculated sample packs were not vacuum sealed before treatment. Studies have been conducted in the past to determine the D_{10}-value for pathogens in various foods using gamma irradiation however; studies on D_{10}-value using electron beam irradiation are limited. Various factors play a role in determining the radiation sensitivity of organisms and hence their D_{10}-value is specific to certain parameters under which it is determined.

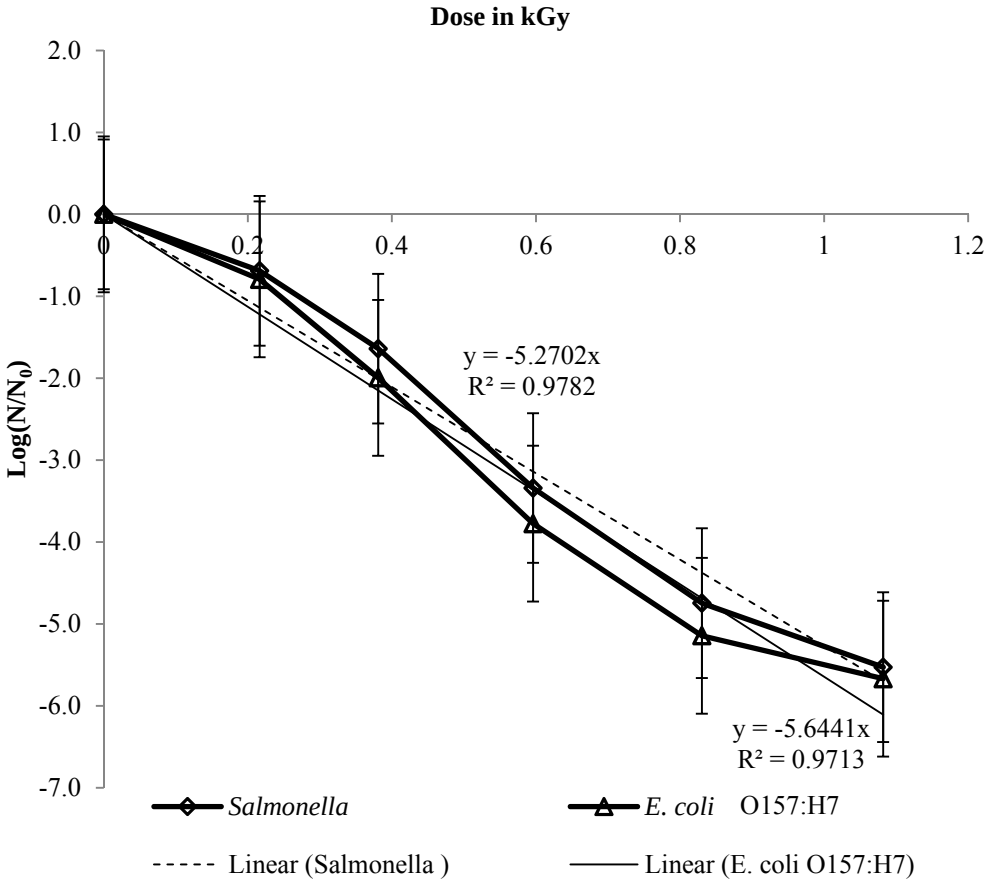

FIGURE 1. *Death curve of* Salmonella *and* E. coli *O157:H7 in baby spinach with increasing doses of irradiation*.

Niemira *(68)* studied effect of gamma radiation on spinach and lettuce with internalized pathogens using a cocktail of 3 isolates of *E. coli* O157:H7and observed a biphasic response for the pathogen on spinach. For a dose range of 0 to 0.75 kGy, the D_{10}-value reported was 0.27 kGy but for the dose range of 0.75 to 1.5 kGy, reduction in pathogen population was marginal. Composition of the medium is the most significant factor along with temperature of the product and moisture content. Lee et al. *(53)* quoted a D_{10}-value of 0.37 kGy for *Salmonella* Typhimurium and 0.42 kGy for *E. coli* O157:H7 on seasoned spinach which is higher than that obtained in this study. Niemira et al. *(71)* found significant increase in D_{10}-value for *E. coli* O157:H7 from 0.19 kGy to 0.43 kGy in romaine lettuce and 0.54 kGy in spinach when treated with gamma radiation. In this case, the inoculated produce was stored for 72 h before treatment and the result was attributed to the formation of a bio-film upon storage, which protects the bacteria and reduces the antimicrobial efficacy of irradiation. This observation may serve as a guide to the processor or packer of leafy green vegetables.

Romaine lettuce

In case of romaine lettuce, the dose survival curve was linear with R^2= 0.99 for *Salmonella* and R^2= 0.99 for *E. coli* O157:H7. A reduction of 4.9 log CFU/g was seen for *Salmonella* with 1.09 kGy. The reduction for *E .coli* O157:H7 was 5.4 CFU/g with 1.09 kGy. The slopes of the lines were -4.3355 and -5.0697 for *Salmonella* and *E. coli* O157:H7 respectively (Fig. 2) and the D_{10}-value obtained was 0.23 kGy and 0.20 kGy for *Salmonella* and *E. coli* O157:H7 respectively. Niemira *(73)* studied effect of gamma

irradiation on romaine lettuce with internalized pathogens using a cocktail of 3 isolates of *E. coli* O157:H7. A D_{10}-value of 0.39 kGy was reported which is higher than the value reported in this study which indicates that leaf internalized *E. coli* O157:H7 is more irradiation resistant than the ones which are attached to the produce surface.

Niemira et al. *(73)* reported an approximately three to fourfold higher value for internalized pathogen than that obtained for surface inoculated *E. coli* O157:H7 on red leaf (0.12 kGy) and green leaf (0.12 kGy) lettuces. However, in that study D_{10}-values were also obtained in homogenized leaf tissue, a model system intended to predict the effect of leaf chemistry on internalized bacteria. In homogenized leaf tissue, the D_{10}-value of *E. coli* O157:H7 was 0.33–0.34 kGy (red leaf, green leaf, and Boston). Radiation sensitivity is largely influenced by the nature of the food substrate in which the pathogen is suspended *(73)*. Niemira et al. *(69, 70)* reported that the lettuce varieties influenced radiation sensitivity however, this phenomenon was not observed by Gomes et al. *(37)* who did not report any significant differences in D_{10}-value of *E. coli* O157:H7 among the lettuce varieties. Effects of X-ray radiation on *E. coli* O157:H7 and *S. enterica* inoculated on shredded iceberg lettuce were studied by Mahmoud *(58)* who found a significant reduction in pathogen population (4.4, and 4.8 log) with 1 kGy treatment. With a 2.0 kGy X-ray, more than a 5 log CFU/g reduction was achieved.

When iceberg lettuce was treated with low energy X-rays which was either dip inoculated or spot inoculated, the D_{10}-values for *E. coli* O157:H7 were much lower - 0.04 kGy and 0.078 kGy respectively.

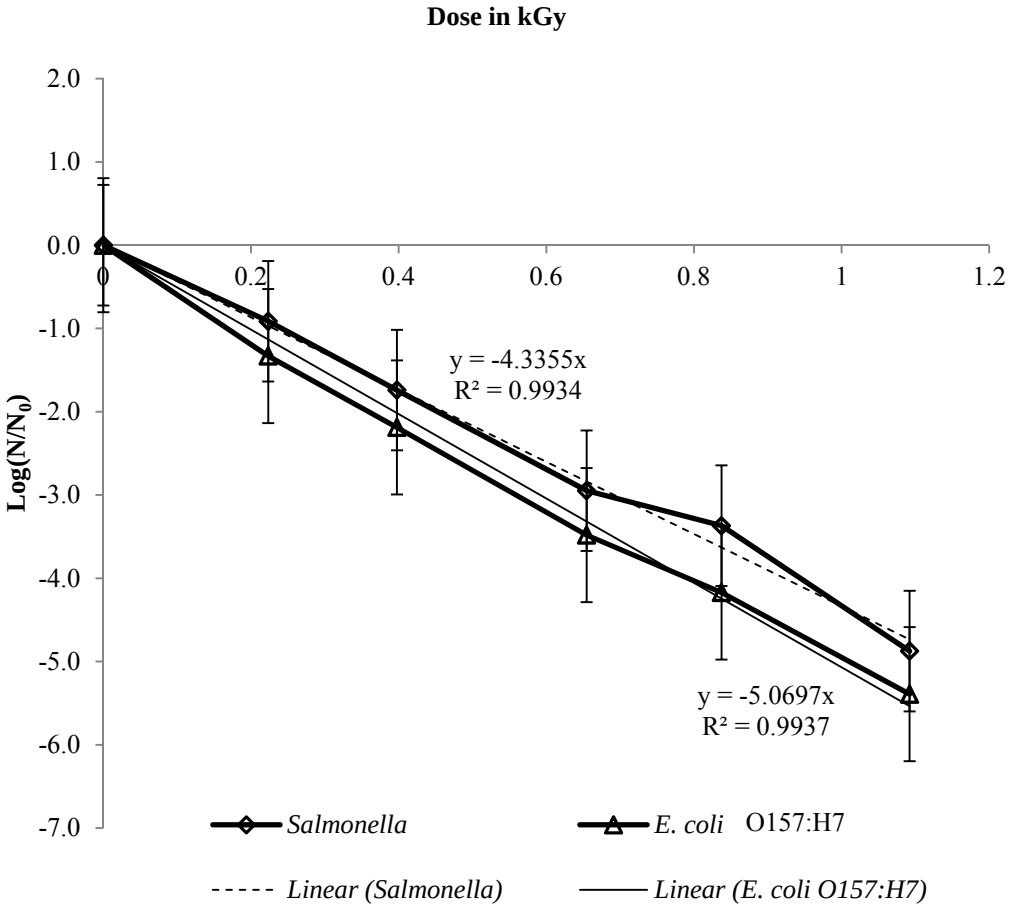

FIGURE 2. *Death curve of* Salmonella *and* E. coli *O157:H7 in romaine lettuce with increasing doses of irradiation.*

Cut cantaloupe

Irradiated cut cantaloupe also yielded a linear plot for *Salmonella* (R^2= 0.97). The graph was plotted with only 4 data points since the results for 0.79 kGy and 1 kGy were inconsistent. The data points used for plotting the graph were representative and

hence did not affect the ability to draw conclusions. The A reduction of 0.9 log CFU/g was seen from control (6.7 log CFU/g) to dose 0.66 kGy (5.8 log CFU/g). The slope was -1.4014 and the D_{10}-value from the reciprocal of slope was found to be 0.71 kGy. For *E. coli* O157:H7, graph was plotted with 3 data points (0 kgy, 0.20 kGy, and 0.66 kGy), since the counts for the remaining doses were inconsistent. The data point for 0.40 kGy was then fitted using the regression equation (Fig. 3). The slope for the fitted line was found to be -1.3682 with R^2= 0.99 and hence a D_{10}-value of 0.73 kGy is proposed for *E. coli* O157:H7 on cut cantaloupe.

Studies on pathogen reduction on cantaloupes using e-beam are very limited. Palekar *(77)* reported a D_{10}-value of 0.211 kGy for *S*. Poona on cut cantaloupe treated with e-beam irradiation in one study and in another study reported a 1.1 log reduction at 0.7 kGy and 3.6 log reduction at 1.5 kGy on cut cantaloupe for *S*. Poona *(77)* illustrating the fact that radiation sensitivity can be a function of product type, dose rate, sample preparation, and type of radiation. For example, the D_{10}-values using e-beam for *E. coli* O157:H7 and *S.* Typhimurium inoculated on broccoli seeds was 0.99 and 0.60 kGy respectively and for red radish seeds was 1.85 and 1.35 kGy respectively. The D_{10}-values using gamma radiation for *E. coli* O157:H7 and *S.* Typhimurium inoculated on broccoli seeds was 1.18 and 0.81 kGy respectively and on red radish seeds was 0.99kGy and 0.80 kGy respectively *(105)*. The same study determined D_{10}-values using e-beam

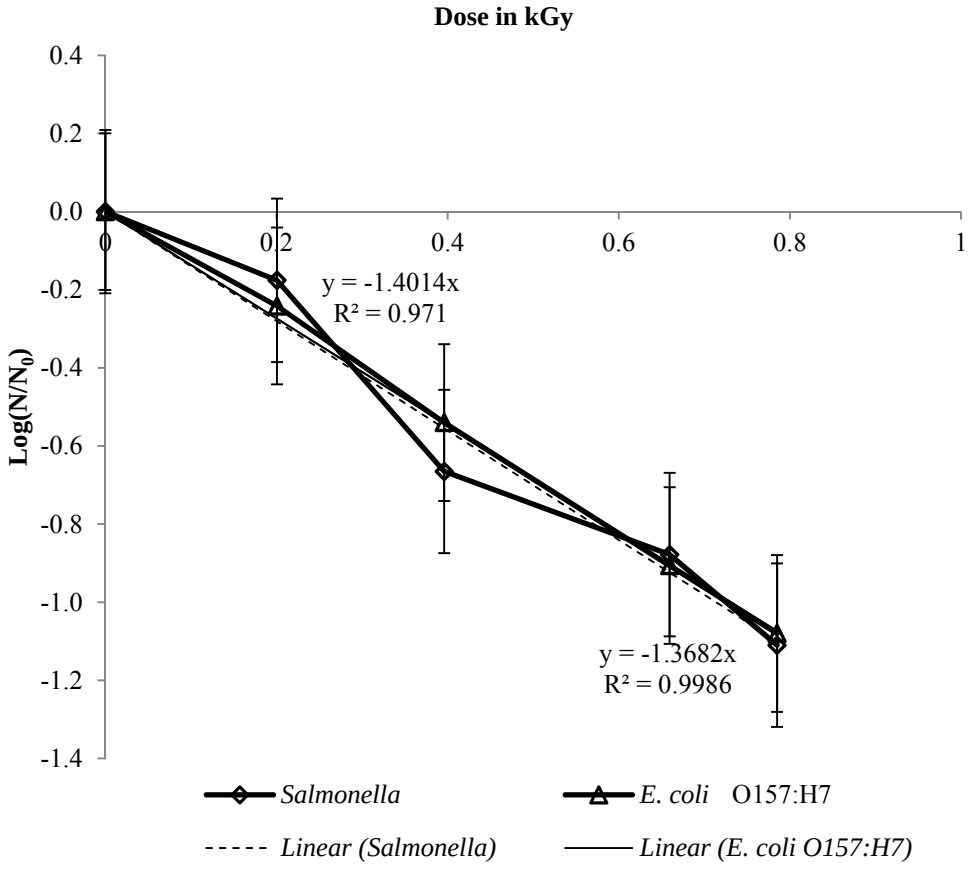

FIGURE 3. *Death curve of* Salmonella *and* E. coli *O157:H7 in cut cantaloupe with increasing doses of irradiation*.

gamma radiation for *E. coli* O157:H7 and *S.* Typhimurium inoculated on broccoli and red radish sprouts red radish sprouts. The values reported for *E. coli* O157:H7 and *S.* Typhimurium for broccoli were 0.73 and 0.3 kGy for e-beam and 0.46 and 0.13 kGy for gamma radiation respectively *(105)*.

Cut roma tomato

Irradiated cut roma tomato yielded a linear plot for dose vs survival for *Salmonella* with a slope -1.5552 (R^2= 0.97) (Fig. 4). A log reduction of 1.5 CFU/g was observed between control (6.6 CFU/g) and 1 kGy (5.1 kGy). Based on the slope obtained, a D_{10}-value of 0.64 kGy is proposed for *Salmonella.* Dose survival plot for *E. coli* O157:H7 was also linear with slope -1.8404 (R^2= 0.91) and a log reduction of 1.7 CFU/g was observed between control (6.5 log CFU/g) and 1 kGy (4.8 log CFU/g). A D_{10}-value of 0.54 kGy is proposed for *E. coli* O157:H7 in cut roma tomato.

In a previous study Prakash et al. *(81)* examined the efficacy of combined irradiation and a 1% calcium chloride dip to reduce the population of *Salmonella enterica* strains on diced tomatoes. The reported D_{10}-values ranged from 0.26 to 0.39 kGy. Another study on cut tomatoes by Schmidt et al. *(92)* evaluated effect of e-beam on two serotypes of *Salmonella*. While *S*. Montevideo showed a 1.8 and 2.2 log CFU/g reduction with 0.7 and 0.95 kGy respectively, *S*. Agona showed a 1.3 and 1.5 log CFU/g reduction at the same doses. James et al. *(46)* studied e-beam effect on nalidixic acid resistant *S*. Montevideo in cooked tomato puree of various pH values and reported that at pH 4.4 and 4.9, the D_{10}-values were 1.07 and 1.50 kGy respectively. This study indicates that pH plays an important role in radiation sensitivity.

Studies focusing on D_{10}-value determination for pathogens in produce are scarce. Grant et al. *(38)* reported D_{10}-values ranging between 0.37 and 0.70 kGy for different *Salmonella* serotypes in commodities such as mashed potato, roast potato, and cauliflower. Rajkowski et al. *(84)* reported D_{10}-values for sprouts which were inoculated

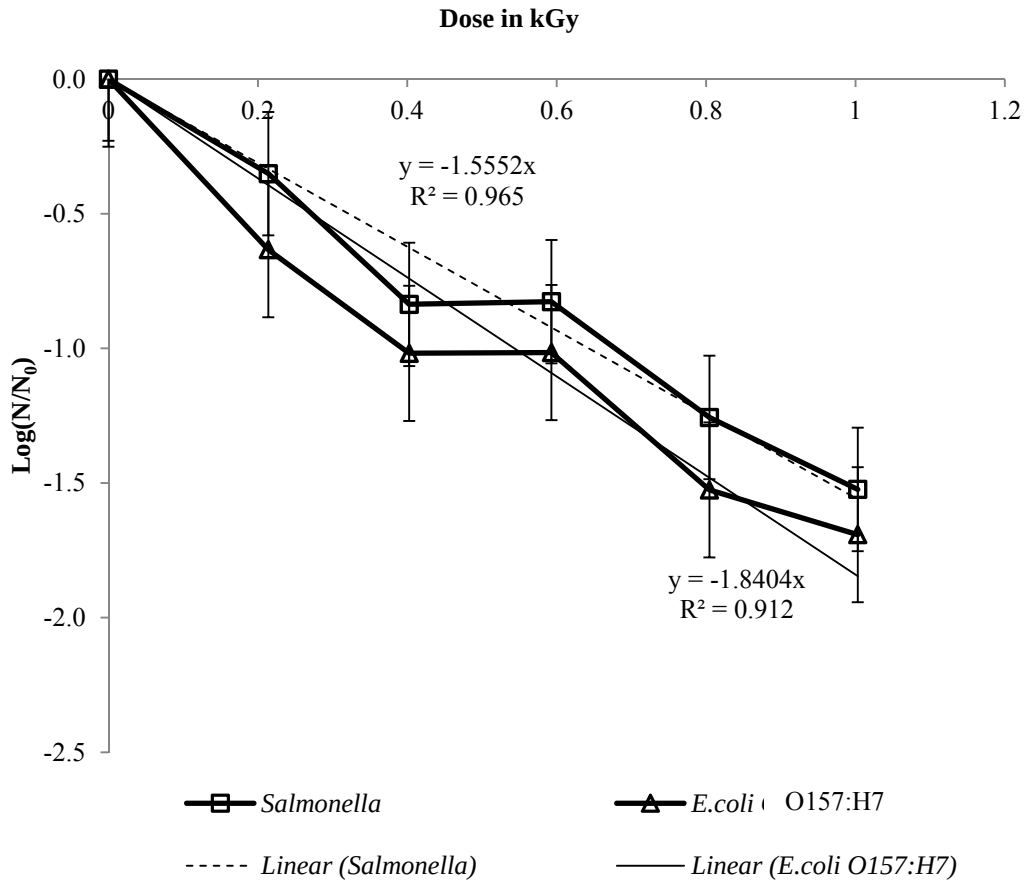

FIGURE 4. *Death curve of* Salmonella *and* E. coli *O157:H7 in cut roma tomato with increasing doses of irradiation.*

either with *Salmonella* or with *E. coli* O157:H7 cocktails made with either meat or vegetable isolates. The D_{10}-values for the *Salmonella* were 0.54 and 0.46 kGy and for *E. coli* O157:H7 were 0.34 and 0.30 kGy, respectively, for the meat and vegetable isolates.

Rodriguez et al. *(89)* used pathogenic and non-pathogenic strains to identify a potential surrogate to describe the radiation sensitivity of the most common pathogens

encountered in fruits. When the pathogen and the surrogate were inoculated in a real fruit cantaloupe, the D_{10}-values obtained were higher than those obtained with the model foods. This indicates that a model food system may not reproduce the variability of the real situation (i.e., pH, composition, and microstructure).

The differences in D_{10}-value of *E. coli* O157:H7 and *Salmonella* seen in this study in leafy green vegetables and cut tomato and cantaloupe may be attributed to the composition of the medium in which the pathogens are suspended. For example, different strains of *Salmonella* tested in orange juice were found to have D_{10}-values ranging from 0.35 to 0.71 kGy, depending on the strain *(72)*.

These differences may also be due to the packaging environment in which the produce are packed for irradiation treatment. According to Farkas *(31)*, oxygen content has a role to play in the radiation resistance of microbes. Presence of oxygen increases the lethal effect of irradiation. In absence of oxygen and in the presence of moisture, the radiation resistance increases 2 folds. In absence of moisture (dry conditions) without oxygen, resistance can increase by a factor of > 8 *(31)*. The microstructure of the surface on which the pathogens were inoculated also needs to be studied closely to understand the radiation resistance of the pathogens in question since the structure of the produce surface varies tremendously in lettuce and spinach to cut tomato and cut cantaloupe. While the leafy greens have a smooth microstructure, the cut tomato and cantaloupe have an uneven surface. The pathogens were inoculated onto the flesh of tomato and cantaloupe, the microstructure of the surface of which is relatively irregular; therefore

the microorganism may have been located in sections with different superficial and geometric characteristics leading to different sensitivity to radiation.

The use of e-beam irradiation for food safety purposes is an evolving research area, and the biological changes that take place during the irradiation of fresh-cut produce are not yet fully understood. D_{10}-values for any foodstuff should be established by taking into consideration all possible physical and environmental parameters beyond the characteristics of the target pathogen.

Quality and shelf life of fresh produce treated with high doses of e-beam radiation

Cut cantaloupe

Firmness

The firmness of cantaloupe irradiated at 1.09, 3.22, and 5.20 kGy was significantly ($P < 0.05$) lower than the control on day 0 (5.26 N), while the firmness value between 1.09, 3.22, and 5.20 kGy was not significantly different ($P > 0.05$) (Table 3). This observation is consistent with the study by Palekar *(76)* who saw lower firmness values for cantaloupe slices treated with e-beam dose 1.4 kGy but the slices treated with 0.7 kGy did not show any difference compared to non-irradiated control. However, a previous study conducted by Castell-Perez *(14)* reported no significant textural changes in fresh cut cantaloupe at low doses of 1 and 1.5 kGy, while at dose 3.1 kGy the cantaloupe had firmer texture. This was attributed to the presence of air in the packages which reduced overall density of the target thus improving penetration.

Boynton et al. *(8)* observed in a study that the inherent variability in the melons used gave no clear trends in differences in the texture of fresh cut cantaloupe packed in MAP treated with e-beam treatment doses of 0.5 and 1.0 kGy. In a second study however, no significant differences between treatments were observed at any storage dates. In another study by Boynton *(9)*, the cantaloupe pieces were treated with 0.3, 0.6, and 0.9 kGy e-beam dose. Firmness value for control cantaloupe cubes were highest on day 0 but softened between day 0 and 2 whereas firmness of all irradiated cantaloupe cubes did not change during storage.

There was an interaction between irradiation dose and storage time for firmness (Fig. 5). Firmness values for the control decreased over storage whereas firmness of irradiated fruits did not change over time (Fig. 5).

Color

Chroma values changed significantly ($P < 0.05$) for irradiated cut cantaloupe samples at all doses tested (1.09, 3.22, 5.20 kGy) compared to the non-irradiated control (Table 3) Chroma is indicative of the intensity/saturation of the color and all irradiated samples showed an increase in chroma values on day 0, the highest value was seen for dose 3.22 kGy (34.74) which is significantly ($P < 0.05$) higher than the control value 28.78. With Boynton et al. *(8)* reported no difference in color throughout storage period of 18 days, regardless of treatment. No specific trends were seen across time within

Table 3. *Least squares means for firmness, chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeast, molds, and LAB for cut cantaloupe as affected by different dosage of electron beam irradiation.*

Effect	Chroma	APC	Yeasts	Mold	LAB
RMSE	3.82	0.58	0.62	N/A	0.56
Dose	<0.0001[a]	<0.0001[a]	<0.0001[a]	N/A	<0.0001[a]
0	28.78[b]	6.3[e]	1.7[c]	<1[b]	6.3[d]
1.09	31.39[c]	2.7[b]	1.0[b]	<1[b]	3.2[c]
3.22	34.74[d]	3.8[d]	<1[b]	<1[b]	2.0[b]
5.20	31.93[c]	3.3[c]	<1[b]	<1[b]	2.4[b]
Days	0.0265[a]	<0.0001[a]	<0.0001[a]	N/A	<0.0001[a]
0	32.77[c]	1.5[b]	<1[b]	<1[b]	1.8[b]
3	31.76[c]	1.8[b]	<1[b]	<1[b]	2.4[c]
6	31.70[bc]	4.5[c]	<1[b]	<1[b]	4.0[d]
9	32.36[c]	5.4[d]	1.0[b]	<1[b]	4.1[d]
12	29.97[b]	6.9[e]	2.1[c]	<1[b]	5.0[e]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcde] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$)

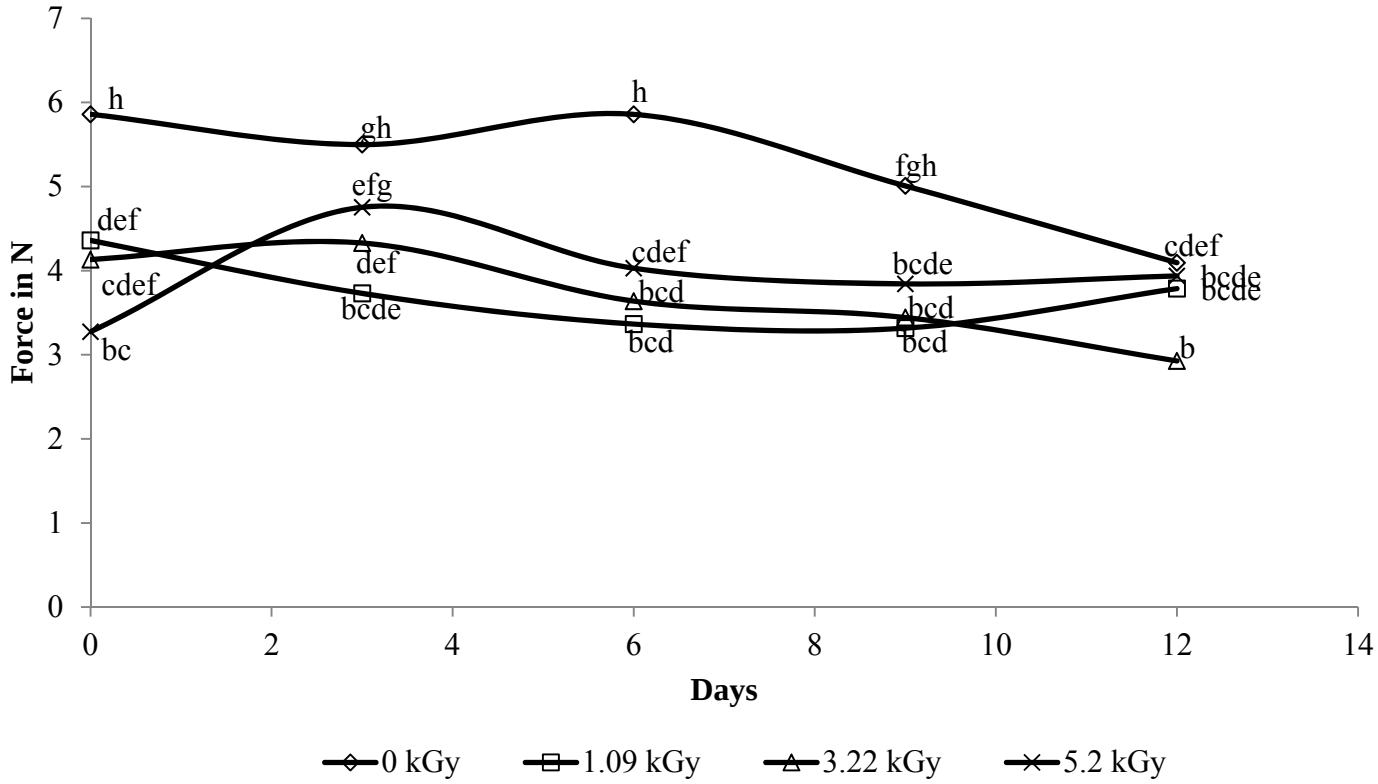

FIGURE 5. *Least squares means of firmness values (N) for cut cantaloupe as affected by interaction between storage days and irradiation dose as obtained using a texture analyzer. Data points with the same letter are not significantly different (P > 0.05).*

treatments between 0, 0.5, and 1.5 kGy and the color for fresh-cut cantaloupe remained stable for 18 days. Palekar et al. *(76)* reported that there was no change in L*, hue or chroma for cut cantaloupes irradiated at 0.7 and 1.4 kGy using e-beam. Fan et al *(28)* studied color changes in fresh cut cantaloupes that were treated with low dose gamma radiation after a hot or cold water wash on the whole fruit. They observed no change in chroma values at 0.5 kGy. There was no significant ($P > 0.05$) time and dose interaction for chroma.

Respiration rate

Respiration rate was higher for all e-beam treated samples on day 0 compared to the non- irradiated control (Table 3). Respiration rate decreased for cut cantaloupe at all doses tested (1.09, 3.22, and 5.20 kGy) including non- irradiated control from day 0 to day 3. From day 3 onwards, all irradiated samples had a lower but stable respiration rate. Respiration rate increased on day 9 and 12 for control indicating that the product underwent further cellular damage or senescence which may have increased the respiration rate. Minimally processed vegetables suffer substantial mechanical injury and tissue damage due to cutting and processing. The resulting increase in respiration rate according to Adam et al. *(1)* may be associated with an activation of glycolysis and sugar consumption. E-beam irradiation is known to injure cells when hit by electrons causing an increase in metabolism and respiration rate. Boynton et al. *(8)* made similar observations on cut cantaloupe treated with low doses of e-beam noting that irradiated samples had a lower and more stable rate of respiration than non-irradiated control

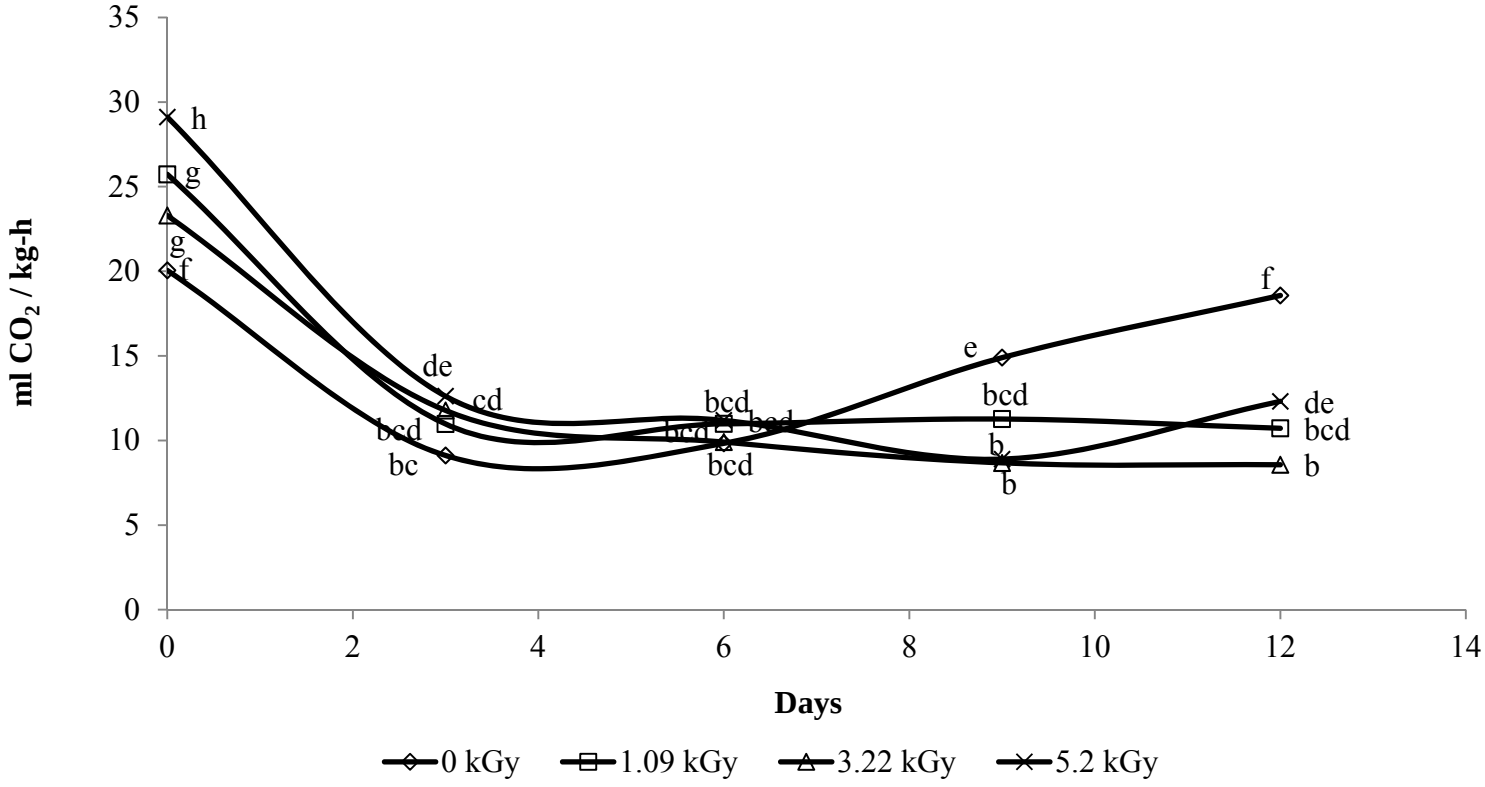

FIGURE 6. *Least square mean respiration rate values (ml CO_2 / kg-h) for cut cantaloupe as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

samples for approximately 20 days under MAP. In another study by Boynton et al. *(9)*, cantaloupes which were not packed in a MAP were treated with low dose e beam.Respiration rates were initially higher in irradiated cantaloupe but over a storage period of 8 days, respiration rate reduced and was comparable to the control. There was an interaction between storage time and irradiation dose for respiration rate ($P < 0.05$) (Fig. 6). Respiration rate for control increased during storage and reached day 0 levels by the end of storage. All irradiated samples had a lower respiration rate throughout storage (Fig. 6)

Microbiological counts

E-beam irradiation reduced the APCs on cut cantaloupe from 6.3 log CFU/g (control) to 2.4 log CFU/g for 1.09 kGy, 3.5 log CFU/g for 3.22 kGy, and 3.0 log CFU/g for 5.20 kGy (Table 3). On day 0, APCs for non-irradiated control was 3.8 log CFU/g and was below detection limit of 1 log CFU/g for all irradiated samples. There was no growth on day 3 for the treated samples whereas the control showed a 1.2 log increase in APC. APCs were detected on treated samples on day 6 - 12. By the end of the storage, APCs were similar for all treated doses but was still lower than the control (8.2 log CFU/g) (Fig. 7). Irradiated samples exhibited lower rate of microbial growth than the non-irradiated control. Boynton et al. *(9)* also had similar findings on e-beam treated fresh cut cantaloupe. Palekar et al. *(76)* tested wash treatments coupled with e-beam irradiation and their findings were also in line with this study. At 0.7 kGy, a reduction of

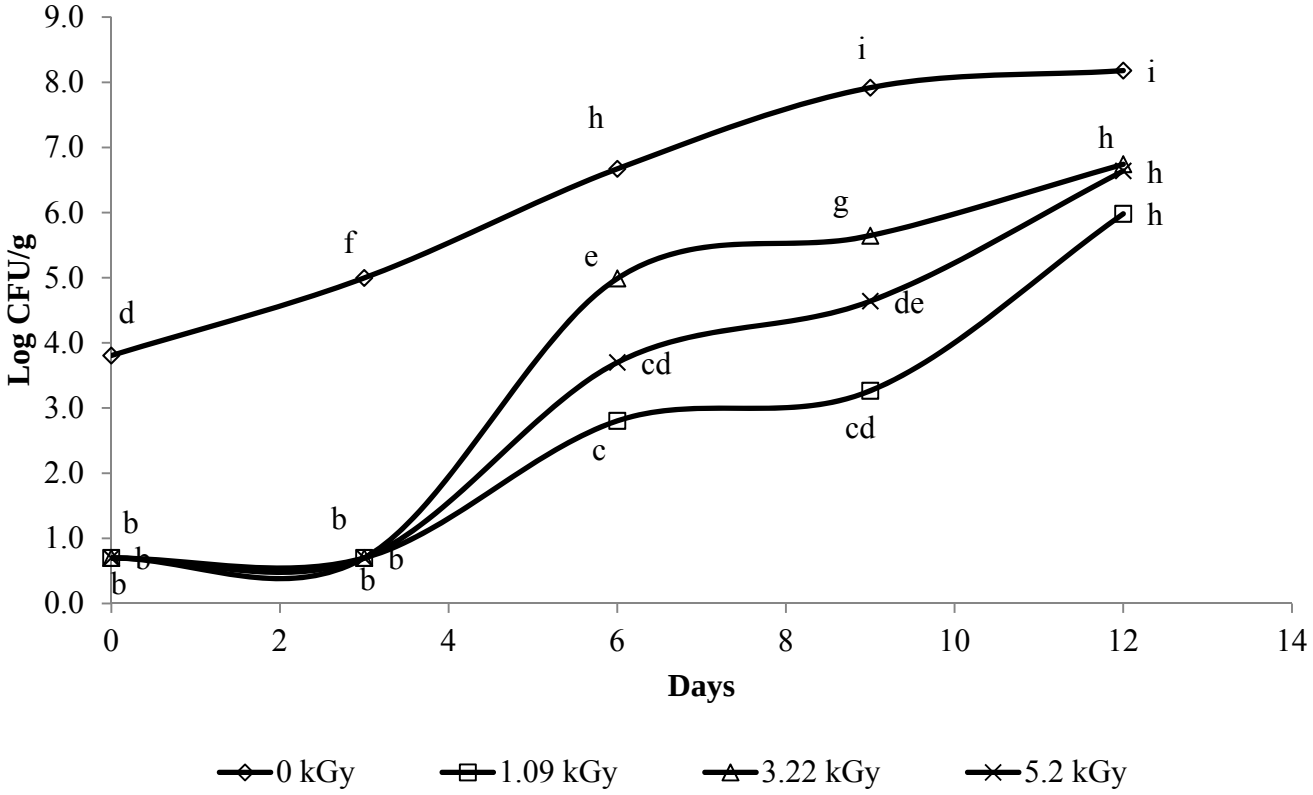

FIGURE 7. *Growth of APCs on irradiated cut cantaloupe stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

2.0 log CFU/g was observed compared to the non-irradiated fruit (2.7 log CFU/g) and APCs were below the detection limit at 1.4 kGy *(76)*.

Yeast counts for cut cantaloupe were below detection limit of 1 log CFU/g for doses 1.09, 3.22 and 5.20 kGy including control on day 0 (Table 3). Yeast counts increased for the control on days 9 – 12. For dose 1.09 kGy, yeast counts increased by 4.7 logs on day 12 (Fig. 8). Mold counts were below detection limit for non-irradiated control and all irradiated samples and no growth was detected in any samples during storage period. Previous studies have reported that e-beam irradiation had less effect on the YNM counts with no differences among treatments at any storage times. YNM counts of all samples increased approximately 4 logs by day 13 *(9)*. Palekar et al. *(76)* reported that e-beam irradiation did not reduce yeasts to a significant extent at either 0.7 or 1.5 kGy. The control exhibited a greater increase in yeast counts over 21 days of storage than cantaloupes irradiated with 0.7 or 1.5 kGy. For both doses, no difference in counts was observed throughout the duration of storage. For molds however, Palekar et al. *(76)* observed that with 0.7 and 1.5 kGy irradiation , a 0.5 and 1.7 log CFU/g reduction seen which was comparable to the control. In this study, no mold growth was noted even for the control. The reason could be due to the use of separate sterile knives for skin peeling and slicing the fruit which resulted in minimal cross contamination.

LAB counts were reduced in all treated samples (3.1 log CFU/g with 1.09 kGy, 4.7 log CFU/g with 3.22 kGy, and 4.3 log CFU/g with 5.20 kGy) (Table 3) compared to the control. There was a significant ($P < 0.05$) reduction in counts between the control and all treated samples through the entire duration of the study. However, no significant

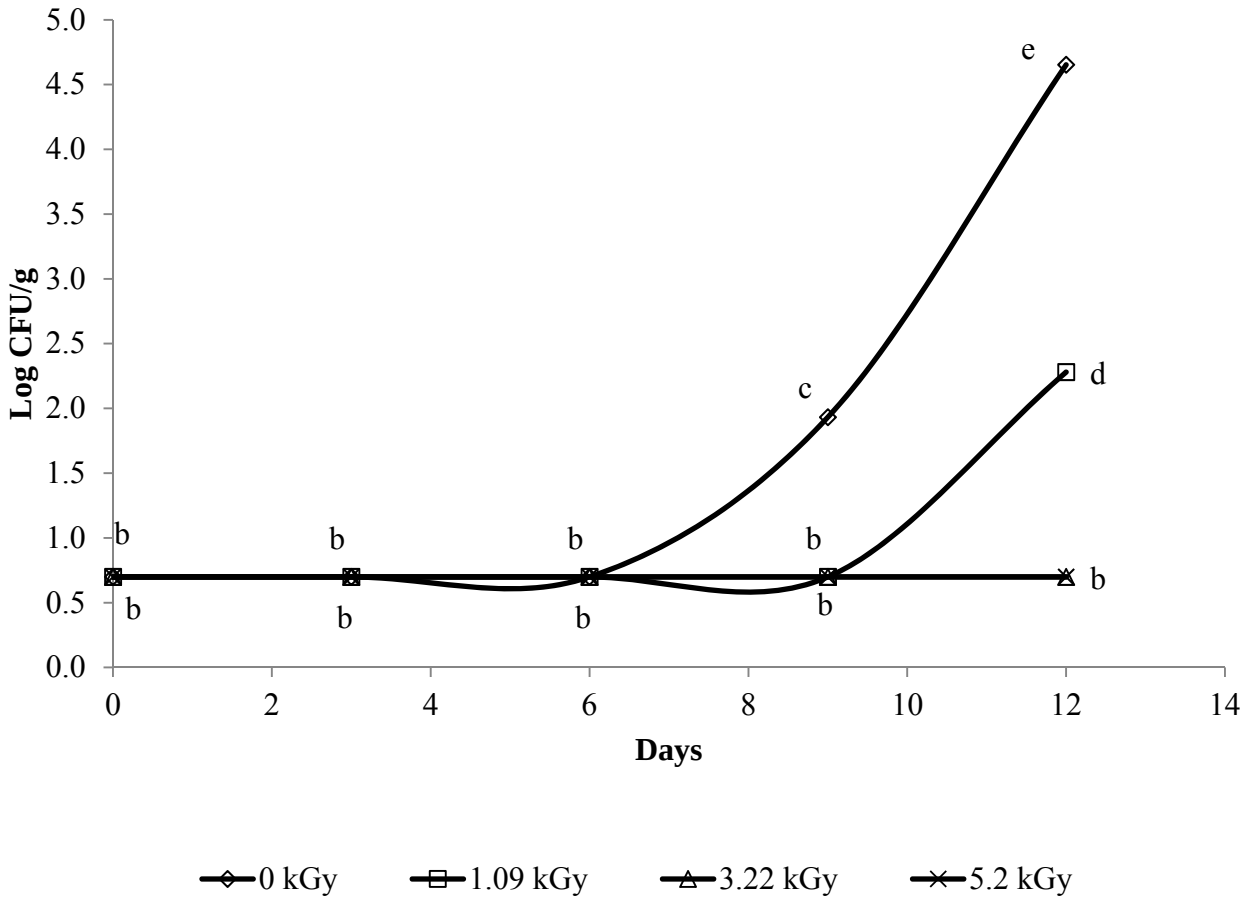

FIGURE 8. *Growth of yeasts on irradiated cut cantaloupe stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

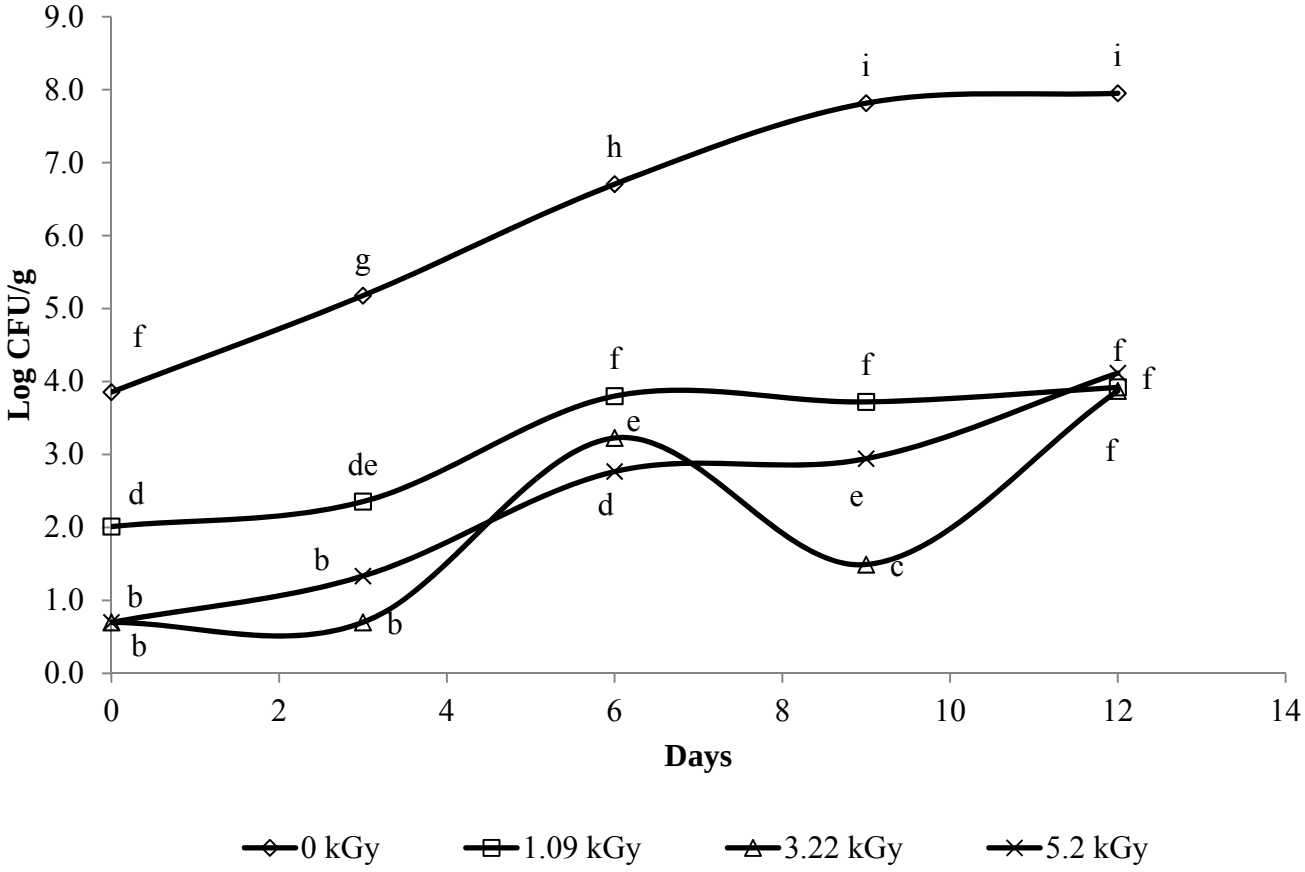

FIGURE 9. *Growth of LAB on irradiated cut cantaloupe stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

($P > 0.05$) difference was noted in counts between 3.22 and 5.20 kGy (Fig. 9). Yeasts, molds, and gram-positive spoilage organisms, such as LAB, are more resistant to irradiation than gram-negative organisms, such as *Salmonella (62)*. Previous studies have shown that LAB grow well at refrigeration temperatures especially in a sugar-rich medium like cantaloupe. They have been shown to increase by 3.5 log CFU/g on fresh-cut cantaloupe stored at 4°C for 14 days *(51)*. No LAB counts were detected at 4°C which was also evidenced by the absence of lactic acid production in the fruit throughout storage. Palekar et al. *(76)* in their study on irradiated sliced cantaloupe reported reduction in LAB counts by 0.2 CFU/g with 0.7 kGy and 2.9 CFU/g with 1.5 kGy on day 0. Also, there was no significant difference in counts between the control and samples irradiated at 0.7 kGy throughout the duration of the study.

Cut roma tomatoes

Firmness

For cut roma tomato, change in firmness was significant ($P < 0.05$) for all doses tested as compared to non-irradiated control (2.91 N). The difference was dose dependent with 1.03 kGy exhibiting least reduction in firmness value (2.17 N) and 5.17 kGy exhibiting a greater decrease (1.28 N) (Table 4). There was no significant interaction between storage time and irradiation dose in case of firmness. Previous studies have shown irradiation effects on whole tomatoes as in case of El Assi *(26)* who saw changes in firmness in irradiated whole tomato and pericarp tissue. These authors reported that the trend in firmness change was dose dependent over storage. In another

study, the textural properties of carrot, potato, and beetroot decreased as the dose of gamma irradiation increased up to 12.0 kGy *(64)*. The histological examination of the plant material confirmed that gamma irradiation resulted in breaking up of cell walls, which resulted in the softening of the tissue but a pre-treatment with calcium was found to reduce the damage. Similar findings were reported by Magee et al. *(57)* for cut roma tomatoes where instrumental firmness reduced for all gamma irradiated all samples; however, when the tomatoes were dipped in 1% calcium chloride and 2% calcium lactate solution, samples remained firmer than the water-dipped control.

Color

E-beam irradiation on cut roma tomatoes decreased chroma values when the dose applied was 5.17 kGy. However, with 1.03 and 3.15 kGy, no significant ($P < 0.05$) difference in chroma values was noted compared to the non-irradiated control (Table 4). On day 0, chroma value for control was 16.95 whereas with 1.03 and 3.15, 5.17 kGy, the values were 19.00, 18.56, and 15.68 respectively. In this study, there was no time and dose interaction for chroma. Chroma values on day 0 were lower for all samples including control compared to day 3 which could be attributed to the variability caused by the colorimeter operator. Though no studies have reported color changes in e-beam irradiated cut tomatoes, effects of gamma radiation in biosynthesis of carotenoids in tomato fruit was studied by Villegas et al. *(103)* who noted that radiation retarded red color development in ripening tomato fruits. The results also indicated that the synthesis

Table 4. *Least squares means for firmness (N), chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeasts, LAB for cut roma tomato as affected by different dosage of electron beam irradiation.*

Effect	Texture (N)	Chroma	APC	Yeasts	Mold	LAB
RMSE	0.66	3.32	0.54	0.33	0.0	0.60
Dose	<0.0001[a]	<0.007[a]	<0.0001[a]	<0.0001[a]	N/A	<0.0001[a]
0	2.91[e]	18.20[c]	6.7[d]	4.9[e]	<1[b]	4.7[d]
1.03	2.17[d]	18.15[c]	3.3[c]	3.2[d]	<1[b]	3.8[c]
3.15	1.62[c]	17.32[bc]	3.4[c]	2.1[c]	<1[b]	2.1[b]
5.17	1.28[b]	16.03[b]	2.8[b]	1.3[b]	<1[b]	1.7[b]
Days	0.0008[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]	N/A	<0.0001[a]
0	2.21[c]	17.34[c]	1.6[b]	1.3[b]	<1[b]	1.3[b]
3	2.20[c]	19.74[d]	1.9[b]	1.3[b]	<1[b]	2.2[c]
6	2.03[c]	17.86[c]	4.7[c]	2.7[c]	<1[b]	3.4[d]
9	1.90[bc]	16.55[bc]	5.3[d]	3.8[d]	<1[b]	3.8[d]
12	1.62[b]	15.66[b]	6.8[e]	5.3[e]	<1[b]	4.6[e]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcdef] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$)

of beta carotene was not affected by increasing the radiation dose but lycopene synthesis, however, appeared to be dependent on the radiation dose.

Respiration rate

Respiration rate was higher for all e-beam treated (1.03, 3.15, and 5.17 kGy) cut roma tomato samples on day 0 when compared to the non-irradiated control. But during storage, treated samples had a significantly ($P < 0.05$) lower but stable respiration rate as compared to the control (Table 4). There was an interaction between storage time and irradiation doses ($P < 0.05$) and the control had a higher respiration rate on day 9 and 12 similar to cut cantaloupe in this study. While with 1.03 kGy, the respiration rate increased during storage, with 3.15 kGy, it did not change and with 5.17 kGy, the rate decreased (Fig. 10). Prakash et al. *(79)* noted that diced tomatoes when treated with 1% calcium chloride, or irradiation, or combination suppressed respiration during storage while the control sample had a brief rise in respiration rate on day 2. The increase in respiration rate of the control sample was similar to the observation made for cut cantaloupe in this study which could be a result of wounding induced by dicing. Mechanical injury accelerates water loss, and increase respiration rate in fruits and vegetables *(6)*.

Microbiological counts

E-beam irradiated roma tomatoes exhibited significant reduction ($P < 0.05$) in APCs with 1.03, 3.15, and 5.17 kGy compared to non-irradiated control (Table 4). A 3.7

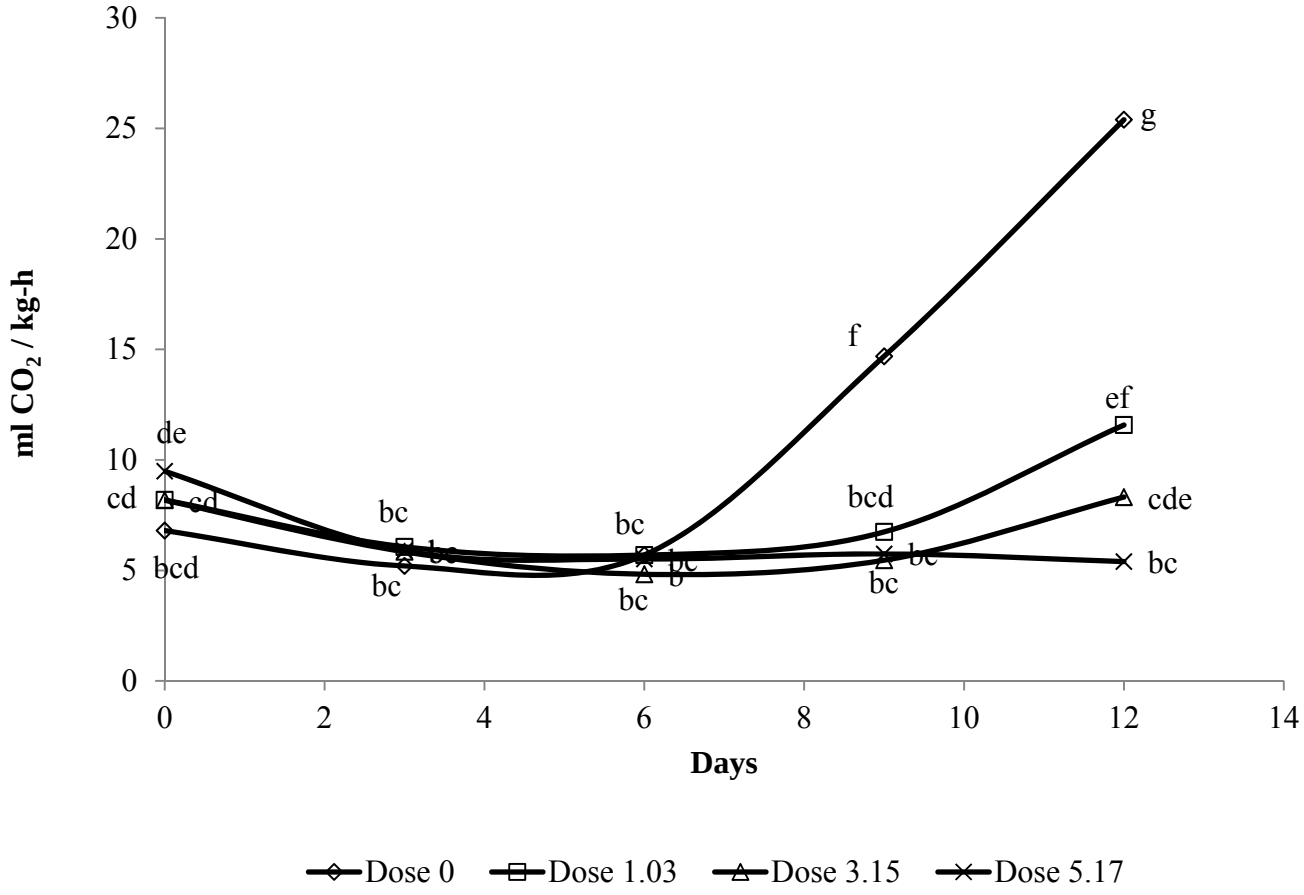

FIGURE 10. *Least squares mean values for respiration rate (ml CO_2 / kg-h) for cut roma tomatoes as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

log CFU/g reduction was noted with 1.03 kGy, a 3.6 log CFU/g reduction with 3.15 kGy and a 4.2 log CFU/g reduction was noted compared to control (6.7 log CFU/g). APCs grew more rapidly with 1.03 and 3.15 kGy compared to 5.17 kGy (Fig. 11). On day 0, APC for non-irradiated control was 3.9 log CFU/g and was below detection limit of 1 log CFU/g for all treated samples. There was no growth on day 3 for the treated samples whereas the control showed a 1.7 log CFU/g increase in APC count (Fig. 11). APCs were detected on treated samples on day 6-12. Results indicate that a low dose of 1.03 kGy is sufficient to reduce APCs on cut tomatoes and maintain the counts lower than the non-irradiated pieces upto 12 days at 5°C. However, Prakash et al. *(79)* observed that irradiation at 1 kGy initially decreased microorganism counts (except YNM) in diced tomatoes by greater than 3 log CFU/g but, the microorganism populations in the irradiated sample reached the initial numbers of total APCs in the control samples only after day 8 for vine variety and day 10 for celebrity tomato.

Yeast counts were reduced significantly ($P < 0.05$) on all irradiated cut tomato samples compared to the non-irradiated control and the reduction was dose dependent (Table 4). Dose 1.03 kGy had the least reduction (1.7 log CFU/g) followed by 3.15 kGy (2.9 log CFU/g) and 5.17 kGy (4.1 log CFU/g) compared to the control (4.9 log CFU/g). Yeast counts were below detection limit of 1 log CFU/g on day 0 with dose 5.17 kGy and increased on days 9 and 12 (Fig. 12). The rate of growth was higher in control samples compared to the irradiated samples which indicate that the yeast cells were killed during irradiation and the residual injured cells start growing at 5°C.

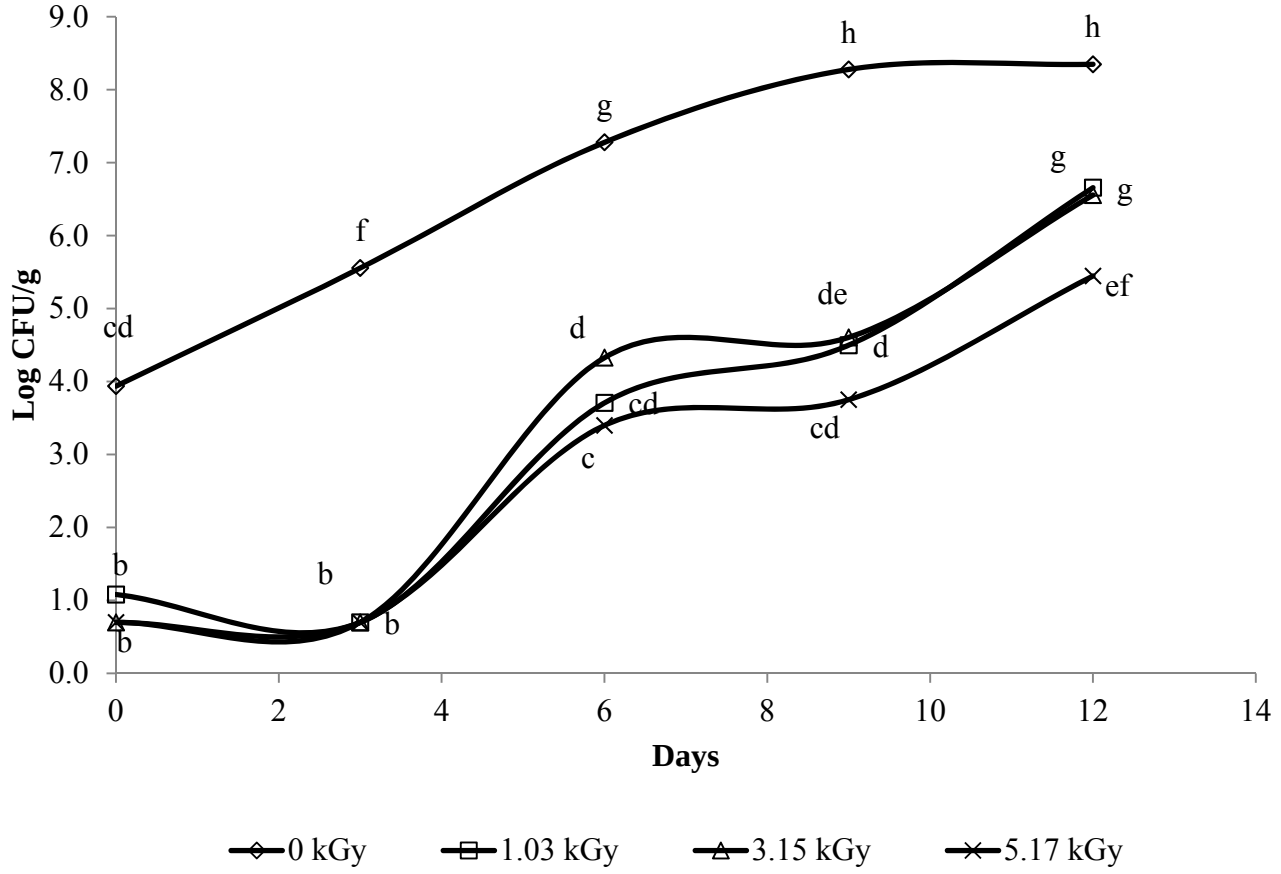

FIGURE 11. *Growth of APCs on irradiated cut roma tomatoes stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

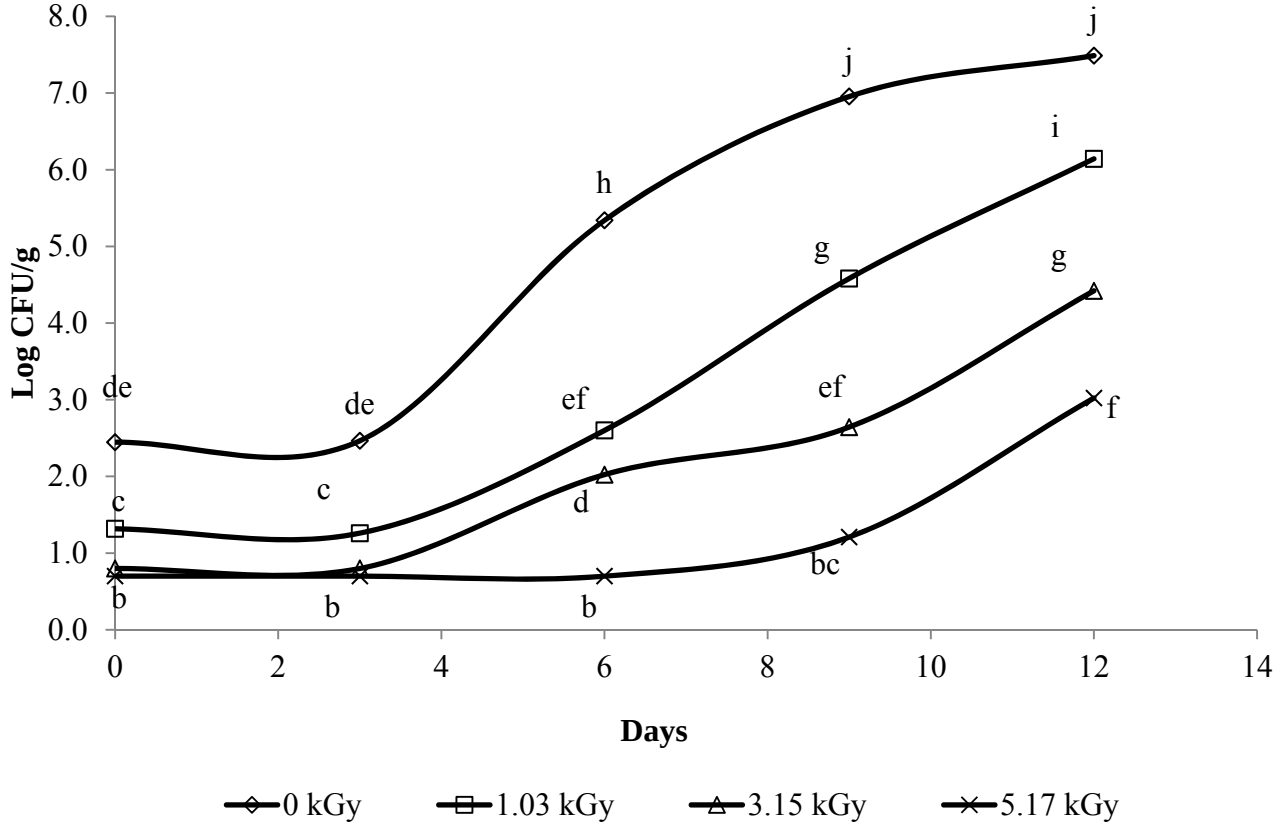

FIGURE 12. *Growth of yeasts on irradiated cut roma tomatoes stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

There was a time and dose interaction (Fig. 12) showing higher rate of growth with 1.03 kGy followed by 3.15 kGy and least growth rate with 5.17 kGy. Similar to cut cantaloupe in this study, no mold growth was noted in any samples including the control throughout the storage period. Tomato slicing was done using sterile knives thus preventing any cross contamination which could explain the mold counts in this study However, in a study where readymade packed slices of tomatoes were treated by 0.5 and 1.24 kGy gamma radiation had 1log CFU/g reduction initially. With 1.24 kGy, a consistently lower YNM counts for days 3-12 was noted but the counts reached similar levels for all samples of tomatoes by day 15 *(82)*. YNM counts on tomato cubes fell below the lowest detection limit after treatment with irradiation with 0.7 and 0.95 kGy in a study by Schmidt et al. *(92)*.

LAB counts were reduced by 1.0 log CFU/g with 1.03 kGy, 2.9 log CFU/g for 3.15 kGy, and 3.4 log CFU/g with 5.17 kGy during storage (Table 4). On day 0, irradiation reduced the counts to below detection limit of 1 log CFU/g for all doses tested compared to control (3.2 log CFU/g). But counts increased for dose 1.03 kGy on day 3 and were as high as counts for control on day 3. By day 12, the counts for control and 1.03 kGy were not different ($P > 0.05$) but were higher than 3.15 and 5.17 kGy (Fig. 13). Schmidt et al. *(92)* reported 1.3- and 2.8-log reduction of LAB on tomato cubes with e-beam doses of 0.7 and 0.95 kGy, respectively but there was no difference in counts between the doses tested *(92)*. LAB counts were variable in tomato cubes throughout the storage period of 15 days at 4°C and the most variable counts were reported with 0.7 kGy *(92)*.

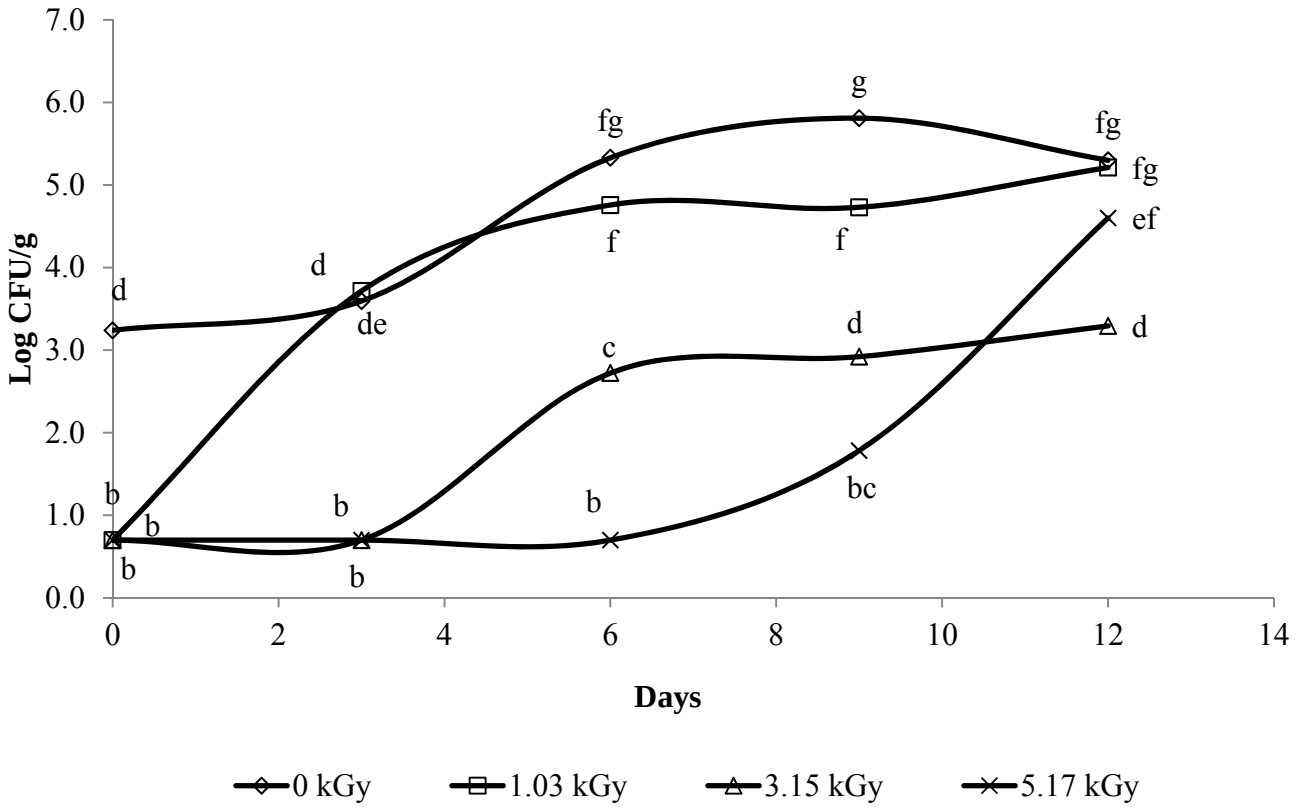

FIGURE 13. *Growth of LAB on irradiated cut roma tomatoes stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

Baby spinach

Firmness

E-beam irradiation significantly ($P < 0.05$) decreased firmness in baby spinach for doses 1.01, 3.05, and 5.16 kGy as compared to non-irradiated control. The change was dose dependent with the least dose showing higher firmness value (158.74 N) and the highest dose showing least firmness value (98.46 N). The control had the maximum firmness (168.91 N) (Table 5). On day 0, firmness values with 1.01, 3.05, and 5.16 kGy were 160.27, 136.79, and 122.70 N respectively. Over the storage period, a dose dependent decrease in firmness was noted (Fig. 14). By day 12, values for firmness between control and 1.01 kGy were comparable and the spinach treated with 5.16 kGy had a soggy appearance. The leakage was prominent and at the end of 12 days, the spinach leaves were bundled up in a lump inside the bag. Irradiation at higher doses (above 1 kGy) is seen to cause an increase in electrolyte leakage of many fresh-cut fruits and vegetables *(29)*. The soggy and wilted appearance of leafy vegetables may be due to increased electrolyte leakage. Electrolyte leakage is often an indication of cell membrane damage. In a study of thirteen vegetables, Fan and Sokorai *(29)* observed that red cabbage, broccoli, endive, celery, carrot, and green onion all had increased electrolyte leakage with cabbage, broccoli, and endive having the lowest increase and celery, carrot, and green onion had the highest increase in leakage. Gomes et al. *(36)* however, did not find any change in texture of baby spinach at 1 kGy e-beam dose.

Table 5. *Least squares means for firmness (N), chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeast and Mold, LAB for baby spinach as affected by different dosage of electron beam irradiation.*

Effect	Chroma	APC	Yeast	Mold	LAB
RMSE	3.26	0.59	0.60	0.20	0.51
Dose	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]
0	28.60[b]	7.6[e]	3.1[d]	2.5[c]	5.8[e]
1.01	32.35[d]	4.9[d]	3.4[d]	2.2[c]	4.6[d]
3.05	30.06[c]	4.3[c]	2.2[c]	<1[b]	2.7[c]
5.16	30.61[c]	1.2[b]	1.1[b]	<1[b]	1.6[b]
Days	0.0192[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]
0	29.00[b]	3.4[b]	1.2[b]	1.0[b]	2.8[b]
3	30.58[c]	3.6[b]	1.6[b]	1.4[c]	3.4[c]
6	31.23[c]	4.9[c]	2.3[c]	1.5[c]	3.7[cd]
9	29.90[bc]	5.1[c]	3.0[d]	1.7[d]	3.9[d]
12	31.32[c]	5.6[d]	4.0[e]	2.1[e]	4.5[e]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcde] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$).

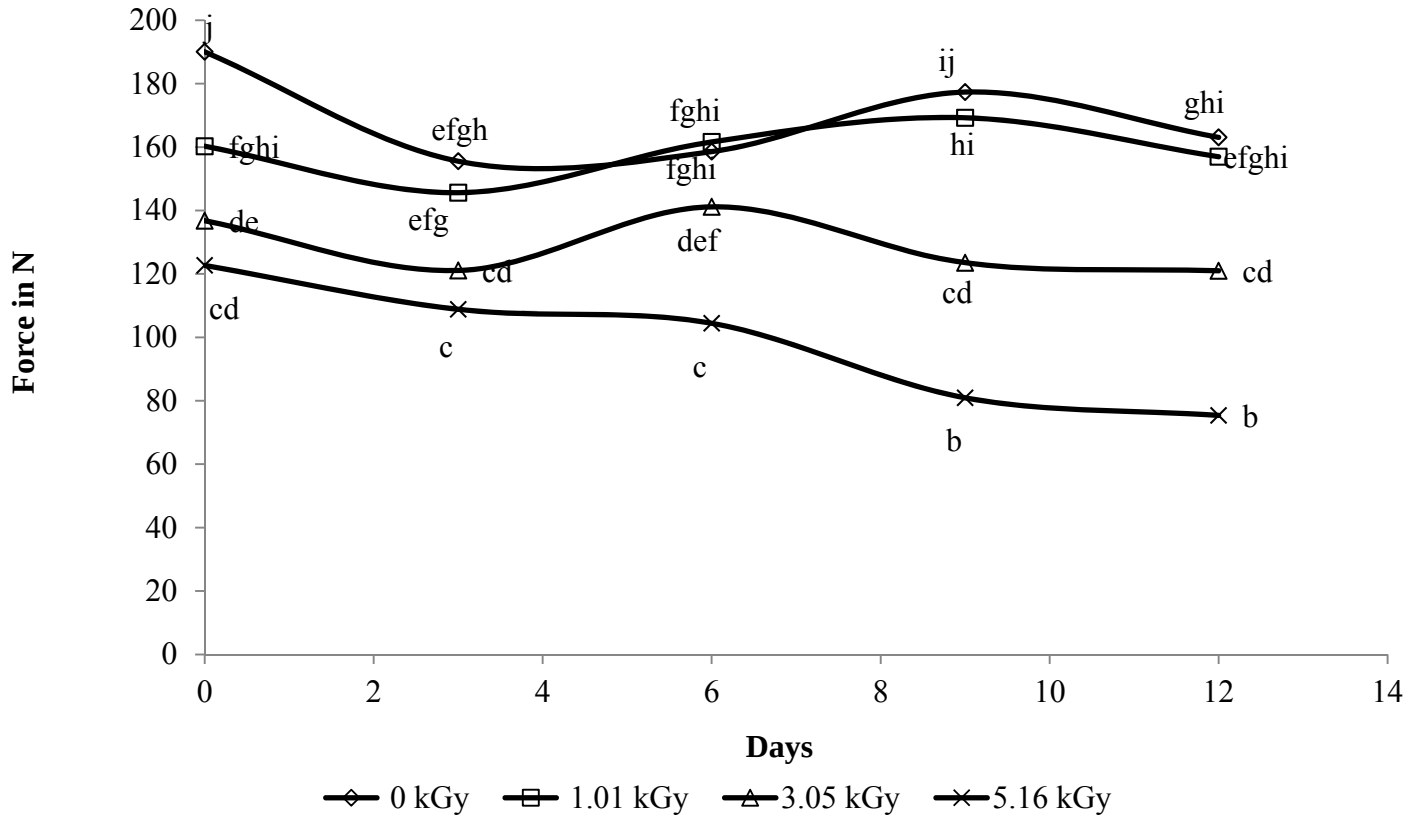

FIGURE 14. *Least Square Mean values for firmness for baby spinach as affected by interaction between storage days and irradiation dose as obtained using a texture analyzer. Data points with the same letter are not significantly different (P > 0.05).*

Color

E-beam doses of 1.01, 3.05, and 5.16 kGy increased chroma values significantly ($P < 0.05$) compared to the non-irradiated control (Table 5). But between doses 3.05 and 5.16, there was no statistically significant ($P < 0.05$) difference. There was no change in chroma during storage over a period of 12 days for all doses except for dose 1.01 which showed a very high chroma value on day 12. Gomes et al. *(36)* concluded that exposure to ionizing radiation up to 1 kGy did not affect the color attributes of the spinach leaves; however, samples treated at 1 kGy were yellowish after 7 days than the rest of the samples. X-ray treatment at 2 kGy did not significantly affect the color of spinach leaves *(59)*. Neal et al. *(65)* reported that the panel for color evaluation found some variation in color of e-beam irradiated spinach, but objective measurements of lightness, hue or chroma values for spinach were not affected by treatment, field, or storage period and interactions between treatments.

Respiration rate

E-beam treated baby spinach at all doses had higher respiration rate compared to control on day 0 (Table 5). Respiration rate decreased for all samples including the control from day 0 to day 3. There was an interaction between storage period and treatment dose ($P < 0.05$) and respiration rate decreased for control as well as all treated samples over storage. This may be due to wound induced injury from irradiation dose increasing respiration rates due to structural damage to the spinach cell walls and pectins

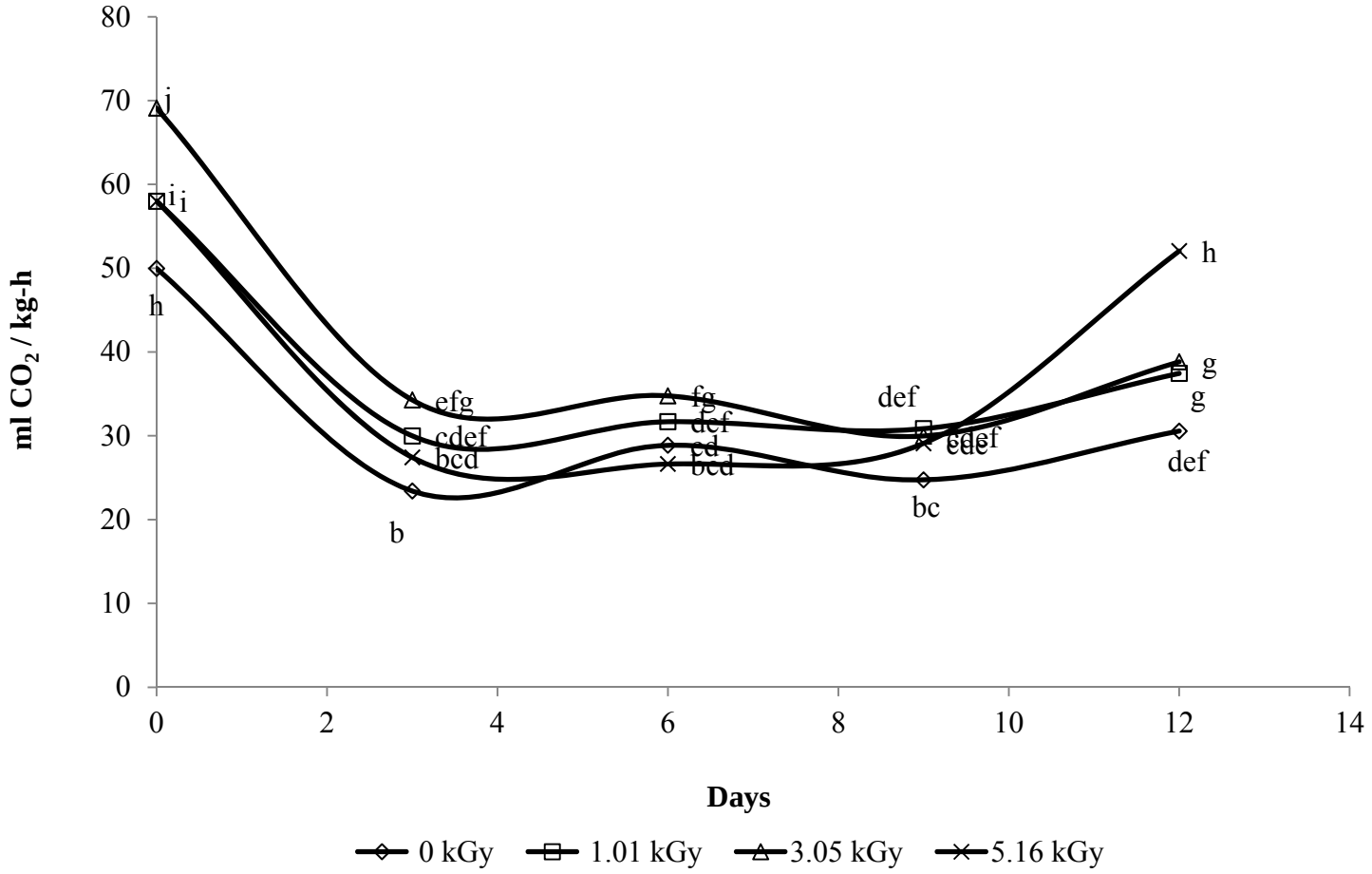

FIGURE 15. *Least square means of respiration rate values (ml CO_2 / kg-h) for baby spinach as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

which provide structural rigidity to the leaf. Neal et al. *(66)* studied effect of e-beam irradiation on commercially available bagged baby spinach at 1.2, 2.1, and 3.2 kGy and reported that the respiration rate of spinach showed no changes after e-beam irradiation up to 2.1 kGy.

Microbiological counts

E-beam irradiation reduced APCs in baby spinach significantly ($P < 0.05$) and the reduction was dose dependent. Highest reduction of 6.5 log CFU/g was seen with 5.16 kGy followed by 3.3 log CFU/g reduction with 3.05 kGy and a 2.7 log CFU/g reduction with 1.01 kGy (Table 5). On day 0, APCs for all irradiated samples were significantly reduced compared to the control (6.6 log CFU/g). While APCs increased with 1.01 and 3.05 kGy on day 6 - 12, spinach irradiated with 5.16 kGy did not show any increase in APCs throughout storage (Fig. 16). This indicates that a very high e-beam dose inflicted irreparable lethal injury to microorganisms and hence they did not proliferate during storage. Neal et al. *(65)* reported APC reduction by 2.6 and 3.2 log CFU/g at 0.7 and 1.4 kGy, respectively in spinach treated with e-beam. They reported an increase of 0.7 log CFU/g in APCs after 14 days of storage at 4°C in control. Also, the irradiation dose of 0.7 kGy resulted in a 2.6 log CFU/g decrease in the microbial load on day 0. After that a reduction of 2 log CFU/g was maintained through 14 days. Samples treated with 1.4 kGy resulted in a 3.2 log CFU/g reduction as compared to control samples on day 0 and maintained a reduction of 3 log CFU/g through a 14 day storage period *(65)*.

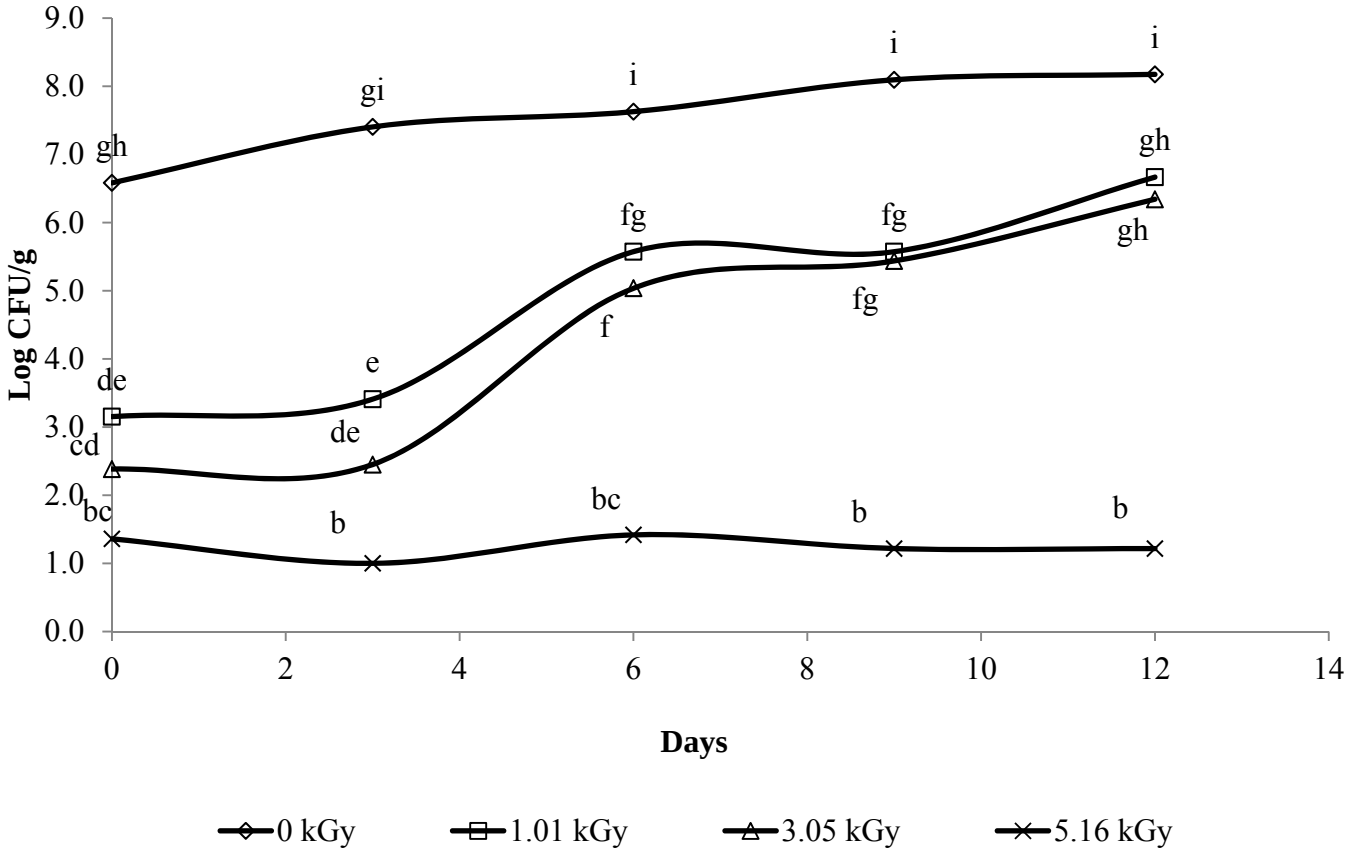

FIGURE 16. *Growth of APCs on irradiated baby spinach stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

Yeast counts were not significantly ($P < 0.05$) different for dose 1.01 kGy compared to the control. A 1.3 log CFU/g reduction was seen with 3.05 kGy and a 2.5log CFU/g reduction was noted with 5.16 kGy over storage (Table 5). On day 0, yeast counts were below detection limit for 3.05 and 5.16 kGy. Yeast counts increased rapidly with 1.01 and 3.05 kGy during storage with counts as high as control on day 12 (Fig. 17). The rate of growth with 5.16 kGy was slower and at day 12 had lower counts than control. The effect on reducing yeast counts was dose dependent with a higher dose resulting in higher reduction and a lower dose resulting in lower reduction. Mold counts were reduced below detection limits with 3.05 and 5.16 kGy on day 0 and continued to remain the same throughout storage (Fig. 18). There was no reduction seen with 1.01 kGy on mold counts and they continued to grow as high as control during storage thus indicating that the storage temperature of 5^{o}C did not appear to suppress growth of yeasts and molds. A majority of studies report both YNM counts as one entity. However, this study enumerated YNM separately and hence a direct comparison may not be valid. In the study completed by Neal et al. *(65)*, they reported that total yeasts and molds were reduced significantly in samples that received 1.4 kGy of e-beam treatment; however, the organisms were able to recover to levels similar to control by day 4 and throughout the duration of storage, both control and irradiated samples continued to show growth with no significant differences in counts between treated and non treated samples.

LAB counts reduced significantly ($P < 0.05$) for all irradiated samples compared to the non-irradiated control. The effect noted in reduction again was dose dependent with high doses showing higher reduction as one would expect. E-beam irradiation dose

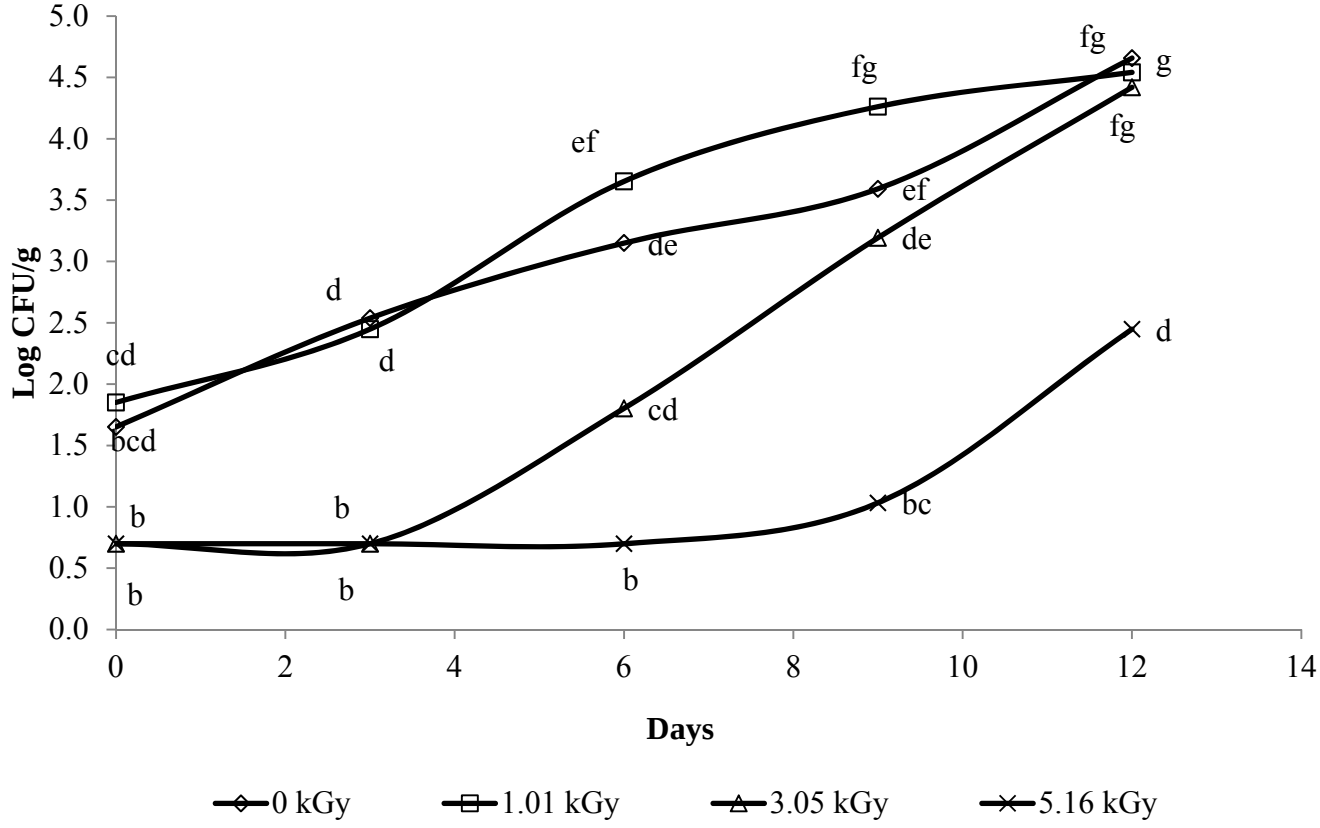

FIGURE 17. *Growth of yeasts on irradiated baby spinach stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

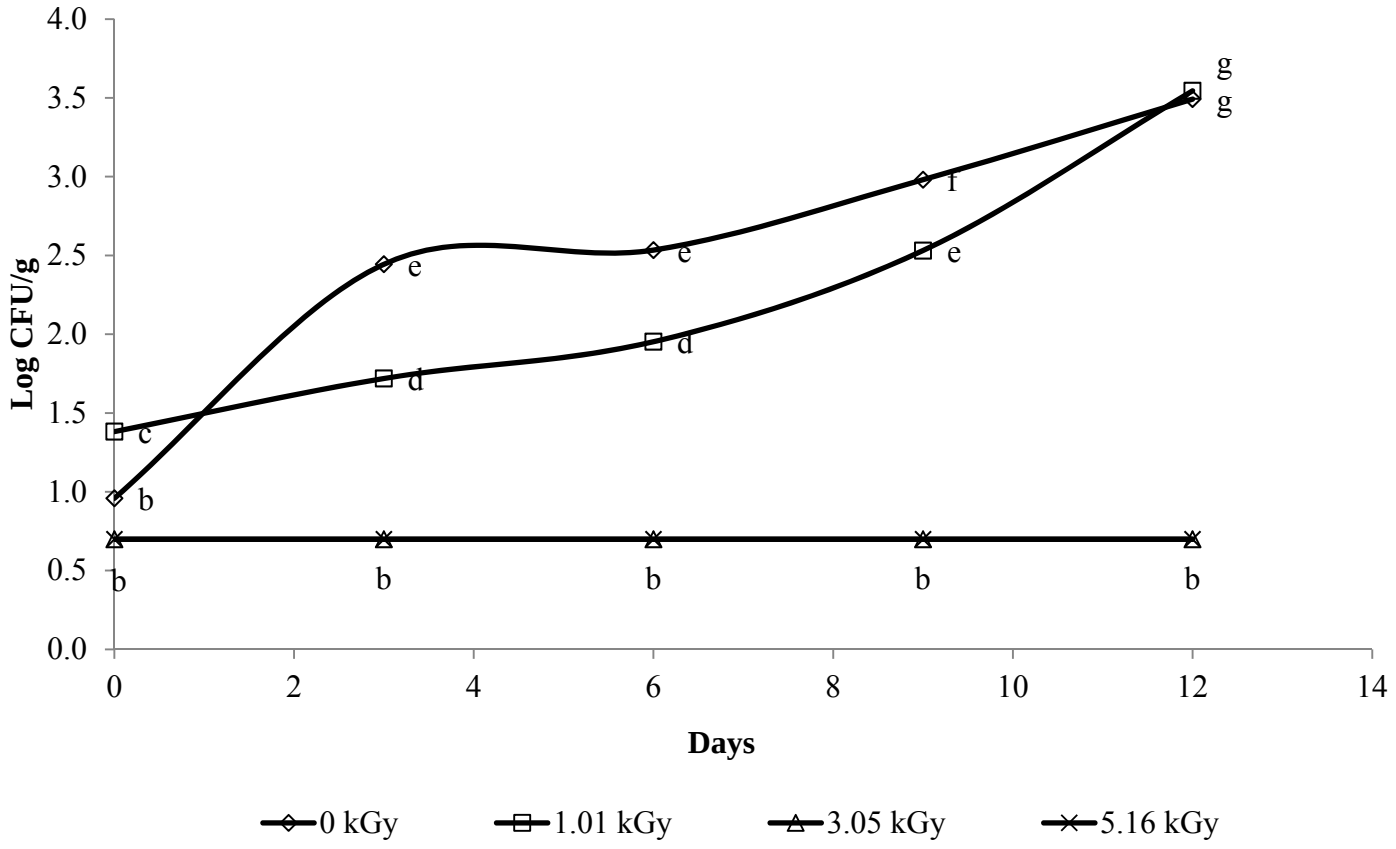

FIGURE 18. *Growth of molds on irradiated baby spinach stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

of 5.16 kGy reduced LAB counts by 4.3 log CFU/g, 3.05 kGy reduced it by 3.1 log CFU/g and the least dose 1.01 kGy by 1.2 log CFU/g compared to the control (4.6 log CFU/g) (Table 5). On day 0, all irradiated spinach showed significant ($P < 0.05$) reduction in LAB counts and the effect was dose dependent. However, during storage, counts did not change significantly ($P < 0.05$) for doses 3.05 and 5.16 kGy (Fig. 19). LAB counts increased for 1.01 kGy over storage and reached the levels of control over storage (Fig. 19). Neal et al. *(65)* reported a higher reduction in LAB counts in their study. A reduction of 2.3 log CFU/g with 0.7 kGy and 2.7 log CFU/g with 1.4 kGy was reported on day 0 compared to control. However, no significant ($P > 0.05$) difference in counts between the 2 irradiation doses were noted on day 0. LAB counts for samples treated with 0.7 and 1.4 kGy doses were 1 log CFU/g and 2.3 log CFU/g less than control samples at day 14 and counts did not increase until 21 days for spinach samples treated with 1.4 kGy *(65)*. Results from the present study suggest that a target low dose of 1 kGy is capable of reducing microbial counts and improving shelf life of baby spinach.

Romaine lettuce

Firmness

Firmness values for romaine lettuce did not change significantly ($P > 0.05$) between control dose 0 and the lowest dose 1.10 kGy. But the changes were significant ($P < 0.05$*)* and dose dependent between control and doses 3.07, and 5.07 kGy. The firmness value was least for dose 5.07 kGy (177.31 N) compared to control (240.34 N)

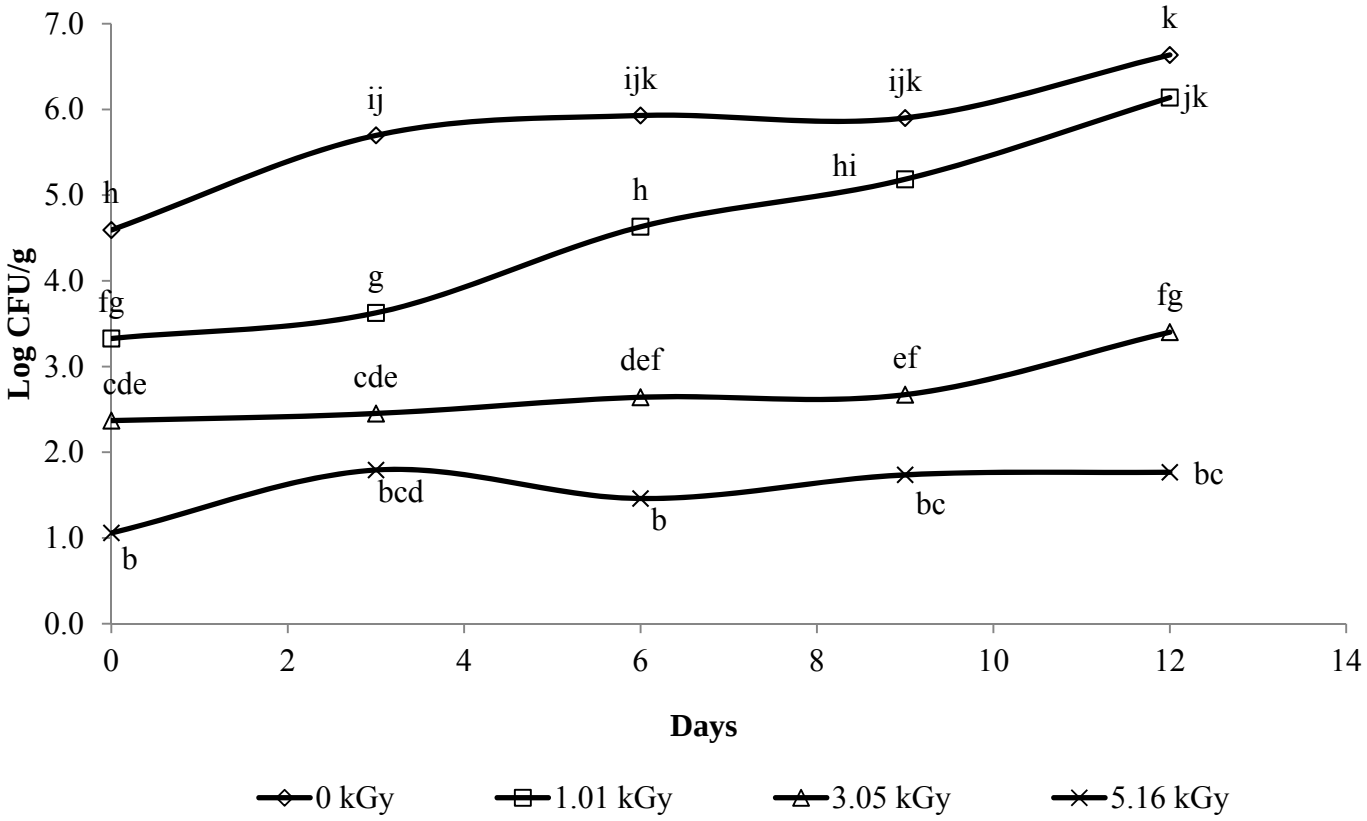

FIGURE 19. *Growth of LAB on irradiated baby spinach stored at 5°C over 12 day. Data points with the same letter are not significantly different (P > 0.05).*

(Table 6). The leaves looked wilted and the midrib looked soft and translucent at the highest dose through the storage period. Han et al. *(40)* observed a marked decrease in firmness of romaine lettuce leaves (49.58%) and ribs (29.13%) when treated with e-beam as the dose level increased from 0 to 3.2 kGy. But Prakash et al. *(80)* observed a 10% loss in firmness at 0.35 kGy gamma radiation which was due to the fact that the texture measurement was done only for the green leaves without the ribs whereas this study measured texture for the entire leaf including the ribs.

Color

For romaine lettuce, e-beam treatment at doses tested (1.1, 3.07, and 5.07 kGy) on day 0 had no effect on the color of the lettuce leaf as color measurement was done on the green leaf. (Table 6) Lettuce leaves treated at dose 3.07 (on day 12), and 5.07 (from day 6 to 12) had a translucent midrib. There was an interaction between storage time and dose ($P < 0.05$) for chroma which increased for control, 1.1 and 3.07 kGy over storage (Fig. 20). But for leaf treated with 5.07 kGy, chroma decreased and the leaf had brown pigmentation on the top side of the green leaf away from the midrib. Due to the small perforations made in the bags, the atmosphere was not depleted of O_2, which might have resulted in the enzymatic browning effect. In accordance with our results, Han et al. *(40)* also observed that chroma values of the romaine lettuce leaves of the control sample remained constant for the entire storage period. However, the leaves of samples irradiated at 1.0kGy showed higher chroma values than the control during the entire

Table 6. *Least squares means for firmness (N), chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeast and Mold, LAB for romaine lettuce as affected by different dosage of electron beam irradiation.*

Effect	Texture (N)	APC	Yeast	Mold	LAB
RMSE	40.91	0.51	0.48	0.30	0.64
Dose	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]
0	240.34[d]	5.2[e]	3.6[e]	2.8[d]	5.2[d]
1.10	230.98[d]	2.9[d]	3.1[d]	2.3[d]	2.9[c]
3.07	215.61[c]	1.9[c]	2.3[c]	1.1[c]	3.0[c]
5.07	177.31[b]	1.3[b]	2.0[b]	<1[b]	2.1[b]
Days	<0.0001[a]	0.0026[a]	<0.0001[a]	0.0110[a]	<0.0001[a]
0	198.61[b]	2.4[b]	1.8[b]	1.5[b]	2.0[b]
3	204.27[b]	2.6[bc]	2.3[c]	1.8[c]	2.6[c]
6	210.08[b]	3.0[c]	2.7[d]	1.7[b]	4.0[d]
9	231.20[c]	2.8[cd]	3.3[e]	1.7[b]	3.8[d]
12	236.14[c]	3.3[d]	3.8[f]	2.0[c]	4.2[d]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcdef] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$).

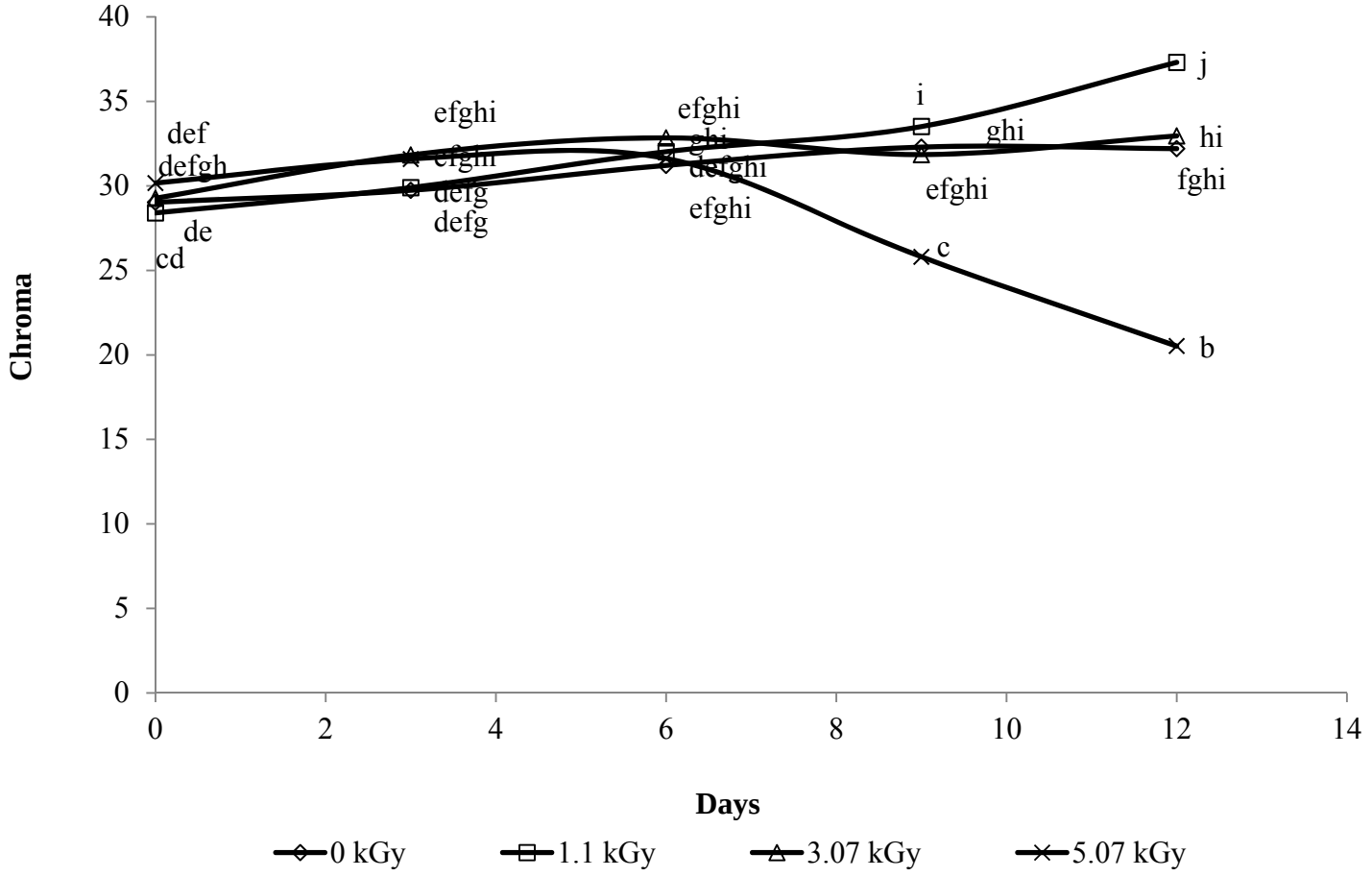

FIGURE 20. *Least square means of chroma values for romaine lettuce as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

storage period. Increased darkening (L value) in the romaine lettuce leaves was also reported by Prakash et al. *(80)* but no difference in the color of the lettuce samples was reported due to treatment with gamma irradiation. Kei et al. *(1)* noted that there is tissue wounding due to irradiation and this wounding triggers enzymatic browning leading to color loss. Furthermore, the oxidation of phenols by polyphenoloxidases and peroxidases to O- quinones results in polymerization leading to formation of dark pigments *(1)*.

Respiration rate

Romaine lettuce leaves treated with e-beam doses of 1.10, 3.07, and 5.07 kGy, exhibited significantly ($P < 0.05$) higher respiration rates compared to control on day 0 which is consistent with the other commodities tested in this experiment (Table 6). Respiration rate decreased for all samples including control from day 0 to day 3 but stabilized during the 12 day storage period. Fig. 21 shows the interaction between storage time and dose. Respiration rate decreased for all samples during storage including control. Irradiation treatment with 1.10 kGy had a lower respiration rate compared to the high doses of 3.07 and 5.07 kGy (Fig. 21). Also, throughout storage, the e-beam treated lettuce had higher respiration rate compared to the control. Han et al. *(40)* treated packaged romaine lettuce with e-beam irradiated at 1.5 and 3.2 kGy and observed that the treated products had significantly higher CO_2 levels than the control after irradiation at day 0 which is consistent with our findings. Hagenmaier and Baker *(39)* reported that the respiration rate for irradiated cut iceberg lettuce was 33% higher than the control 1 day after treatment, the same after 8 days, and slightly lower after 13 days.

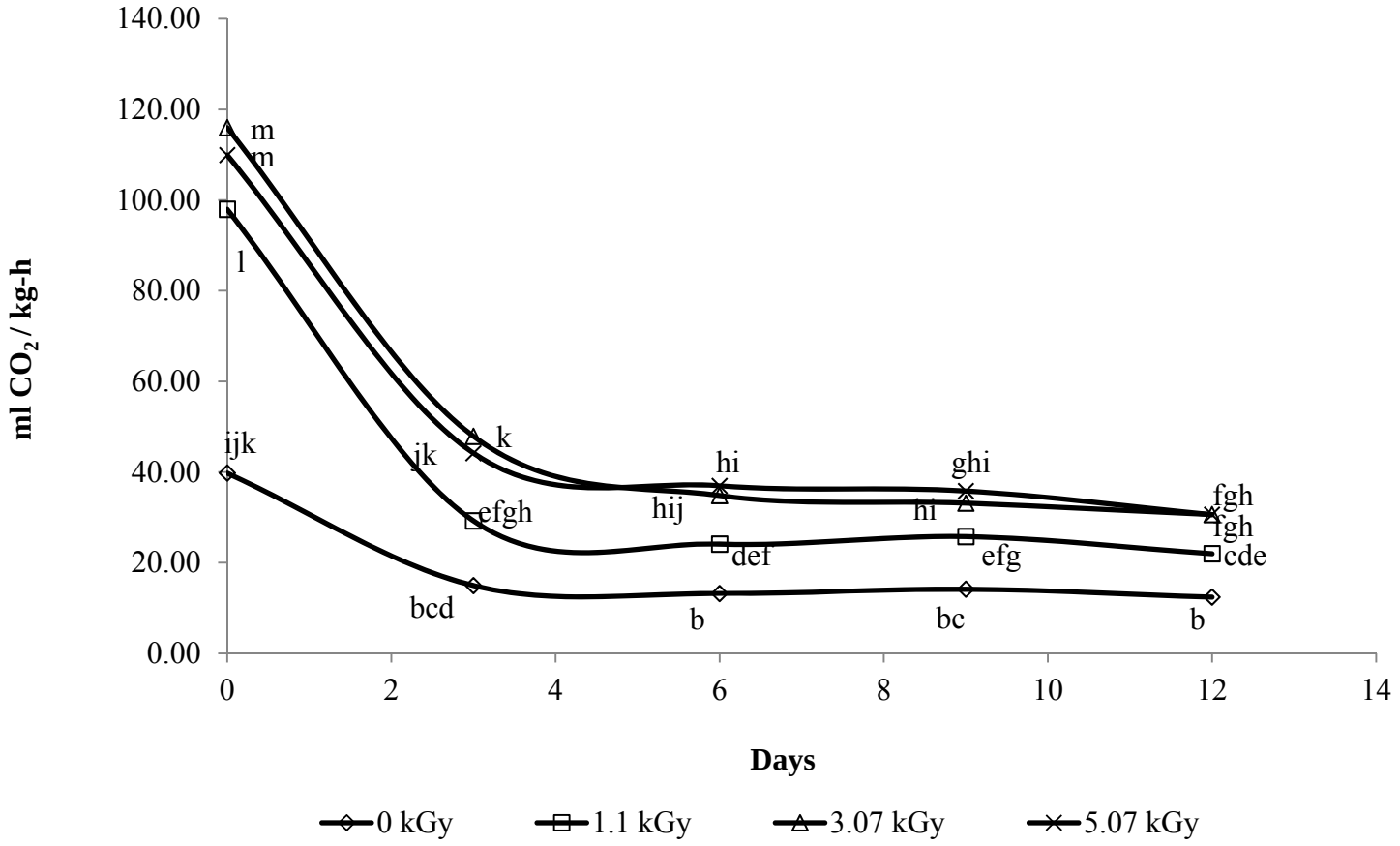

FIGURE 21. *Least square means of respiration rate values (ml of CO_2 / kg-h) for romaine lettuce as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

Microbiological counts

APCs in e-beam irradiated romaine lettuce showed significant ($P < 0.05$) reduction compared to control and the effect was dose dependent. The highest dose 5.07 kGy resulted in the highest reduction of 4.2 log CFU/g whereas 3.07 kGy resulted in a 3.5 log CFU/g reduction and 1.10 kGy gave the least reduction of 2.4 log CFU/g (Table 6). Irradiated samples had significantly ($P < 0.05$) lower APCs on day 0 compared to the non-irradiated control (4.7 log CFU/g). During storage, APCs increased in both the irradiated and control samples (Fig. 22) with an exception of samples treated with 3.07 kGy which did not show any change in APCs. At the end of 12 days of storage, the irradiated samples had significantly ($P < 0.05$) lower APCs compared to control. E-beam irradiation at dose 1.10 kGy appears to be promising for enhancing shelf life of romaine lettuce since irradiation reduced APCs significantly compared to the conventional de-contamination techniques.

Prakash et al. *(80)* studied effects of low dose gamma irradiation on cut romaine lettuce packed under MAP and found that the microbial load was reduced by approximately 1.5 logs with a dose of 0.35 kGy throughout the study. Gamma radiation dose of 0.15 kGy resulted in smaller reduction. Results of this study are in agreement with Zhang et al. *(113)* who found a dose dependent reduction in APCs on fresh cut lettuce. A 3.1 log CFU/g reduction in APCs with 1.5 kGy gamma radiation was reported and the counts increased over storage of 8 days.

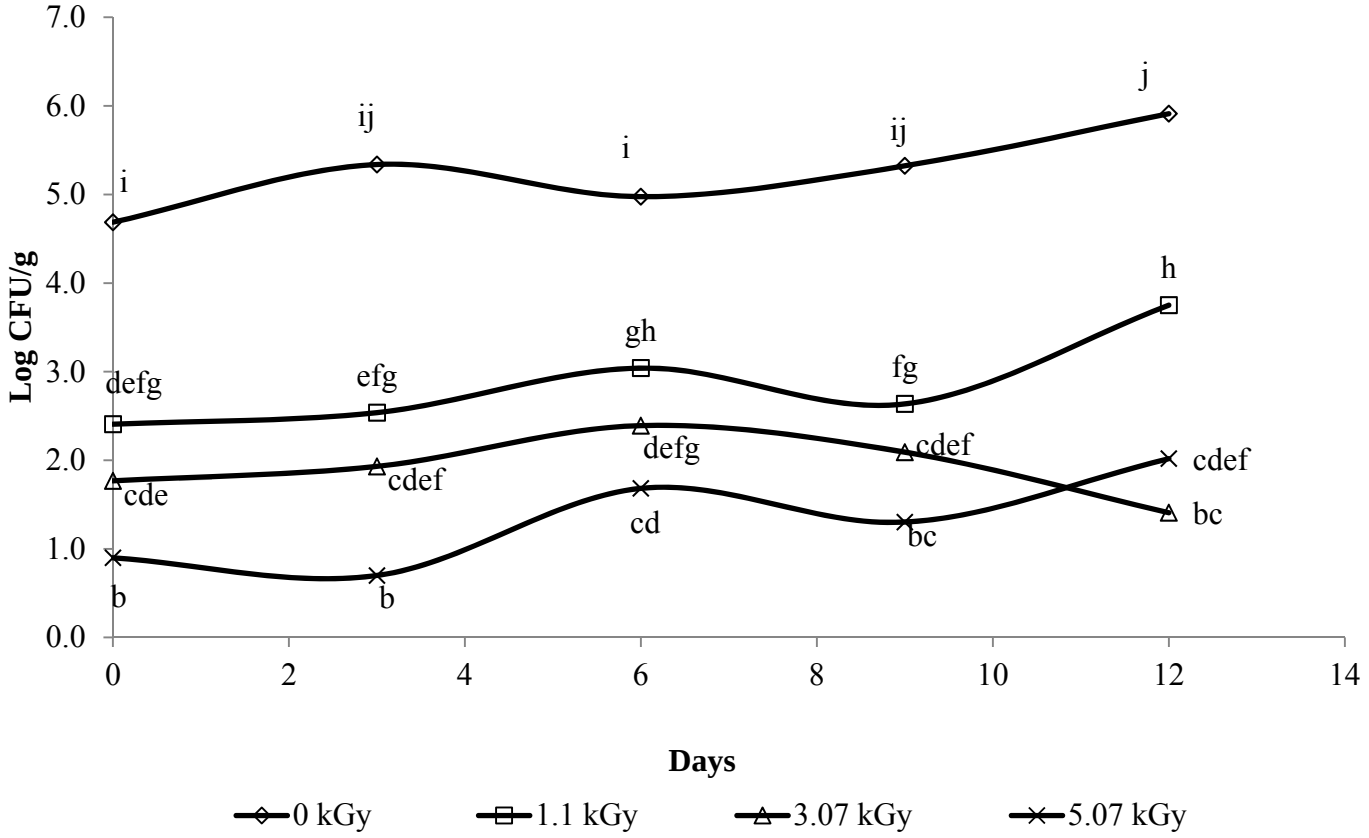

FIGURE 22. *Growth of APCs on irradiated romaine lettuce stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

Yeast population on irradiated romaine lettuce reduced significantly ($P < 0.05$) compared to the control. As in case of APCs, the reduction was dose dependent. A 1.8 log CFU/g reduction was found with 5.07 kGy followed by a 1.3 log CFU/g reduction with 3.07 kGy and a 0.5 log CFU/g reduction with the least dose 1.10 kGy (Table 6). Yeast counts for all the irradiated samples increased during storage (Fig. 23). Yeast counts increased at a higher rate for samples treated with 3.07 and 5.07 kGy than with 1.10 kGy during storage. Counts for control samples did not change during storage (Fig. 23). At a lower dose of 0.35 kGy of gamma radiation, Prakash et al. *(80)* observed a reduction of 1 log CFU/g in the initial YNM counts. At 0.15 kGy, there was no significant reduction in YNM counts. During storage, the YNM counts increased but were still lower than the non-irradiated control *(80)*. Also, it was noted that mold counts were not detected consistently during storage, so the total counts were mostly representing yeast colonies *(80)*. In the present study, mold counts were taken separately and a reduction could be easily noted. Dose 1.10 kGy did not result in any significant ($P > 0.05$) reduction but with 3.07 and 5.07 kGy, significant reduction ($P < 0.05$) in mold counts was noted. Mold counts were below detectable limit with irradiation dose of 5.07 kGy and did not change during storage (Fig. 24). Mold counts increased only in control and 3.07 kGy but not with 1.10 and 5.07 kGy during storage.

LAB counts were reduced significantly ($P < 0.05$) for all irradiated samples compared to the non-irradiated control. The highest reduction of 3.2 log CFU/g was achieved with 5.07 kGy (Table 6). On day 0, there was no significant reduction in LAB counts with 1.10 and 3.07 kGy, but counts increased rapidly for control during storage

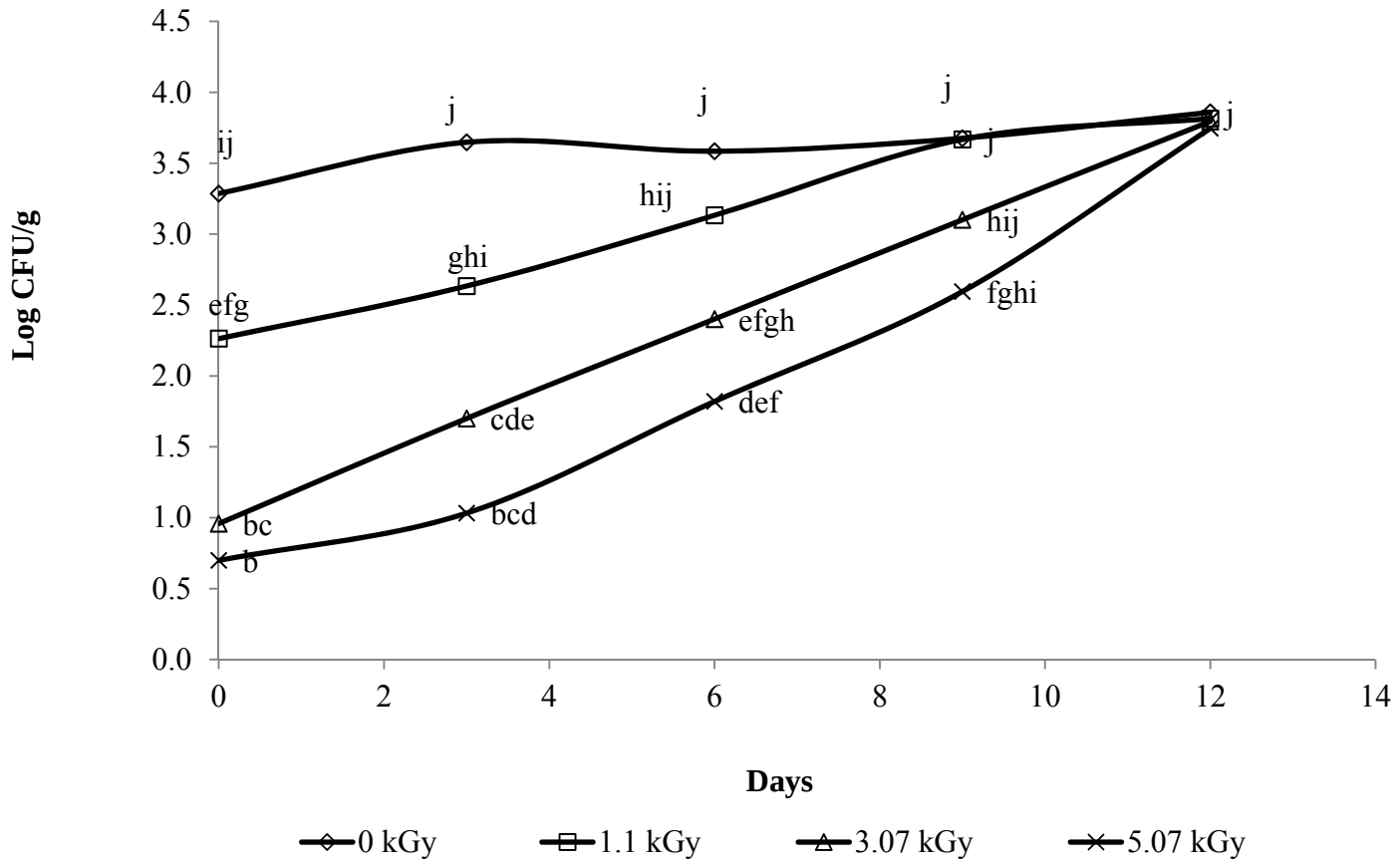

FIGURE 23. *Growth of yeasts on irradiated romaine lettuce stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

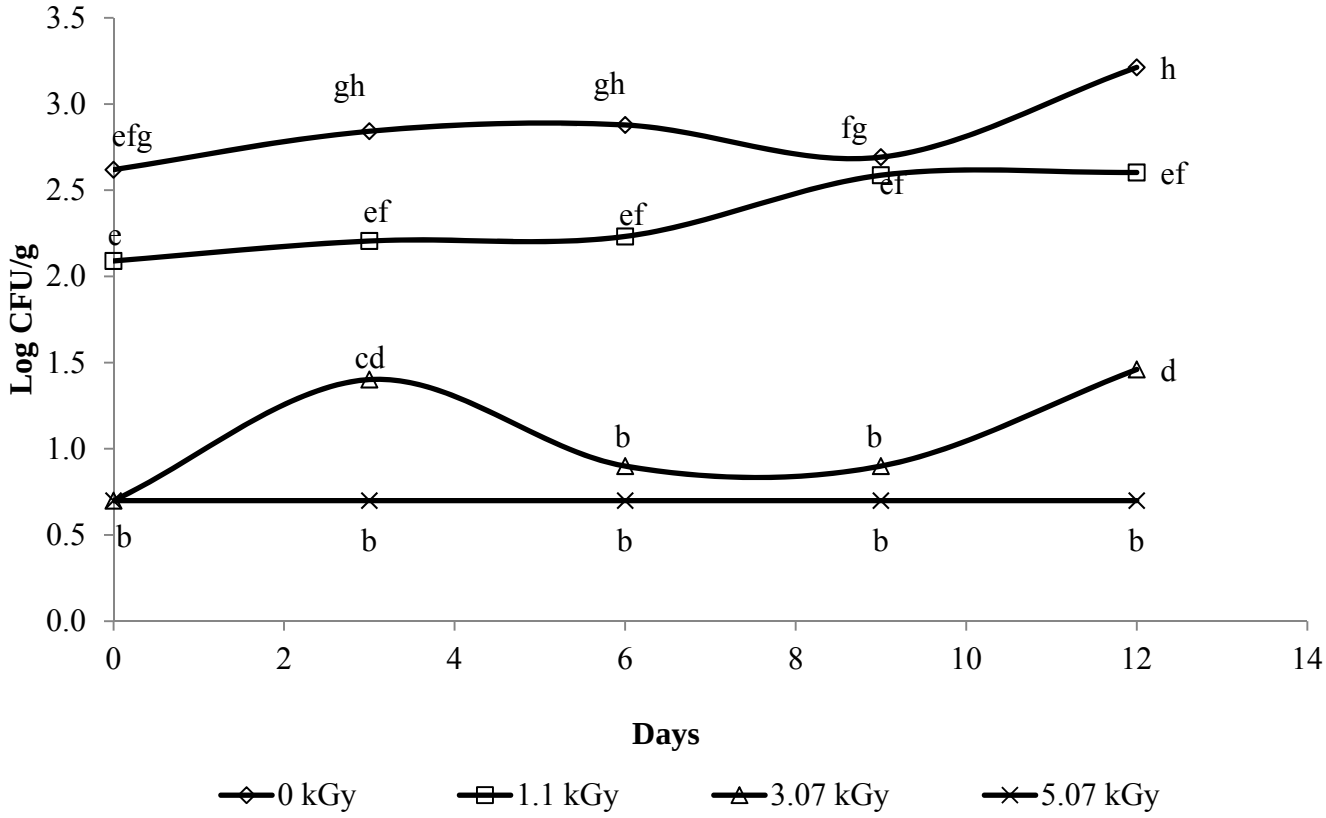

FIGURE 24. *Growth of molds on irradiated romaine lettuce stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

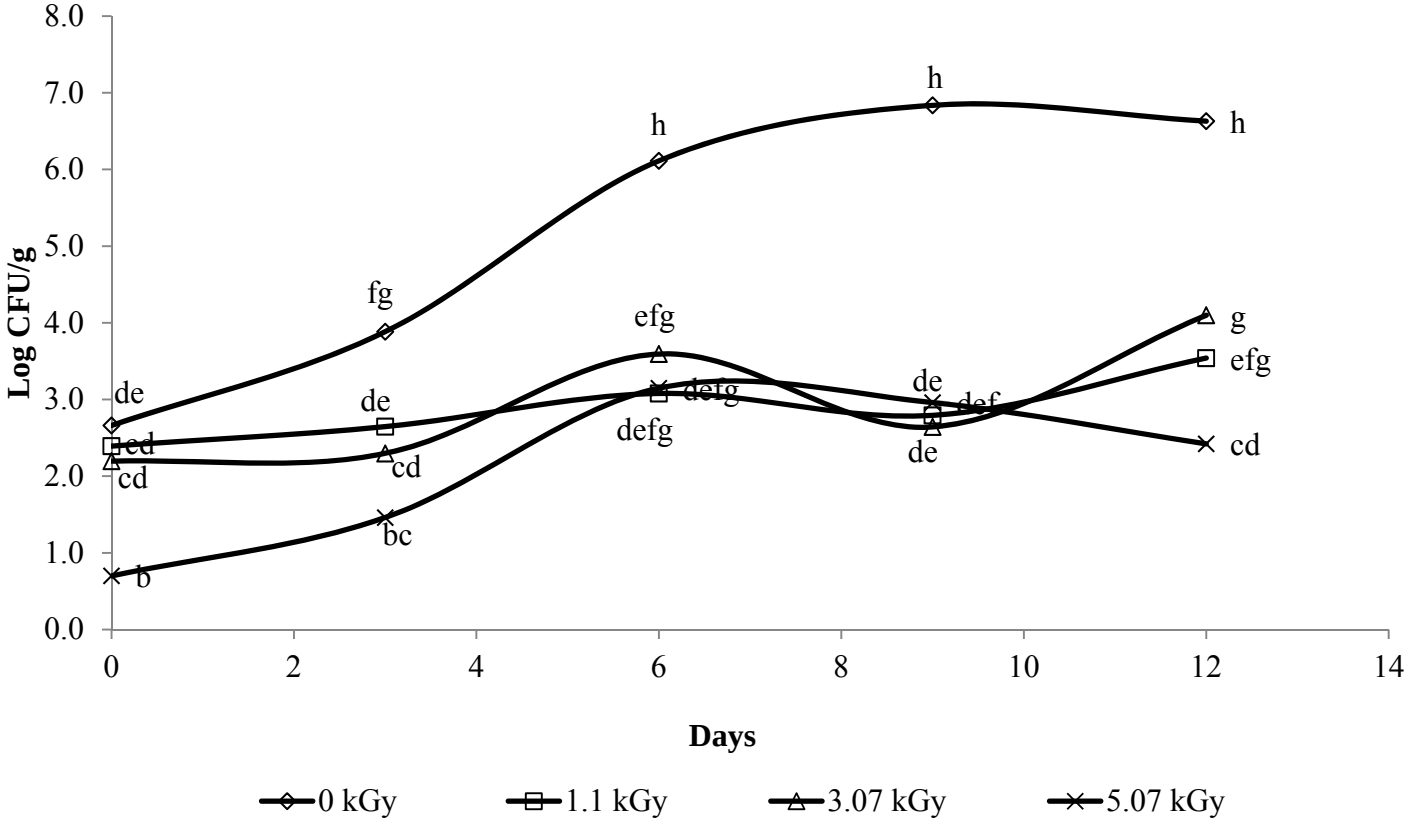

FIGURE 25. *Growth of LAB on irradiated romaine lettuce stored at 5°C over 12 days Data points with the same letter are not significantly different (P > 0.05).*

(Fig. 25). Although counts for irradiated samples also increased during storage, it was at a much slower rate. Both 1.10 and 3.07 kGy had similar counts at day 12 (Fig. 25). This may be due to the inability of the sub-lethally injured LAB to proliferate on romaine lettuce under low temperature of 5^{o}C. Kim et al. *(50)* evaluated effect of low dose gamma radiation on survival of LAB on shredded iceberg lettuce. At 0.5 kGy, LAB counts were reduced to below the limit of detection and did not grow throughout the 10 day storage period. The control non-irradiated sample was 3.61 log CFU/g on day 0 and increased to 4.25 log CFU/g at the end of storage.

Strawberries

Firmness

Effect on texture changes was very prominent in strawberries treated with 1.07, 2.92, and 5.43 kGy e-beam dose. Firmness value for non-irradiated control (3.39 N) was significantly ($P < 0.05$) higher than dose 1.07 kGy (1.95 N), 2.92 kGy (1.32 N), and 5.43 kGy (0.64 N) (Table 7). Strawberries treated with 5.43 kGy e-beam were very mushy and at the end of storage, about 7 ml of fluid was collected in the Ziploc® bag containing 4 medium sized fruits. Damage to cell membranes results in loss of intracellular water leading to loss of cell turgescence *(104)*. Yu et al. *(110)* found a significant correlation between the firmness and oxalate-soluble pectin in irradiated strawberries, but no correlation between water-soluble pectin and firmness. Irradiation induced texture change has been associated with changes in pectic substances *(44)*. According to

Table 7. *Least squares means for firmness (N), chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeast and Mold, LAB for strawberries as affected by different dosage of electron beam irradiation.*

Effect	APC	Yeast	Mold	LAB
RMSE	0.96	0.58	0.48	1.09
Dose	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]
0	3.5[d]	4.3[e]	3.9[d]	4.0[d]
1.07	2.5[c]	3.8[d]	3.7[d]	2.3[c]
2.92	2.2[c]	3.4[c]	2.2[c]	1.0[b]
5.43	1.4[b]	2.2[b]	<1[b]	1.3[b]
Days	<0.0001[a]	<0.0001[a]	0.0077[a]	0.0314[a]
0	1.4[b]	2.7[b]	2.2[b]	1.3[b]
3	1.3[b]	3.0[b]	2.6[c]	2.1[bc]
6	3.2[c]	3.4[c]	2.7[c]	2.2[bc]
9	3.5[c]	3.8[cd]	2.9[c]	2.7[c]
12	2.7[c]	4.1[d]	2.7[c]	2.5[c]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcde] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$).

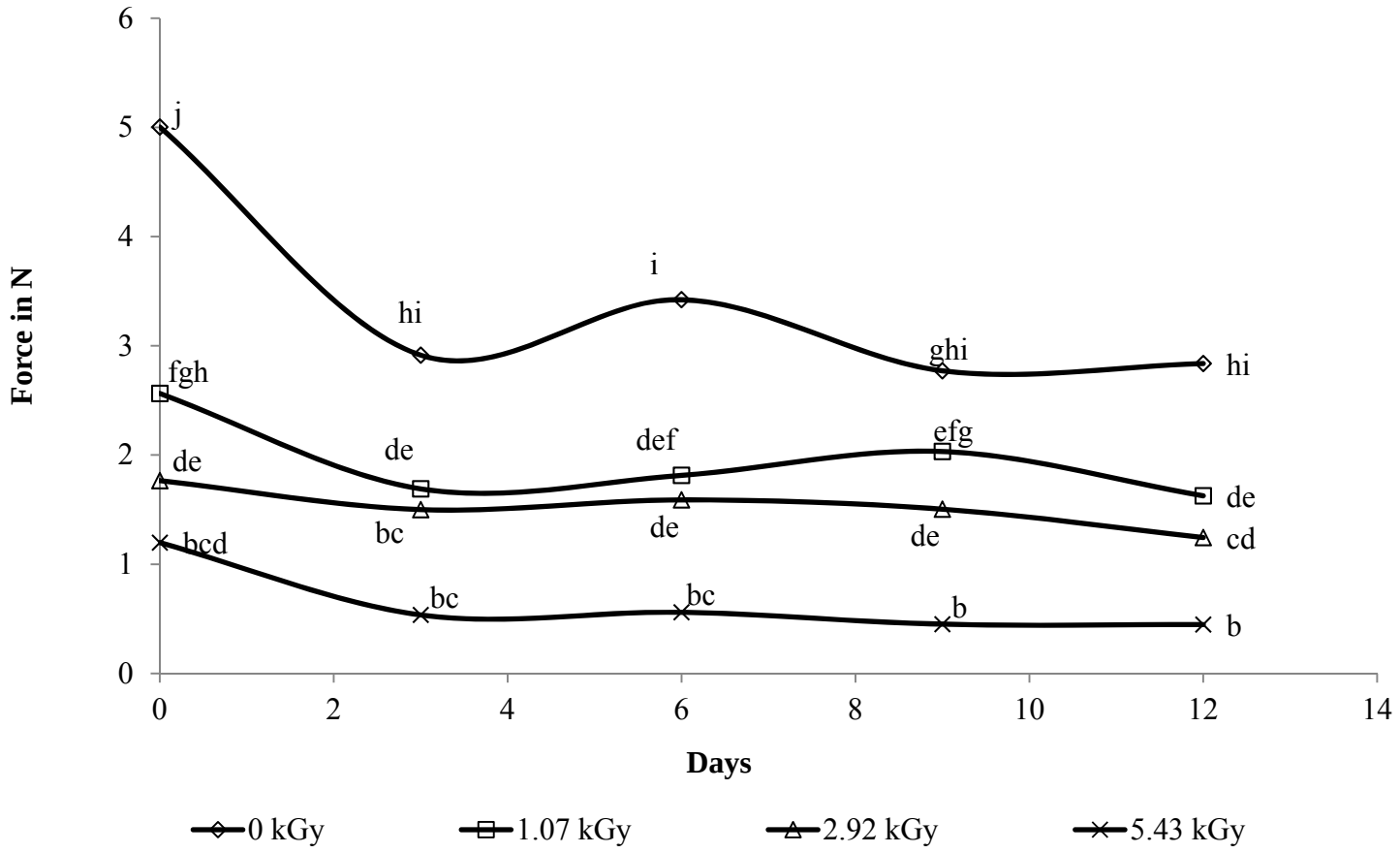

FIGURE 26. *Least square means of firmness values (N) for strawberries as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

D'Amour et al. *(21)*, this tissue softening may be caused by partial depolymerization of cell wall polysaccharides, mainly cellulose and pectins. There was an interaction between time and dose for firmness in strawberries (Fig. 26) where the control and fruits treated with 1.07 kGy exhibited a decrease in firmness during storage. However, with 2.92 and 5.43 kGy, there was no change in firmness noted during storage (Fig. 26).

Color

Chroma values did not change for all doses tested (1.07, 2.92, and 5.43 kGy) as compared to control on day 0 (Table 7). The interaction between storage time and dose ($P < 0.05$) is shown in Fig. 27. There was no change in chroma values for control and with 2.92 kGy during storage, but for dose 1.07 and 5.43 kGy, chroma value dropped significantly ($P < 0.05$) (Fig. 27) more for a high dose (5.43 kGy). Strawberries may tolerate gamma irradiation up to 2 kGy *(97)*. Doses in excess of 2 kGy caused texture and color changes of strawberries *(97)*. Zegota *(112)* noted the loss of color of strawberries after treatment with 2.5 kGy and 3.0 kGy irradiation. Yu et al. *(109)* observed that Hunter *L* values increased with irradiation dose for e-beam.

Respiration rate

Respiration rates for strawberries followed the same pattern as for the other commodities tested. All treated samples (1.07, 2.92, and 5.43 kGy) had higher respiration rates on day 0 compared to the non-irradiated control and all samples

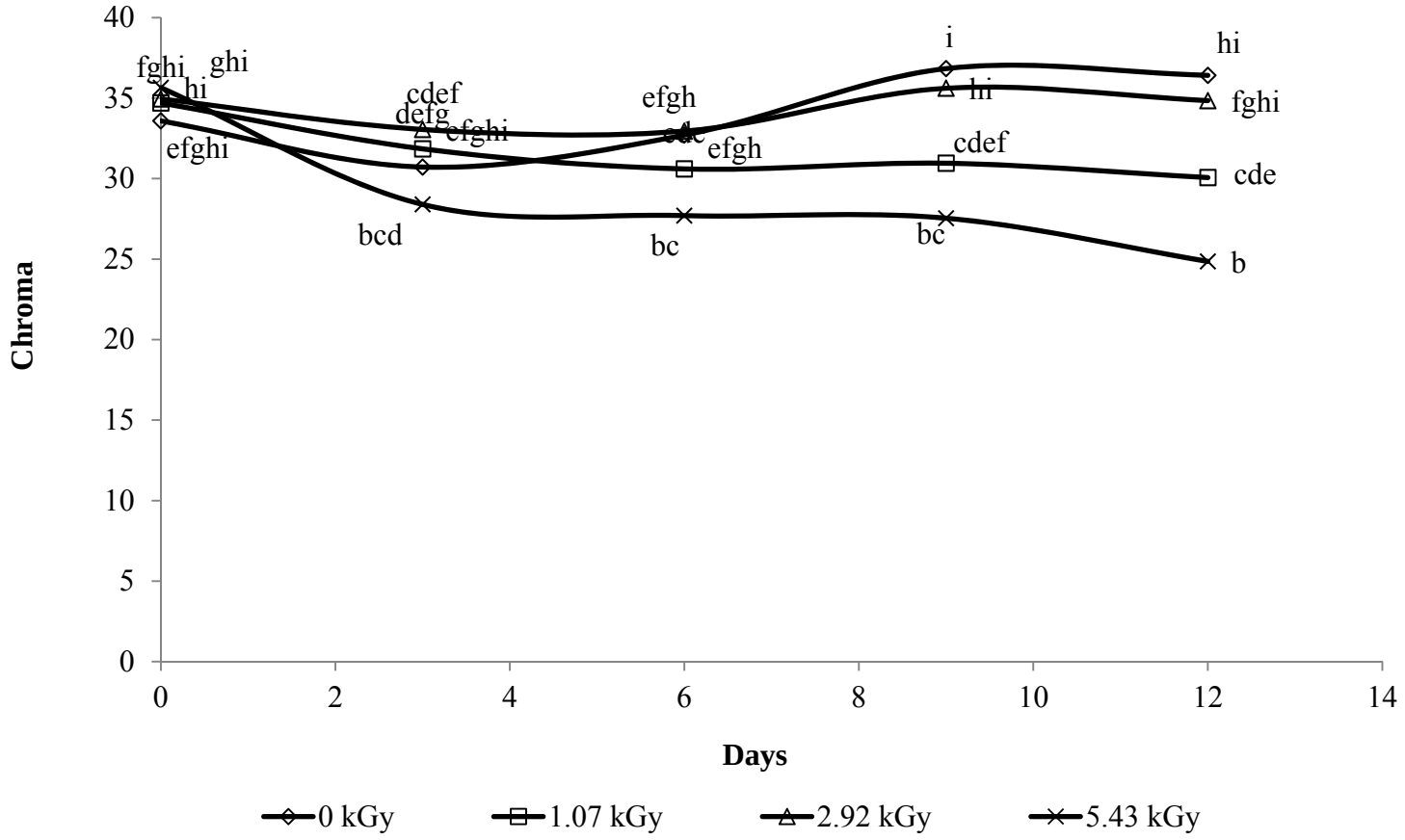

FIGURE 27. *Least square means of chroma values for strawberries as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

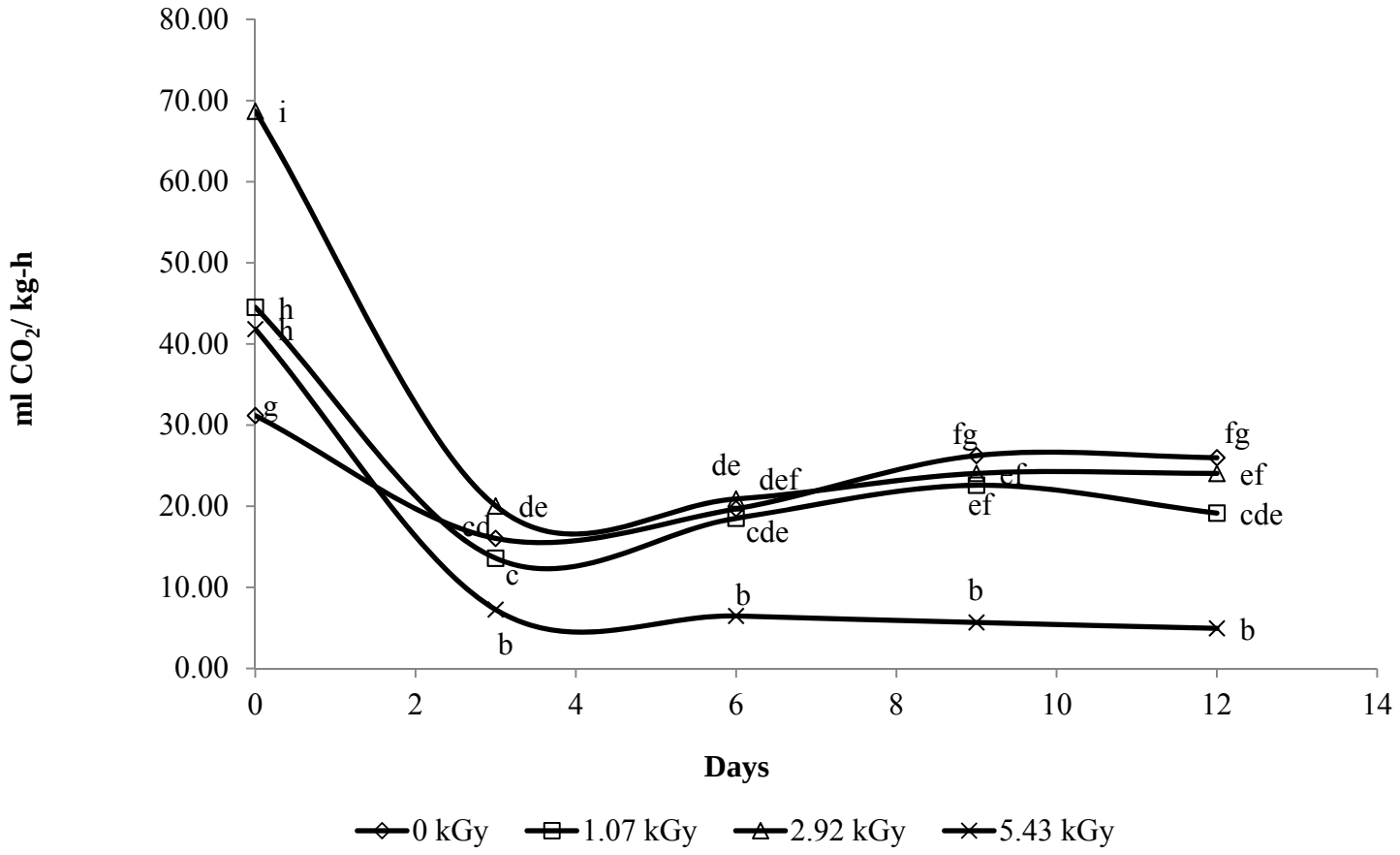

FIGURE 28. *Least square means of respiration rate (ml CO_2 / kg-h) for strawberries as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

including control had lower respiration rates on day 3 compared to day 0 (Table 7). Over the storage period, an interaction with dose was observed (Fig. 28) and respiration rate decreased for all samples including control on day3, but remained stable through day 12. The reduction was highest in dose 5.43 kGy. There are no studies reporting effect on respiration rate in irradiated strawberries but Moreno et al. *(63)* reported that the respiration rate of blueberries treated with e-beam doses of 1.1, 1.6, and 3.2 kGy was high right after irradiation. The CO_2 concentration was significantly higher for treated samples compared to control over storage.

Microbiological counts

Strawberries when treated with e-beam at doses 1.07, 2.92, and 5.43 kGy showed reduction in APCs compared to the control. Significant ($P < 0.05$) reduction was seen for all doses but the highest reduction of 2.4 log CFU/g was seen with 5.43 kGy (Table 7). With 1.07 and 2.92 kGy the reduction in APC was 1 log CFU/g and 1.6 log CFU/g respectively. APC reduction was dose dependent and on day 0, the counts were below detection limit of 1 log CFU/g with 2.92 and 5.43 kGy. However, no significant ($P < 0.05$) reduction was noted with 1.07 kGy compared to control on day 0. Although, during storage counts in all treated samples increased drastically reaching levels of control by day 12 (Fig. 29).

Most studies involving irradiation of strawberries have focused on YNM counts since strawberries are a commodity which supports the growth of YNM which are the main spoilage microorganisms found in strawberries. Yeast counts were significantly (*P*

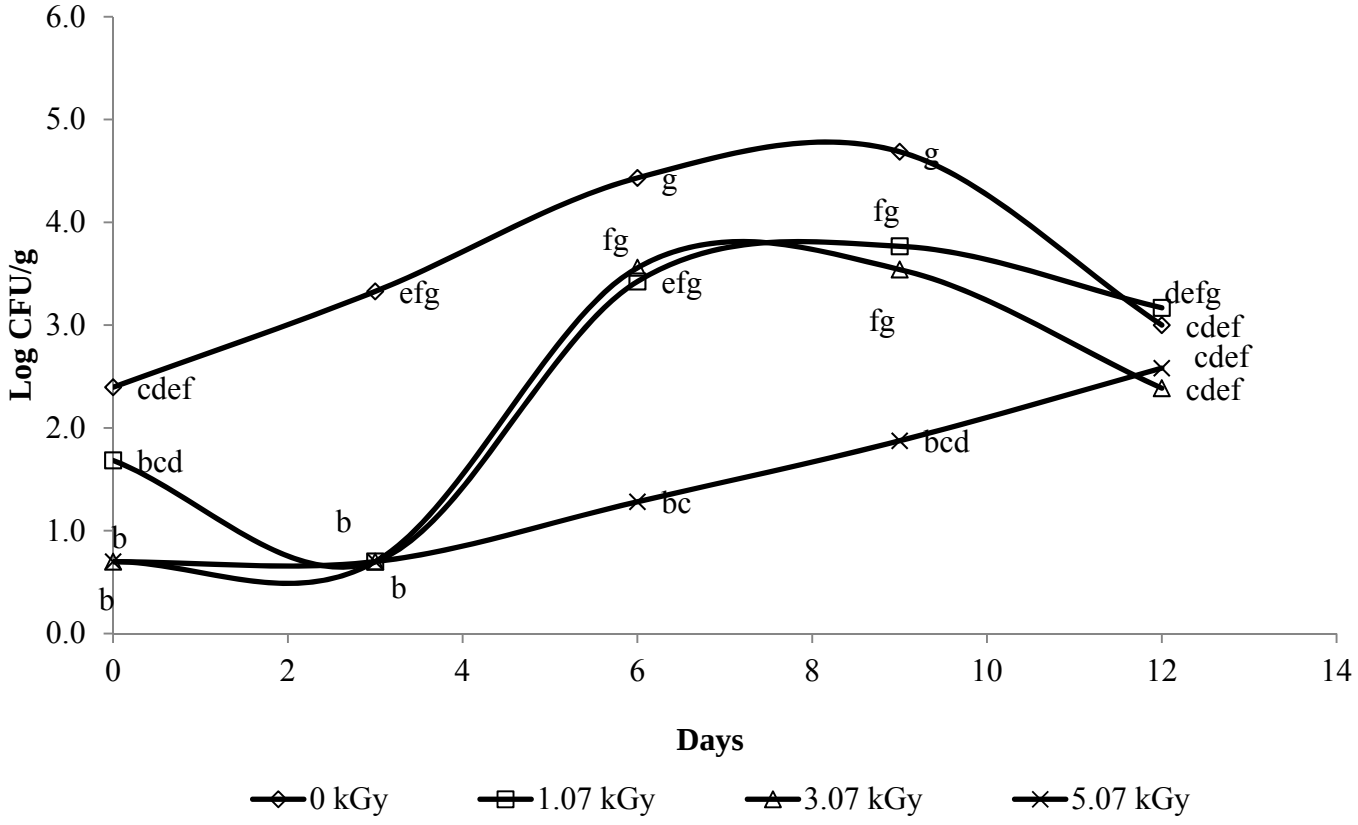

FIGURE 29. *Growth of APCs on irradiated strawberries stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

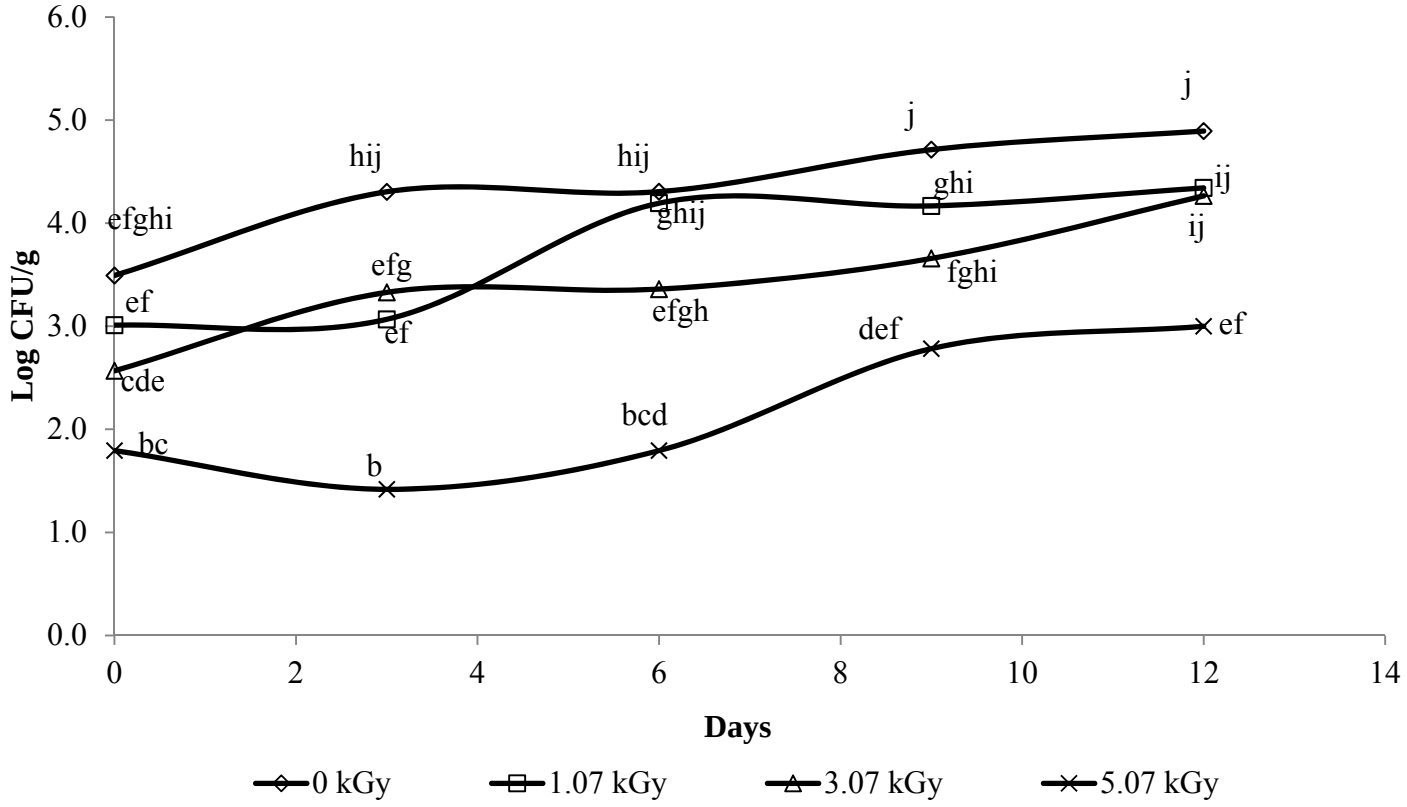

FIGURE 30. *Growth of yeasts on irradiated strawberries stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

< 0.05) reduced for all irradiated samples compared to control and the reduction was dose dependent. Control samples had an initial yeast count of 4.3 log CFU/g which was reduced by 2.2 log CFU/g with 5.43 kGy, by 2.2 log CFU/g with 2.92 kGy and by 1.1 log CFU/g with 1.07 kGy (Table 7). On day 0, yeast counts were lower for irradiated samples, the least for 5.43 kGy. During storage, the counts increased to levels of control with 1.07 and 2.92 kGy at a faster rate than for 5.43 kGy (Fig. 30). Mold counts were significantly ($P < 0.05$) reduced for 2.92 and 5.43 kGy and highest reduction as expected was seen with 5.43 kGy with counts below detection limits throughout storage. No reduction in counts was noted for 1.07 kGy on day 0 and counts did not increase during storage. But for control and with 2.92 kGy, counts increased during storage (Fig. 31). A dose of 2.92 kGy is suitable for reducing YNM counts in strawberries according to this study. Yu et al. *(109)* tested quality changes in e-beam irradiated Tristar strawberries and reported that irradiation suppressed fungal growth but during storage, the counts increased for the irradiated products. The shelf life of strawberries was extended as irradiation dose increased.

Botrytis cinerea is necrotrophic fungus that affects soft fruits like strawberries and grapes and Barkai-Golan et al. *(5)* who studied effect of gamma radiation on the keeping quality of strawberries reported that although a dose of 1 kGy caused a slight reduction of rot incidence initially, during storage of 4 days facilitated fungal growth and recovery resulting in a considerable rise in rot incidence.

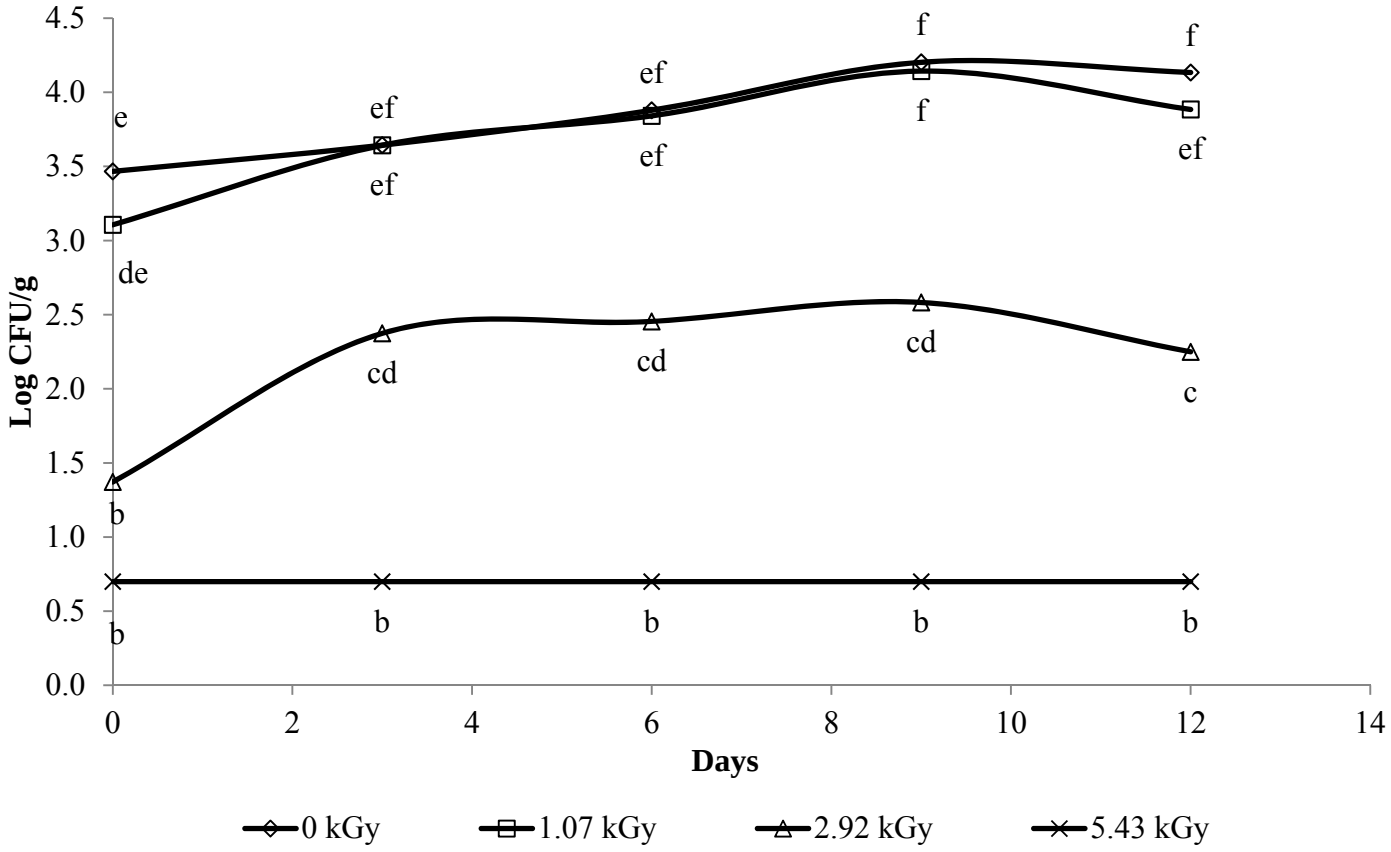

FIGURE 31. *Growth of molds on irradiated strawberries stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

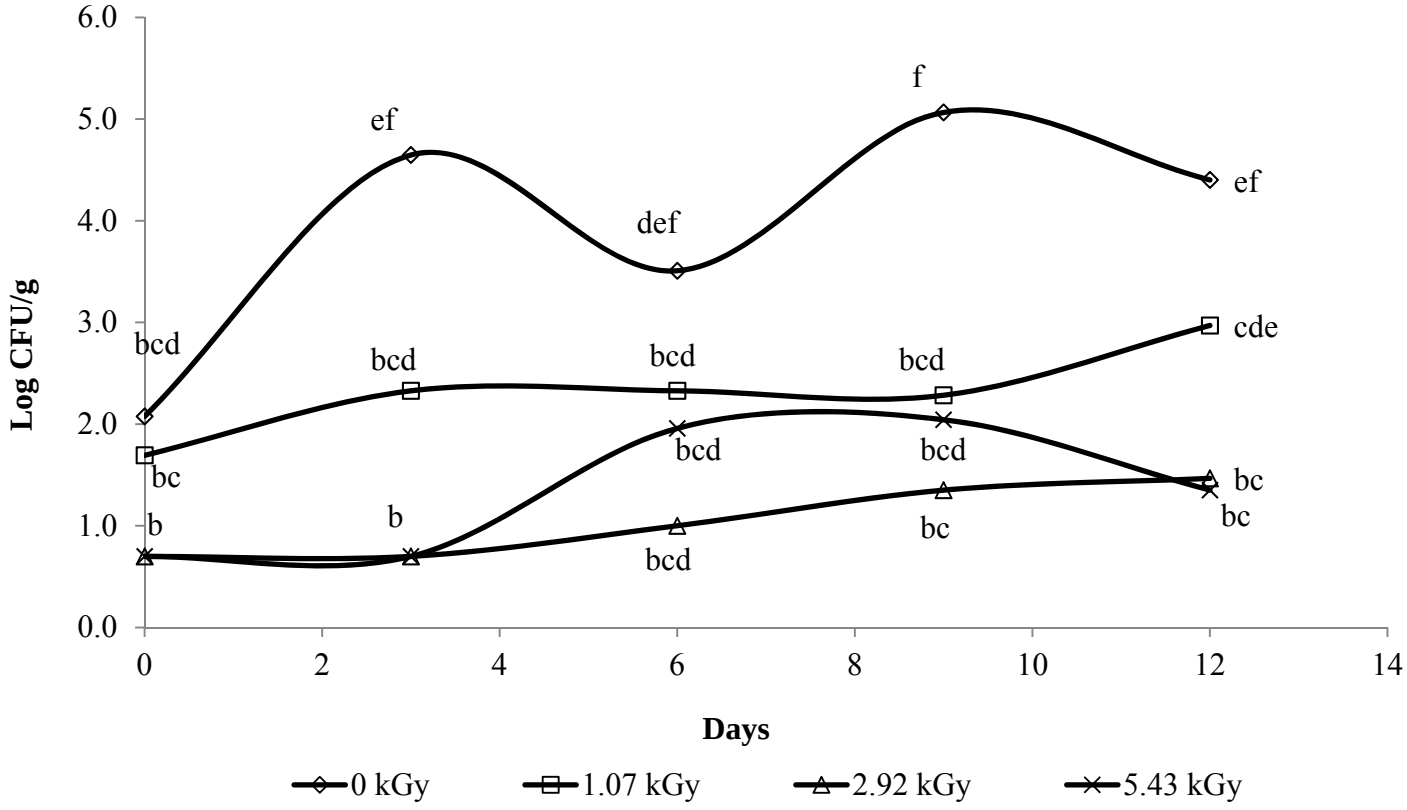

FIGURE 32. *Growth of LAB on irradiated strawberries stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

LAB counts were reduced in all irradiated fruits when compared to the non-irradiated control with the highest reduction of 3.4 log CFU/g with 2.92 kGy and 3.0 log CFU/g with 5.43 kGy. With 1.07 kGy, the reduction was 1.6 log CFU/g (Table 7). LAB counts did not increase for all irradiated samples during storage in contrast with control where counts increased significantly during storage (Fig. 32). LAB counts were below detection limits with 2.92 and 5.43 kGy on day 0. LAB thrive under reduced oxygen content and have the property of producing lactic acid from sugars by fermentation. Strawberries treated with e-beam probably were sub lethally injured and did not have a conducive atmosphere for rapid growth.

Green onion

Firmness

E-beam doses of 1.03, 3.05, and 5.11 kGy in green onion showed significant ($P < 0.05$) reduction in firmness as compared to non-irradiated control (415.06 N). The effect was dose dependent with lower dose having higher firmness value (370.73 N) and higher doses have lower firmness value (326.49 N for 3.05 kGy, and 279.83 N for 5.11 kGy) (Table 8). Like in the case of baby spinach, green onion treated at the highest dose of 5.11 kGy showed soggy appearance and this is in accord with Fan and Sokorai *(29)* who reported that celery, carrot, and green onion had the highest increase in leakage when treated with radiation dose above 1 kGy. However, Kim et al. *(48)* observed no consistent effect on the texture of fresh-cut green onions at a dose 1.5 kGy when treated with gamma irradiation. Another study on gamma irradiation on green onion conducted

Table 8. *Least squares means for firmness (N), chroma, respiration rate (ml CO_2/ kg-h), APCs, Yeast and Mold, LAB for green onion as affected by different dosage of electron beam irradiation.*

Effect	Chroma	Respiration rate (ml CO_2/ kg-h)	APC	Yeasts	Mold	LAB
RMSE	4.75	3.79	0.56	0.74	N/A	0.69
Dose	0.056[a]	0.0005[a]	<0.0001[a]	<0.0001[a]	<1	<0.0001[a]
0	26.93[b]	28.34[b]	7.2[e]	5.9[e]	<1	4.0[d]
1.03	27.63[b]	30.44[bc]	2.7[d]	4.5[d]	<1	3.2[c]
3.15	29.62[c]	34.38[d]	1.9[c]	2.3[c]	<1	2.8[c]
5.17	27.99[b]	32.78[cd]	1.0[b]	1.5[b]	<1	2.1[b]
Days	0.0126[a]	<0.0001[a]	0.0201[a]	<0.0001[a]	N/A	0.0010[a]
0	26.68[b]	46.04[c]	3.1[bc]	2.5[b]	<1	2.3[b]
3	27.06[bc]	28.52[b]	2.8[b]	2.7[b]	<1	2.8[bc]
6	27.29[bc]	27.16[b]	3.4[cd]	3.7[c]	<1	3.5[d]
9	29.94[d]	28.65[b]	3.1[bcd]	4.4[d]	<1	3.2[cd]
12	29.25[cd]	27.06[b]	3.6[d]	4.4[cd]	<1	3.4[d]

[a] is the P-value from Analysis of Variance table
RMSE = Root Mean Square Error
[bcde] Means within the same column and effect that have a common letter are not significantly different ($P > 0.05$).

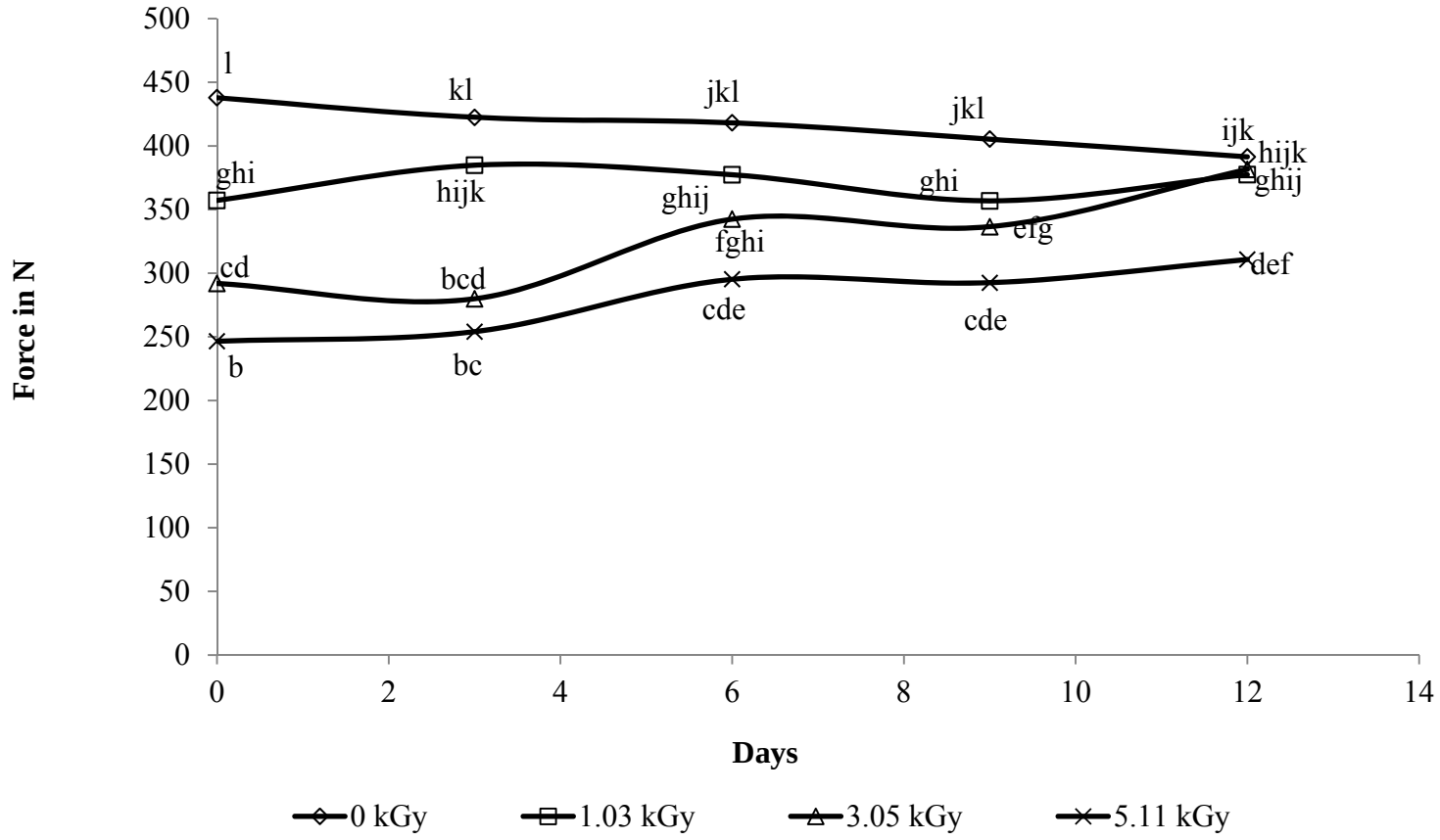

FIGURE 33. *Least square means of firmness values (N) for green onion as affected by interaction between storage days and irradiation dose. Data points with the same letter are not significantly different (P > 0.05).*

by Fan et al. *(27)* noted that the firmness of fresh-cut green onions irradiated at doses 1, 2, and 3 kGy was similar to the non-irradiated controls at 1, 5, or 9 day of storage. There was a dose and time interaction and during storage, control samples reduced in firmness. Samples treated with 1.03 kGy did not show any changes in firmness over storage but with 3.05 and 5.11 kGy however, firmness increased during storage (Fig. 33).

Color

Green onion showed significant ($P < 0.05$) increase in chroma value only for dose 3.05 kGy compared to control but for the other doses tested (1.03 and 5.11 kGy) did not show any significant ($P > 0.05$) difference (Table 8). The chroma values increased only for dose 3.05 kGy over 9 days and reduced on day 12 which might be attributed to piece to piece variation and may not be linked to dose effect. The other doses did not exhibit any change in chroma values over storage. This is in agreement with Kim et al. *(48)* who reported that compared with the control, irradiation treatment at 0.5, 1, and 1.5 kGy doses did not significantly change chroma values. This however, is different from the observations of Fan et al. *(27)* who found that fresh cut green onion irradiated at 1 kGy had similar color values as control during the 14days storage period. However, samples treated at 2, and 3 kGy had increased chroma values during storage.

Respiration rate

Green onion treated with e-beam at doses 1.03, 3.05, and 5.11 kGy had higher respiration rates when compared to control. However, the increase was not significant (*P*

< 0.05) for dose 1.03 kGy. Dose 3.05 and 5.11 kGy had the highest respiration rate over the storage period and they were comparable to each other (Table 8). Irradiated samples had a higher respiration rate compared to non-irradiated control on day 0 and respiration rate for all samples including control decreased on day 3. During storage this trend continued and all samples had a stable respiration rate with irradiated samples slightly higher than the control. Respiration is an indicator of metabolic activity of all living produce and plays a role in the keeping quality of fresh produce. The rate of deterioration is generally proportional to their respiration rate *(83)*.

Microbiological counts

E-beam irradiation reduced APCs in green onion significantly ($P < 0.05$) compared to control (Table 8). From an initial count of 7.2 log CFU/g with dose 0 (control), a 4.5 log CFU/g reduction was noted for the lowest dose of 1.03 kGy. However, with 3.05 kGy, the reduction was 5.6 log CFU/g and the counts were below detectable limit of 1 log CFU/g with 5.11 kGy (Fig. 34). APCs for control increased during storage but all treated samples did not show any increase in counts during storage. The results are in agreement with Kim et al. *(48)* who also found an initial reduction of 3 to 3.5 log CFU/g in APCs compared to control for cut green onions which were dipped in warm water followed by a low dose gamma irradiation (0.5 to 1.5 kGy). Fan et al *(27)* tested gamma radiation doses of 1, 2, and 3 kGy and found that there was deterioration in quality of green onion at 2 and 3 kGy although at these doses, the microbial counts were not detectable. However, samples treated with 1 kGy retained

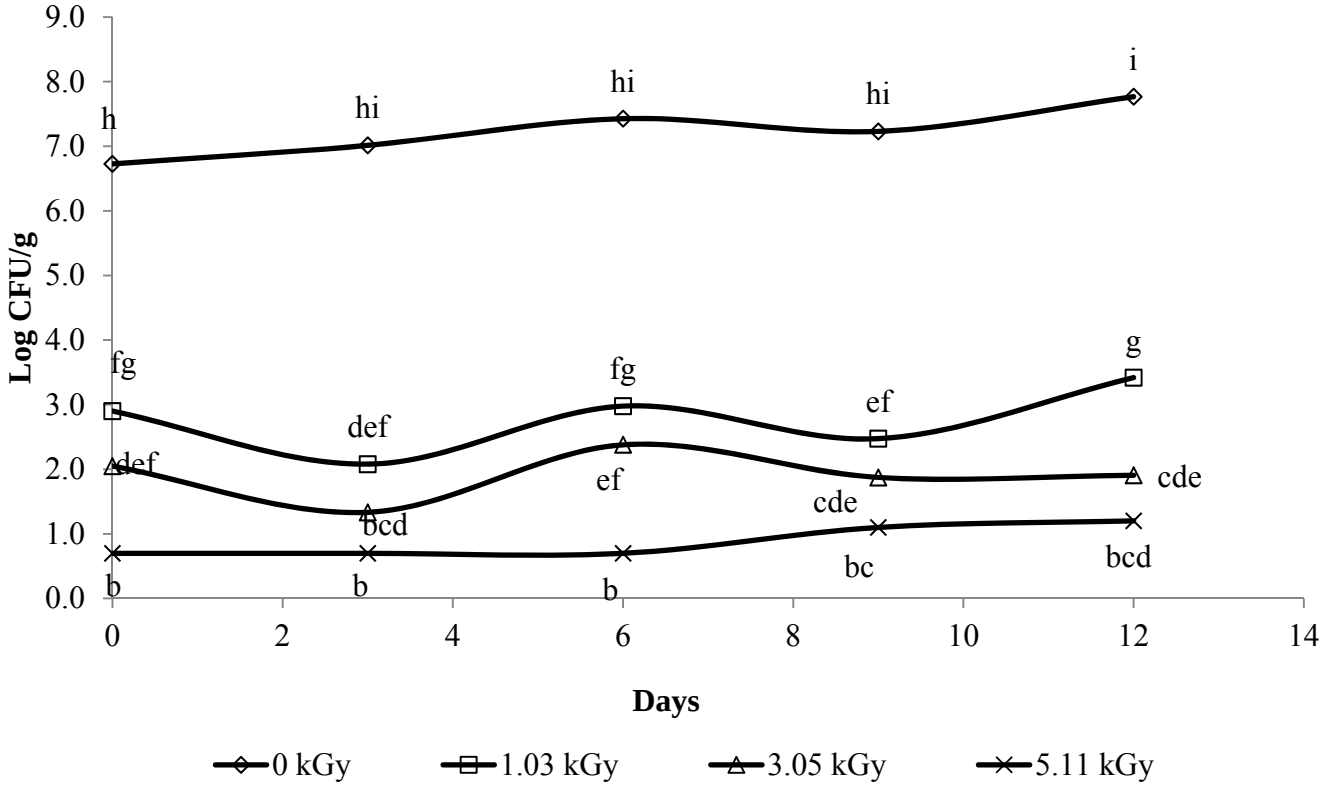

FIGURE 34. *Growth of APCs on irradiated green onion stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

quality and had a reduced microbial load throughout the 14 days storage *(27)*.

Yeast counts were reduced significantly ($P < 0.05$) for all irradiated samples compared to counts for non-irradiated control (5.9 log CFU/g) (Table 8). Highest reduction of 4.8 log CFU/g was found for the highest dose of 5.11 kGy followed by a 3.9 log CFU/g reduction with 3.05 kGy and the least reduction of 1.3 log CFU/g was noted with 1.03 kGy. Yeast counts were below detection limits of 1 log CFU/g on day 0 with 3.05 and 5.11 kGy (Fig. 35) but increased during storage. Counts increased for samples treated with 1.03 kGy during storage, however; control samples did not show any growth throughout storage (Fig. 35). The present study enumerated YNM separately and mold counts were below detection limits for all samples including control throughout storage. Butris et al. *(12)* studied the effect of gamma and e-beam irradiation on microbial counts on green onion and recorded reduction in YNM counts by 3-5 log CFU/g due to irradiation.

LAB counts were reduced due to irradiation at all doses (Table 8). No significant difference ($P > 0.05$) in counts was found for samples treated with 1.03 and 3.05 kGy. E-beam treatment with the highest dose 5.11 kGy showed a reduction of 1.9 log CFU/g. Reduction in counts for LAB with 1.03 and 3.05 kGy was 0.8 log CFU/g and 1.2 log CFU/g respectively. LAB counts increased during storage for control, 1.03 and 5.11 kGy but with 3.05 kGy, no increase in counts was observed. At the end of storage period, counts with 1.03 kGy were as high as control. LAB thrive in decayed fruits and vegetables due to the acid production leading to low pH. Untreated control was seen to

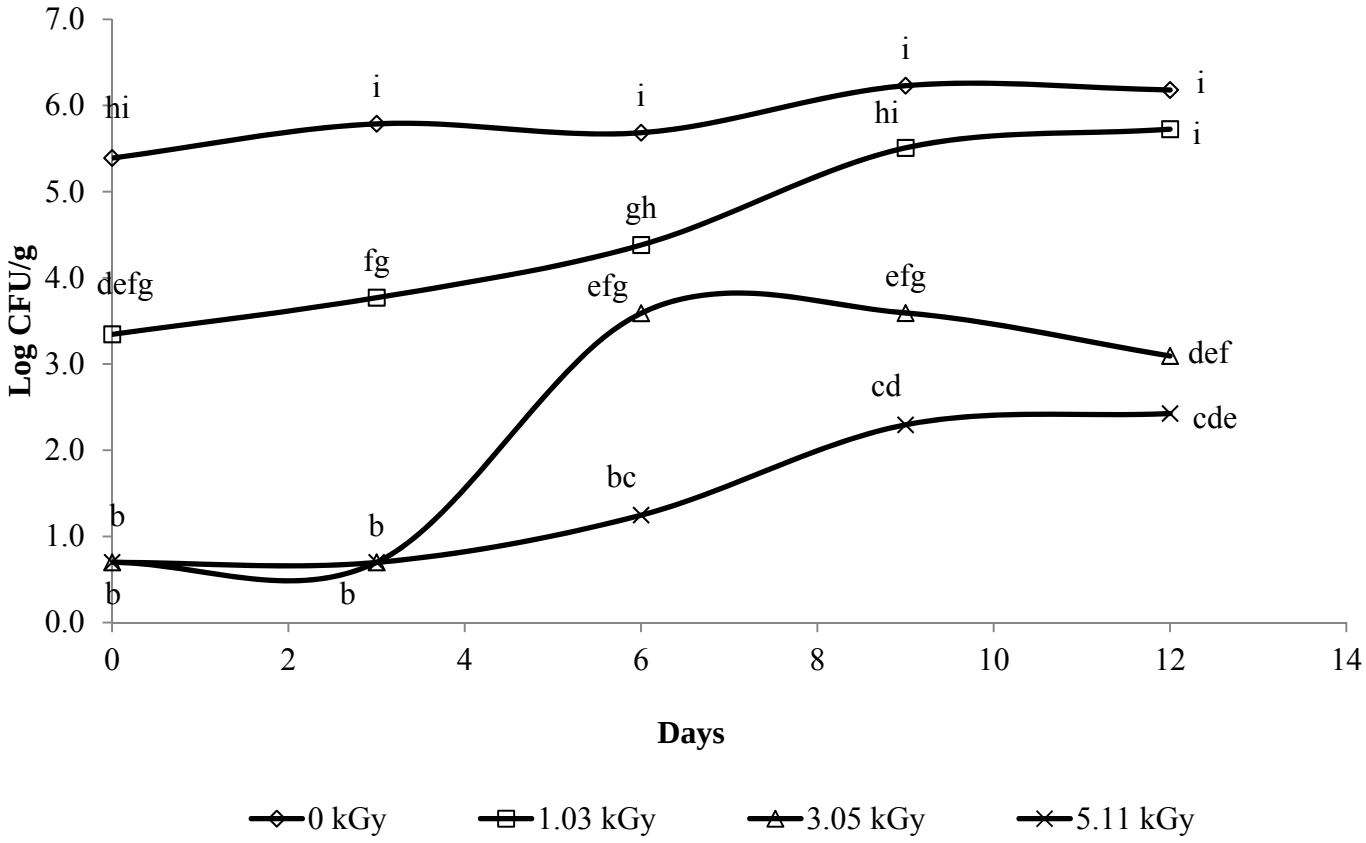

FIGURE 35. *Growth of yeasts on irradiated green onion stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

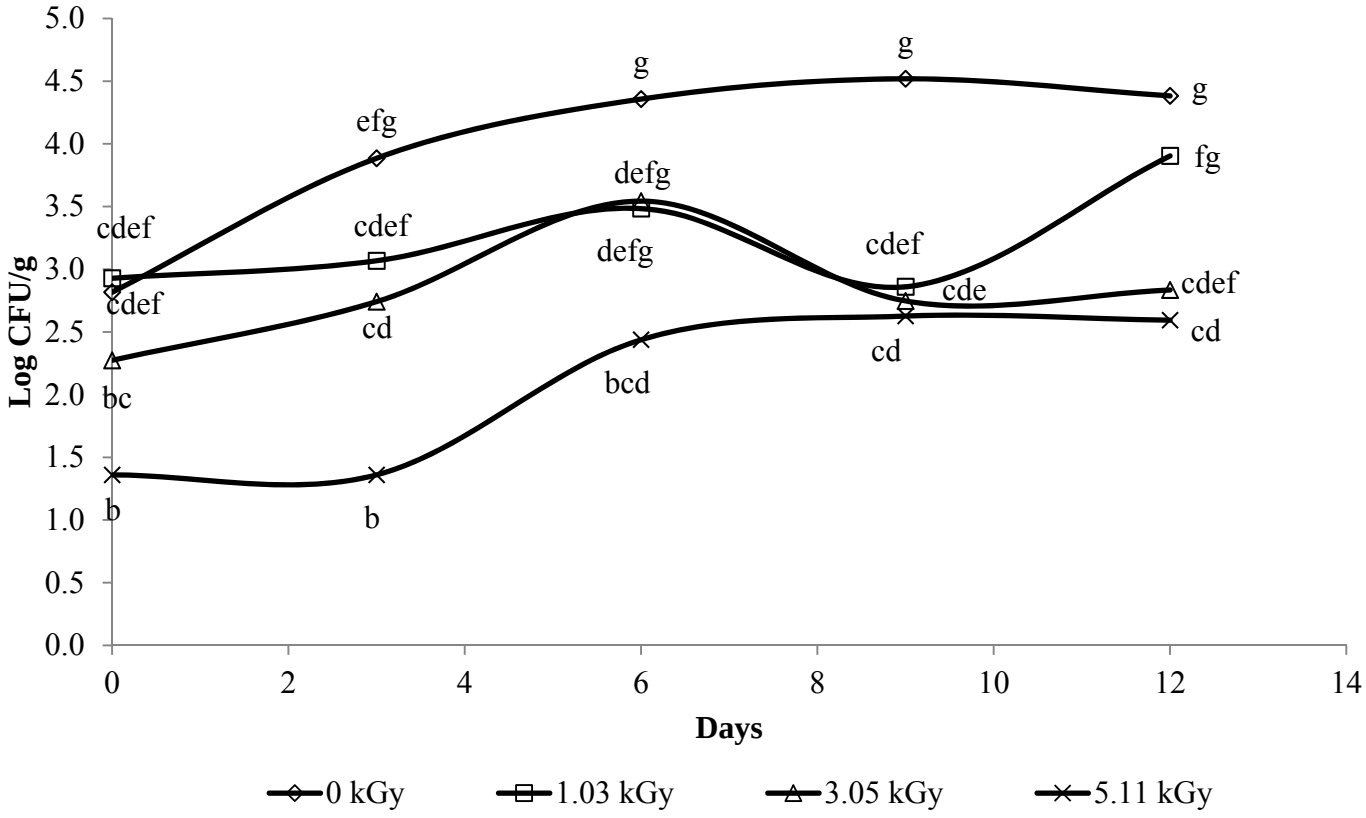

FIGURE 36. *Growth of LAB on irradiated green onion stored at 5°C over 12 days. Data points with the same letter are not significantly different (P > 0.05).*

Have the highest count of LAB by the end of storage indicating that irradiation helps in controlling LAB growth in green onion for 12 days. There were no studies found which focused on effects of irradiation on LAB in green onion.

CONCLUSIONS

E-beam irradiation at low doses was found to be an efficient tool for decontamination of fresh produce. The present study shows that irradiation can effectively reduce levels of *Salmonella* and *E. coli* O157:H7 increasingly at 0.2, 0.4, 0.6, 0.8, and 1.0 kGy in fresh cut cantaloupe, fresh cut roma tomatoes, baby spinach, and romaine lettuce. The D_{10}-value for *Salmonella* on e-beam irradiated cut cantaloupe, cut roma tomatoes, baby spinach, and romaine lettuce was found to be 0.71 kGy, 0.64 kGy, 0.19 kGy, and 0.23 kGy respectively. The D_{10}-value for *E. coli* O157:H7 on the same was found to be 0.73 kGy, 0.54 kGy, 0.18 kGy, and 0.20 kGy respectively. Factors such as shape and size of the commodities being irradiated affect the uniformity and hence effectiveness of absorbed dose. Thus, it is important to suggest D_{10}-values for pathogens after taking into consideration several physical, chemical and processing parameters of the commodity of interest.

Low dose e-beam irradiation was found to be an excellent tool for ensuring the reduction of spoilage organisms and extension of shelf life in cut cantaloupe, cut roma tomatoes, baby spinach, romaine lettuce, strawberries, and green onion. Irradiation followed by storage at 5°C for 12 days was efficient at reducing the microbial load of the tested produce. Spoilage organisms varied in their response to irradiation. Changes in color, texture, and respiration rate were significant with 5 kGy treatment. Strawberries treated with 5 kGy had a mushy appearance by the end of the storage. Baby spinach and green onion displayed a wet and soggy appearance whereas romaine lettuce leaves were wilted had a translucent midrib and brown pigmentation on the leaf with 5 kGy. Color in

cut tomatoes was redder (higher chroma value) by the end of storage with 5 kGy. The impact on color was lowest with target dose 1 kGy compared to target doses 3 kGy and 5 kGy for all commodities tested.

Firmness reduced appreciably for cut roma tomatoes, baby spinach, strawberries, and green onion with increasing doses. High dose imparted a mushy and soggy appearance due to loss of firmness. Romaine lettuce showed large reduction in firmness at 3.07 and 5.07 kGy but had no effect with 1.10 kGy. Cut cantaloupe was low in firmness but was not dose dependent.

E-beam irradiation increased respiration rate for all samples on day 0 compared to non irradiated control irrespective of the commodity type. The effect was dose dependent and low dose had least increase in respiration rate.

APCs, yeast counts, and LAB were reduced appreciably at all doses tested on all commodities. Molds did not grow on any samples including control for cut cantaloupe, cut tomatoes, and green onion but for the other commodities, molds were reduced as effectively as vegetative bacteria. LAB were reduced at all doses while the reduction was highest with 5 kGy in all commodities, there was no significant difference noted in counts between 1 kGy and 3 kGy in green onion and romaine lettuce. In cut cantaloupes and cut roma tomatoes, no difference was noted between 3 kGy and 5 kGy.

From this research, it is proposed that electron beam irradiation can be effective as a decontamination strategy in the processing of packaged fresh-cut produce. A low dose of 1 kGy is effective in reducing microbial counts while having least effect on the qualityfor all commodities tested. In addition, a high dose of 3 kGy is suitable for cut

cantaloupe and cut roma tomatoes according to the results. For these two commodities, e-beam dose of 2 kGy should be applied to test the effect on quality. But a high dose of 5 kGy has a detrimental effect on produce quality. However, a sensory evaluation of the irradiated produce against the non-irradiated produce must be carried out to correlate the instrumental findings. The proposed technology for shelf life extension may have the potential to be applied to various other produce commodities to address quality and shelf life issues faced by the fresh-cut produce industry.

REFERENCES

1. Adams, P. B., and K. S. Rowan. 1970. Glycolytic control of respiration during aging of carrot root tissue. *Plant Physiol*. 45:490-494.

2. Alvarado-Casillas, S., S. Ibarra-Sánchez, O. Rodríguez-Garcia, N. Martínez-Gonzáles, and A. Castillo. 2007. Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *J. Food Prot.* 70:655-660.

3. Andress, E., K. Delaplane, and G. Schuler 2000. Food Irradiation. Cooperative Extension Service, Institute of Food and Agricultural Sciences. University of Florida.

4. Attix, F. H. 1986. Introduction to radiological physics and radiation dosimetry. Wiley-Interscience, New York.

5. Barkai-Golan, R., S. Ben-Yehoshua, N. Aharoni, E. Eisenberg, and R. S. Kahan. 1971. The development of Botrytis cinerea in irradiated strawberries during storage. *Int. J. Appl. Radiat. Is*. 22:155-158.

6. Barrett, D. M., L. P. Somogyi, and H. S. Ramaswamy (ed.). 2005. Processing fruits: science and technology CRC Press, Boca Raton, FL.

7. Beuchat, L. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot*. 59:204-216.

8. Boynton, B. B., B. A. Welt, C. A. Sims, M. O. Balaban, J. K. Brecht, and M. R. Marshall. 2006. Effects of low-dose electron beam irradiation on respiration, microbiology, texture, color, and sensory characteristics of fresh-cut cantaloupe stored in modified-atmosphere packages. *J. Food Sci*. 71:149-155.

9. Boynton, B. B., B. A. Welt, C. A. Sims, J. K. Brecht, M. O. Balaban, and M. R. Marshall. 2005. Effects of low-dose electron beam irradiation on respiration, microbiology, color, and texture of fresh-cut cantaloupe. *Horttechnol*. 15:802-807.

10. Brandl, M. T. 2006. Fitness of human enteric pathogens on plants and implications for food safety1. *Annu. Rev. Phytopathol*. 44:367-392.

11. Burnett, S. L., and L. R. Beuchat. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *J. Ind. Microbiol. Biotechnol*. 27:104-110.

12. Butris, S., M. Foley, F. Caporaso, and A. Prakash. 2003. Effect of low dose gamma and electron beam irradiation on the quality characteristics of fresh cut green onions. *IFT Annual Meeting Chicago* 104-105.

13. Casteel, M. J., C. E. Schmidt, and M. D. Sobsey. 2009. Chlorine inactivation of coliphage MS2 on strawberries by industrial-scale water washing units. *J. Water Health* 7:244-250.

14. Castell-Perez, E., M. Moreno, O. Rodriguez, and R. G. Moreira. 2004. Electron beam irradiation treatment of cantaloupes: effect on product quality. *Food Sci. Technol. Int*. 10:383-390.

15. Castillo, A., L.M.Lucia, K.JGoodson, J.W.Savell, and G.R.Acuff. 1998. Use of hot water for beef carcass decontamination. *J. Food Prot*. 61:19-25.

16. Centers for Disease Control and Prevention. 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico – United States and Canada, 2000–2002. *Morb. Mortal. Wkly. Rep.* 51:1044-1047.

17. Centers for Disease Control and Prevention. 2003. Hepatitis A outbreak associated with green onions at a restaurant—Monaca, Pennsylvania. *Morb. Mortal. Wkly. Rep.* 52:1155-1157.

18. Centers for Disease Control and Prevention. 2005. Outbreaks of *Salmonella* infections associated with eating roma tomatoes—United States and Canada. *Morb. Mortal. Wkly. Rep*. 54:325–328.

19. Centers for Disease Control and Prevention. 2008. Outbreak of Salmonella serotype Saintpaul infections associated with multiple raw produce items – United States. *Morb. Mortal. Wkly. Rep.* 57:929-934.

20. Cliver, D. O. 1997. Virus transmission via food: scientific status summary. *Food Technol.* 51:71-78.

21. D'Amour, J., C. Gosselin, J. Arul, F. Castaigne, and C. Willemot. 1993. Gamma-radiation affects cell wall composition of strawberries. *J. Food Sci*. 58:182-185.

22. Delaquis, P., S. Bach, and L.-D. Dinu. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. *J. Food Prot*. 70:1966-1974.

23. DelRosario, B. A., and L. R. Beuchat. 1995. Survival and growth of *Escherichia coli* O157:H7 in cantaloupe and watermelon. *J. Food Prot*. 58:105-107.

24. Dentinger, C. M., W. A. Bower, O. V. Nainan, S. M. Cotter, G. Myers, L. M. Dubusky, S. Fowler, E. D. P. Salehi, and B. P. Bell. 2001. An outbreak of hepatitis A associated with green onions. *J. Infect. Dis*. 183:1273-1276.

25. Doyle, M., and M. Erickson. 2008. Summer meeting 2007 – the problems with fresh produce: an overview. *J. Appl. Microbiol*. 105:317-330.

26. El Assi, N., D. J. Huber, and J. K. Brecht. 1997. Irradiation-induced changes in tomato fruit and pericarp firmness, electrolyte efflux, and cell wall enzyme activity as influenced by ripening stage. *J. Am. Soc. Hortic. Sci*. 122:100-106.

27. Fan, X., B. A. Niemira, and K. J. B. Sokorai. 2003. Use of ionizing radiation to improve sensory and microbial quality of fresh-cut green onion leaves. *J. Food Sci*. 68:1478-1483.

28. Fan, X., Niemira, B.A., Doona, C.J., Feeherry, F.E., Gravani, R.B. (ed.). 2009. Microbial safety of fresh produce. Wiley-Blackwell, Ames, IA.

29. Fan, X., and K. J. B. Sokorai. 2008. Retention of quality and nutritional value of 13 fresh-cut vegetables treated with low-dose radiation [electronic resource]. *J. Food Prot*. 73:367-372.

30. Fan, X. T., B. A. Annous, K. J. B. Sokorai, A. Burke, and J. P. Mattheis. 2006. Combination of hot-water surface pasteurization of whole fruit and low-dose gamma irradiation of fresh-cut cantaloupe. *J. Food Prot*. 69:912-919.

31. Farkas, J., M. P. Doyle, L. R. Beuchat, and T. J. Montville (ed.). 2001. Food microbiology: fundamentals and frontiers. ASM Publication Washington, DC.

32. Farkas, J., T. Saray, C. Mohacsi-Farkas, K. Horti, and E. Andrassy. 1997. Effect of low dose gamma radiation on shelf-life and microbiological safety of pre-cut/prepared vegetables. *Adv. Food Sci.* 19:111-119.

33. Feng, P. 1995. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerg Infect Dis*. 1:47-52.

34. Food Marketing Institute. 2007. Perishables group: facts, figures and the future. Available http://www.factsfiguresfuture.com/archive/april_2007.htm Accessed 21 May 2010.

35. Gil, M. I., M. V. Selma, F. López-Gálvez, and A. Allende. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *Int. J. Food Microbiol*. 134:37-45.

36. Gomes, C., P. Da Silva, A. Castillo, J. Kim, R. G. Moreira, and M. E. Castell-Perez. 2008. E-Beam irradiation of bagged, ready-to-eat spinach leaves (Spinacea oleracea): an engineering approach. *J. Food Sci*. 73:95-102.

37. Gomes, C., P. Da Silva, R. G. Moreira, E. Castell-Perez, E. A. Ellis, and M. Pendleton. 2009. Understanding *E. coli* internalization in lettuce leaves for optimization of irradiation treatment. *Int. J. Food Microbiol*. 135:238-247.

38. Grant, I. R., M. F. Patterson., and 1992. Sensitivity of foodborne pathogens to irradiation in the components of a chilled ready meal. *Food Microbiol.* 9:95–103.

39. Hagenmaier, R. D., and B. R. A. 1997. Low-dose irradiation of cut iceberg lettuce in modified atmosphere packaging. *J. Agric. Food Chem*. 45:2864-2868.

40. Han, J., C. L. Gomes-Feitosa, E. Castell-Perez, R. G. Moreira, and P. F. Silva. 2004. Quality of packaged romaine lettuce hearts exposed to low-dose electron beam irradiation. *Lebensm. Wiss. Technol*. 37:705-715.

41. Harris, L., J. Farber, L. Beuchat, M. Parish, T. Suslow, E. Garrett, and F. Busta. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Safety* 2:78-141.

42. Herwaldt, B. L., and M.-L. Ackers. 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *New Engl J Med*. 336:1548-1556.

43. Hitti, M. Date, 2007, FDA sets fresh-produce safety rules. Available at: http://www.webmd.com/food-recipes/food-poisoning/news/20070312/fda-sets-fresh-produce-safety-rules. Accessed 10 October, 2010.

44. Howard, L. R., and R. W. Buescher. 1989. Cell wall characteristics of gamma-radiated refrigerated cucumber pickles. *J. Food Sci*. 54:1266-1268.

45. Ibarra-Sánchez, L. S., S. Alvarado-Casillas, M. O. Rodríguez-García, N. E. Martínez-Gonzáles, and A. Castillo. 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. *J. Food Prot*. 67:1353-1358.

46. James, D., J. Jaczynski, and K. Matak. 2010. Electron beam irradiation on nalidixic acid-resistant *Salmonella* Montevideo in cooked tomato puree of various pH values. *J. Food Safety* 30:515-525.

47. Ke, D., and M. E. Saltveit. 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in Iceberg lettuce. *Plant Physiol*. 88:1136–1140.

48. Kim, H. J., H. Feng, S. A. Toshkov, and X. Fan. 2005. Effect of sequential treatment of warm water dip and low-dose gamma irradiation on the quality of fresh-cut green onions. *J. Food Sci.* 70:179-185.

.

49. Kim, J., R. Moreira, and E. Castell-Perez. 2010. Simulation of pathogen inactivation in whole and fresh-cut cantaloupe (Cucumis melo) using electron beam treatment. *J. Food Eng*. 97:425-433.

50. Kim, J. H., J. W. Lee, J. H. Kim, J. H. Seo, S. B. Han, H. J. Chung, and M. W. Byun. 2006. Effect of gamma irradiation on *Listeria ivanovii* inoculated to iceberg lettuce stored at cold temperature. *Food Control*. 17:397-401.

51. Lamikanra, O., C. J. C, B. D, and H. P. A. 2000. Biochemical and microbial changes during the storage of minimally processed cantaloupe. *J. Agric. Food Chem*. 48:5955-5961.

52. Le Guyader, F. S., C. Mittelholzer, L. Haugarreau, K.-O. Hedlund, R. Alsterlund, M. Pommepuy, and L. Svensson. 2004. Detection of noroviruses in raspberries associated with a gastroenteritis outbreak. *Int J Food Microbiol*. 97:179-186.

53. Lee, N., C. Job, D. Shin, W. Kim, and M. Byun. 2006. Effect of gamma irradiation on pathogens inoculated into ready-to-use vegetables. *Food Microbiol*. 23:649-656.

54. Lin, C. M., S. S. Moon, M. P. Doyle, and K. H. McWatters. 2002. Inactivation of *Escherichia coli* O157 : H7, *Salmonella* enterica serotype enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot*. 65:1215-1220.

55. Lopez-Galvez, F., A. Allende, P. Truchado, A. Martinez-Sanchez, J. A. Tudela, M. V. Selma, and M. I. Gil. 2010. Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biol. Technol.* 55:53-60.

56. Lynch, M. 2007. *Escherichia coli* O157:H7 outbreaks due to raw leafy green vegetables. Memorandum by the US Center of Disease Control and Prevention.1: 3.

57. Magee, R. L., F. Caporaso, and A. Prakash. 2003. Effects of exogenous calcium salt treatments on inhibiting irradiation-induced softening in diced roma tomatoes. *J. Food Sci*. 68:2430-2435.

58. Mahmoud, B. S. M. 2010. Effects of X-ray radiation on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* enterica and *Shigella* flexneri inoculated on shredded iceberg lettuce. *Food Microbiol*. 27:109-114.

59. Mahmoud, B. S. M., G. Bachman, and R. H. Linton. 2010. Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri* on spinach leaves by X-ray. *Food Microbiol*. 27:24-28.

60. Mele, A., T. Stroffolini, F. Palumbo, G. Gallo, P. Ragni, E. Balocchini, M. E. Tosti, R. Corona, A. Marzolini, and A. Moiraghi. 1997. Incidence of and risk factors for hepatitis A in Italy: public health indications from a 10-year surveillance. *J. Hepatol*. 26:743-747.

61. Mohle-Boetani, J. C., R. Reporter, S. B. Werner, A. Sharon, J. Farrar, S. H. Waterman, and D. J. Vugia. 1999. An outbreak of Salmonella serogroup Saphra due to cantaloupes from Mexico. *J. Infect. Dis*. 180:1361-1364.

62. Monk, J. D., L. R. Beuchat, and M. P. Doyle. 1995. Irradiation inactivation of food-borne microorganisms. *J. Food Prot*. 58:197-208.

63. Moreno, M. A., M. Elena Castell-Perez, C. Gomes, P. F. Da Silva, and R. G. Moreira. 2007. Quality of electron beam irradiation of blueberries (Vaccinium corymbosum L.) at medium dose levels (1.0-3.2 kGy). *Food Sci. Technol*. 40:1123-1132.

64. Nayak, C. A., K. Suguna, K. Narasimhamurthy, and N. K. Rastogi. 2007. Effect of gamma irradiation on histological and textural properties of carrot, potato and beetroot. *J. Food Eng*. 79:765-770.

65. Neal, J. A., B. Booren, L. Cisneros-Zevallos, R. K. Miller, L. M. Lucia, J. E. Maxim, and A. Castillo. 2010. Shelf life and sensory characteristics of baby spinach subjected to electron beam irradiation. *J. Food Sci*. 75:319-326.

66. Neal, J. A., E. Cabrera-Diaz, M. Márquez-Gonzaález, J. E. Maxim, and A. Castillo. 2008. Reduction of *Escherichia coli* O157:H7 and *Salmonella* on baby spinach, using electron beam radiation. *J. Food Prot*. 71:2415-2420.

67. Nguyenthe, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci*. 34:371-401.

68. Niemira, A. B. 2007. Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate *Escherichia coli* O157:H7 internalized in leaves of romaine lettuce and baby spinach. *J. Food Prot*. 70:2526-2532.

69. Niemira, B. A. 2003. Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on 4 lettuce types. *J. Food Sci*. 68:2784-2787.

70. Niemira, B. A. 2008. Irradiation compared with chlorination for elimination of *Escherichia coli* O157:H7 internalized in lettuce leaves: influence of lettuce variety. *J. Food Sci*. 73:208-213.

71. Niemira, B. A., and P. H. Cooke. 2010. *Escherichia coli*O157:H7 biofilm formation on romaine lettuce and spinach leaf surfaces reduces efficacy of irradiation and sodium hypochlorite washes. *J. Food Sci*. 75:270-277.

72. Niemira, B. A., C. H. Sommers, and G. Boyd. 2001. Irradiation inactivation of four *Salmonella* serotypes in orange juices with various turbidities. *J. Food Prot*. 64:614-617.

73. Niemira, B. A., C. H. Sommers, and X. Fan. 2002. Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157:H7. *J. Food Prot*. 65:1388-1393.

74. Niu, M. T., L. B. Polish, B. H. Robertson, B. K. Khanna, B. A. Woodruff, C. N. Shapiro, M. A. Miller, J. D. Smith, J. K. Gedrose, M. J. Alter, and H. S. Margolis. 1992. Multistate outbreak of hepatitis A associated with frozen strawberries. *J. Infect Dis*. 166:518-524.

75. Olson, D. (ed.). 1995. Food irradiation: a sourcebook. Iowa State University Press., Ames, IA

76. Palekar, M., E. Cabrera-Diaz, A. Kalbasi-Ashtari, J. Maxim, R. Miller, L. Cisneros-Zevallos, and A. Castillo. 2004. Effect of electron beam irradiation on the bacterial load and sensorial quality of sliced cantaloupe. *J. Food Sci*. 69:267-273.

77. Palekar, M. P. 2004. Attachment of *Salmonella* on cantaloupe and effect of electron beam irradiation on quality and safety of sliced cantaloupe. Ph.D. dissertation. Texas A&M University, College Station.

.

78. Pönkä, A., L. Maulula, C. H. v. Bonsdorff, and O. Lyytikäinen. 1999. An outbreak of calicivirus associated with consumption of frozen raspberries. *Epidem. Inf.* 123:469-474.

79. Prakash, A., P. C. Chen, R. L. Pilling, N. Johnson, and D. Foley. 2007. 1% calcium chloride treatment in combination with gamma irradiation improves microbial and physicochemical properties of diced tomatoes. *Foodborne Patho. Dis*. 4:89-98.

80. Prakash, A., A. Guner, F. Caporaso, and D. Foley. 2000. Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. *J. Food Sci*. 65:549-553.

81. Prakash, A., N. Johnson, and D. Foley. 2007. Irradiation D values of Salmonella spp. in diced tomatoes dipped in 1% calcium chloride. *Foodborne Patho. Dis*. 4:84-88.

82. Prakash, A., J. Manley, S. DeCosta, F. Caporaso, and D. Foley. 2002. The effects of gamma irradiation on the microbiological, physical and sensory qualities of diced tomatoes. *Radiat. Phys. Chem*. 63:387-390.

83. Rahman, M. S. (ed.). 2007. Handbook of food preservation CRC Press, Boca Raton, FL.

84. Rajkowski, K. T., and D. W. Thayer. 2000. Reduction of *Salmonella* spp. and strains of *Escherichia coli* O157:H7 by gamma irradiation of inoculated sprouts. *J. Food Prot.* 3:871–875.

85. Ramsay, C. N., and P. A. Upton. 1989. Hepatitis A and frozen raspberries. *Lancet* 333:43-44.

86. Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States,1982–2002. *Emerg. Infect. Dis*. 11:603-609.

87. Reid, T. M., and H. G. Robinson. 1987. Frozen raspberries and hepatitis A. *Epidem. Inf.* 98:109-112.

88. Reller, M. E., J. M. Nelson, K. Molbak, D. M. Ackman, D. J., and T. P. R. Schoonmaker-Bopp, and E. D. Mintz. 2006. A large, multiple-restaurant outbreak of infection with Shigella flexneri serotype 2a traced to tomatoes. *Clin. Infect. Dis*. 42:163-169.

89. Rodriguez, O., M. E. Castell-Perez, N. Ekpanyaskun, R. G. Moreira, and A. Castillo. 2006. Surrogates for validation of electron beam irradiation of foods. *Int. J. Food Microbiol*. 110:117-122.

90. Sanghyup, J., M. P. Bradley, R. T. Elliot, and M. R. Scott. 2010. Inactivation of *Escherichia coli* O157:H7 on lettuce, using low-energy X-ray irradiation. *J. Food Prot*. 73:547-551.

91. Sapers, G. M., and J. E. Sites. 2003. Efficacy of 1% hydrogen peroxide wash in decontaminating apples and cantaloupe melons. *J. Food Sci*. 68:1793-1797.

92. Schmidt, H., M. Palekar, J. Maxim, and A. Castillo. 2006. Improving the microbiological quality and safety of fresh-cut tomatoes by low-dose electron beam irradiation. *J. Food Prot*. 69:575-581.

93. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot*. 67:2342-2353.

94. Solomon, E. B., S. Yaron, K. R. Mathews. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68:397-400.

95. Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, , and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce a preliminary report to the national advisory committee on microbiological criteria for foods. *J. Food Prot*. 60:1400-1408.

96. Thayer, D. W., K. T. Rajkowski, G. Boyd, P. H. Cooke, and D. S. Soroka. 2003. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* by gamma irradiation of alfalfa seed intended for production of food sprouts. *J. Food Prot.* . 66:175-181.

97. Thomas, P. 1986. Radiation preservation of foods of plant origin. part V. temperate fruits: pome fruits, stone fruit and berries. *Crit. Rev. Food Sci. Nutr.* . 24:357-400.

98. U.S. Food and Drug Administration. 2006. FDA Finalizes Report on 2006 Spinach Outbreak. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108873.htm. Accessed 10 September 2010.

99. U.S. Food and drug Administration. 1998. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. Available at www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/UCM169112.pdf. Accessed 8 August 2010.

100. U.S. Food and drug Administration. 2009. Effect of preservation technologies and microbiological inactivation in foods. Available at http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFood Processes/ucm094151.htm. Accessed 2 January, 2011.

101. U.S. Food and drug Administration. 2010. Irradiation in the production, processing and handling of food. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=179&showFR=1. Accessed 30 October, 2010.

102. Ukuku, D. O. 2006. Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with Salmonella. *Food Microbiol*. 23:289-293.

103. Villegas, C. N., C. O. Chichester, L. C. Raymundo, and K. L. Simpson. 1972. Effect of gamma irradiation on the biosynthesis of carotenoids in the tomato fruit. *Plant Physiol*. 50:694-697.

104. Voisine, R., L. P. Vézina, and C. Willemot. 1993. Modification of phospholipid catabolism in microsomal membranes of gamma-irradiated cauliflower (*Brassica oleracea*). *Plant Physiol*. 102:213-218.

105. Waje, C. K., S. Y. Jun, Y. K. Lee, B. N. Kim, D. H. Han, C. Jo, and J. H. Kwon. 2009. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. *Food Control* 20:200-204.

106. Wei, C. I., T. S. Huang, J. M. Kim, W. F. Lin, M. L. Tamplin, and J. A. Bartz. 1995. Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. *J. Food Prot*. 58:829-836.

107. Wheeler, C., T. M. Vogt, G. L. Armstrong, G. Vaughan, A. Weltman, O. V. Nainan, V. Dato, G. Xia, K. Waller, J. Amon, T. M. Lee, A. Highbaugh-Battle, C. Hembree, S. Evenson, M. A. Ruta, I. T. Williams, A. E. Fiore, and B. P. Bell. 2005. An outbreak of hepatitis A associated with green onions. *New Engl. J. Med*. 353:890-897.

108. Yaguang, L. Date, 2009, Improving produce safety by stabilizing chlorine in washing solutions with high organic loads. Available at: cps.ucdavis.edu/.../Improving_produce_safety_by_stabilizing_chlorine_in_washing_solutions_with_high_organic_loads.html. Accessed 12 December, 2010.

109. Yu, L., C. Reitmeier, M. Gleason, G. Nonnecke, D. Olson, and R. Gladon. 1995. Quality of electron beam irradiated strawberries. *J. Food Sci*. 60:1084-1087.

110. Yu, L., C. Reitmeier, and M. Love. 1996. Strawberries texture and pectin content as affected by electron beam irradiation. *J. Food Sci*. 61:844-846.

111. Yuk, H. G., M. Y. Yoo, J. W. Yoon, K. D. Moon, D. L. Marshall, and D. H. Oh. 2006. Effect of combined ozone and organic acid treatment for control of Escherichia coli O157:H7 and Listeria monocytogenes on lettuce. *J. Food Sci*. 71:83-87.

112. Żegota, H. 1988. Suitability of Dukat strawberries for studying effects on shelf life of irradiation combined with cold storage. *Z. Lebensm. Unters. Forsch*. 187:111-114.

113. Zhang, L., Z. Lu, and H. Wang. 2006. Effect of gamma irradiation on microbial growth and sensory quality of fresh-cut lettuce. *Int. J. Food Microbiol*. 106:348-351.

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