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Effect of Electron Beam Irradiation on Survival of Selected Gram Positive and Gram Negative Bacteria in Pork Salami Stored at Refrigeration Temperature

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A B S T R A C T

The study was carried out to assess and optimize the effect of electron beam doses on inactivation/reduction of selected gram positive and gram negative bacteria inoculated in sterile pork salami samples stored at refrigeration temperature (0-4^{0}C). Pork salami samples were purchased from reputed HACCP accredited and ISO 22000 certified pork processing plant, sterilized, inoculated with 10^8 CFU/mL of selected gram positive and gram negative bacteria, packaged in sterile low density polyethylene pouches and subsequently irradiated at the dose rate of 1, 2 and 3 kGy. The packaged irradiated and non-irradiated (control) samples were stored at 0-4^{0}C and analyzed for selected gram positive and gram negative bacteria at 0, 2nd, 4th, 6th, 8th and 10th day of refrigerated storage. The study revealed that microbial log reduction was found to be increased with the increase of electron beam irradiation doses and period of storage. However, no viable cells of *Salmonella enterica* were detected in the pork salami samples irradiated at 1 to 3 kGy of doses. Thus, the study concluded that amongst all the electron beam irradiation doses used under study, 3 kGy was found to be more effective in microbial log reduction.

Introduction

India possesses one of the largest livestock wealth in the world and a quarter of the agricultural gross domestic product is contributed by the livestock sector. Pigs form a very important component of the Indian livestock sector and it's a cheap source of healthy animal protein. Pig population in India is estimated to be 10.29 million and it ranks 5th in the world (Sulabh *et al.*, 2017). The global demand for pork continues to rise and it remains the most widely consumed meat protein in the world. Meat and meat products have been incriminated as predominant cause of many food-borne infections, zoonoses and death in many parts of world (Banerjee *et al.*, 2001). Food-borne pathogens are a major

contributor to human illnesses, hospitalizations, and deaths each year. The Centers for Disease Control and Prevention (CDC) estimates that 47.8 million illnesses and 3000 deaths are caused by food-borne pathogens each year. These pathogens are well-documented as being present in pigs or pork products, making pork a potential contributor to food-borne illness (CDC, 2011).Salmonellosis is well recognized as a major health threat to consumers of pork and pork products (Beloeil *et al.,* 2004), causing80.3 million cases of foodborne salmonellosis occur annually in the world (Majowicz *et al.,* 2010). *Bacillus* species are notable agents of human infection frequently implicated in the spoilage of foods preserved by appertisation and responsible for many outbreaks due to the synthesis of two types of toxins (De-Lara *et al.,* 2002). *Pseudomonas* spp. are responsible for off-odor and off flavor development in meat, leading to cause serious economic losses in meat industry (Farkas, 1998). *Klebsiella pneumoniae* is a colonizing opportunistic pathogen of humans and animals, and a common contaminant of retail meat (Kim *et al.,* 2005).

Ionizing radiation is a non-thermal treatment used to enhance microbial food safety and it extends the shelf-life of meat products (Mohamed *et al.,* 2011). In several studies, irradiation significantly reduced food-borne pathogen concentrations (Fu *et al.,* 1995; Molins *et al.,* 2001; Satin *et al.,* 2002). Irradiation is known as an effective way to eliminate foodborne pathogens. Electron-beam irradiation has been shown to destroy 99.9% of the major food pathogenic bacteria (Rodriguez *et al.,* 2006) and it has less influence on the quality of food because of its low penetrating power (Lewis *et al.,* 2002). Furthermore, it does not generate radioisotope concern (Black and Jaczynski, 2006), as it has short processing time, low temperature rise which makes the method more environment friendly and highly acceptable to consumers (Hong *et al.,* 2008). Thus, the present study was therefore undertaken to assess and optimize the doses of electron beam irradiation on inactivation/reduction of selected gram positive and gram negative bacteria inoculated in pork salami.

Materials and Methods

Procurement of samples and sterilization

Freshly prepared pork salami samples were procured from HACCP accredited and ISO 22000 certified processing plants in Mumbai, Maharashtra. Sterilization of samples was carried out by autoclaving at 121^0C (15 lbs pressure) for 15 min.

Test pathogens and inoculation

A reference strains of gram positive bacteria viz. *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-441) and gram negative bacteria viz., *Salmonella enterica* (MTCC-3218), *Klebsiella pneumoniae* (MTCC-432) and *Pseudomonas aeruginosa* (MTCC-2453) were procured from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh, India were used to prepare the inoculum to test in pork salami. The colonies of the selected gram positive and gram negative standard bacterial isolates at 10^8CFU/mL were inoculated in tryptic soy broth (TSB) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37^0C for 24 h. After incubation, the culture suspension was poured into sterile centrifuge tubes and was centrifuged at 5,000×*g* for 10 min and then the supernatant was discarded, and the pellet was resuspended in 10 mL of sterile distilled water and centrifuged again as previously described. The final supernatant was discarded, and the pellet was resuspended in 1 mL of 3% TSB with 30% glycerol solution in a 2-mL cryovial. Stock cultures were stored at −20°C until ready for use (Sarjeant *et al.,* 2005).

A sterile bacteriological loop was used to transfer thawed stock cultures to test tubes containing 10 mL of 3% TSB. The tubes were incubated at 37^0C for 24 h. After incubation, serial dilutions of the culture were prepared in 0.1% peptone water and plated on selective Agar. The plates were incubated at 37°C for 24 h and colony-forming units of selected gram positiveand gram negative bacteria were counted. Approximately 10^8CFU/mL of the selected bacterial isolates grown in TSB were recovered on the selective Agar after 24 h of incubation at 37°C.

Each sample was inoculated with approximately 1 mL of test bacteria with 10^8 CFU/mL. The standard culture suspension was uniformly and aseptically inoculated in whole area of pork salami by pipette. The inoculum was then spread over the pork salami with sterile glass rod and kept for 20 min at room temperature to allow for bacterial attachment and then inoculated samples were packed separately in sterile low density polyethylene (LDPE) pouches, each containing 100 gm of product. The pouches were heat sealed and individually labeled. Each sample was stacked with the thickness of 3.0 cm and taken to electron beam (EB) facility of Isotope and Radiation Application Division, BARC, VashiNavi Mumbai for exposure to varying doses of electron beam irradiation.

Electron-beam irradiation

All these pork salami samples were divided into 5 separate groups, of which one was kept as inoculated non-irradiated control and other as uninoculated non-irradiated control and remaining three groups were exposed to 1, 2 and 3 kGy doses of electron beam irradiation. For electron beam irradiation, the pouches were arranged in aluminium boxes and irradiated on both sides in a ILU EB machine (Energy 4.5 MeV, beam power 15 kW).Irradiation was performed with a conveyer velocity of 1.8m/min (3cm/sec). Dosimetry for these irradiation of the sample was carried out using radiochromic film dosimeter (B-3). Double sided irradiation was carried out in order to ensure uniform dose. During the irradiation treatment, chilled temperature was maintained by filling the aluminium boxes with ice packs. All the irradiated samples along with their corresponding controls were brought to the laboratory in the ice box and stored at temperature of 0-4^0C, until further analysis.

Microbiological analysis

Microbial analysis was done at the 0, 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th} days of refrigerationstorage. Each sample (10 g) was aseptically homogenized for 2 min in a sterile stomacher bags containing 90 ml of sterile 0.1% peptone water using stomacher (Seward Stomacher 80, Fisher Scientific, U.K.) at normal speed for 60 sec. Then, samples were serially diluted in sterile 0.1% peptone water and each diluent (0.1 mL) was spread on selective bacterial media by direct plating. The plates were incubated at 37^0C for 24 h, and microbial counts were expressed as log CFU/g.

Colonies typical of selected bacteria were counted and were identified by gram stain. Media for the enumeration of thegram positive bacteria viz. *Bacillus cereus, Bacillus subtilis* and gram negative bacteria viz. *Salmonella enterica*, *Klebsiella pneumoniae* and *Pseudmonas aeruginosa*were *Bacillus cereus* agar base, *Bacillus* differentiation agar, xylose lysine deoxycholate agar, MacConkey agarand *Pseudomonas* isolation agar base, respectively. All the media used in the study were procured from M/s. HiMedia Laboratories Pvt. Ltd., Mumbai, India. Populations of Selective bacteria in unsterilized uninoculated non-irradiated pork salami samples were also determined.

Statistical analysis

The data generated for microbiological quality during the experiment was compiled and analyzed by Randomized Block Design within the treatments on each day of storage using software "WASP-Web Agree Stat Package- 2.0" developed at ICAR research complex, Goa, India.

Results and Discussion

Effect of electron beam irradiation on survival of selected gram positive bacteria inoculated in Pork salami

Bacillus cereus

All the pork salami samplesinoculated with *Bacillus cereus* at the concentration of 10^8 CFU/g were irradiated at 1, 2 and 3kGy and were analyzed for the presence of *Bacillus cereus* (Table 1). From the Table 1 it is indicated that *Bacillus cereus* was found to beincreased in control uninoculated non-irradiated group and control inoculated non-irradiated group up to storage period of 2^{nd} and 10^{th} day of refrigeration storage, respectively. The control inoculated non-irradiated group showed 5.68±0.61level of *Bacillus cereus* (log CFU/g) on 0^{th} day whichincreasedto the level of 6.04±0.03on 10^{th} day of refrigeration storage. The samples treated with electron beam irradiation doses of 1, 2 and 3 kGy showed the average *Bacillus cereus* count(log CFU/g) in pork salami as 4.14±0.81, 2.86±2.48 and 2.85±2.48 on 0^{th} day, respectively and further subsequently decreased to 3.23±0.28, 1.20±2.07 and 1.27±2.20 on 10^{th} and 4^{th} day, respectively (Table 1 and Figure 1).

The log reduction in the *Bacillus cereus* count was observed after treating the pork salami samples with 1, 2 and 3 kGy of electron beam irradiation as compared to control inoculated non-irradiated pork salami samples. Among all the irradiation doses used, the maximum log reduction in the *Bacillus cereus* count was observed in pork salami samples treated with 3 kGy of electron beam irradiation.

Similar observations are reported by Hong *et al.,* (2008) who stated that electron-beam irradiation inhibits the growth of *Bacillus cereus* in powdered weaning food. Valero *et al.,* (2006)studied the effect of electron beam irradiation doses at 1.3 and 3.1 kGy followed by heating at 90^0C on heat resistance of *Bacillus cereus* spore and noted the reduction of *Bacillus cereus* spore strain to 1.3 and 2.5 times, respectively.

Bacillus subtilis

The count of *Bacillus subtilis* in pork salami treated with the doses of 1, 2 and 3kGy showed significant reduction throughout the storage at refrigeration temperature in all the samples (Table 2). The *Bacillus subtilis* was found to be increased upto 10^{th} day in control inoculated non- irradiated group. However, no viable cells were detected in control uninoculated non-irradiated group. The level of *Bacillus subtilis* (log CFU/g) into pork salami in control inoculated non-irradiated group was 5.5±0.03 on 0^{th}day which was increased to the level of 6.10±0.01 on 10^{th} day.The samples treated with electron beam irradiation doses of 1 and 2 kGy showed the average concentration of *Bacillus subtilis* (log CFU/g) in pork salami as 3.61±0.16 and 3.59±0.06 on 0^{th}day, respectively. As the storage period advanced, the bacterial count decreased to 3.06±0.17 on 6^{th} day after electron beam irradiation dose of 1 kGy. Irradiation dose of 2 kGy showed 3.06±0.17 level of *Bacillus subtilis* on 4^{th} day. However, no single sample showed presence of *Bacillus subtilis* in pork salami samples irradiated at 3

kGy and stored at refrigeration temperature (Table 2 and Figure 2).

The *Bacillus subtilis* count was reduced after treating the pork salami samples with 1, 2 and 3 kGy of electron beam irradiation as compared tocontrol inoculated non-irradiated pork salami samples. Among all the irradiation doses used, the maximum log reduction of *Bacillus subtilis* was noticed in pork salami samples treated with 3 kGy of electron beam irradiation.

De-Lara *et al.,* (2002) reportedsimilar observation regarding the initial irradiation of *Bacillus subtilis* spores with the electron beam prior to heat treatment increased the sensitivity of the spores to high temperatures. In addition, the D_T values (>3.3 kGy) of *B. subtilis* spores were reduced by 3-folds. Ohki (1990) examined the relative sensitivities of endospores of *B. subtilis* to electron beam in order to determine the sterilization condition. The electron beam irradiation sensitivity of the strain was almost equivalent to gamma-rays and X-rays.

Effect of electron beam irradiation on survival of selected gram negative bacteria inoculated in Pork salami

Pseudomonas aeruginosa

The effect of electron beam irradiation on *Pseudomonas aeruginosa* inoculated into pork salamiat the concentration of 10^8 CFU/g and irradiated at 1, 2 and 3kGy is shown in Table 3. The count of *Pseudomonas aeruginosa* was increased with the increased storage period under refrigeration temperature in control inoculated non-irradiated group. However, no viable cells were noticed in control uninoculated non-irradiated group. The control inoculated non-irradiated group showed 5.72±0.01 level of *Pseudomonas aeruginosa* (log CFU/g) on 0^{th} day which was increased to the level of 6.18±0.01 on 10^{th} day under refrigeration temperature. The number of *Pseudomonas aeruginosa* (log CFU/g) colonies in the samples exposed to 1 and 2 kGy irradiation were observed as 3.83±0.11and 1.19±2.05 on 0^{th}day, respectively. The *Pseudomonas aeruginosa* (log CFU/g) count was reduced to 1.84±1.67 on 10^{th} and 0.56±0.9 on 6^{th} day after electron beam irradiation dose of 1 and 2 kGy, respectively. However, 3 kGy of electron beam irradiation dose eliminated the population of the microorganism in all the samples stored throughout refrigeration storage (Table 3 and Figure 3).

When the pork salami samples were treated with 1, 2 and 3 kGy of electron beam irradiation, the reduction in *Pseudomonas aeruginosa*count was observed more in electron beam irradiated groups as compared to control inoculated non-irradiated pork salami group. The maximum log reduction in the *Pseudomonas aeruginosa*count was observed in pork salami samples treated with 3 kGy of electron beam irradiationamong all the irradiation doses used.

The results of this research concerned with the reduction of *Pseudomonas aeruginosa* count in irradiated pork salami are in accordance with Chung *et al.,* (2000) who observed that the initial level of *P. fluorescens* was 6.1 log CFU/g in the beef sample. When *P. fluorescens* in beef samples was irradiated by electron beam at the dose of 1.5 kGy, the level of *P. fluorescens* reduced to 4.0 log CFU/g which was evaluated after two days of interval. Sarjeant *et al.,* (2005) determined that electron beam irradiation of chicken breast samples with 2 and 3 kGy resulted in lower count of *P. fluorescens* when compared with the control samples and samples irradiated with 1.0 kGy and also stated that the most effective irradiation treatment was 3.0 kGy.

Klebsiella pneumoniae

All the pork salami samples analyzed for the presence *Klebsiella pneumoniae* after spiking at the concentration of 10^8 CFU/g and treated withelectron beam irradiation doses of 1, 2 and 3kGy. From the Table 4 it is indicated that *Klebsiella pneumoniae* was found to be increased with the increased storage up to 10^{th} day under refrigeration temperature in control inoculated non-irradiated group. The control inoculated non-irradiated group showed 5.68±0.03 level of *Klebsiella pneumoniae* (log CFU/g) on 0^{th}day which was increased to the level of 6.12±0.02 on 10^{th} dayunder refrigeration condition. The sample treated with electron beam irradiation doses of 1, 2 and 3 kGy revealed the average concentration of *Klebsiella pneumoniae* (log CFU/g) count in pork salami as 4.26±0.39, 1.38±2.39and non-detected level on 0^{th}day, respectively. Average *Klebsiella pneumoniae* count (log CFU/g) decreased with period of storage from 4.26±0.39to 3.78±0.07 on 10^{th} day after electron beam irradiation dose of 1 kGy, whereas the dose of 2kGy showed 1.29±2.23 level of *Klebsiella pneumoniae* on 4^{th} day and thereafter no growth was observed. However, all the pork salami samples treated with electron beam dose of 3kGy did not showed presence of *Klebsiella pneumoniae* throughout the storage period (Table 4 and Figure 4).

The pork salami samples in irradiated group treated with 1, 2 and 3 kGy of electron beam irradiation witnessed log reduction in the *Klebsiella pneumoniae* as compared to control inoculated non-irradiated pork salami samples. *Klebsiella pneumoniae* showed maximum log reduction count in pork salami samples treated with 3 kGy of electron beam irradiation among all the irradiation doses used.

Similar observation was recorded by Sarjeant *et al.,* (2005) who mentioned that electron beam irradiation of chicken breast samples with 2 and 3 kGy resulted in reduction of *Klebsiella pneumoniae* count when compared with the control samples and samples irradiated with 1.0 kGy they further stated that the most effective irradiation treatment was 3.0 kGy.

Salmonella enterica

None of the pork salami sample inoculated at 10^8 (CFU/g) and irradiated at 1, 2 and 3 kGy showed presence of *Salmonella enterica*. The control inoculated non-irradiated group showed 7.69log CFU/g level of *Salmonella enterica* at 0^{th} day. These results indicate that *Salmonella enterica* is very sensitive to electron beam irradiation treatment.

Various scientists have examined the effect of electron beam irradiation on *Salmonella* in different foods. Fu *et al.,* (1995) reported that irradiation at medium-dose (1.8 or 2.0 kGy) eliminated *Salmonella* from hams that were inoculated at 5 log CFU/g under aerobic conditions at 7^0C for 7 days of storage. Kang *et al.,* (2012) observed the effect of electron beam irradiation at 3 and 4 kGy doses on pork jerky inoculated with 8 log CFU/g of *Salmonella typhimurium* stored at 25°C and the study revealed no viable counts of *Salmonella typhimurium* in pork jerky samples. Kim *et al.,* (2014) also reported that no viable counts for *Salmonella typhimurium* in pork jerky samples exposed to 1.5, 2 and 3 kGy electron beam irradiation dose.

Salmonella enterica (Gram-negative) was found to be the most sensitive to irradiation treatment, as compared to *Bacillus cereus* (Gram-positive). These differences are attributed to the structural differences of these bacteria (Davidson, 1997; Nikaido, 1996). Nikaido (1996) demonstrated that the cell wall of Gram-negative bacteria consists of lipopolysaccharides, which are hydrophilic, whereas the cell wall of Gram-positive bacteria mainly contains a thick layer of a unique peptidoglycan that is important for their survival.

Table.1 Effect of electron beam irradiation on the survival of *Bacillus cereus* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculat	Treatment group	Average microbial count (log CFU/g) observed on different storage period (Days) at refrigeration temperature (0-4^{0}C)					
		0	2	4	6	8	10
B. cereus	Control uninoculated non irradiated	1.22±2.11^{c}	1.32±2.28^{b}	ND	ND	ND	ND
	Control inoculated non irradiated	5.68±0.61^{a}	5.71±0.59^{a}	5.76±0.21^{a}	5.86±0.01^{a}	5.93±0.02^{a}	6.04±0.03^{a}
	Pork salami inoculated and exposed to 1 kGy	4.14±0.81^{ab}	3.81±0.03^{a}	3.72±0.66^{a}	3.64±0.61^{b}	3.29±0.32^{b}	3.23±0.28^{b}
	Pork salami inoculated and exposed to 2 kGy	2.86±2.48^{bc}	1.42±2.46^{b}	1.32±2.28^{b}	1.30±2.26^{c}	1.28±2.21^{c}	1.20±2.07^{c}
	Pork salami inoculated and exposed to 3 kGy	2.85±2.48^{bc}	1.32±2.28^{b}	1.27±2.20^{b}	ND	ND	ND

a-c - Means with different letters within the same column differ significantly ($p \leq 0.05$).
ND - Not detected

Table.2 Effect of electron beam irradiation on the survival of *Bacillus subtilis* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculat	Treatment group	Average microbial count(log CFU/g) on different storage period (Days) at refrigeration temperature (0-4^{0}C)					
		0	2	4	6	8	10
B. subtilis	Controluninoculated non irradiated	ND	ND	ND	ND	ND	ND
	Control inoculated non irradiated	5.5±0.03^{c}	5.65±0.01^{a}	5.78±0.01^{a}	5.89±0.01^{a}	5.96±0.01^{b}	6.1±0.01^{a}
	Pork salami inoculated and exposed to 1 kGy	3.61±0.16^{b}	3.59±0.06^{b}	3.49±0.21^{b}	3.06±0.17^{b}	ND	ND
	Pork salami inoculated and exposed to 2 kGy	3.59±0.06^{b}	3.22±0.24^{b}	3.06±0.17^{c}	ND	ND	ND
	Pork salami inoculated and exposed to 3 kGy	ND	ND	ND	ND	ND	ND

a-c - Means with different letters within the same column differ significantly ($p \leq 0.05$).
ND - Not detected

Table.3 Effect of electron beam irradiation on the survival of *Pseudomonas aeruginosa* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculat	Treatment group	Average microbial count (log CFU/g) observed on different storage period (Days) at refrigeration temperature (0-4^{0}C)					
		0	2	4	6	8	10
P.aeruginosa	Control uninoculated non irradiated	ND	ND	ND	ND	ND	ND
	Control inoculated non irradiated	5.72±0.01^a	5.77±0.01^a	5.86±0.01^a	5.96±0.01^a	6.09±0.03^a	6.18±0.01^a
	Pork salami inoculated and exposed to 1 kGy	3.83±0.11^a	3.66±0.62^b	3.56±0.21^b	2.27±1.9^b	2.17± 1.90^b	1.84±1.67^b
	Pork salami inoculated and exposed to 2 kGy	1.19±2.05^b	1.11±1.93^c	0.98±1.71^c	0.56±0.9^{bc}	ND	ND
	Pork salami inoculated and exposed to 3 kGy	ND	ND	ND	ND	ND	ND

a-c - Means with different letters within the same column differ significantly ($p \leq 0.05$).
ND - Not detected

Table.4 Effect of electron beam irradiation on the survival of *Klebsiella pneumoniae* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculated food pathogen	Treatment group	Average microbial count (log CFU/g) observed on different Storage period (Days) at refrigeration temperature (0-4^{0}C)					
		0	2	4	6	8	10
K.pnemoniae	Control uninoculated non irradiated	ND	ND	ND	ND	ND	ND
	Control inoculated non irradiated	5.68±0.03^a	5.72±0.01^a	5.78±0.01^a	5.98±0.02^a	6.01±0.02^a	6.12±0.02^a
	Pork salami inoculated and exposed to 1 kGy	4.26±0.39^a	4.02±0.38^a	3.98±0.44^a	3.91±0.15^b	3.86±0.06^b	3.78±0.07^b
	Pork salami inoculated and exposed to 2 kGy	1.38±2.39^b	1.32±2.28^b	1.29±2.23^b	ND	ND	ND
	Pork salami inoculated and exposed to 3 kGy	ND	ND	ND	ND	ND	ND

a-b - Means with different letters within the same column differ significantly ($p \leq 0.05$).
ND - Not detected

Figure.1 Effect of electron beam irradiation on the survival of *Bacillus cereus* inoculated in porksalami and stored at refrigeration temperature (0-4^{0}C)

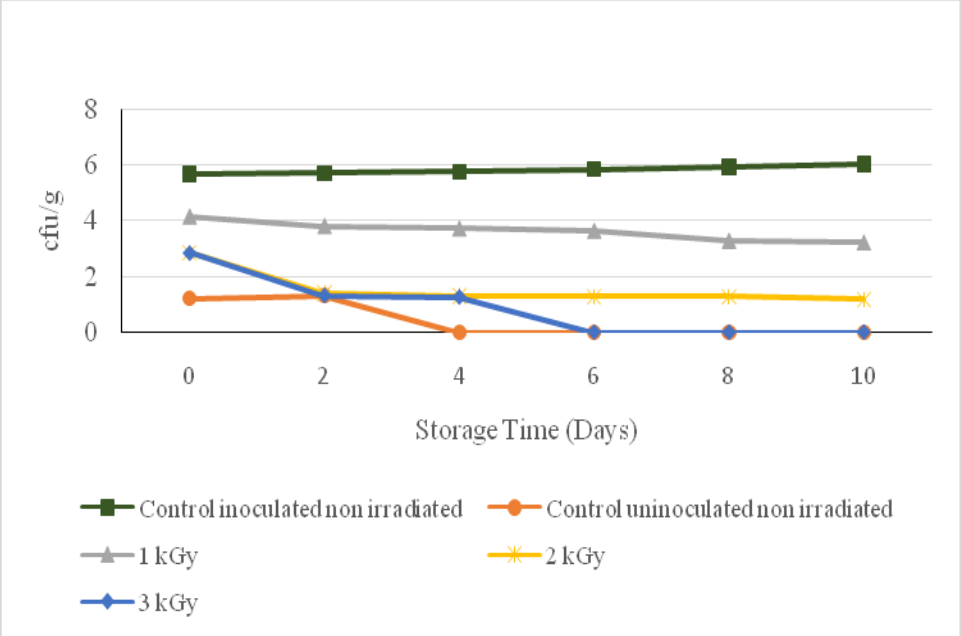


Figure.2 Effect of electron beam irradiation on the survival of *Bacillus subtilis* inoculated in porksalami and stored at refrigeration temperature (0-4^{0}C)

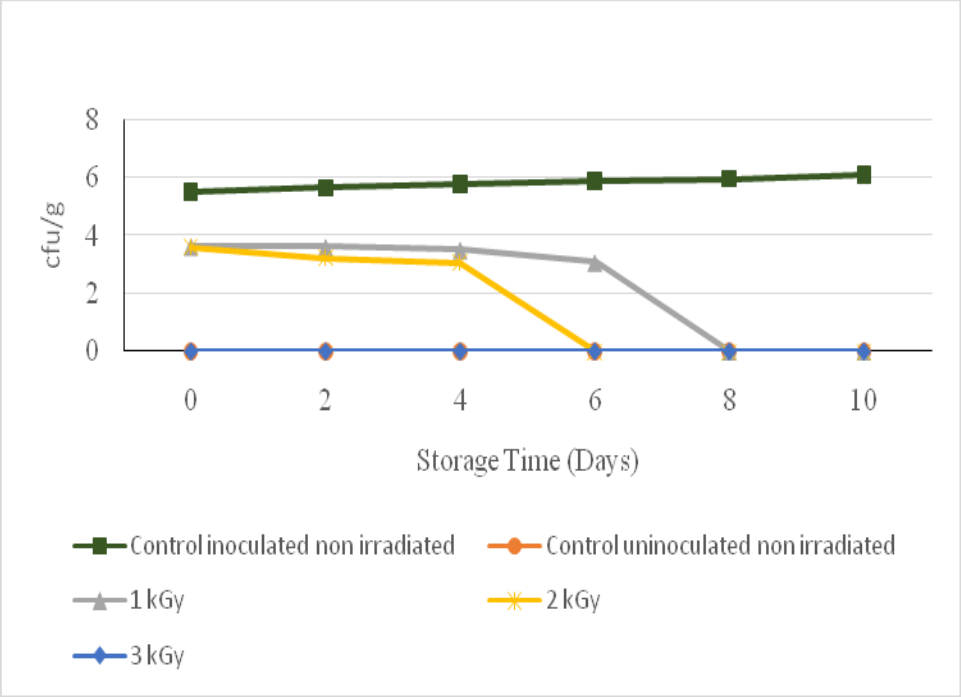


Figure.3 Effect of electron beam irradiation on the survival of *Pseudomonas aeruginosa* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

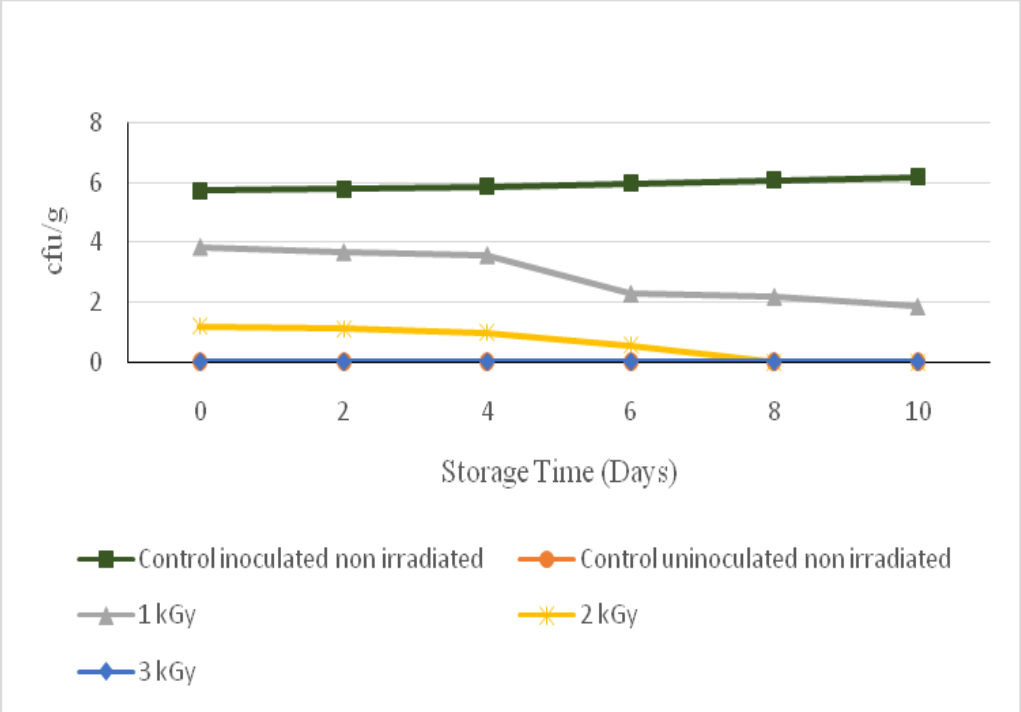
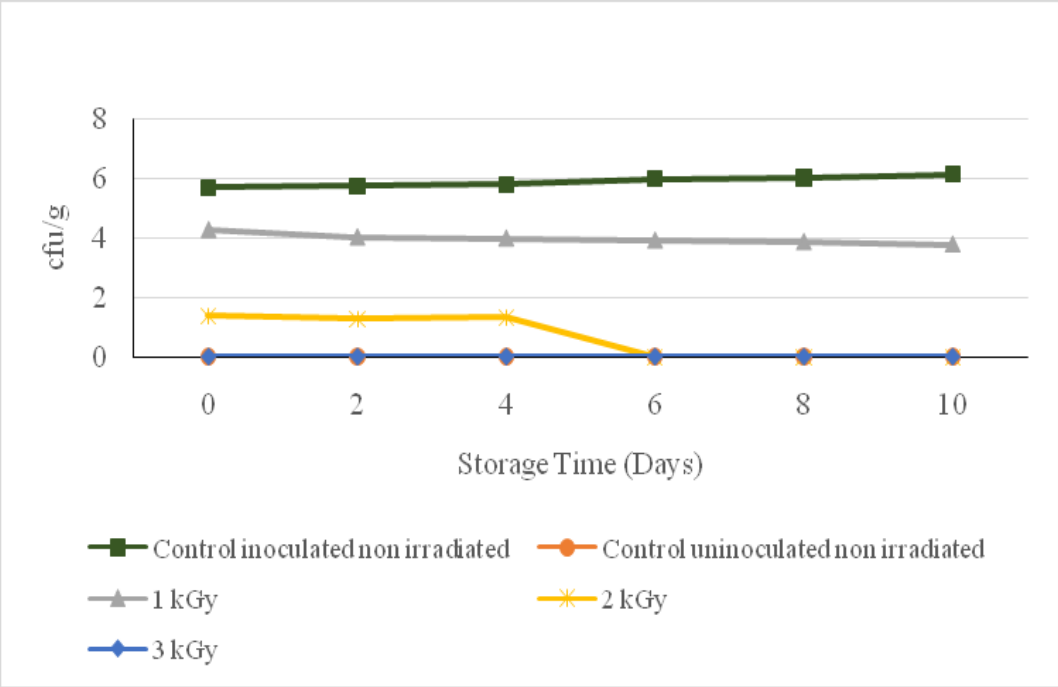


Figure.4 Effect of electron beam irradiation on the survival of *Klebsiella pneumoniae* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)



In addition, some of the constituents of complex food system, such as proteins, are thought to compete with cells for interactions with radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organisms more radiation-resistant (Jo *et al.,* 2004).The failure of the radiation injured cells of *Salmonella*spp.to grow during storage at refrigeration condition has been reported before (Thayer *et al.,* 1992). *Salmonella enteritidis* neither able to grow at refrigeration temperatures nor is the risk as high under conditions of temperature abuse occurs compared with that of *L. monocytogenes* (Marquez *et al.,* 2012). Lucht *et al.,* (1998) demonstrated that the temperature of 14-22^{0}C is optimal for the recovery of irradiation-injured pathogens. Sublethal damage to cells caused by irradiation is likely to increase their sensitivity to the environmental stress factors. An extension of the lag time in the growth of the surviving cells in foods with radiation related injuries has also been reported (Grant and Patterson, 1992). Thus, 1, 2 and 3kGy doses used in this study reduced population of *Salmonella enterica* to non-detectable levels in pork salami.

In conclusion, the present study revealed that doses of 1, 2 and 3kGy electron beam irradiation can effectively enhance the microbial safety of pork salami and reduce the hazards of common foodborne pathogens accompanied by refrigeration storage. Gram positive and Gram-negative bacteria were affected differently by the electron beam irradiation. No viable cells of *Salmonella enterica* were detected in the pork salami samples irradiated at 1 to 3 kGy of doses. Thus, *Salmonella enterica* was found to be the most sensitive bacteria to the electron beam irradiation. Amongst all the electron beam irradiation doses used under study, 3kGy was found to be more effective in reducing the microbial count when compared to other irradiation doses.

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