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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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°C). Pork salami samples were purchased from reputed HACCP accredited and ISO 22000 certified pork processing plant, sterilized, inoculated with 10⁸ 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°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.

Keywords
Electron beam, Gram positive, Gram negative, Irradiation, Refrigeration, Sterilization

Abstract
The inactivation/reduction of selected gram positive and gram negative bacteria inoculated in sterile pork salami samples stored at refrigeration temperature (0-4°C). Pork salami samples were purchased from reputed HACCP accredited and ISO 22000 certified pork processing plant, sterilized, inoculated with 10⁸ 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°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), causing 80.3 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°C (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 10^8 CFU/mL were inoculated in tryptic soy broth (TSB) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37°C 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 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°C 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 positive and gram negative bacteria were counted. Approximately 10^8 CFU/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°C, until further analysis.

**Microbiological analysis**

Microbial analysis was done at the 0, 2nd, 4th, 6th, 8th and 10th days of refrigeration storage. 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°C 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 the gram positive bacteria viz. *Bacillus cereus*, *Bacillus subtilis* and gram negative bacteria viz. *Salmonella enterica*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosawere Bacillus cereus* agar base, *Bacillus* differentiation agar, xylose lysine deoxycholate agar, MacConkey agar and *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 samples inoculated with *Bacillus cereus* at the concentration of $10^8$ CFU/g were irradiated at 1, 2 and 3 kGy and were analyzed for the presence of *Bacillus cereus* (Table 1). From the Table 1 it is indicated that *Bacillus cereus* was found to be increased in control uninoculated non-irradiated group and control inoculated non-irradiated group up to storage period of 2nd and 10th day of refrigeration storage, respectively. The control inoculated non-irradiated group 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 0th day, respectively and further subsequently decreased to 3.23±0.28, 1.20±2.07 and 1.27±2.20 on 10th and 6th 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°C 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 3 kGy showed significant reduction throughout the storage at refrigeration temperature in all the samples (Table 2). The *Bacillus subtilis* was found to be increased upto 10th 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 0th day which was increased to the level of 6.10±0.01 on 10th day. The samples treated with electron beam irradiation doses of 1 and 2 kGy showed the average *Bacillus subtilis* concentration (log CFU/g) in pork salami as 3.61±0.16 and 3.59±0.06 on 0th day, respectively. As the storage period advanced, the bacterial count decreased to 3.06±0.17 on 6th day after electron beam irradiation dose of 1 kGy. Irradiation dose of 2 kGy showed 3.06±0.17 level of *Bacillus subtilis* on 4th 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 to control 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) reported similar 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 DT 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 salami at the concentration of 10^8 CFU/g and irradiated at 1, 2 and 3 kGy 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 0th day which was increased to the level of 6.18±0.01 on 10th 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±0.11 and 1.19±2.05 on 0th day, respectively. The Pseudomonas aeruginosa (log CFU/g) count was reduced to 1.84±1.67 on 10th and 0.56±0.9 on 6th 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 irradiation among 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 with electron beam irradiation doses of 1, 2 and 3 kGy. From the Table 4 it is indicated that *Klebsiella pneumoniae* was found to be increased with the increased storage up to 10th 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 0th day which was increased to the level of 6.12±0.02 on 10th day under 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±2.39 and non-detected level on 0th day, respectively. Average *Klebsiella pneumoniae* count (log CFU/g) decreased with period of storage from 4.26±0.39 to 3.78±0.07 on 10th day after electron beam irradiation dose of 1 kGy, whereas the dose of 2 kGy showed 1.29±2.23 level of *Klebsiella pneumoniae* on 4th day and thereafter no growth was observed. However, all the pork salami samples treated with electron beam dose of 3 kGy 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 7.69 log CFU/g level of *Salmonella enterica* at 0th 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°C 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°C)

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

<sup>a-c</sup> - Means with different letters within the same column differ significantly (*p* ≤ 0.05).

<sup>ND</sup> - 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°C)

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

<sup>a-c</sup> - Means with different letters within the same column differ significantly (*p* ≤ 0.05).

<sup>ND</sup> - 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°C)

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

<sup>a-c</sup> - Means with different letters within the same column differ significantly (*p* ≤ 0.05).

<sup>ND</sup> - 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°C)

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

<sup>a-b</sup> - Means with different letters within the same column differ significantly (*p* ≤ 0.05).

<sup>ND</sup> - Not detected
Figure 1 Effect of electron beam irradiation on the survival of *Bacillus cereus* inoculated in pork salami and stored at refrigeration temperature (0-4°C).

![Graph showing the effect of electron beam irradiation on *Bacillus cereus* survival.](image1)

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

![Graph showing the effect of electron beam irradiation on *Bacillus subtilis* survival.](image2)
**Figure 3** Effect of electron beam irradiation on the survival of *Pseudomonas aeruginosa* inoculated in pork salami and stored at refrigeration temperature (0-4°C)

![Graph showing effect of electron beam irradiation on Pseudomonas aeruginosa](image)

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

![Graph showing effect of electron beam irradiation on Klebsiella pneumoniae](image)
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°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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