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Comparative effect of gamma and electron beam irradiation on some food borne pathogenic bacteria contaminating meat products

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ABSTRACT

In terms of food irradiation, ionizing radiation in the form of gamma radiation or electron beam is currently allowed and employed as a non-thermal procedure for ensuring food safety and quality. The purpose of this study is to investigate the effect of radiation on viability of certain isolated food borne pathogenic bacteria like E. coli, Staphylococcus aureus, Proteus mirabilis, Listeria monocytogenes, and Enterococcus faecalis in meat products. In food irradiation, the requested dose of D₁₀ value to inactivate 90% of microbial population was 0.39, 0.49, 0.45, 0.54, and 0.57 kGy, being exposed to gamma radiation, and 0.41, 0.52, 0.48, 0.58, and 0.63 kGy for electron beam respectively suggesting that gamma radiation is more efficient than electron beam irradiation. The effect of radiation on the bacterial load have been assessed by injecting the smoky turkey samples with a cocktail of above mentioned bacteria in presence of natural microflora, and then subjected to 2.0, 4.0, and 6.0 kGy. These bacteria were inhibited to undetectable levels (<10 CFU/g) and total bacterial counts were greatly reduced at 4.0 kGy from either gamma or an electron beam radiation, indicating that this irradiation dose can be used to control some foodborne pathogenic bacteria of public health concern. E. coli was the most sensitive tested bacteria to irradiation, whereas Enterococcus faecalis was the most resistant.

1. Introduction

Food, particularly those of animal origin, are prone to spoilage and harmful microorganisms which may come from different sources. Food contaminated with those microbes have a short shelf life, even at refrigeration temperatures, and can lead to public health issues. Large-scale foodborne outbreaks originating from the consumption of pathogenic bacteria-contaminated food remain a constant hazard to public health, particularly for children, old, pregnant women, and immunocompromised persons ^[1].

Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, and E. coli 0157:H7 are at the top of the list causing the most outbreaks, cases, and deaths^[2].

In addition to causing urinary tract infection, rheumatoid arthritis, and meningitis in babies, the presence of *Proteus mirabilis* in meat and poultry causes health concerns ^[3]. When *Enterococcus faecalis* contaminates food, it produces a toxin called cytolysin, which causes hemolysis ^[4].

Considering the present global pandemic, more effective and long-lasting preservation procedures are required to assure the safety of food goods for longer periods of time than usual. Food irradiation is one of several processing techniques that have become increasingly popular in recent years for the preservation of food and food products. Irradiation of food is a non-thermal technology that is used to reduce the number of spoilage microorganisms, eliminate foodborne pathogenic bacteria, control insect infection, and delay or eliminate natural biological processes in fresh food such as ripening, germination, and sprouting ^[5].

Currently the main ionizing radiation commonly used in the application of food irradiation are gamma radiation from radioisotopes (Co-60 or Cs-137) and electron beams generated by electricity from accelerators with energies less than 10 MeV. High dosage penetrating power and significant homogeneity in food products are two advantages of Co-60 gamma radiation facilities. However, these facilities have drawbacks as they require a far Co-60 source that can never be switched off ^[6]. Electron beam accelerators, on the other hand, have several advantages, including the ability to turn it on and off as needed, the lack of a need to replace the source, the absence of radioactive waste, and higher throughput and reduced operating costs [6 - 8]. Several national, regional, and worldwide organizations/authorities, such as FAO, WHO, IAEA and CODEX have authorized and endorsed food irradiation as a safe and effective technology ^[9].

Since 1999, the market for irradiated fresh and frozen meats in the USA has grown increasingly and irradiated ground beef and hamburger are available in thousands of major retail outlet and restaurants across the country ^{[10].} Irradiated foods produced under established good manufacture practices (GMP) are safe and nutritionally adequate ^[11, 12].

Microorganisms' resistance to ionizing radiation varies greatly. The decimal reduction dose is used to determine a microbe's resistance to irradiation. The D_{10} -value is defined as the irradiation dosage required to lower the starting number of microbes by one log10 cycle or to kill 90% of them. Although gamma and electron beam accelerators are currently utilized in Egypt to treat food products, there have been a few research evaluating the effects of gamma and electron

beam irradiation on foodborne pathogenic bacteria infecting food of animal origin. The goal of this study is to isolate and identify some foodborne pathogenic bacteria from various animal-derived foods, determine the D₁₀-value of the identified pathogenic bacteria, and compare the effects of gamma and electron beam irradiation on these pathogens after inoculation into smoked turkey slices.

2. Materials ad Methods

Sixty samples of smoked turkey, chicken fillets, chicken luncheon, minced meat, beef luncheon, and raw sausage were obtained from different stores in Cairo, Egypt. Ten samples of each product (each weighing 25g) were packed in clean, dry, and irradiated-sterilized polyethylene bags and maintained at 4°C. Within an hour, samples were sent to the National Center for Radiation Research and Technology (NCRRT), Food Microbiology Laboratory for microbiological investigation.

2.1 Isolation of certain non-spore forming pathogenic bacteria

- *E. coli* was discovered and isolated on the Charm peel plate (EC) microbiological test (kit: code; PP EC look). This test has been certified as a performance test method O7150 by the Association of Analytical Community's Research Institute ^[13].

- Using the surface spreading approach, *Staphylococcus aureus* was isolated on Baird-Parker ager media ^[14].

- On xylose-lysin decarboxylate agar media, *Proteus mirabilis* was isolated ^[15].

- On listeria selective base medium, *Listeria monocytogenes* was isolated ^[16].

- On kanamycin esculin azide agar medium, *Enterococcus faecalis* was isolated ^[17].

2.2 Identification of the isolates

On their selective media, each pathogen's colonies were isolated based on their morphological traits. They were identified using Gram (staining) and routine biochemical assays.

2.3 Confirmation

The VITEK2 system (version 0801) was used to confirm the detected bacterial isolates (Biomerieux, Inc, USA, Hazelwood, MO, USA).

3.4. Preparation of bacterial-cell suspension

Each pathogenic bacterium's stock culture was grown overnight in 100 mL of nutrient broth (NB) medium

(Oxoid) in a 500 mL conical flask and incubated at 37°C for 24 hours. The culture broth contained a bacterialcell suspension of approximately 10⁹ CFU/ml. 2.5 Inoculation in smoked turkey meat

Using the immersion method, ten grams of irradiated decontaminated smoked turkey (10 kGy) samples were inoculated with 1.0 ml of each microbial-cell suspension.

2.6 Gamma irradiation process

The inoculated smoked turkey samples were packed and sealed in polyethylene bags, then they have been exposed to the following available irradiation doses 0.5, 1.5, 2.0, and 3.0 kGy for gamma irradiation and 0.5, 1.5 and 2.5 kGy for electron beam irradiation in addition to a zero control samples (0.0 kGy) as a reference sample. For each dose, three replicates were employed. The irradiation was carried out in the Indian Co-60 Gamma Chamber, 4000A, at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The dose rate of this source during the time of irradiation was 1.178kGy/h, and the irradiation was done at room temperature.

2.7 Electron beam irradiation (EBI)

Electron beam irradiation was carried out at the NCRRT using an electron beam accelerator (model: ICT, VIVIRAD CO, France). This accelerator has a maximum energy of 3 MeV, a beam current of 30 MA, a beam power of 90 KW, a scan width of 90 cm, and a distance between the scanner and the conveyor system of 53.0 cm. To calibrate the source and establish the average absorbed dosage in the samples, alanine dosimeters (traceable to the National Physical Laboratory in the United Kingdom) were employed. The Department of Radiation Protection and Dosimetry at the National Center for Radiological Research and Technology (NCRRT) did extensive dose mapping in compliance with Egyptian requirements.

2.8 Plating and counting

Serial dilutions were generated for each tube to count the surviving colonies for each tested pathogen following irradiation. On a duplicate petri dish plate, one ml of each of the three suitable dilutions was placed in the center and poured with plate count agar (PCA). The inoculated plates were incubated for 24 – 48 hours at 37°C. The number of survivors was calculated and represented as Log CFU/g.

2.9 Calculation of D₁₀-value

The slope of the dose-response curve, produced by plotting Log survival counts against the irradiation doses utilized, was used to calculate the D₁₀- value for each pathogen ^[18]. The slope of the dose-response curves was computed using Excel Microsoft Office Professional plus and a linear regression according to the equation:

 D_{10} -value = -1/b b = Exy-nx⁻y⁻/Ex²-nx²; Where:

x = dose level (kGy),

y = Log number survival count after receiving X amount of radiation,

n = number of calculated points.

2.10 Effect of gamma and electron beam irradiation on the cocktail of the selected pathogenic bacteria inoculated into smoked turkey

2.10.1 Smoked turkey slices samples

Twenty-five samples of smoked turkey slices (size 5x10 cm and weight 10 gm) were purchased from a local grocery market in foam dishes rapped with sticky sheet. The samples dishes were put in ice box and transferred to Food Microbiology lab at NCRRT within two hours.

2.10.2 Preparation of the inoculums

Each pathogenic bacteria culture (*E. coli, Proteus mirabilis, Staphylococcus aureus, Listeria monocytogenes,* and *Enterococcus faecalis*) was streaked onto nutrient agar plates and incubated for 24 hours at 37°C. A single colony of each pathogenic bacteria was transferred to a 250 ml conical flask containing 50 ml of sterile nutrient broth (in triplicate) and incubated at $37^{\circ}C\pm1$ for 24 hours to generate an inoculum. The inoculation mixture solution was produced from a cocktail of the targeted five pathogenic bacteria in a 2.0 L sterile beaker.

2.10.3 Inoculation of smoked turkey slices

Each sample (10g) of smoked turkey slices containing natural microflora was immersed in this mixed solution for 15.0 minutes under aseptic conditions. The surplus solution on the surface of the samples was air-dried (35-38°C) for 1.0 hour under aseptic conditions on sterilized filter paper. The inoculated smoked turkey slices were kept in heat-sealed polyethylene bags.

2.10.4 Irradiation process

The inoculated samples were separated into four sets

and packed. The first, second, and third sets were exposed to gamma and electron beam irradiation dosages of 2.0, 4.0, and 6.0 kGy, respectively. The fourth set was not irradiated, to be used as controlled samples. For each dose, three replicates were employed.

2.10.5 Microbiological analysis

Microbiological testing was performed immediately following the irradiation. Each package has been opened aseptically with alcohol sterilized scissors. In 0.1 percent sterile peptone saline water, the samples were diluted 10 times. The entire surviving bacterial population was then counted using non-selective media (PCA), while the survivors of each foodborne pathogenic bacterium were counted on their specific selective medium to determine their lethal and sublethal dose. The number of colonies was measured and represented as Log CFU/g.

2.11. Statistical analysis

The significance of the data was determined using a one-way ANOVA analysis (IBM SPSS statistics 22).

3. Results and discussion

The consumption of animal-origin foods has increased in recent years, but little is known about their contamination with the principal non-spore producing pathogenic bacteria commonly found in food. Our current study has been conducted on specific meat products to assess the control of foodborne pathogenic microorganisms with public health concern. For the isolation and identification of various foodborne pathogens, ten samples of each tested product (smoked turkey, chicken fillets, chicken luncheon, minced meat, beef luncheon, and uncooked sausage) were collected from different retail markets in Cairo.

E. coli and Enterococcus faecalis were found in all tested samples of each product, according to Table 1. Staphylococcus aureus was found in 5 (50%) of the smoked turkey slices, 4 (40%) of the chicken fillet and chicken luncheon, and 3 (30%) of the minced meat, raw sausage, and beef luncheon samples. Proetus mirabilis was also found in two (20%) samples of smoked turkey slices, chicken luncheon, beef luncheon, and raw sausage, while it was found only in one (10%) sample of chicken fillet and minced meat. Listeria monocytogenes was found in 3 (30%) of the smoked turkey samples and 2 (20%) of the chicken fillets, minced meat, beef luncheon, and raw sausage samples. For all the chicken luncheon samples there was no any Listeria monocytogenes found. Many other papers came to the same conclusion. Listeria monocytogenes is a pathogen of concern in ready-to-eat meat products because it is abundant and persistent in meat processing plants ^[19].

Salmonella, Shiga-toxigenic E. coli, Listeria monocytogenes, and to a lesser extent Staphylococcus aureus, Bacillus aureus, Clostridium perfringens, and Clostridium botulinum are the most common causal agents of meat-related outbreaks ^[20].

Campylobacter, Salmonella, Yersinia enterocolitica, pathogenic E. coli, and *Listeria monocytogenes* were the most common causes of human foodborne infection in the EU in 2018, with over 350,000 reported cases ^[21].





3.1 *D*₁₀ -values of the selected foodborne pathogenic bacteria

Each pathogen's D₁₀-value was obtained from an irradiation dose-response curve.

3.1.1 E. coli

The D₁₀-value indicates a microbe's resistance or sensitivity to irradiation. We can forecast or calculate the irradiation dose required to kill bacteria based on their D₁₀-value. The viable count of *E. coli* decreases as the gamma and electron beam irradiation dose increased, as shown in Fig. **1**. The count of *E. coli* was reduced from 8.5 logs to 1.0 log after a 3 kGy irradiation dosage. The D₁₀-value calculated was 0.39 kGy. In addition, 2.5 kGy of electron beam irradiation (EBI) reduced *E. coli* levels from 8.5 logs (the initial count) to 2.5 logs. With EBI, the computed D₁₀-value was 0.41 kGy, showing that gamma irradiation was more effective in comparison with electron beam irradiation.

 D_{10^-} value of *E. coli* exposed to gamma irradiation and EBI was established by a number of other researchers. *E. coli* in cultured media and blueberries have EBI D_{10} -value of 0.43 and 0.37 kGy ^[5]. *E. coli* D_{10} -values in ground beef ranged from 0.24 to 0.63 kGy with EBI ^[7]. The D_{10} -value varies depending on the source of isolation, strains, temperature, irradiation, oxygen present, and food matrix ^[22].

3.1.2 Staphylococcus aureus

Staph. aureus counts were reduced from 8.83 logs to be 2.84 logs at 3.0 kGy gamma irradiation dosage, the D₁₀-value was 0.52 kGy. EBI dose of 2.5 kGy reduced the original count (8.83 logs) to be only 4.32 logs. The D_{10} - value in this case was 0.49 kGy (Fig. 2). These findings demonstrated that gamma irradiation was more effective than EBI in controlling Staph. aureus. Other results showed that the mean D₁₀value of EBI for Staph. aureus was 0.85 kGy, which was greater than our results. This could be attributable to the strain of Staph. aureus, the dietary composition, or the irradiation condition ^[23]. The effects of gamma irradiation and EBI on Staph. aureus inoculated into salted seafood, and fermented oysters were compared ^[24]. The scientists discovered that gamma irradiation was more effective than EBI, with a D₁₀-value of 0.71 kGy compared to 0.94 kGy for EBI and concluded that gamma irradiation was more effective in reducing Staph. aureus' D10-value. D10-value in minced pork at room temperature utilizing EBI of Staph. aureus was 0.58 kGy, according to other findings, these variations could be attributable to the dose rate of ionizing radiation, strains, feeding matrix, isolation source, and other variables ^[18].



Fig. 1 Irradiation-dose response curves (D₁₀-value) of E. coli in smoked turkey slices



Fig. 2 Irradiation-dose response curves (D10-value) of Staphylococcus aureus in smoked turkey slices

3.1.3 Proteus mirabilis

Fig. 3 shows that the viable counts of *P. mirabilis* decrease dramatically as the gamma irradiation dose increased. The initial count (8.28 logs) was decreased to 1.82 logs with 3.0 kGy gamma irradiation dose, D_{10} -value of *P. mirabilis* was 0.45 kGy. While the initial count of *P. mirabilis* decreased to 1.82 kGy at 2.5 kGy EB irradiation dose and the D_{10} -value was 0.47 kGy, showing that gamma irradiation was more effective than EBI.

Different study for *P. mirabilis* in beef flesh, showed that D_{10} -value was 0.44 kGy for gamma irradiation treatment ^[25]. In another study the D_{10} -values measured for *P. mirabilis* were within the previously reported range (0.24 to 0.5 kGy) ^[26].

3.1.4 Listeria monocytogenes

The initial count of *L. monocytogenes* decreased at 3.0 kGy gamma irradiation from 8.04 logs to be 2.96 logs, the calculated D_{10} -value was 0.54 kGy, while it was reduced to be 4.04 logs with 2.5 kGy irradiation dose of EBI, demonstrating that gamma irradiation was also more effective than EBI in reducing the viable counts. In the case of EBI, the D_{10} -value was 0.58 kGy, as shown in Fig. 4.

Almost similar D₁₀-value for *L. monocytogenes* has been reported by several investigators, the effects of gamma and EB irradiation on *L. monocytogenes* inoculated into salted seafood, and fermented oysters were compared. The D₁₀-values for gamma and EB irradiation were 0.6 kGy and 0.89 kGy, respectively ^[20]. The D₁₀-value of *L. monocytogenes* ranged from 0.42 to 0.49 kGy in inoculated chicken meat at 10°C ^[27].

3.1.5 Enterococcus faecalis

The viable count of *E. faecalis* was reduced by increasing gamma irradiation dose as show in Fig. 5. *E. faecalis*' D_{10} -value was 0.57 kGy, but it was higher (0.63 kGy) with EBI.

In another study, D₁₀-value of *E. faecalis* was ranging from 0.65 to 1.1 kGy, indicating that it is more irradiation resistant than other harmful examined bacteria. For the same bacterial species, the source of isolation, strain, temperature during irradiation of food composition, and presence of oxygen may all play a role in affecting the D₁₀-value ^[28].

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Fig. 3 Irradiation-dose response curves (D10-value) of Proteus mirabilis in smoked turkey slices



Fig. 4 Irradiation-dose response curves (D10-value) of Listeria monocytogenes in smoked turkey slices



Fig. 5 Irradiation-dose response curves (D10-value) of Enterococcus faecalis in smoked turkey slices

3.2 Effect of gamma and electron beam irradiation on the foodborne pathogenic bacteria inoculated into smoked turkey slices

A mixture of five pathogens were inoculated into smoked turkey slices: E. coli, Staph. aureus, P. mirabilis, L. monocytogenes, and E. faecalis. Infected samples were exposed to gamma and EB irradiation at dosages of 2.0, 4.0, and 6.0 kGy, in addition to reference control samples (without irradiation). The total bacterial survival counts, as well as the survival counts of each pathogen, were counted before and after irradiation. Total Bacterial Count (TBC) of inoculated smoked turkey samples before gamma irradiation (0.0 kGy) was 9.2 logs, according to Table 2. Before irradiation, the counts of the pathogens: such as E. coli, Staph. aureus, P. mirabilis, L. monocytogenes, and E. faecalis, were 7.2, 7.4, 7.9, 7.7, and 7.4 logs, respectively. TBC and all pathogen counts decreased by gamma irradiation from 9.2 logs to 3.0 logs at the highest dose of 6.0 kGy. A 4 kGy dosage of gamma irradiation drastically reduced all the investigated pathogens to undetectable levels (10² CFU/g). Furthermore, the initial TBC decreased from 9.2 logs to 4.8 logs after this irradiation dose.

In a similar study, foodborne pathogens like *L. monocytogenes, E. coli,* and *Salmonella typhimurium*) decreased to undetectable levels in ready-to-cook Iranian grilled chicken meat samples by gamma irradiation doses of 4.5 kGy ^[29]. While other researches recorded that the most prevalent pathogenic bacteria have D₁₀-values ranging from 0.11 to 0.7 kGy ^[30].

Table 3 shows that a 4.0 kGy EBI dose reduced TBC of inoculated smoked turkey samples from 9.2 to 5.3

logs, a difference of just 4 logs compared to 6.0 logs with gamma irradiation. Furthermore, the survivor's count of all tested pathogens decreased to be below the detectable limit by 4.0 kGy.

In other investigation, the doses of EBI greater than 2.0 kGy reduced bacterial counts in chilled turkey flesh by at least 100 times, with dosage ratios ranging from 1 Gy/sec to 100 Gy/sec ^[31]. Moreover, EBI of 4.0 kGy reduced the amount of *L. monocytogenes* and *E. coli* inoculated in beef loin by 6.7 logs, the population of these pathogens was below the detection limit (10 CFU/g) after irradiation at 5.0 kGy ^[32]. The effect of gamma and EB irradiation on a three-strain cocktail of *Listeria monocytogenes, Staphylococcus aureus,* and *Vibrio parahaemolyticus* inoculated into salted seafood and fermented oysters, have been studied showing that over 5 kGy gamma or EB irradiation, no live cells were observed ^[20].

There are direct and indirect effects for both gamma and EB irradiation which can inactivate germs in food. Direct effect of delivered energy within microbial cells, resulting in the disruption of chemical and molecular bonds (e.g., DNA breakage). The indirect consequence of water molecules being ionized to form free radicals, particularly the hydroxyl radical (OH•). These free radicals can harm cellular metabolic pathways, causing cell injury and death by promoting intercellular oxidation ^[33]. Gramnegative bacteria (such as *E. coli and Proteus mirabilis*) were shown to be more sensitive to irradiation than gram-positive bacteria (*L. monocytogens, Staph. aureus* and *E. Faecalis*). There are several researches approved that gram- positive bacteria are more resistance than gram-negative bacteria ^[34,35].

Gamma Irradiation dose (kGy)	Microbial count (Log N)							
	TBC	E. coli	Staph. aureus	Proteus mirabilis	Listeria mono.	Entero. faecalis		
0	9.2 ^a ± 0.1	7.2ª±0.2	7.4 ^a ±0.4	7.9 ^ª ± 0.05	7.7ª±0.2	7.4ª ± 0.05		
2	$7.8^{b} \pm 0.2$	$2.0^{b} \pm 0.2$	$3.0^{b} \pm 0.5$	$3.5^{b} \pm 0.5$	$3.8^{b} \pm 0.05$	$3.8^{b} \pm 0.2$		
4	$4.8^{\circ} \pm 0.2$	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²		
6	$3.0^{d} \pm 0.2$	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²		

 Table 2 Effect of gamma irradiation doses on microbial count and pathogenic bacteria inoculated into smoked turkey

Mean value \pm SD of three samples followed by different superscript mean significantly different (P<0.05) <10² below detectable level

Electron beam	Microbial count (Log N)							
irradiation dose	TBC	E. coli	Staph. aureus	Proteus mirabilis	Listeria mono.	Entero. faecalis		
(kGy)								
0	$9.2^{a} \pm 0.08$	$7.2^{a} \pm 0.05$	$7.4^{a} \pm 0.4$	$7.9^{a} \pm 0.05$	7.7 ^a ±0.1	$7.4^{a} \pm 0.03$		
2	$8.4^{b} \pm 0.2$	$2.9^{b} \pm 0.03$	$3.8^{b} \pm 0.03$	$4.1b \pm 0.1$	4.3 ^b ± 0.03	$4.3^{b} \pm 0.2$		
4	$5.3^{c} \pm 0.1$	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²		
6	$4.1^{d} \pm 0.05$	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²		

Table 3 Effect of electron beam doses on microbial count and pathogenic bacteria inoculated to smoked turkey

Mean value \pm SD of three samples followed by different superscript mean significantly different (*P*<0.05) <10² below detectable level

4. Conclusion

Gamma and Electron Beam Irradiation can be used in microbiological safety of meat products. In the tested foodborne pathogenic bacteria, gamma irradiation was found to be more effective than electron beam irradiation. All of the examined pathogens D₁₀-values for gamma irradiation varied from 0.39 to 0.57 kGy, while D₁₀-values for electron beam irradiation ranged from 0.41 to 0.63 kGy. Calculated D₁₀-values were within the range of most foodborne pathogenic bacteria previously reported. Gram-negative bacteria (such as *E. coli and Proteus mirabilis*) were shown to be more sensitive to irradiation than Gram-positive bacteria (*L. monocytogens, Staph. aureus* and *E. faecalis*)

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