Fishery Technology 62 (2025) : 254 - 261

Research Note

Gamma irradiation: A Novel Approach to Mitigate *Klebsiella pneumoniae* Contamination in Seafood

Srinu Nayak Banavath[1], Manjanaik Bojayanaik[1]*, Sachin Dnyanoba Chavan[1], Maga Raju Siravati[1], Sagar Naik Chandra Naik[1], Veena Shetty Alandur[2], and Somashekarappa Hiriur[3]

[1] *Department of Fish Processing Technology, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore - 575002, India.*

[2] *Department of Microbiology, K. S. Hegde Medical Academy, Nitte (DU), Mangalore- 575018, India.*

[3] *Centre for Application of Radioisotopes and Radiation Technology (CARRT), Mangalore University Konaje, Mangalore-574199, India.*

Abstract

Klebsiella pneumoniae, a known causative agent of community-acquired bacterial pneumonia, is an emerging pathogen associated with severe morbidity and mortality, particularly in newborns and immunocompromised individuals. Recent reports have indicated the presence of *Klebsiella* spp. in contaminated seafood, raising concerns about food safety. This investigation evaluated the radiation sensitivity of *K. pneumoniae* (ATCC 700603) in various seafood samples. The D_{10} values, representing the radiation dose required to achieve a one-log reduction in bacterial population, were determined in both saline and nutrient broth as 0.119±0.004 kGy and 0.1255±0.002 kGy, respectively. Seafood samples including shrimp, clams, and squid were experimentally contaminated with *K. pneumoniae* at a concentration of 6.85×10^8 cfu/g and exposed to gamma irradiation at incremental doses of 0, 1, 2, 3, and 4 kGy. The D_{10} values observed for *K. pneumoniae* in shrimp, clams, and squid were 0.1995±0.0015 kGy, 0.208±0.0019 kGy, and 0.2015±0.0005 kGy, respectively. Notably, no viable *K. pneumoniae* was recovered from samples treated with 3 kGy and stored at 4°C for 12 days, even after enrichment and selective plating. These results demonstrate that a gamma irradiation dose of 3 kGy is effective for the complete elimination of *K. pneumoniae* in seafood, highlighting its potential application for enhancing seafood safety.

Keywords: *Klebsiella pneumoniae, gamma irradiation, D_{10} value, shrimp, clams and squid*

Introduction

Fish and fishery products are at the forefront of food safety and quality improvement due to their prominence in international trade. Shrimp and cephalopods (squid and cuttlefish) are among the major seafood species exported from India, significantly contributing to the country's foreign exchange earnings (MPEDA, 2024). The primary importers of Indian seafood are the USA, the European Union, and East Asia (MPEDA, 2024). In the year 2023-24, India exported 17,81,602 metric tons of seafood, reaching an all-time high value of USD 7.38 billion. However, cross-contamination with bacterial pathogens presents a significant barrier to global shrimp trade (Mahto, Ghosh, Das, & Das, 2015). Indian seafood consignments have been rejected by importing countries due to the presence of antibiotic residues, potential bacterial pathogens such as *Salmonella* and *Escherichia coli* (O157), and unhygienic processing conditions (EIC, 2021). Foodborne diseases, caused by biological agents consumed with food, pose a constant threat to human health. Contamination can occur at any stage along the production to consumption chain and may result from environmental factors such as polluted water, soil, or air (WHO, 2007).

Received 4 November 2024; Revised 19 April 2025; Accepted 21 April 2025

Handling Editor: Dr. B. Madhusudana Rao

*E-mail: manjanaikb@rediffmail.com

Klebsiella pneumoniae, a gram-negative, rod-shaped, non-motile, facultative anaerobic bacterium from the family Enterobacteriaceae, is a well-known cause of community-acquired bacterial pneumonia (Puspanadan et al., 2012). It is also an emerging opportunistic pathogen that causes severe morbidity and mortality among newborns and immunocompromised individuals (Vernet et al., 1995; WHO, 2014). Infections caused by this bacterium can lead to meningitis, bronchitis, bacteraemia, pneumonia, and urinary tract infections in both humans and animals (Siri, Sithebe, & Ateba, 2011). The detection of *K. pneumoniae* in fish sold in Indian retail markets and ready-to-eat fish products raises serious concerns regarding food safety and public health. (Sharma, Bedi, Gill, Aulakh, & Sharma, 2006; Tambekar, Kulkarni, Shirsat, & Bhadange, 2011; Diana & Manjulatha, 2012). Traditional physical and chemical methods for eliminating foodborne pathogens have been proven to be ineffective under experimental conditions (Nagar & Bandekar, 2011). In contrast, irradiation processing, a cold treatment method, have been effective in eliminating foodborne pathogens in sprouts (Bari et al., 2004; Saroj et al., 2006) and flesh products such as meat and fish (Meng & Doyle, 2002). Gamma irradiation, known for its high penetration power, can inactivate pathogens that have infiltrated the tissues of sprouts or flesh. This technique ensures microbiological safety without compromising the sensory and nutritional qualities of meat, poultry, and fresh produce, and its use is increasing globally (Hashim, Resurreccion, & McWalters, 1995; Abu-Tarboush et al., 1997; Thayer, Rajkowski, Boyd, Cooke, & Soroka, 2003; Ahn et al., 2004; Hajare et al., 2006).

Gamma irradiation at doses up to 5 kGy can serve as an additional tool alongside freezing to eliminate pathogens and reduce the overall microbial load (Youssef, 1994).

However, there is a paucity of information on the radiation sensitivity of *K. pneumoniae* in seafood, indicating a gap in the current literature. Therefore, the present study aims to evaluate the effectiveness of gamma irradiation in eliminating *K. pneumoniae* from seafood samples such as shrimp, clams, and squid.

Materials and Methods

The American type culture collections (ATCC 700603) strain of *K. pneumoniae* obtained from the Central Research Laboratory, K.S. Hegde Medical Academy, NITTE (Deemed to be University), Deralakatte, Mangalore, India was maintained at 4°C on tryptic soya agar (TSA) slants and in glycerol broth, and stored at -80°C for further analysis.

The dehydrated bacteriological media used in this study were procured from Hi-media laboratories, Mumbai, India. Chemicals and analytical reagents were obtained from Thermo Scientific and Merck Laboratories, India.

The ATCC culture strain of *K. pneumoniae* was inoculated into 25 mL of Tryptic Soy Broth (TSB) and incubated in a shaker incubator at 37°C (100 rpm) for 12-16 hours. The overnight-grown culture, containing approximately 10^8 cfu/mL, was harvested and centrifuged (Thermo Scientific, India) at 8000 rpm for 2-3 minutes to obtain a pellet. The pellet was rinsed twice with saline to remove excess media and then resuspended in 1.5 mL of sterile saline (0.85% NaCl) and nutrient broth. The suspension was further diluted to obtain a bacterial concentration of 10^8 cfu/mL, from which 1.2 mL of the bacterial suspension was transferred into 1.5 mL microfuge tubes.

The tubes were placed on ice and irradiated at the doses of 0, 1, 2, 3, and 4 kGy at 0-4°C in a cobalt-60 gamma irradiator (Gamma Chamber 5000, Board of Radiation and Isotopes Technology, Mumbai, India) at a dose rate of 3.916 kGy/h. After irradiation, the total viable count (TVC) of *K. pneumoniae* was determined using the spread plating technique (Plate count agar) with appropriate dilutions. The plates were incubated at 37°C for 18-24 hours, and the bacterial colonies were counted and expressed as cfu/mL (Gautam, Nagar, & Shashidhar, 2015). Each experiment was performed in triplicate.

The average number of surviving viable cells, both in saline and nutrient broth, was plotted against the gamma radiation dose (kGy). The slopes of the individual survival curves were calculated using linear regression (Microsoft Excel Office 2022). The D_{10} value was calculated by taking the negative reciprocal of the slope of the survival curve.

K. pneumoniae was cultured in 25 mL of TSB at 37°C and 150 rpm for 12-16 hours in a shaker incubator. A 2 mL aliquot of the culture was then centrifuged at 8000 rpm for 5 minutes to collect the pellets. The pellets were resuspended in 2 mL of sterile saline.

The bacterial inoculum, with a cell density of 10^8 cfu/mL, was used to inoculate seafood samples (shrimp, clams, and squid).

The seafood samples, including shrimp (*Metapenaeus monoceros*), clams (*Meretrix meretrix*), and squid (*Loligo duvauceli*), were procured from the major landing centre located in Mangalore, Karnataka, India. The samples were transported to the laboratory under aseptic condition with ice and were dressed. The shrimp, clams, and squid samples were used for the determination of D_{10} values and inoculated pack studies. Ten gram each of shrimp, clams, and squid meat were packed separately in autoclavable polypropelene bags (Hi-media Laboratories, Mumbai, India). The packed shrimp, clams, and squid samples were decontaminated by autoclaving at 121°C for 15 minutes using a digital autoclave (Rotek, India) to eliminate native microflora for subsequent inoculated pack and storage studies.

The decimal reduction dose (D_{10}) of *K. pneumoniae* was determined in shrimp, clams, and squid samples in triplicate. The desired number of cells (approximately 10^8 cfu/g) of *K. pneumoniae* attached to shrimp, clams, and squid were standardized. *K. pneumoniae* was cultured independently in Tryptic Soy Broth (TSB) and allowed to grow for 12-16 hours at 37°C in a shaker incubator at 150 rpm. The incubated culture was serially diluted to obtain an appropriate of the bacterial titer. The culture suspension containing approximately 10^8 cfu/ml was used to contaminate shrimp, clams, and squid samples. The packs were divided into control (non-irradiated) and four different doses of irradiation. After inoculation, the packs were sealed under aseptic conditions and incubated at 37°C to allow cell adhesion to the samples. The inoculated samples of shrimp, clams, and squid were exposed to radiation doses of 0, 1, 2, 3, and 4 kGy using a gamma irradiator (Gamma Chamber 5000, BRIT, Mumbai, India). After irradiation, the samples were kept refrigerated until further analysis.

The irradiated shrimp, clams, and squid samples were aseptically homogenized in a homogenizer (Rotek, India) with 90 mL of sterile saline. Serial dilutions of the homogenate were prepared, and appropriate dilutions were used to determine the total viable counts using TSA. The plates were incubated at 37°C for 24-48 hours, and counts were expressed as cfu/g. The experiment was carried out in triplicate, and the average number of surviving viable cells in the samples was plotted against the radiation dose (kGy). The slope of the individual survival curves was calculated by linear regression using Microsoft Excel Office 2022. The D_{10} values were calculated as the negative reciprocal of the survival curve slope.

After decontamination, shrimp, clams and squid samples (10g each) were inoculated with *K. pneumoniae* (8 log cfu/g). The inoculated samples (10^8 cfu/gm of *K. pneumoniae*) prepared in triplicate, were irradiated at 0, 1, 2, and 3 kGy using a cobalt -60 gamma irradiator (Gamma Chamber-5000, BRIT, Mumbai, India). The surviving bacterial population was determined by plating the serial dilutions on TSA, followed by incubation at 37°C for 18-24 hr.

The inoculated samples of shrimp, clams, and squid were irradiated on ice with doses of 0, 1, 2, and 3 kGy using a cobalt-60 irradiator (GC-5000, BRIT, Mumbai, India) and then stored at 4°C. After irradiation, the samples were screened for the presence of *K. pneumoniae* on different storage days (0th, 3rd, 6th, 9th, and 12th day). Enrichment and selective plating methods were carried out to confirm the complete elimination of *K. pneumoniae*. The experiment was conducted in triplicate.

Approximately 10 g of irradiated sample was placed in a sterilized homogenizer with 90 ml of lactose broth (Hi-media, Laboratories, Mumbai, India) and homogenized thoroughly. After homogenization, the samples were incubated overnight at 37°C. A loopful of the pre-enriched sample was then streaked onto Bismuth Sulphite Agar (Hi-media, Laboratories, Mumbai, India), and the plates were incubated at 37°C for 24-48 hours. Characteristic colonies (black-brownish with a metallic sheen) were observed. These colonies were sub-cultured and subjected to confirmatory biochemical tests, such as Indole production, Methyl Red-Voges Proskauer, Citrate utilization and Lysine Iron Agar test.

The data obtained for the D_{10} value of *K. pneumoniae* in saline, nutrient broth, and seafood samples were statistically analyzed using MS Office Excel, 2022.

Results and Discussion

The Decimal Reduction Value (D_{10}) of *K. pneumoniae* in saline, nutrient broth, shrimp, clams, and squid homogenates were sensitive to gamma irradiation,

with values ranging from 0.1245 kGy to 0.2105 kGy (Fig. 1 and Fig. 2). The mean D_{10} values in saline, nutrient broth, shrimp, clams, and squid homogenates were 0.119±0.004 kGy, 0.1245±0.002 kGy, 0.1995±0.0015 kGy, 0.2087±0.0019 kGy, and 0.2105±0.0005 kGy, respectively (Fig. 1 and Fig. 2). There are limited reports available on the gamma irradiation sensitivity of *K. pneumoniae*. However, D_{10} values for bacterial groups like *K. pneumoniae* in sprout homogenate, poultry homogenate, and fish homogenate ranged from 0.116 kGy to 0.227 kGy (Gautam et al., 2015). Rocelle et al. (1994) reported that the radiation sensitivity of *E. coli* and *Salmonella enterica* in ground beef ranged from 0.241 to 0.307 kGy and 0.618 to 0.800 kGy, respectively. The highest D_{10} values for *K. pneumoniae* were observed in seafood homogenates; shrimp (0.1995 kGy), clams (0.2087 kGy), and squid (0.2105 kGy). D_{10} values for *K. pneumoniae* in shrimp, clams, and squid homogenates were higher than those observed in saline and nutrient broth at 4°C (Gautam et al., 2015). Differences in D_{10} values among bacterial pathogens such as *Staphylococcus aureus, Vibrio* spp., *Aeromonas hydrophila, Salmonella Typhimurium, S. typhi, S. paratyphi A, S. paratyphi B, Bacillus cereus,* and *Listeria monocytogenes* in various seafood products (shrimp, fish paste, and oyster homogenate) at ambient and atmospheric temperatures were reported by Venugopal, Doke, and Thomas (1999).

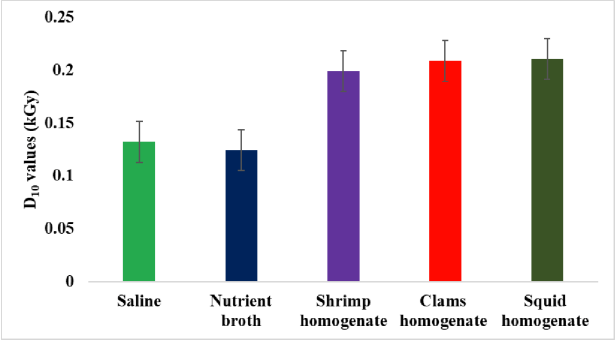

Fig. 1. D_{10} values of *Klebsiella pneumoniae* (ATCC 700608) in saline, nutrient broth, shrimps, clams and squids after gamma irradiation.

Dion, Charbonneau, and Thibault (1994) found that bacteria were more radiosensitive when irradiated in a saline solution (0.85% NaCl) compared to a poultry meat suspension. Nagar and Bandekar (2011) elucidated that the intrinsic properties of food products, such as water activity, nature, irradiation temperature, and the presence of oxygen, can affect the D_{10} values of bacteria in food (Dhokane, Hajare, Shashidhar, Sharma, & Bandekar, 2006).

Saroj et al. (2006) reported significant variation in the D_{10} values of *Salmonella Typhimurium* and *L. monocytogenes*, likely due to the intrinsic properties

Table 1. Effect of gamma irradiation processing on the growth of *Klebsiella pneumoniae* (ATCC 700608) in shrimp, clams and squid samples during 12 days storage period at 4°C.

Medium	Dose (kGy)	Recovery of *Klebsiella pneumoniae* ATCC (700603)														
		0 day			3days			6 days			9 days			12 days		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Shrimps	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clams	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Squids	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

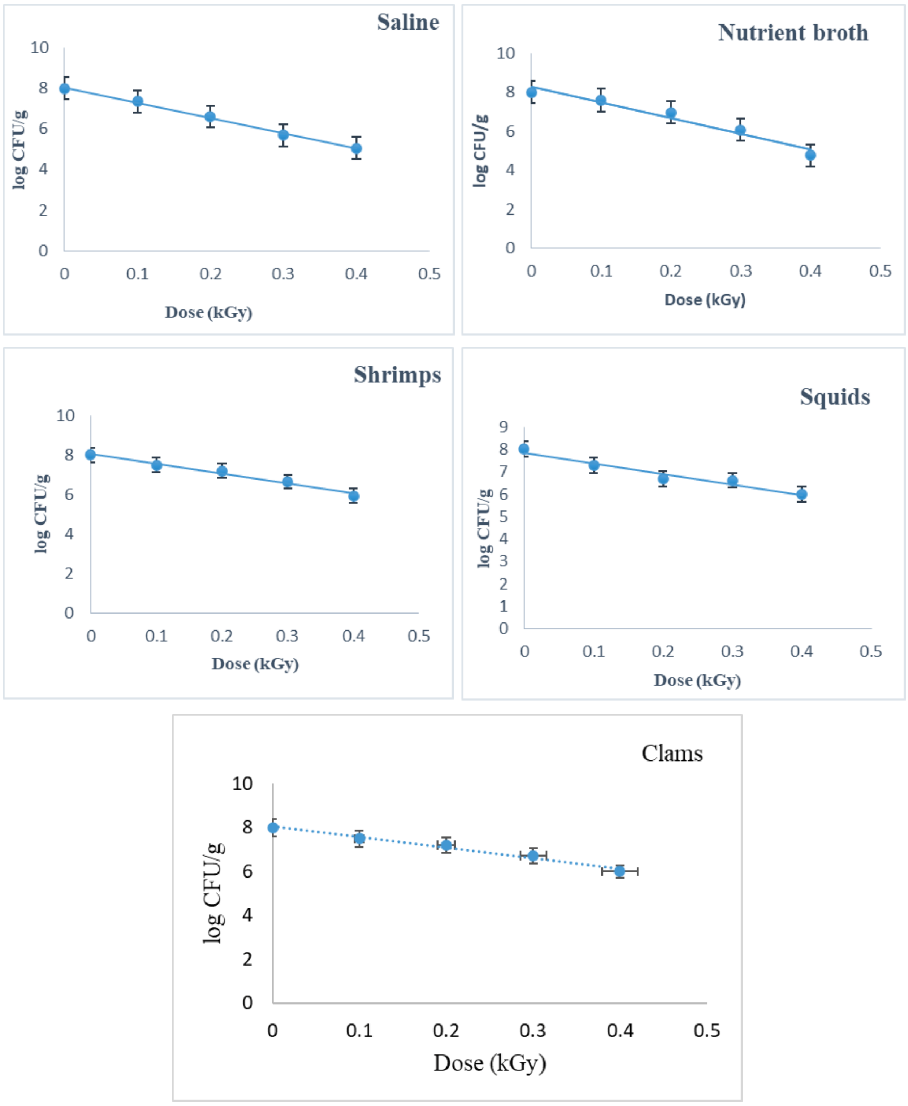

Fig. 2. D_{10} value of *Klebsiella pneumoniae* (ATCC 700608) in saline (D_{10} = 0.119±0.004 kGy), y = -8.336x +8.8254, r^2 = 0.9562), Nutrient Broth (D_{10}=0.125±0.0025 kGy, y = -8x +8.2735, r^2 = 0.953),Shrimp(D_{10} = 0.0.200±0.0015 kGy, y = -4.9925x+8.0527, r^2 = 0.9798) clams D_{10}=0.208±0.0019 kGy, y = -4.7905x + 8.0389, r^2 = 0.98) and Squid (D_{10} = 0.2105±0.0005 kGy, y = -4.7271x + 7.8676, r^2 = 0.9508) after gamma irradiation. The mean values of the three experiments are plotted along with standard deviation.

of sprouts and the irradiation conditions. The radiation sensitivity of *E. coli* (O157: H7) inoculated in lettuce was studied by Niemira, Sommers, and Fan (2002). Urbain (1986) noted that the presence of proteins in the food matrix can reduce radiation damage by competing with bacterial cells for interaction with radiolytic free radicals. Nagar and Bandekar (2011) investigated the effectiveness of gamma irradiation in eliminating a cocktail of the five most resistant *Aeromonas* isolates, such as *A. salmonicida, A. caviae, A. jandaei, A. hydrophila* CECT, and *A. veronii*. The D_{10} values of the *Aeromonas* cocktail in mixed sprouts, chicken, and fish samples were found to be 0.0817±0.001 kGy, 0.0897±0.003 kGy, and 0.0917±0.003 kGy, respectively.

The effect of gamma irradiation on the survival of *K. pneumoniae* was investigated in shrimp, clams, and squid samples. The inoculation of *K. pneumoniae* in these samples achieved a concentration of $6.85x10^8$ cfu/mL. A radiation dose of 3 kGy resulted in the reduction of *K. pneumoniae* from 10^8 cfu/mL

to undetectable levels in shrimp, clams, and squid samples, with the survival and recovery of the pathogen evaluated immediately after irradiation. However, recovery of *K. pneumoniae* was observed in the 0, 1, 2, and 3 kGy treated samples after enrichment in TSB for 24 hours, followed by selective plating on Bismuth Sulphite Agar (Table 1).

Nagar and Bandekar (2011) reported that radiation-induced damage may be repaired during enrichment in a cocktail of *Aeromonas* spp. A similar study conducted by Lamuka, Sunki, Chawan, Rao, and Shackelford (1992) also reported the resuscitation of gamma radiation- injured bacterial cells when placed under favourable growth conditions. In this study, all the samples were enriched prior to plating on selective media to check for recovery of *K. pneumoniae* cells that might have been metabolically injured during the irradiation process. In this experiment, no recovery of *K. pneumoniae* was observed in the 3 kGy treated samples after enrichment and selective plating (Table 1). Many research studies have shown that a radiation dose in the range of 1 to 2 kGy does not significantly affect the nutritional and sensory attributes of various food products, including fish, shrimp, squid, poultry, and mixed sprouts (Venugopal et al., 1999; Hajare, Saroj, Dhokane, Shashidhar, & Bandekar, 2007; Kanatt, Rao, Chawla, & Sharma, 2010; Manjanaik, Kavya, Shetty, Somashekarappa, & Rajashekar, 2018).

Seafood samples such as shrimp, clams, and squid were inoculated with 8 log (10^8 cfu/g) of *K. pneumoniae* and exposed to gamma irradiation at different doses (0, 1, 2, and 3 kGy). The control (non-irradiated) and irradiated samples were analyzed for the recovery and survival of the inoculated bacterial pathogen up to 12 days at 4°C using enrichment and selective plating. The survival and recovery of *K. pneumoniae* were observed in all control samples throughout the experiment, which lasted 12 days at 4°C. During the storage studies, variable recovery of *K. pneumoniae* was observed in some replicates of the 0, 1, 2, and 3 kGy treated shrimp, clams, and squid samples (Table 1). However, the results of this investigation showed that no recovery of *K. pneumoniae* was observed in the 3 kGy irradiated shrimp, clams, and squid samples during the 12-day storage period at 4°C. These findings suggest that gamma irradiation treatment could ensure microbial safety with respect to *K. pneumoniae* in different seafood samples.

The results from this investigation indicate that *K. pneumoniae* (ATCC 700603) is sensitive to gamma irradiation at a dose of 3 kGy. The decimal reduction values (D_{10}) for *K. pneumoniae* were 0.119±0.004 kGy in saline, 0.1245 kGy in nutrient broth, 0.1995 kGy in shrimp, 0.2087 kGy in clams, and 0.2105 kGy in squid. A 3 kGy gamma irradiation dose was effective in achieving an 8-log reduction in *K. pneumoniae*. No recovery was observed in any of the samples treated with 3 kGy radiation, even after 12 days of storage at 4°C. This study reports on the gamma irradiation sensitivity and inoculated pack studies of *K. pneumoniae* in various seafood samples, including shrimp, clams, and squid.

Acknowledgments

The financial assistance from the Board of Research in Nuclear Sciences (BRNS), Mumbai, Department of Atomic Energy, Government of India (Grant number 2013/35/4/BRNS) for carrying out this work is gratefully acknowledged. The support and encouragement extended by the Centre for Application of Radioisotope and Radiation Technology (CARRT), Mangalore University, Konaje, India is also appreciated and acknowledged.

References

Abu-Tarboush, H. M., Al-Kahtani, H. A., Atia, M., Abou-Arab, A. A., Bajaber, A. S., & El-Mojaddid, M. A. (1997). Sensory and microbial quality of chicken as affected by irradiation and post irradiation storage at 4^0C. *Journal of Food Protection*, *60*(7), 761-770. https://doi.org/10.4315/0362-028X-60.7.761.

Ahn, H. J., Kim, J. H., Jo, C., Lee, J. W., Yook, H. S., Kim, H. Y., & Byun, M. W. (2004). Combined effects of gamma irradiation and a modified atmospheric packaging on the physicochemical characteristics of sausage. *Radiation Physics and Chemistry*, *71*(1-2), 53-56. https://doi.org/10.1016/j.radphyschem.2004.04.006.

Bari, M. L., Al-Haq, M. I., Kawasaki, T., Nakauma, M., Todoriki, S., Kawamoto, S., & Isshiki, K. (2004). Irradiation to kill *Escherichia coli* O157: H7 and *Salmonella* on ready-to-eat radish and mung bean sprouts. *Journal of Food Protection*, *67*(10), 2263-2268. https://doi.org/10.4315/0362-028X-67.10.2263.

Dhokane, V. S., Hajare, S., Shashidhar, R., Sharma, A., & Bandekar, J. R. (2006). Radiation processing to ensure safety of minimally processed carrot (*Daucus carota*) and cucumber (*Cucumis sativus*): optimization of dose for the elimination of *Salmonella Typhimurium* and *Listeria monocytogenes*. *Journal of Food Protection*, *69*(2), 444-448. https://doi.org/10.4315/0362-028X-69.2.444.

Diana, T. C., & Manjulatha, C. (2012). Incidence and identification of *Klebsiella pneumoniae* inmucosal buccal polyp of *Nemipterus japonicas* of Vishakapatnam coast. *Journal of Fisheries and Aquatic Science*, *7*(6), 454-460. https://doi.org/10.3923/jfas.2012.454.460.

Dion, P., Charbonneau, R., & Thibault, C. (1994). Effect of ionizing dose rate on the radioresistance of some food pathogenic bacteria. *Canadian Journal of Microbiology*, *40*(5), 369-374. https://doi.org/10.1139/m94-060.

Export Inspection Council of India (EIC). (2021). *Annual Report 2020-21.* Ministry of Commerce and Industry, Government of India, New Delhi.

Gautam, R. K., Nagar, V., & Shashidhar, R. (2015). Effect of radiation processing in elimination of *Klebsiella pneumoniae* from food. *Radiation Physics and Chemistry*, *115*, 107-111. https://doi.org/10.1016/j.radphyschem.2015.06.016.

Hajare, S. N., Dhokane, V. S., Shashidhar, R., Saroj, S., Sharma, A., & Bandekar, J. R. (2006). Radiation processing of minimally processed pineapple (*Ananas comosus* Merr.): effect on nutritional and sensory quality. *Journal of Food Science*, *71*(6), S501-S505. https://doi.org/10.1111/j.1750-3841.2006.00116.x.

Hajare, S. N., Saroj, S. D., Dhokane, V. S., Shashidhar, R., & Bandekar, J. R. (2007). Effect of radiation processing on nutritional and sensory quality of minimally processed green gram and garden pea sprouts. *Radiation Physics and Chemistry*, *76*(10), 1642-1649. https://doi.org/10.1016/j.radphyschem.2007.01.007.

Hashim, I. B., Resurreccion, A. V. A., & McWalters, K. H. (1995). Descriptive sensory analysis of irradiated frozen or refrigerated chicken. *Journal of Food Science*, *60*(4), 664-666. https://doi.org/10.1111/j.1365-2621.1995.tb06202.x.

Kanatt, S. R., Rao, M. S., Chawla, S. P., & Sharma, A. (2010). Shelf-life extension of convenience meat products sold in Indian supermarkets by radiation processing. *Radiation Physics and Chemistry*, *79*(12), 1259-1263. https://doi.org/10.1016/j.radphyschem.2010.07.008.

Lamuka, P. O., Sunki, G. R., Chawan, C. B., Rao, D. R., & Shackelford, L. A. (1992). Bacteriological quality of freshly processed broiler chickens as affected by carcass pretreatment and gamma irradiation. *Journal of Food Science*, *57*(2), 330-332. https://doi.org/10.1111/j.1365-2621.1992.tb05487.x.

Mahto, R., Ghosh, S., Das, M. K., & Das, M. (2015). Effect of gamma irradiation and frozen storage on the quality of fresh water prawn (*Macrobrachium rosenbergii*) and tiger prawn (*Penaeus monodon*). *LWT-Food Science and Technology*, *61*(2), 573-582. https://doi.org/10.1016/j.lwt.2014.12.028.

Manjanaik, B., Kavya, N., Shetty, V., Somashekarappa, H., & Rajashekar, P. (2018). Influence of gamma irradiation and low temperature storage on the quality and shelf life of squid (*Doryteuthis sibogae*). *Food Research*, *2*(1), 39-45. https://doi.org/10.26656/fr.2017.2(1).170.

Meng, J., & Doyle, M. P. (2002). Introduction: Microbiological food safety. *Microbes and Infection*, *4*(4), 395-397. https://doi.org/10.1016/S1286-4579(02)01552-6.

MPEDA. (2024). *Annual report 2023-2024*. Marine products Export Development Authority, Kochi, India.

Nagar, V., & Bandekar, J. R. (2011). Effectiveness of radiation processing in elimination of *Aeromonas* from food. *Radiation Physics and Chemistry*, *80*(8), 911-916. https://doi.org/10.1016/j.radphyschem.2011.04.002.

Niemira, B. A., Sommers, C. H., & Fan, X. (2002). Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157: H7. *Journal of Food Protection*, *65*(9), 1388-1393. https://doi.org/10.4315/0362-028X-65.9.1388.

Puspanadan, S., Afsah-Hejri, L., Loo, Y. Y., Nillian, E., Kuan, C. H., Goh, S. G., Chang, W. S., Lye, Y. L., John, Y. H. T., Rukayadi, Y., Yoshitsugu, N., Nishibuchi, M & Son, R. (2012). Detection of *Klebsiella pneumoniae* in raw vegetables using most probable number-polymerase chain reaction (MPN-PCR). *International Food Research Journal*, *19*(4), 1757-1762.

Rocelle, M., Clavero, S., Monk, J. D., Beuchat, L. R., Doyle, M. P., & Brackett, R. E. (1994). Inactivation of *Escherichia coli* 0157:H7, *Salmonella*, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Applied and Environmental Microbiology*, *60*(6), 2069–2075. https://doi.org/10.1128/aem.60.6.2069-2075.1994.

Saroj, S. D., Shashidhar, R., Pandey, M., Dhokane, V., Hajare, S., Sharma, A., & Bandekar, J. R. (2006). Effectiveness of radiation processing in elimination of *Salmonella Typhimurium* and *Listeria monocytogenes* from sprouts. *Journal of Food Protection*, *69*(8), 1858-1864. https://doi.org/10.4315/0362-028X-69.8.1858.

Sharma, C. S., Bedi, S. K., Gill, J. P. S., Aulakh, R. H., & Sharma, J. K. (2006). Prevalence of enteropathogens of zoonotic significance in fish and fishery products from Ludhiana. *Indian Journal of Fisheries*, *53*(3), 341-344.

Siri, G. P., Sithebe, N. P., & Ateba, C. N. (2011). Identification of *Klebsiella* species isolated from modimola dam (mafikeng) north west province South Africa. *African Journal of Microbiology Research*, *5*(23), 3958-3963.

Tambekar, D. H., Kulkarni, R. V., Shirsat, S. D., & Bhadange, D. C. (2011). Bacetriological quality of street vended food panipuri: A case study of Amravati city. India. Bioscience Discovery, *2*(3), 350-354.

Thayer, D. W., Rajkowski, K. T., Boyd, G., Cooke, P. H., & Soroka, D. S. (2003). Inactivation of *Escherichia coli*

O157: H7 and *Salmonella* by gamma irradiation of alfalfa seed intended for production of food sprouts. *Journal of Food Protection*, *66*(2), 175-181. https://doi.org/10.4315/0362-028X-66.2.175.

Urbain, W. M. (1986). Biological effects of ionizing radiation. In W. M. Urbain (Ed.), *Food Irradiation* (pp. 83-117). Academic Press, Orlando.

Venugopal, V., Doke, S. N., & Thomas, P. (1999). Radiation processing to improve the quality of fishery products. *Critical Reviews in Food Science and Nutrition*, *39*(5), 391-440. https://doi.org/10.1080/10408699991279222.

Vernet, V., Philippon, A., Madoulet, C., Vistelle, R., Jaussaud, R., & Chippaux, C. (1995). Virulence factors (aerobactin and mucoid phenotype) in *Klebsiella pneumoniae* and *Escherichia coli* blood culture isolates. *FEMS Microbiology Letters*, *130*(1), 51-57. https://doi.org/10.1111/j.1574-6968.1995.tb07697.x.

WHO. (2007). *WHO food safety and food-borne illnesses*. Retrieved from. http://www.who.int/mediacentre/factsheets/fs237/en/.

WHO. (2014). *Food safety and foodborne illness* (Fact sheet 237). World Health Organization, Geneva.

Youssef, B. M. (1994). Microbial flora of frozen beefburger as affected by gamma radiation. *Egyptian Journal of Microbiology*, *29*(2), 105-113.