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Survival of *Salmonella* spp. in Pork Salami at Refrigeration Temperature after Exposure to Lower Doses of Electron Beam Irradiation

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The study was carried out to assess and optimize the effect of electron beam doses on inactivation/reduction of *Salmonella typhimurium* and *Salmonella enterica* inoculated in sterile pork salami samples stored at refrigeration temperature (0-4ºC). Pork salami samples were procured from reputed HACCP accredited and ISO 22000 certified pork processing plant, sterilized, inoculated with 10^8 CFU/mL of *Salmonella typhimurium* and *Salmonella enterica*, packaged in sterile low density polyethylene pouches and subsequently irradiated at the dose rate of 0.5, 0.75, 0.90 and1 kGy. The packaged irradiated and non-irradiated (control) samples were stored at 0-4ºC and analyzed for *Salmonella typhimurium* and *Salmonella enterica* 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 typhimurium* and *Salmonella enterica* were detected in the pork salami samples irradiated at 1 kGy of dose. Thus, the study concluded that amongst all the electron beam irradiation doses used under study, 1kGy was found to be more effective in elimination of *Salmonella typhimurium* and *Salmonella enterica*.

Introduction

Salmonella is one of the most important food borne pathogens worldwide, and products of animal origin constitute common infection sources. Salmonellosis is well recognized as a major health threat to consumers of pork and pork products (Beloeil *et al.*, 2004) and predominant spoilage bacteria in pork products (Liu *et al.*, 2006). Food safety is a defining issue in the competitive global pork market today and *Salmonella* is a major concern for the swine industry all over the world. It is estimated that 80.3 million cases of food borne salmonellosis occur annually in the world (Majowicz *et al.*, 2010). An increase in demand for convenient ready-to-eat processed meat products such as sausages

and salami is due to the changing habits of consumers. Survival of organisms in ready-to-eat products has the potential to cause illness and pork salami has been identified as the food vehicle for *Salmonella typhimurium* (Pontello, 1998).

Irradiation, one of the cold sterilization processing method and a promising technology used for preservation of meat without affecting the nutritional as well as sensory attributes (Grolichova *et al.*, 2004).Ionizing radiations enhance the shelf-life and quality of meat by killing the pathogens by targeting their DNA (Akram and Kwon, 2010). Irradiation has been studied extensively for improving the safety of meat products. Olson (1998) indicated that low-dose (<10 kGy) irradiation can kill at least 99.9% of *Salmonella* in poultry. Low dose of electron beam irradiation can extend the shelf-life of meat by decontaminating microorganisms (Arvanitoyannis *et al.*, 2009).Thus, the present study was therefore undertaken to assess and optimize the doses of electron beam irradiation on inactivation/reduction of *Salmonella typhimurium* and *Salmonella enterica* 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 pork 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 *Salmonella typhimurium* (MTCC-98), *Salmonella enterica* (MTCC-3218) were procured from Microbial Type Culture Collection (MTCC) and Gene Bank Chandigarh, India were used to prepare the inoculum to test in pork salami. The colonies of the *Salmonella typhimurium* and *Salmonella enterica* isolates at10^8CFU/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 *Salmonella typhimurium*, *Salmonella enterica* were counted. Approximately 10^8 CFU/mL of *Salmonella typhimurium*, *Salmonella enterica* 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 the whole area of pork salami by pipette. The inoculum was then spread over the pork salami with a 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, Vashi, Navi Mumbai for exposure to varying doses of electron beam irradiation.

Electron-beam irradiation

All these pork salami samples were divided into 6 separate groups, of which one was kept as uninoculated non-irradiated control and other as inoculated non-irradiated control and remaining four groups were exposed to 0.5, 0.75, 0.90 and 1 kGy doses of electron beam irradiation. For electron beam irradiation, the pouches were arranged in aluminium boxes and irradiated on both sides in an ILU EB machine (Energy 4.5 MeV, beam power 15 kW). Irradiation was performed with a conveyor velocity of 1.8m/min (3cm/sec). Dosimetry for this irradiation of the sample was carried out using radiochromic film dosimeter (B-3). Double sided irradiation was carried out in order to ensure a 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 icebox and stored at a temperature of 0-4^{0}C, until further analysis.

Microbiological analysis

Microbial analysis was done at the 0, 2nd, 4th, 6th, 8th and 10thdays 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 Xylose-Lysine Deoxycholate agar (XLD) by direct plating. The plates were incubated at 37^{0}C for 24 h, and microbial counts were expressed as log CFU/g.Colonies typical of *Salmonella typhimurium* and *Salmonella enterica* were counted and were identified by Gram stain.Media used in the study were procured from M/s. HiMedia Laboratories Pvt. Ltd., Mumbai, India. Count of *Salmonella typhimurium* and *Salmonella* Enterica, in 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 *Salmonella typhimurium* and *Salmonella enterica* inoculated in Pork salami

Salmonella typhimurium

All the pork salami samples inoculated with *Salmonella typhimurium* at the concentration of 10^8 CFU/g were irradiated at 0.5, 0.75, 0.90 and 1kGy and analyzed for the presence of *Salmonella typhimurium* (Table 1). From Table 1 it is indicated that *Salmonella typhimurium* count was found to be increased in control inoculated non-irradiated group throughout the refrigeration storage period of 10 days. However, all the pork salami samples in control uninoculated non-irradiated group did not show the presence of

Salmonella typhimurium throughout the storage period. The control inoculated non-irradiated group showed average *Salmonella typhimurium* (log CFU/g) count as 7.72±0.002 on 0 day, which was increased to the level of 7.98±0.001 on 10^{th} day of refrigeration storage.

The samples treated with electron beam irradiation doses of 0.5, 0.75 and 0.90kGy showed the average *Salmonella typhimurium* count (log CFU/g)in pork salami as 7.37±0.02, 7.33±0.02 and 7.19±0.02 on 0 day, respectively and further subsequently decreased to 7.12±0.09, 7.01±0.02 and 6.87±0.04 on 10^{th} day, respectively (Table 1 and Figure 1). None of the pork salami sample inoculated at 10^{8} (CFU/g) and irradiated at 1 kGy showed the presence of *Salmonella typhimurium*.

The log reduction in the *Salmonella typhimurium* count was observed after treating the pork salami samples with 0.5, 0.75, 0.90 and 1 kGy of electron beam irradiation as compared to control inoculated non-irradiated pork salami samples. Amongst all the irradiation doses used, total elimination in the *Salmonella typhimurium* count was observed in pork salami samples treated with 1 kGy of electron beam irradiation.

The reduction levels of *Salmonella typhimurium* found in pork salami in the present study are lower (0.35, 0.39 and 0.52 log CFU/g for 0.5, 0.75 and 0.90 kGy respectively) than those reported by Fu *et al.*, (1995) who reported that *Salmonella typhimurium* levels was reduced by 1 log on pork chops and 3 log for hams after E-beam irradiation at 0.90 kGy. Kang *et al.*, (2012) also reported that the number of *Salmonella typhimurium* colonies in the samples exposed to 0.5 kGy irradiation reduced by approximately 1.74 log CFU/g in the pork jerky. Kim *et al.*, (2014) reported that the number of *Salmonella typhimurium* count in the samples exposed to 0.5 kGy irradiation reduced by 2.02 log CFU/g in the pork jerky.

Salmonella enterica

The effect of electron beam irradiation on *Salmonella enterica* inoculated into pork salamiat the concentration of 10^{8} CFU/g and irradiated at 0.5, 0.75, 0.90 and 1kGy are presented in Table 2. The *Salmonella enterica* count was increased with the increased storage period under refrigeration temperature in control inoculated non-irradiated group. The control inoculated non-irradiated group showed 7.72±0.004 level of *Salmonella enterica* (log CFU/g) on 0 day which was increased to the level of 7.96±0.02 on 10^{th} day under refrigeration temperature. However, no viable cells were noticed in control uninoculated non-irradiated group.The number of *Salmonella enterica* (log CFU/g) colonies in the samples exposed to 0.5, 0.75 and 0.90 kGy irradiation were observed as 7.39±0.01, 7.33±0.005 and 7.14±0.07 on 0^{th} day, respectively. The *Salmonella enterica* (log CFU/g) count was reduced to 7.19±0.02, 7.16±0.01 and 6.92±0.02 on 10^{th} day after electron beam irradiation dose of 0.5, 0.75 and 0.90 kGy, respectively (Table 2 and Figure 2). None of the pork salami sample inoculated at 10^{8} (CFU/g) and irradiated at 1 kGy showed the presence of *Salmonella* Enterica.

When the pork salami samples were treated with 0.5, 0.75, 0.90 and 1k Gy of electron beam irradiation, the reduction in *Salmonella enterica* count was observed more in electron beam irradiated groups as compared to control inoculated non-irradiated pork salami group. Total elimination in the *Salmonella enterica* count was observed in pork salami samples treated with 1 kGy of electron beam irradiation than other three irradiation doses used.

Table.1 Effect of electron beam irradiation on the survival (log CFU/g) of *Salmonella typhimurium* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculated *Salmonella* spp.	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
S. Typhimurium	Control uninoculated non irradiated	ND	ND	ND	ND	ND	ND
	Control inoculated non irradiated	7.72±0.002^a	7.78±0.027^a	7.83±0.003^a	7.89±0.02^a	7.92±0.02^a	7.98±0.001^a
	Pork salami inoculated and exposed to 0.5 kGy	7.37±0.02^b	7.33±0.01^b	7.31±0.01^b	7.28±0.01^b	7.24±0.01^b	7.12±0.09^b
	Pork salami inoculated and exposed to 0.75 kGy	7.33±0.02^c	7.31±0.02^b	7.29±0.01^b	7.26±0.004^b	7.22±0.02^b	7.01±0.02^c
	Pork salami inoculated and exposed to 0.90 kGy	7.19±0.02^d	7.13±0.01^c	7.09±0.01^c	7.07±0.02^c	6.93±0.03^c	6.87±0.04^d
	Pork salami inoculated and exposed to 1 kGy	ND	ND	ND	ND	ND	ND

Note: a-d - 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 (log CFU/g) of *Salmonella enterica* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)

Inoculated *Salmonella* spp.	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
S. Enterica	Control uninoculated non irradiated	ND	ND	ND	ND	ND	ND
	Control inoculated non irradiated	7.72±0.004^a	7.76±0.01^a	7.81±0.005^a	7.85±0.02^a	7.91±0.01^a	7.96±0.02^a
	Pork salami inoculated and exposed to 0.5 kGy	7.39±0.01^b	7.35±0.04^b	7.29±0.01^b	7.27±0.01^b	7.22±0.01^b	7.19±0.02^b
	Pork salami inoculated and exposed to 0.75 kGy	7.33±0.005^c	7.29±0.01^c	7.26±0.01^b	7.23±0.01^b	7.20±0.02^b	7.16±0.01^c
	Pork salami inoculated and exposed to 0.90 kGy	7.14±0.07^d	7.12±0.01^d	7.10±0.05^c	7.07±0.05^c	6.98±0.02^c	6.92±0.02^d
	Pork salami inoculated and exposed to 1 kGy	ND	ND	ND	ND	ND	ND

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

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

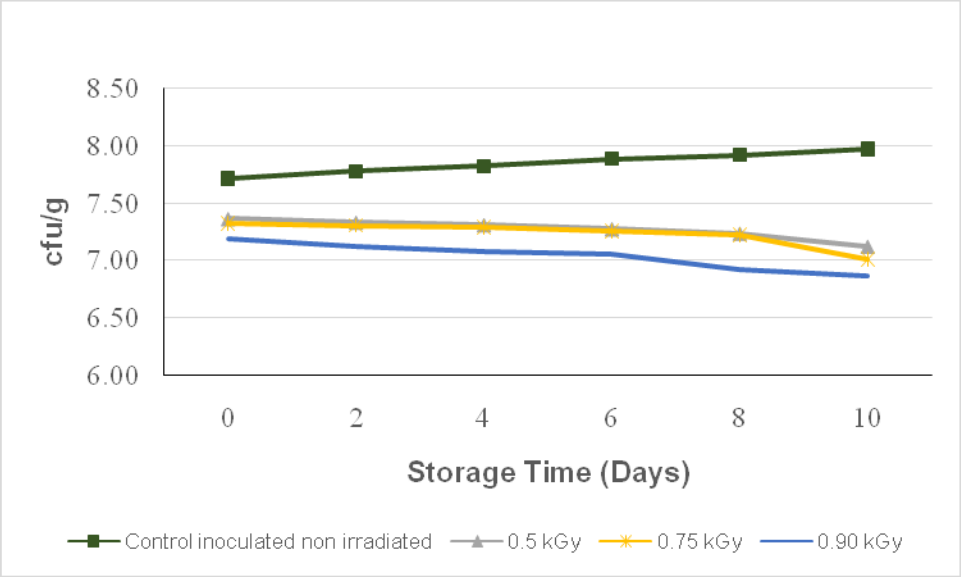
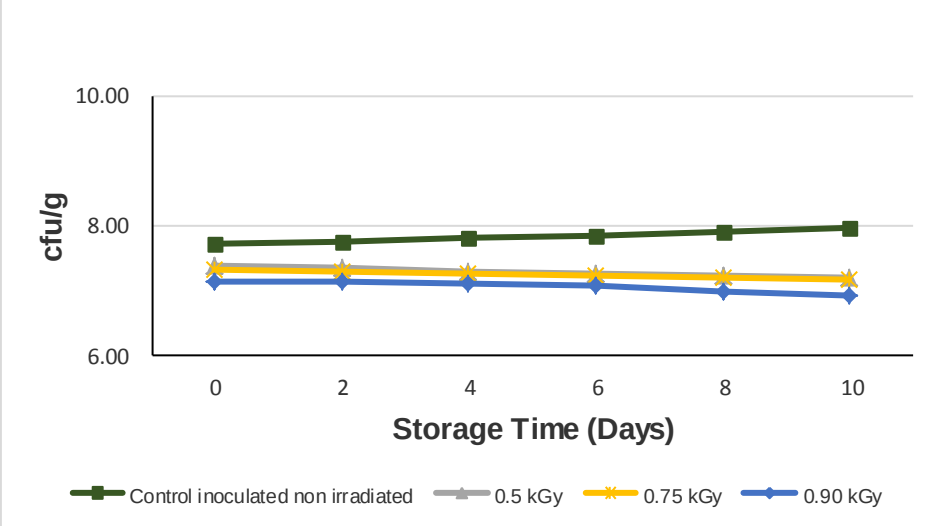


Fig.2 Effect of electron beam irradiation on the survival of *Salmonella enterica* inoculated in pork salami and stored at refrigeration temperature (0-4^{0}C)



The *Salmonella typhimurium* and *Salmonella enterica* were very sensitive to electron beam irradiation treatment. Various scientists have examined the effect of electron beam irradiation on *Salmonella* in different foods. Lewis *et al.*, (2002) reported that electron beam irradiation dose of 1.0 kGy, is effective in eliminating *Salmonella* spp. from poultry meat. Fu *et al.*, (1995) reported that irradiation at medium-dose (1.8 kGy) eliminated *Salmonella* from hams that were inoculated at 5 log CFU/g under aerobic conditions at 7^{0}C for 7 days of storage. Kim *et al.*, (2014) also reported that no viable counts for *Salmonella typhimurium* in pork jerky samples exposed to 1.5kGy electron beam irradiation dose.

Salmonella typhimurium and *Salmonella enterica* (Gram-negative) were found to be the most sensitive to irradiation treatment. This may be due 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 unique peptidoglycan that is important for their survival. 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, total elimination of *Salmonella typhimurium* and *Salmonella enterica* occurred in the pork salami samples irradiated at 1 kGy of dose.

In conclusion, the present study revealed that electron beam irradiation doses of 0.5,0.75,0.95 and1 kGy can effectively enhance the microbial safety of pork salami and reduce the hazards of *Salmonella* spp. accompanied by refrigeration storage. No viable cells of *Salmonella typhimurium* and *Salmonella enterica* were detected in the pork salami samples irradiated at 1 kGy of dose. Amongst all the electron beam irradiation doses used under study, 1kGy was found to be more effective in total elimination of *Salmonella typhimurium* and *Salmonella enterica* when compared to other irradiation doses.

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