**Efficacy of Gamma Irradiation in Eliminating Salmonella Typhimurium contamination in Seafood**

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## Abstract

*Salmonella* is a foodborne pathogen known to cause gastrointestinal disturbances. Contamination of seafood with *Salmonella* is a major public health concern, often occurring through cross-contamination. The natural habitat of *Salmonella* is the gastrointestinal tract of animals, including birds and humans. The current research study was carried out to investigate the gamma radiation sensitivity of *Salmonella Typhimurium* (NCIM 2501) in shrimp, squid, and clam samples. The decimal reduction dose ($D_{10}$) values of *Salmonella Typhimurium* in saline and nutrient broth were 0.119 ± 0.004 kGy and 0.139 ± 0.0014 kGy, respectively.Seafood samples such as shrimp, squid, and clams were inoculated with *Salmonella Typhimurium* and exposed to gamma irradiation at doses of 0, 0.1, 0.2, 0.3, and 0.4 kGy to evaluate the effectiveness of irradiation in the complete elimination of *Salmonella Typhimurium*. The $D_{10}$ values of *Salmonella Typhimurium* in shrimp, squid, and clam samples were found to be 0.182 ± 0.0007 kGy, 0.209 ± 0.0014 kGy, and 0.192 ± 0.004 kGy, respectively. The results of this study reveals that gamma radiation treatment at a dose of 5 kGy resulted in the complete reduction of 6.85 × 10□ CFU/g of *Salmonella Typhimurium* from shrimp, squid, and clam samples. Additionally, no recovery of *Salmonella Typhimurium* was observed in 5 kGy-treated shrimp, squid, and clam samples stored at 4°C for up to 12 days, even after enrichment and selective plating.


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**Keywords:** ***Salmonella Typhimurium,Gamma irradiation, $D_{10}$ value, Shrimp,Squid, Clams.***


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## I. Introduction

Awareness of the nutritional and health benefits of seafood consumption has significantly increased consumer demand for fish and shellfish across the globe. One effective way to meet this rising demand while also supplying higher-quality seafood is by reducing post-harvest losses, which also improves profitability for fish producers and processors. Post-harvest losses can occur at any stage, from the point of harvest to reaching the consumer, with major losses often due to quality degradation caused by improper handling and storage. Quality

losses in fish are primarily related to microbial spoilage, and various preservation and processing methods are employed to extend shelf life and maintain quality by slowing microbial activity. Seafood is increasingly recognized as a valuable dietary component due to its high nutritional value compared to other foods. It provides high-quality protein, omega-3 fatty acids, essential micronutrients, and minerals, which contribute to its growing popularity among health-conscious consumers worldwide.However, biological contamination remains a significant concern in seafood safety, with bacteria, viruses, and parasites capable of causing illnesses that range from mild gastroenteritis to life-threatening diseases. These pathogens can be naturally present in the aquatic environment or introduced through animal or human faecal contamination, such as sewage pollution. For instance, fish and shellfish can become contaminated with *Salmonella* from polluted waters or during storage and processing (Panisello et al., 2000). *Salmonella* contamination in seafood is a major public health concern, as it can cause various infections in humans.

*Salmonella* naturally resides in the gastrointestinal tracts of animals, including birds and humans (Pelczer, 1989). The genus *Salmonella* consists of two species: *S. enterica*, which is subdivided into over 2,000 serovars, and*S. bongori*. Some serovars of *S. enterica*, such as *S. Typhi*, cause systemic infections like typhoid fever, while others, like *S. Typhimurium*, cause gastroenteritis. Additionally, certain serovars are host-specific, such as *S. Typhi*, which infects only humans, while others, like *S. Typhimurium*, are host generalists and can infect humans as well as many other mammalian species. *Salmonella* is a Gram-negative, facultative anaerobic bacillus belonging to the family *Enterobacteriaceae*, with 2,579 different serotypes (Grimont and Weill, 2007).

Salmonella is a prominent foodborne pathogen and ranks second after *Campylobacter spp.* in causing gastrointestinal infections in humans. Among foodborne pathogens, *Salmonella* is one of the most frequent causes of foodborne illness, particularly gastroenteritis (Majowicz et al., 2010). There are nearly 2,500 documented serotypes of *Salmonella*, with *Salmonella Typhimurium* being frequently associated with salmonellosis worldwide (Galanis et al., 2006). *S. Typhimurium* is primarily transmitted through the consumption of raw or undercooked eggs, vegetables, fruits, and poultry (Crum-Cianflone & Nancy, 2008), leading to foodborne illnesses in human and animal hosts globally (Brandt et al., 2013). The presence of this organism in food poses a significant threat to public health. Lipopolysaccharides (LPS), which are endotoxins and integral components of the outer membrane of Gram-negative bacteria like *S. Typhimurium*, can cause

UNDER PEER REVIEW

septic shock (Ding et al., 2007), pyrogenic reactions (Murai et al., 1987), hypotension, diarrhoea, and vascular blood clotting (Kotani et al., 1985).

*Salmonella* serovars are widely distributed in nature and can enter aquatic environments through wild animals, domestic livestock, poor sanitation, and improper disposal of human and animal waste (FAO, 2010). Notably, once *Salmonella* reaches soil or aquatic environments, it can survive for extended periods, lasting months or even years (Winfield & Groisman, 2003), ensuring its transmission to new hosts. Studies have reported contamination in Indian seafood, including fish (30.5%), shrimp (29%), and clams (34.1%) (Kumar et al., 2008).

During 2021-22, India exported 13.69 million tons of seafood, generating an all-time high revenue of US $7.76 billion. The USA, European Union, and Southeast Asia continued to be the major importers (MPEDA, 2016). However, cross-contamination with bacterial pathogens presents a significant barrier to global shrimp trade (Mahto et al., 2015). There have been instances of seafood consignments from India being rejected by importing countries due to the detection of antibiotic residues, the presence of potential bacterial pathogens (including *Salmonella* and *E. coli* O157), and poor hygienic conditions. Both the FDA and European Union common marketing standards require zero tolerance for *Salmonella* in fishery products (Rina et al., 2015).

Radiation processing, a cold method, has been demonstrated to be effective in eliminating foodborne pathogens in various food items, such as sprouts (Saroj et al., 2006; Bari et al., 2004) and flesh products like meat and fish (Meng & Doyle, 2002). Gamma radiation, with its high penetration power, can inactivate pathogens that may have penetrated tissues. Irradiation ensures microbiological safety without compromising the sensory and nutritional properties of meat, poultry (Abu-Tarboush et al., 1997; Hashim et al., 1995), and fresh produce (Hajare et al., 2006; Thayer et al., 2003). Food irradiation has proven to be successful in enhancing both the safety and shelf life of fresh meats by effectively inactivating pathogens without deteriorating product quality (Mahapatra et al., 2005).The aim of this study was to assess the effectiveness of gamma irradiation in eliminating *Salmonella Typhimurium* from seafood, specifically shrimp, squid, and clams.

## 2. Materials and Methods

### 2.1 Bacterial Strain

UNDER PEER REVIEW

The standard bacterial strain of *Salmonella Typhimurium* (NCIM 2501) was obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. The strain was maintained at 4°C on Tryptic Soy Agar (TSA) slants and as a glycerol stock, stored at -80°C. Preliminary radiation sensitivity studies were conducted using *Salmonella Typhimurium* isolates from seafood and clinical sources. No significant difference in radiation sensitivity was observed between the seafood isolates and the standard strain *Salmonella Typhimurium* (NCIM 2501) used in this study.

**2.2 Bacteriological Media and Chemicals**

The bacteriological media used in this study were procured from HiMedia Laboratories, Mumbai, India. All chemicals and analytical reagents were sourced from Thermo Scientific and Merck Laboratories, Mumbai, India.

**2.3 Decimal Reduction Dose (D10)**

The culture of *Salmonella Typhimurium* (NCIM 2501) was inoculated into 25 mL of Tryptic Soy Broth (TSB) (HiMedia, India) and incubated at 37°C in a shaker incubator at 100 rpm for 12-16 hours. After incubation, the overnight-grown culture, containing approximately 10810^8108 cfu/mL, was harvested by centrifugation at 8,000 rpm for 2-3 minutes (Thermo Scientific, India). The resulting pellet was washed twice with sterile saline (0.85% NaCl) to remove residual media. The washed pellet was resuspended in 1.5 mL of sterile saline and nutrient broth, then further diluted to achieve a bacterial concentration of 10810^8108 cfu/mL.Subsequently, 1.2 mL of this bacterial suspension was transferred into 1.5 mL Eppendorf tubes, which were then placed on ice. The samples were irradiated at 0 kGy, 0.1 kGy, 0.2 kGy, 0.3 kGy, and 0.4 kGy at 4°C using a Cobalt-60 gamma irradiator (Gamma Chamber 5000, Board of Radiation and Isotope Technology, Mumbai, India) at a dose rate of 3.916 kGy/hour.After irradiation, total viable counts of *Salmonella Typhimurium* were determined using the spread plate technique with appropriate dilutions. Plates were incubated at 37°C for 24 hours, and the bacterial colonies were counted and expressed as cfu/mL. Each experiment was performed in triplicate. The average number of surviving viable cells in both saline and nutrient broth was plotted against radiation dose (kGy). The slope of each individual survival curve was determined using linear regression analysis. D10 values were calculated as the negative reciprocal of the slope of the survival curve.

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**2.4. Preparation of Bacterial Inocula**

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Fresh *Salmonella Typhimurium* (NCIM 2501) was cultured in 25 ml of TSB and incubated at 37°C at 150 rpm for 12–16 hours in a shaker incubator. Two milliliters of culture were aliquoted and centrifuged at 8000 rpm for 5 minutes to obtain a pellet. The pellet was resuspended in 2 ml of sterilized saline. The bacterial inoculum, with a cell density of 10□ cfu/ml, was used to inoculate shrimp, squid, and clam samples.

**2.5. Preparation of Samples**

Seafood samples such as shrimp (*Metapenaeus monoceros*), squid (*Loligoduvaucelli*), and clams (*Meretrix meretrix*) were procured from a major landing centre located in Mangalore, Karnataka, India. The samples were brought to the laboratory in aseptic conditions with ice and dressed. Shrimp, squid, and clam samples were used for the determination of D□□ values and inoculated pack studies. Ten grams each of shrimp, squid, and clam meat were packed separately in autoclavable polyethylene bags (Hi-Media, Mumbai, India). Decontamination of the packed shrimp and squid samples was carried out by autoclaving at 121°C for 15 minutes.

**2.6. Determination of D□□ Values in Shrimp, Squid, and Clam Samples**

The decimal reduction dose (D□□) values of *Salmonella Typhimurium* (NCIM 2501) were determined in shrimp, squid, and clam samples in triplicate. The culture suspension containing approximately 10□ cfu/ml was used to contaminate the shrimp, squid, and clam samples, as mentioned in section 2.4. The packs were divided into control (non-irradiated) and four different doses of irradiation. After inoculation, the packs were sealed in aseptic conditions and stored at 37°C in an incubator so that the cells adhered to the samples. The inoculated shrimp, squid, and clam samples were exposed to radiation doses of 0, 0.1, 0.2, 0.3, and 0.4 kGy (GC 5000, BRIT, Mumbai, India) at a dose rate of 3.908 kGy/hr. After irradiation, the shrimp and squid samples were kept in refrigeration until further analysis. The irradiated shrimp and squid samples were aseptically homogenized in a homogenizer (Rotek, India) containing 90 ml of sterilized saline. Serial dilutions of the homogenate were prepared, and the appropriate dilutions were plated to determine the total viable counts using tryptic soya agar (TSA). The plates were incubated at 37°C for 16–24 hours, and the counts were expressed as cfu/g. The experiment was done in triplicate. The average number of surviving viable cells (cfu/g) in the samples was plotted against the radiation dose. The slopes of the individual survivor curves were calculated by linear regression. D□□ values were calculated by taking the negative reciprocal of the survivor curve slopes.

**2.7. Determination of Radiation Dose Required to Eliminate 8 Log cfu/g of the Inoculated Bacterial Cells**

After decontamination, the shrimp, squid, and clam samples (10g) were inoculated with *Salmonella*

UNDER PEER REVIEW

*Typhimurium* (8 log cfu/g) as described in section 2.6. The inoculated samples (10□ cfu/g of *Salmonella Typhimurium*) in triplicate were irradiated at 0, 1, 2, 3, 4, and 5 kGy in a cobalt-60 gamma irradiator (GC-5000, BRIT, Mumbai, India), and the surviving bacterial population was determined by plating the serial dilutions on TSA with an incubation period of 18–24 hours at 37°C.

**2.8. Storage Studies of Irradiated Shrimp, Squid, and Clam Samples Inoculated with *Salmonella Typhimurium* (NCIM 2501)**

The decontaminated shrimp, squid, and clam samples, inoculated with 10□ cfu/g of *Salmonella Typhimurium*, as explained in section 2.6, were irradiated at doses of 0, 1, 2, 3, 4, and 5 kGy while stored in ice (4°C) in a cobalt-60 irradiator (GC-5000, BRIT, Mumbai, India). The irradiated shrimp, squid, and clam samples were stored at 4°C and screened for the presence of *Salmonella Typhimurium* on different storage days (0, 3, 6, 9, and 12 days). Enrichment and selective plating were carried out to confirm the complete elimination of *Salmonella Typhimurium*. The experiment was done in triplicate. About 10 g of the irradiated sample was placed in a sterilized homogenizer with 90 ml of lactose broth (HiMedia, India) and homogenized well. After homogenization, the samples were incubated overnight at 37°C. After incubation, a loopful of the pre-enriched sample was streaked onto Bismuth Sulphite Agar (BSI) (HiMedia, India) and incubated at 37°C for 24–48 hours. Positive colonies (black or brownish with or without a black center) were observed. The characteristic positive colonies were subcultured and subjected to a battery of biochemical tests, such as indole production, MR-VP, citrate utilization test, lysine iron agar, and triple sugar iron agar (HiMedia, India), for further confirmation.

**2.9. Statistical Analysis**

The data obtained from this investigation were analyzed statistically using Microsoft Excel, 2022. Linear regression was used to determine the slope of the survivor curve (Microsoft Corporation, WA).4o

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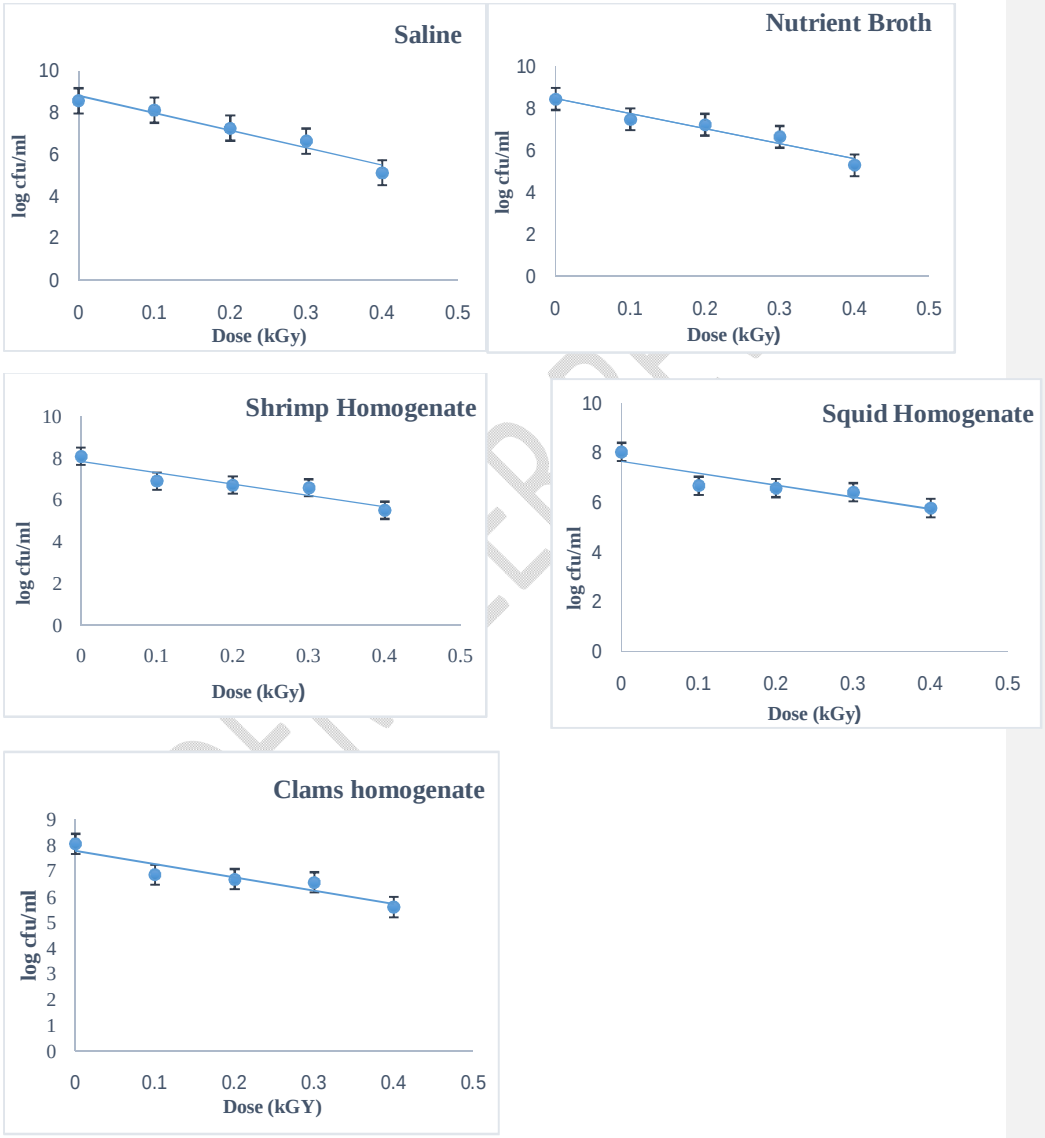
## 3. Results and Discussion

### 3.1. Decimal reduction value (D10) of *Salmonella Typhimurium* (NCIM 2501) in saline, nutrient broth, shrimp, squid, and clam homogenates

*Salmonella Typhimurium* (NCIM 2501) exhibited limited sensitivity to gamma irradiation, with mean decimal reduction values (D10) in various media, such as saline, nutrient broth, shrimp, squid, and clam homogenates, ranging from 0.119 kGy to 0.209 kGy**(Fig. 1& 2).** The mean D10 values in saline, nutrient broth, shrimp, squid,

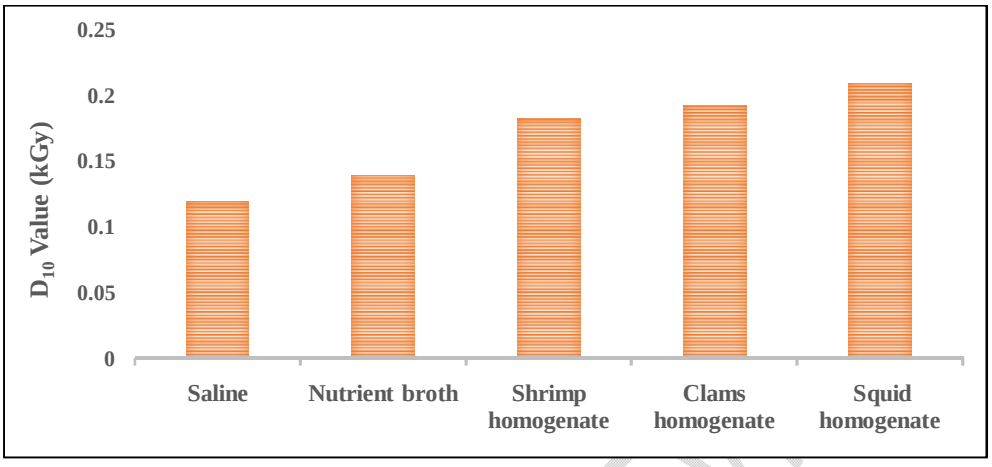
and clam homogenates were 0.119 ± 0.004 kGy, 0.139 ± 0.0014 kGy, 0.182 ± 0.0007 kGy, 0.209 ± 0.0014 kGy, and 0.192 ± 0.004 kGy, respectively **(Fig. 2).**

Fig.1. Decimal reduction ($D_{10}$) values of *Salmonella Typhimurium (*NCIM 2501) in saline, Nutrient broth, Shrimp homogenate, Clam homogenate and Squid Homogenate after gamma irradiation.




The mean values of three experiments are plotted along with standard deviation.

Fig.2. $D_{10}$ value of *Salmonella Typhimurium* (NCIM 2501) in Saline, Nutrient Broth, Shrimp, squid and clams after gamma irradiation.




UNDER PEER REVIEW

There are limited reports available regarding the gamma irradiation sensitivity of *Salmonella Typhimurium* in seafood. However, D10 values for other bacterial groups, such as *Klebsiella pneumoniae*, in sprout homogenates, poultry homogenates, and fish homogenates, ranged from 0.116 kGy to 0.227 kGy (Gautam et al., 2015). Rocelle et al. (1994) also reported the radiation sensitivity of *Escherichia coli* and *Salmonella enterica* in ground beef, which ranged from 0.241 to 0.307 kGy and 0.618 to 0.800 kGy, respectively. The highest D10 values for *Salmonella Typhimurium* were observed in shrimp and squid homogenates, measuring 0.182 ± 0.0007 kGy and 0.209 ± 0.0014 kGy, respectively.

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The D10 values for *Salmonella Typhimurium* (NCIM 2501) in shrimp and squid homogenates were higher than in saline and nutrient broth at 4°C (Gautam et al., 2015). Differences in the D10 values of bacterial pathogens like *Staphylococcus aureus*, *Vibrio* spp., *Aeromonas hydrophila*, *Salmonella Typhimurium*, *S. typhi*, *S.*

*paratyphi*, *Bacillus cereus*, and *Listeria monocytogenes* in various fish and shellfish at ambient and atmospheric temperatures have been reported by Venugopal et al. (1999).

A study conducted by Dion et al. (1994) reported that, at all dose rates, bacteria were more radiosensitive when irradiated in saline solution (0.85% NaCl) than in a poultry meat suspension. Nagar and Bandekar (2011) also elucidated those intrinsic properties of food products, such as water activity, food composition, irradiation temperature, and the presence of oxygen, affect the D10 values of bacteria in food (Dhokane et al., 2006). Saroj et al. (2006) reported a wide variation in the D10 values of *Salmonella Typhimurium* and *L. monocytogenes*, likely due to the intrinsic properties of sprouts as well as irradiation conditions.

The radiation sensitivity of *E. coli* (O157) inoculated in lettuce was reported by Neimira et al. (2003). Urbain (1986) mentioned that the presence of proteins in food systems could reduce radiation-induced damage, as these proteins compete with cells for interaction with radiolytic free radicals. The effectiveness of gamma irradiation in eliminating a cocktail of five resistant *Aeromonas* isolates, such as *A. salmonicida*, *A. caviae*, *A. jandaei*, *A. hydrophila*, and *A. veronii*, was demonstrated, with D10 values in mixed sprouts, chicken, and fish samples recorded as 0.0817 ± 0.001, 0.0897 ± 0.003, and 0.0917 ± 0.003 kGy, respectively, as reported by Nagar and Bandekar (2011).

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**3.2 Gamma Irradiation Dose for 8-log Elimination of *Salmonella Typhimurium* from Shrimp, Squid, and Clam Samples**

The effect of gamma irradiation on the survival of *Salmonella Typhimurium* was investigated in shrimp, squid, and clam samples. The inoculation of *Salmonella Typhimurium* in shrimp, clam, and squid samples was achieved at a level of 6.85 × 10□ cfu/ml. A radiation dose of 5 kGy resulted in the reduction of *Salmonella Typhimurium* from 10□ cfu/ml to undetectable levels in shrimp, squid, and clam samples, where the survival and recovery of the pathogen were evaluated immediately after irradiation. However, recovery of *Salmonella Typhimurium* was observed in the 1, 2, 3, 4, and 5 kGy treated samples after enrichment in Tryptic Soy Broth for 24 hours, followed by selective plating on Bismuth Sulphite Agar (Table 1). Nagar and Bandekar (2011) reported that radiation-induced damage may repair during enrichment in a cocktail of *Aeromonas* spp. A similar study conducted by Lamuka et al. (1992) reported the resuscitation of gamma radiation-induced injured bacterial cells when placed under favorable growth conditions. In this study, all the samples were enriched prior to plating on selective media to check the recovery of *Salmonella Typhimurium* cells that might have been metabolically injured during the irradiation process. In the present study, no such recovery of *Salmonella Typhimurium* was observed in the 5 kGy treated samples after enrichment and selective plating (Table 1).

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Several studies have elucidated that radiation doses in the range of 1 to 2 kGy do not significantly affect the

| Medium | Dose (kGy) | Recovery of S*almonella Typhimurium* (NCIM 2501) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $0^{th}$ day | | | $3^{rd}$ day | | | $6^{th}$ day | | | $9^{th}$ day | | | $12^{th}$ day | | |
| | | I | II | III | I | II | III | I | II | III | I | II | III | I | II | III |
| Shrimp homogenate | 0 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Squid homogenate | 0 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

nutritional and sensorial attributes of different kinds of food products, including fish, shrimp, squid, poultry, and mixed sprouts (Venugopal et al., 1999; Kanatt et al., 2010; Hajare et al., 2007; Manjanaik et al., 2018).

UNDER PEER REVIEW

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 0 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Clams | 1 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| homoge | 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| nate | 3 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Table 1. Recovery of *SalmonellaTyhphimurium* (NCIM 2501) from shrimp, squid and clams samples during 12 days storage period at 4□C.

+ = Viable cells detected on different days of storage.

- = No viable cells detected on different days of storage.

**3.3. Storage Studies of GammaIrradiated Shrimp, Squid, and Clam Samples Inoculated with *Salmonella Typhimurium* (NCIM 2501)**

Seafood samples such as shrimp, squid, and clams were inoculated with *Salmonella Typhimurium* at a concentration of 8 log cfu/g and subjected to gamma irradiation at different doses (1, 2, 3, 4, and 5 kGy). Both control (non-irradiated) and irradiated samples were analyzed for the recovery and survival of the inoculated pathogen over a storage period of 12 days at 4°C, using enrichment and selective plating methods.

In the control samples, *Salmonella Typhimurium* was recovered throughout the entire 12-day storage period. Variable recovery of *Salmonella Typhimurium* was observed in some replicates of shrimp and squid samples treated with 1, 2, 3, and 4 kGy, suggesting that these doses were not sufficient for the complete elimination of *Salmonella Typhimurium* at the inoculated concentration of 8 log cfu/g.However, no viable growth of *Salmonella Typhimurium* was detected in shrimp, squid, or clam samples irradiated with 5 kGy throughout the 12-day storage period. These results indicate that gamma irradiation at a dose of 5 kGy effectively ensured the microbial safety of these seafood samples with respect to *Salmonella Typhimurium*.

**Conclusions**

The results from this study indicate that *Salmonella Typhimurium* is susceptible to gamma irradiation, with an effective dose of 5 kGy. The Decimal Reduction Values (D10) for *Salmonella Typhimurium* ranged between 0.119 kGy and 0.205 kGy across different mediums. Specifically, the D10 values for saline, nutrient broth, shrimp, squid, and clams were 0.119 kGy, 0.139 kGy, 0.182 kGy, 0.209 kGy, and 0.192 kGy, respectively. A gamma irradiation dose of 5 kGy resulted in an 8-log reduction in *Salmonella Typhimurium*, and no recovery of the pathogen was observed in any of the treated samples even after 12 days of storage at 4°C.

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**Disclaimer**

This article is true as result of pure research without being engineered and doesn't use AI technology

**Ethical approval**

Not applicable

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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