Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 869 – 883 (2022)
www.ejabf.journals.ekb.eg

Gamma-irradiation induced effects on histamine-forming bacteria isolated from the chilled mackerel fish

Mohamed S. Abd El-AL[1,*], Ali M. Saeed [2], Ali A. Hammad [1], Hesham M. Swailam [1], Mohamed A. Abouzeid [2]

1. Radiation Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (AEA), Cairo, Egypt.
2. Microbiology Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt.

*__Corresponding author: Mohamed.Salah@eaea.org.eg__

ARTICLE INFO

Article History:
Received: March 18, 2022
Accepted: June 19, 2022
Online: June 30, 2022

Keywords:
Histamine-forming bacteria;
Gamma radiation;
D_{10}-value;
mackerel fish;
Pantoea agglumerance

ABSTRACT

Scombroid food poisoning is caused by the consumption of histamine-containing foods. Since histamine is thermally stable, controlling histamine-forming bacteria in seafood is an effective technique for limiting histamine generation. The aim of this study was to investigate the induced effects of gamma radiation on the development of histamine-forming bacteria, specifically *Pantoea agglumerance*, in both mackerel flesh and tryptone soya broth. *Klebsiella pneumoniae*, *Pseudomonas fluoresense*, *Pantoea agglumerance*, and *Bacillus* sp. are histamine-forming bacteria were cultured in tryptone soy broth and exposed to gamma irradiation at doses of 0.25–5 kGy. In the meantime, mackerel meat was injected with the histamine-forming bacteria and then gamma-irradiated at 0.25-5.0 kGy. *K. pneumonia* and *Pseudomonas fluoresense* were particularly susceptible to gamma irradiation, and at 1.5 kGy, they were completely destroyed. *Pantoea agglumerance* and *Bacillus* sp. were found to be more resistant, with total inactivation at 3.0 and 5.0 kGy, respectively. The D_{10}-values to inactivate 90% of the bacterial populations in tryptone soya broth and inoculated mackerel ranged from 0.13 to 1.03 kGy and 0.14 to 1.12 kGy, respectively, depending on the kind of bacteria. These findings imply that gamma irradiation can efficiently eradicate histamine-forming bacteria, lowering the risk of histamine poisoning from seafood. However, because modest doses of irradiation are ineffective in decreasing *Bacillus* sp, the early counts of *Bacillus* sp in fish samples should be closely monitored.

INTRODUCTION

Fish is a significant part of the human diet, and there has been a rise in fish intake globally **(Jong, 2016; Arulkumar *et al.*, 2021)**. It is widely known that fish and fish products are frequently linked to human illnesses. Thus, it is necessary to study the prevalence of pathogens in fish to ensure their safety **(Arvanitoyannis and Tserkezou, 2014)**. Decarboxylation of amino acids by bacteria results in the production of biogenic amines in various foods **(Hungerford, 2021)**. Foodborne illnesses caused by eating foods high in biogenic amines especially histamine, has gained a lot of attention in recent years because of the illnesses, health issues, and economic concerns that it creates. Histamine, a chemical concern and the primary cause of scombroid poisoning **(FDA, 2020)**.

Histamine is a post-mortem product found in fish muscle, but only in very small amounts in fresh fish. However, heat or bacterial enzymatic decarboxylation of free histidine in muscle might cause it to accumulate in fish during storage **(Hungerford, 2021)**. Scombroid fish such as tuna and mackerel, which have a lot of free histidine in their muscles, are frequently linked to histamine poisoning **(Mercogliano and Santonicola, 2019)**. Fresh fish comprises low quantities of histamine; however, inappropriate handling and storage of fish can enhance histamine formation through the development of histidine decarboxylating bacteria **(Özogul and Özogul, 2020)**.

The majority of bacteria involved in histamine production belong to the Enterobacteriaceae family. The most well-known members of this family include *Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Enterobacter erogenes*, and *Pantoea agglumerance* **(Kim *et al*., 2000; Tsai *et al*., 2005; Russo *et al*., 2020)**. *Clostridium* sp., *Vibrio alginolyticus, Bacillus thuringiensis, Acinetobacter lowffi*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* sp., and *Photobacterium* have all been implicated with histamine poisoning **(Russo *et al*., 2020)**. As a result, decreasing the incidence of histamine poisoning requires measures that regulate the production and accumulation of histamine in fish and fish products. Histamine, are formed when bacteria decarboxylate amino acids **(Hungerford, 2021)**, and suppressing bacterial development is a successful strategy to lower the risk of histamine illness. Since histamine formation is temperature dependent, storing fish at low temperatures is a frequent approach of reducing histamine formation. **(Mercogliano and Santonicola, 2019)**. Nevertheless, certain bacteria, like *Photobacterium* sp., may survive and produce histidine decarboxylase at cold temperatures **(Emborg and Dalgaard, 2006)**. Consequently, preventing the production of histamine is not always possible with temperature management alone. Additionally, keeping low temperatures throughout the food chain is challenging, because temperature abuse is common across distribution **(Nei *et al.*, 2005)**.

Food irradiation is an appealing approach for lowering the incidence of biogenic amine poisoning **(Arvanitoyannis and Tserkezou, 2014)**. Food irradiation is the process of subjecting foods to ionising radiation in order to destroy microorganisms and improve shelf life while lowering health risks. Irradiation of various foods, including meat products, fish and shellfish, and fresh vegetables, is generally accepted and permitted in more than 50 countries

(Nishihira, 2020). Irradiation lowers the incidence of histamine poisoning, making it an appealing approach for enhancing food quality **(Arvanitoyannis and Tserkezou, 2014)**. Damage to nucleic acids is the primary mechanism of ionizing radiation-induced microbial inactivation, through water radiolysis-derived oxidative radicals either directly or indirectly damaging the body. Gamma irradiation is a clean, effective, ecofriendly, and energy-efficient decontamination method **(Nishihira, 2020)**. Gamma radiation treatment of fish may diminish or eradicate prolific histamine producers and natural spoilage microbes, reducing microbiological spoiling during storage **(Arvanitoyannis and Tserkezou, 2014)**. As a result, studies on the effectiveness of irradiation in regulating biogenic amines and biogenic amine-producing have been published **(Nei *et al*., 2012)**. However, there is little information on the resistance of other histamine-producing bacteria, such as *Pantoea agglumerance* to gamma ray. The primary goals of this work were to assess the survival and resistance of histamine-forming bacteria to gamma irradiation in pure cultures and mackerel fish.

MATERIALS AND METHODS

Bacterial isolates

Histamine-forming bacterial isolates (*Klebsiella pneumoniae*, *Pseudomonas fluoresense*, *Pantoea agglumerance*, and *Bacillus* sp) were kindly supplied by the food microbiology laboratory at the National Center for Radiation Research and Technology (NCRRT). The tested bacterial isolates were confirmed for histamine-producing ability on Niven's medium **(Niven *et al*., 1981)**.

Preparation of bacterial-cell suspension

The stock cultures of each bacterium was separately cultured in 100 mL tryptone soy broth (TSB; Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 24 h. The initial bacterial counts were determined as total plate counts on tryptic soy agar (TSA; Oxoid Ltd., Basingstoke, Hampshire, England). The culture broths were 10-fold serially diluted in physiological saline (0.85% NaCl, w/v), and a 0.1-mL aliquot from each dilution was spread plated on tryptic soy agar in duplicate and incubated at 37°C for 24 h to obtain cell suspension of approximately 10^7–10^8 CFU/mL.

Bacterial inoculation in pure culture and on mackerel meat

Mackerel fish samples were obtained from an Egyptian fish market and chopped into "1.3 cm × 1.3 cm × 1.3" cm pieces. To eliminate background microflora, 10 g of each sample was put in sealed polyethylene bags and treated to 20 kGy of gamma radiation under refrigerated conditions **(Ramakrishna Reddy *et al*., 2020)**. Then 0.5 mL of the culture suspension was aseptically inoculated onto sterilized mackerel muscle, and then incubated for 1.0 h at room temperature to allow surface attachment of the inoculated bacteria. The inoculated mackerel samples were immediately treated with irradiation after drying. Three tubes (each containing 9 mL of the work culture suspension of each bacteria) were used. For each irradiation dose, three tubes from each bacterial isolate were used **(Ramakrishna Reddy *et al*., 2020)**.

Gamma irradiation

Survivability studies of selected histamine formers both in broth and on surface-inoculated mackerel muscle were performed according to **Gautam *et al*. (2015)**. Briefly 3 test tubes from each bacterial strain containing 9 mL of tryptone soy broth suspensions were made as previously mentioned, then irradiated at ambient temperature. Irradiation dosages of 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, or 2.5 kGy were applied to broth suspension and mackerel meat spiked with *Klebsiella pneumoniae* and *Pseudomonas fluoresense* bacteria. While broth suspensions and mackerel meat inoculated with *Pantoea agglumerance* and *Bacillus* sp. were subjected to irradiation dosages of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or 4.5 kGy. Control samples (0.0 kGy) evaluated for all cultures were not exposed to irradiation. Each histamine-producing bacterial strain was tested in three replicates at each dose. Irradiation was carried out using a cobalt-60 gamma source (Gamma Chamber 4000, India), 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 0.950 kGy/h. The dose rate was established using alanine transfer dosimeter (Bruker Instruments, Rheinstetten, Germany). Radiation dose variations in test samples were reduced by placing them in a uniform area of the radiation field.

Microbial analysis

Following irradiation, the samples were tested for surviving bacteria using standard microbiological techniques. Serial dilutions were prepared for each tube to count the surviving colonies for each histamine-forming bacteria in the tryptone soya broth. For detection of the surviving bacteria in the mackerel meat, 90 mL of sterile physiological saline (0.85% NaCl, w/v) was added to sample bags that contained 10 g of inoculated sample, and the sample was mixed by stomaching for 90 s. Decimal dilutions were done (10-fold dilutions). Diluted samples (1.0 mL) were plated onto Petri dishes and poured by tryptone soya agar (TSA; Oxoid Ltd., Basingstoke, Hampshire, England) medium and incubated at 37°C for 24 h and then counted **(Sommers and Rajkowski, 2011)**.

Decimal reduction dose (D_{10}-value)

When the bacterial counts dropped to the detection thresholds (1.0 log CFU/g for mackerel meats and 1.0 log CFU/mL for tryptone soya broth), the survival of histamine-producing bacteria was confirmed. The radiation-decimal reduction curves were constructed by plotting the average number of surviving viable cells against the radiation doses. Linear regression was used to determine the slope of each survival curve using Origin 2018 (OriginLab Corporation). By using the negative reciprocal of the survival curve slope, the D_{10}-value was computed **(Sommers and Rajkowski, 2011)**.

Statistical analysis

Using Origin 2018 (OriginLab Corporation), the experimental findings and the slope of the various survival curves were examined and determined. The experiments were carried out three times, and the D_{10}-values are presented as means and standard deviations.

RESULTS

Radiation sensitivity of *K. pneumoniae*

K. pneumoniae are the most important members of the *Enterobacteriaceae* family that are predominantly implicated in histamine poisoning. A known count of bacteria, ranging from 8.48 log CFU/mL in TSB to 8.70 log CFU/g in mackerel meat, was recovered prior to the irradiation procedure. Gamma irradiation reduced the count of *K. pneumoniae*, and the reduction was increased by higher doses of irradiation. At 1.0 and 1.25 kGy, respectively, the bacterial count in tryptone soya broth was reduced by 7.30 and 6.05 log CFU/mL. However, *K. pneumoniae* was entirely destroyed at 1.5 kGy. While in mackerel meat, *K. pneumoniae* displayed radiation resistance compared to the tryptone soya broth, as the bacterial count was fewer than the detection limits (1.0 log CFU/g) when spiked mackerel was irradiated at 1.75 kGy. *K. pneumoniae* was susceptible to gamma radiation, with mean D_{10}-values in tryptone soya broth and mackerel flesh, respectively, ranging from 0.17 to 0.27 kGy as shown in **Table 1** and **Fig. 1**. The D_{10}-values for *K. pneumoniae* in mackerel fish was higher than tryptone soya broth.

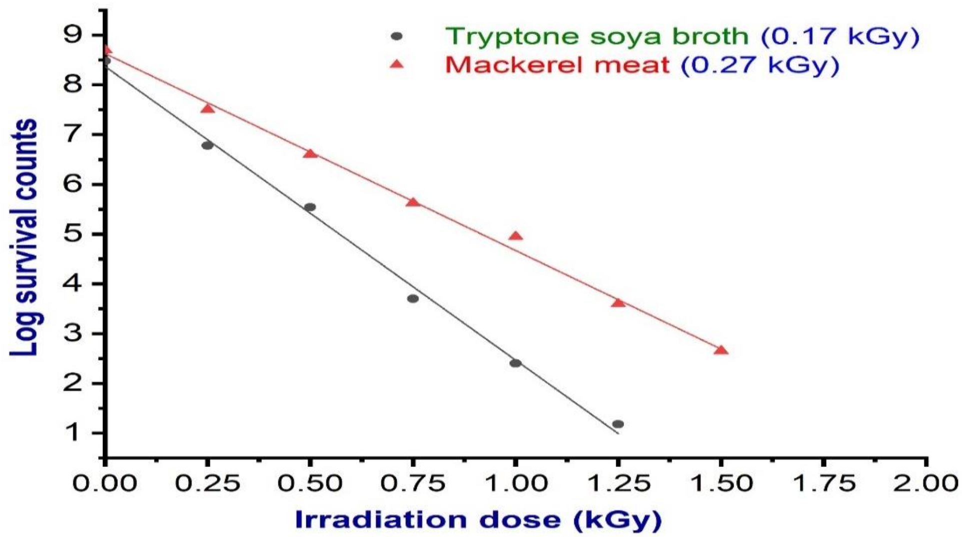

Fig. (1): Radiation-dose response curves of *K. pneumoniae* suspended in tryptone soya broth and in mackerel fish

Radiation sensitivity of *P. fluoresense*

The impact of gamma irradiation on *P. fluoresense* bacterial counts in tryptone soya broth and mackerel meat was studied. The non-inoculated mackerel samples were examined for bacterial count on the tryptone soya agar before the irradiation treatment, and no bacterium count was detected. The initial counts of bacteria were ranged from 8.60 to 8.69 log CFU in both tryptone soya broth and mackerel muscles, respectively. After 1.0 kGy, the bacterial counts of *P. fluoresense* in tryptone soya broth and mackerel fish were reduced by 7.4 log CFU/mL and 7.2 CFU/g, respectively. This indicates the sensitivity of *P. fluoresense* to gamma irradiation. Additionally, the dosage-related log decreases in bacterial counts were linear, and *P. fluoresense* was completely eradicated at doses lower than 1.5 kGy. The bacterial cells were enriched in tryptone soya broth before counting after 1.5 kGy to ensure the resuscitation of those that had been damaged during the irradiation procedure. After enrichment, no *P. fluoresense* recovery was detected in 1.5 kGy or 2.0 kGy treated samples. **Table 1** and **Fig 2** illustrate the radiation sensitivity of *P. fluoresense* calculated from the survival curves. The D_{10}-values were ranged from 0.13 to 0.14 kGy indicating a variation depending on the type of substrate. The survival curves obtained were linear in their nature (R= 0.9973 and 0.9995 for tryptone soya broth and mackerel fish meat, respectively) as shown in **Fig. 2**.

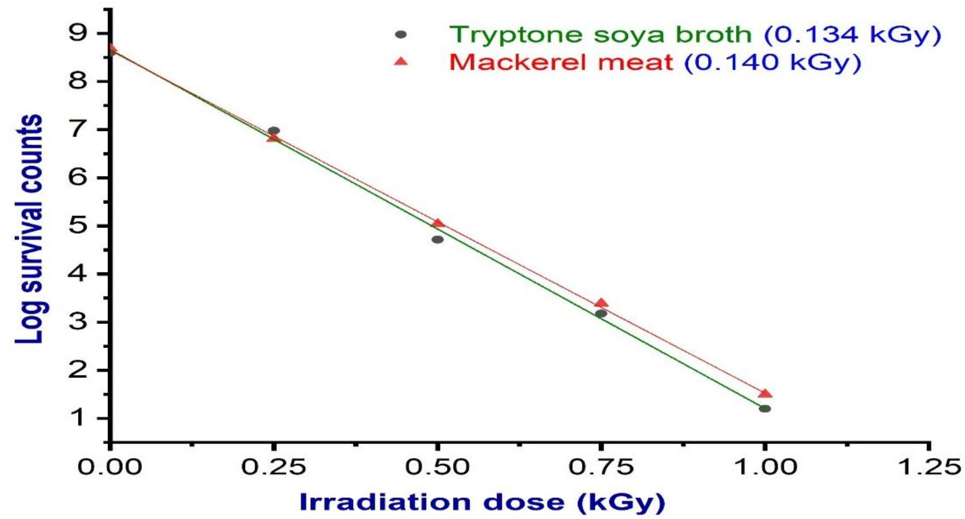

Fig. (2): Radiation-dose response curves of *P. fluorescens* suspended in tryptone soya broth and in mackerel fish

Radiation sensitivity of *P. agglumerance*

The initial count of *P. agglumerance* decreased from 8.60 to 1.34 log CFU/mL and 2.3 log CFU/g in both tryptone soya broth and mackerel muscle, respectively. 2.0 kGy was sufficient to reduce the population of this organism by 6.2 log CFU/mL in tryptone soya broth, and 5.15 log CFU/g in mackerel meat. However, 3.0 kGy completely eliminated this species. After enrichment, mackerel samples inoculated with *P. agglumerance* showed 100% elimination at a dosage of 3.0 kGy. The dose necessary to decrease 1 bacterial log was about 0.34 and 0.39 kGy in tryptone soya broth and mackerel meat, respectively as shown in **Table 1** and **Fig. 3.**

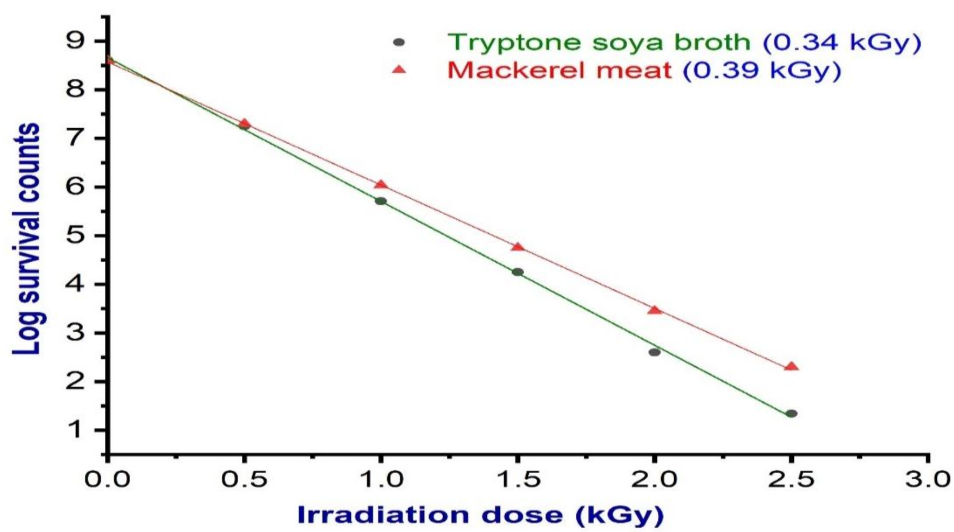

Fig. (3): Radiation-dose response curves of *P. agglumerance* suspended in tryptone soya broth and in mackerel fish

Radiation sensitivity of *Bacillus* sp.

Nearly 5.04-5.0 log CFU of *Bacillus* sp was recovered before irradiation. *Bacillus* sp. displayed resistance to gamma rays, with reductions of 3.34 and 3.26 log CFU at a 3.5 kGy irradiation dosage in both tryptone soya broth and mackerel flesh. Therefore, even after irradiation at 4.0 kGy, it was difficult to eliminate *Bacillus* sp, and greater doses would be necessary to ensure total eradication. Until 4.0 kGy, there was a definite inverse connection between the dosage and the level of surviving bacteria. At 4.5 kGy, the growth of the bacteria in tryptone soya broth and mackerel flesh was totally suppressed. However, after enrichment at 4.5 kGy, bacteria were recovered from treated mackerel samples, but recovery was not detected at 5.0 kGy. The D_{10}-values for *Bacillus* sp. in tryptone soya broth (1.03 kGy) and mackerel muscle (1.12 kGy) were nearly identical as illustrated in **Table 1** and **Fig. 3**. The histamine-producing bacteria on mackerel meats were less sensitive to radiation than those in tryptone soya broth. Although variations in water and oxygen conditions were given as possible reasons since these factors have a significant influence on bacteria radiation sensitivities, the difference in radiation sensitivities between tryptone soya broth and mackerel meats could not be explained.

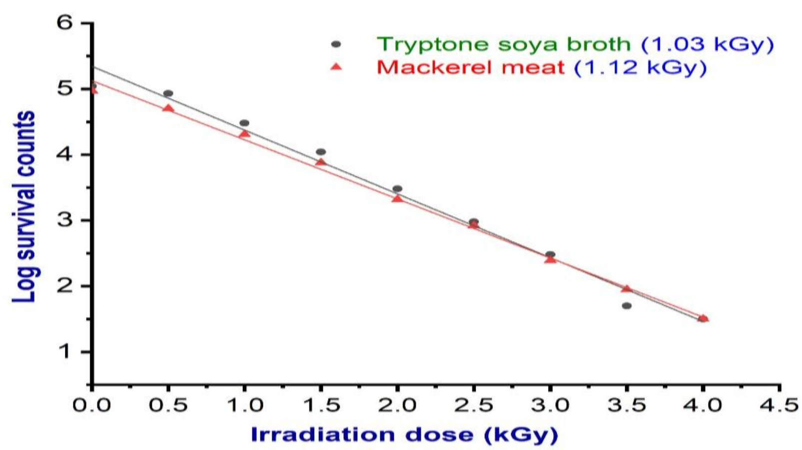

Fig. (4): Radiation-dose response curves of *Bacillus* sp. suspended in tryptone soya broth and in mackerel fish

Table 1: The radiation D_{10}-values of histamine-producing bacteria

	D_{10}-value*			
	K. pneumoniae	*P. fluoresense*	*P. agglumerance*	*Bacillus* sp.
Tryptone soya broth	0.17 ± 0.04	0.13 ± 0.02	0.34 ± 0.01	1.03 ± 0.02
Mackerel meat	0.27 ± 0.01	0.14 ± 0.02	0.39 ± 0.02	1.12 ± 0.04

***** The radiation D_{10}-values are shown by the average of three separate trials and their standard deviations (n = 3).

DISCUSSION

Histamine poisoning is most commonly connected with scombroid fish such as mackerel, which has high quantities of free histidine and is most likely the optimal species for histamine formation **(Özogul and Özogul, 2020; Hungerford, 2021)**. As a result, approaches for limiting histamine development as well as histamine-forming bacteria in fish play an essential role in lowering the incidence of illness and scombroid poisoning. Since histamine are formed as a result of decarboxylation of histidine by decarboxylating bacteria, inhibiting the growth of these bacteria is an efficient strategy to lower the risk of getting sick **(Křížek *et al*., 2012; Russo *et al.*, 2020; Houicher *et al*., 2021).**

K. pneumoniae, Morganella morganii and *Raoultella planticola* are well-known bacterial strains that have been linked to histamine formation **(Russo *et al.*, 2020)**. For controlling the production of histamine, it is crucial to determine these species' radiation sensitivity or resistance. Most early investigations focused on the radiosensitivity of histamine producers that produce large amounts of histamine, as *Morganella morganii, Klebsiella variicola,* and *Raoultella planticola* **(Nei *et al*., 2012; Ramakrishna Reddy *et al*., 2020)**. For inistance, **Nei *et al.* (2012)** reported the D_{10}-value of *Morganella morganii, Enterobacter aerogenes,* and *Raoultella planticola* bacteria within range of 0.32 to 0.42 kGy. Human health may be jeopardized by other species, even if they only generate trace levels of histamine. Along with Gram-positive bacteria like *Bacillus* sp., which has a well-established history of producing histamine. For example, *Bacillus coagulans* and *Bacillus megaterium* were identified as the main histamine-producing bacteria **(Tsai *et al*., 2006)**. The radiation sensitivity of other histamine-forming bacteria, such as *Pantoea agglomerans* has not been described. Thus, the survivability of four HIS-forming bacteria exposed to the radiation were studied in both tryptone soya broth and artificially inoculated mackerel meat.

The initial counts of *K. pneumoniae, P. fluorescens, P. agglumerance*, and *Bacillus* sp., were ranged from 8.48 to 8.70, 8.60 to 8.69, 8.60 to 8.61, and 5.04 to 4.97 log CFU, respectively. The bacterial count of *K. pneumoniae* was significantly decreased after gamma irradiation. *K. pneumoniae* was extremely susceptible to gamma radiation, with a mean D_{10}-value of 0.17 kGy in tryptone soya broth and 0.27 kGy in mackerel flesh. At 1.5 kGy, complete reduction was accomplished. Its growth was fully halted with a radiation dose of 1.5 kGy. **Gautam *et al*. (2015)** reported the D_{10}-value of *K. pneumoniae* in mixed sprouts, poultry and fish samples *w*ithin 0.142, 0.125 and 0.277 kGy, respectively. In both nutrient broth and tuna flesh, *K. variicola* was suppressed at a dose of 1.5 kGy **(Ramakrishna Reddy *et al*., 2020)**.

The growth of *P. fluoresense,* on the other hand, were reduced by 7.4 log CFU/mL and 7.2 log CFU/g after 1.0 kGy in tryptone soya broth and mackerel fish, respectively. In tryptone soya broth and mackerel flesh, the mean D_{10}-values of *P. fluoresense* were 0.13 and 0.14 kGy, respectively. The D_{10}-value of *P. fluoresense* in buffered peptone water was 0.12 kGy **(Olanya *et al*., 2015)**. Significant reduction also have been noted in case of *P. agglumerance*; 6.20 log CFU/mL and 5.15 log CFU/g reduction was obtained at 2.0 kGy. However, 3.0 kGy completely eliminated this species. The D_{10}-values for *P. agglumerance* in tryptone soya broth (0.34 kGy) and mackerel muscle (0.39 kGy) were significantly different. To the best of our knowledge, there are no reports available for the radiation sensitivity of *P. agglumerance.* Despite the fact that ground beef included *Escherichia coli* and *Salmonella enterica*, two closely related genera of Enterobacteriaceae, their individual D_{10}-values ranged from 0.24 to 0.31 kGy and 0.62 to 0.80 kGy **(Begum *et al*., 2020)**.

In the current study, *Bacillus* sp. exhibited greater gamma radiation resistance, with a mean D_{10}-value ranging from 1.03 to 1.12 kGy in tryptone soya broth and mackerel flesh, respectively. In a previous study, *Bacillus* sp. T61 was shown to be extremely resistant to gamma radiation, with a D_{10}-value of 10%, significantly greater than that of radiation-sensitive *E. coli* K12 **(Huijuan *et al*., 2014)**. However, it was difficult to eliminate *Bacillus* sp. even after irradiation at 4.0 kGy, and higher doses (5.0 kGy) would be required to achieve complete elimination. Fish irradiation has been offered as an attractive method of preservation for prolonging the shelf life of refrigerated and stored fish as well as qualitatively and quantitatively lowering the microbial counts in fish and fish products. The shelf-life of fish was prolonged by irradiating it at doses of 3.0-4.0 kGy without any discernible temperature increase and without changing the fish's organoleptic properties **(Arvanitoyannis and Tserkezou, 2014; Nishihira, 2020)**. *S. aureus*, *E. coli*, and *Listeria monocytogenes* may all be eradicated with radiation doses of 3.0 kGy and 4.0 kGy, respectively, without affecting the physicochemical properties of the fish **(Mohamed *et al*., 2009)**.

However, high doses of radiation (above 4.5 kGy) can cause oxidative damage in fish, a decrease in fatty acid content, and an increase in thiobarbituricacid production, which can result in the generation of radiolysis products and the destruction of thiamine (B1) while retaining riboflavin (B2) **(Erkan, 2014)**. A maximum dose of absorbed radiation of up to 10 kGy has been set by Codex Alimentarius. While the highest allowable dose for processing fish products USA, Canada, and Brazil is 2.2 kGy, it is 3.0 kGy in England, France, and the Netherlands **(Chauhan *et al*., 2009)**.

Histamine-producing bacteria on mackerel meats were less sensitive to radiation than those in tryptone soya broth. This could be attributed to the variation in intrinsic and extrinsic parameters, such as pH, oxygen tension, protein composition, and bacterial rate of growth, which might influence the sensitivity of various bacteria in a specific medium **(Thayer *et al*., 1995; Sommers and Boyd, 2006; Gomes *et al*., 2011)**. it has been reported that the radiation sensitivity of *K. pneumoniae* MTCC 109 was significantly greater *(P* < 0.05) in aqueous media than on sprout homogenate, poultry homogenate, and fish homogenate **(Gautam *et al*., 2015)**. In that study, the radiation sensitivity of *K. pneumoniae* MTCC 109 was 0.116 kGy in saline, 0.136 kGy in nutrient broth, 0.142 kGy in sprout homogenate, 0.125 kGy in poultry homogenate, and 0.277 kGy in fish homogenate. The strain, temperature, pH, and water content of foods were also shown to have a significant impact on radiation sensitivity **(Buchanan *et al*., 1999; Gautam *et al*., 2015; Olanya *et al*., 2015; Osaili *et al*., 2018)**.

Novoslavskij *et al*. (2016) have reported differences in D_{10}-values of pathogens like *S. aureus*, *Vibrio* sp., *A. hydrophila*, *S. typhimurium*, *S. typhi*, *S. fecalis*, *B. cereus*, *L. monocytogenes*, and *Y. enterocolitica* in different fish/shellfish media (surface of prawns, shrimp and fish paste, shrimp, fish, and oyster homogenate) at varied temperatures (ambient temperature to –20ºC) and atmosphere (air/vacuum). High quantities of histamine have been detected in fish as well as liquid food items like milk and fish sauce, thus techniques of regulating these bacteria must be developed in order to keep perishable food products safe **(Magwamba *et al*., 2010; Ma *et al*., 2022)**. This study's findings show that irradiation is a successful strategy for lowering the histamine-forming bacteria. Since early microbial population has a substantial influence on the accumulation of biogenic amines, gamma irradiation should be considered as an intervention approach to reduce the danger of histamine poisoning in fish and fish products **(Křížek *et al*., 2012)**. The radiation dose required to limit the development of histamine-forming bacteria is determined by the bacterial resistance, which may necessitate combined treatment.

CONCLUSION

The impacts of gamma rays on histamine-forming bacteria were investigated in this study. The findings of this investigation demonstrated that gamma irradiation significantly decreased histamine-producing bacteria that were artificially inoculated in mackerel meat. In both *K. pneumoniae* and *P. fluorescens*, the lower dosage (1.5 kGy) significantly decreased the microbial load in mackerel muscle by 4 to 5 log CFU/g, which would be adequate to eradicate naturally occurring histamine formers in fish muscles. To the best of our knowledge, the radiation sensitivity of *P. agglumerance* in tryptone soya broth and mackerel meat injected with bacterial cells is reported for the first time, with D_{10}-values of 0.34 and 0.39 kGy, respectively. However, because low-dose irradiation is inefficient in reducing *Bacillus* sp, early counts of this bacteria in fish samples should be regularly monitored. Given that, with the exception of *Bacillus* sp., histamine-forming bacteria in fish muscle are typically radiation sensitive, gamma irradiation may successfully inhibit histamine formation in mackerel, ensuring its safety for human intake.

REFERENCES

Arulkumar, A.; Paramithiotis, S. and Paramasivam, S. (2021). Biogenic amines in fresh fish and fishery products and emerging control. *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2021.02.001.

Arvanitoyannis, I. S. and Tserkezou, P. (2014). Irradiation of Fish and Seafood. In *Seafood Processing*, (pp. 83–127).

Begum, T.; Follett, P. A.; Hossain, F.; Christopher, L.; Salmieri, S. and Lacroix, M. (2020). Microbicidal effectiveness of irradiation from Gamma and X-ray sources at different dose rates against the foodborne illness pathogens *Escherichia coli, Salmonella Typhimurium* and *Listeria monocytogenes* in rice. *LWT*, 132: 109841. https://doi.org/10.1016/j.lwt.2020.109841.

Buchanan, R. L.; Edelson, S. G. and Boyd, G. (1999). Effects of pH and acid resistance on the radiation resistance of Enterohemorrhagic *Escherichia coli*. *Journal of Food Protection*, 62(3): 219–228. https://doi.org/10.4315/0362-028X-62.3.219.

Chauhan, S. K.; Kumar, R.; Nadanasabapathy, S. and Bawa, A. S. (2009). Detection Methods for Irradiated Foods. *Comprehensive Reviews in Food Science and Food Safety*, 8(1): 4–16. https://doi.org/10.1111/j.1541-4337.2008.00063.x.

Emborg, J. and Dalgaard, P. (2006). Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protection*, 69(4): 897–906. https://doi.org/10.4315/0362-028X-69.4.897.

Erkan, N. (2014). Alternative seafood preservation technologies: ionizing radiation and high pressure processing. *Journal of FisheriesSciences.com*, 8(3): 238-251. https://doi.org/10.3153/jfscom.201430.

FDA. (2020). Fish and Fishery Products Hazards and Controls Guidance, fourth ed. https. www.fda.gov/media/80637/download. (Accessed 5 August 2021).

Gautam, R. K.; Nagar, V. and 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.

Gomes, C.; Moreira, R. G. and Castell-Perez, E. (2011). Radiosensitization of *Salmonella* spp. and *Listeria* spp. in ready-to-eat baby spinach leaves. *Journal of Food Science*, 76(1): 141–148. https://doi.org/10.1111/j.1750-3841.2010.01904.x.

Houicher, A.; Bensid, A.; Regenstein, J. M. and Özogul, F. (2021). Control of biogenic amine production and bacterial growth in fish and seafood products using phytochemicals as biopreservatives: A review. *Food Bioscience*, 39: 100807. https://doi.org/10.1016/j.fbio.2020.100807.

Huijuan, X.; Li, L.; Mingyan, B.; Yunfeng, S.; Ping, W. and Zhiwei, Y. (2014). Radiation resistance of *Bacillus* sp T61 and analysis of cellular protein changes after gamma-ray irradiation. *Journal of Radiation Research and Radiation Processing*, 32(3): 8. https://doi.org/10.11889/j.1000-3436.2014.rrj.32.030204.

Hungerford, J. M. (2021). Histamine and Scombrotoxins. *Toxicon*, 201: 115–126. https://doi.org/10.1016/j.toxicon.2021.08.013.

Jong, E. C. (2016). Fish and shellfish poisoning: toxic syndromes. Travel and tropical medicine manual. Edited by Sanford CA, Pottinger PS, Jong EC. Fifth Ed. Section, 4: 451-456. https://doi.org/10.1016/B978-0-323-37506-1.00034-9.

Kim, S. H.; Barros-Velázquez, J.; Ben-Gigirey, B.; Eun, J. B.; Jun, S. H.; Wei, C. I. and An, H. (2003). Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science and Biotechnology*, 12(4): 451-460.

Kim, S. H.; Ben-Gigirey, B.; Barros-Velázquez, J.; Price, R. J. and An, H. (2000). Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. *Journal of Food Protection*, 63(2): 244–251. https://doi.org/10.4315/0362-028X-63.2.244.

Křížek, M.; Matějková, K.; Vácha, F. and Dadáková, E. (2012). Effect of low-dose irradiation on biogenic amines formation in vacuum-packed trout flesh *(*Oncorhynchus mykiss). *Food Chemistry*, 132(1): 367–372. https://doi.org/10.1016/j.foodchem.2011.10.094.

Ma, X.; Zhang, Y.; Li, X.; Bi, J.; Zhang, G.; Hao, H. and Hou, H. (2022). Impacts of salt-tolerant *Staphylococcus nepalensis* 5-5 on bacterial composition and biogenic amines accumulation in fish sauce fermentation. *International Journal of Food Microbiology*, 361: 109464. https://doi.org/10.1016/j.ijfoodmicro.2021.109464.

Magwamba, C.; Matsheka, M. I.; Mpuchane, S. and Gashe, B. A. (2010). Detection and Quantification of Biogenic Amines in Fermented Food Products Sold in Botswana. *Journal of Food Protection*, 73(9): 1703–1708. https://doi.org/10.4315/0362-028X-73.9.1703.

Mercogliano, R. and Santonicola, S. (2019). Scombroid fish poisoning: Factors influencing the production of histamine in tuna supply chain. A review. *LWT*, 114:

108374. https://doi.org/10.1016/j.lwt.2019.108374.

Mohamed, W. S.; EL-Mossalami, E. I. and Nosier, S. M. (2009). Evaluation of sanitary status of imported frozen fish fillets and its improvement by gamma radiation. *Journal of Radiation Research and Applied Sciences*, 2(5): 921–931.

Nei, D.; Kawasaki, S.; Inatsu, Y.; Yamamoto, K. and Satomi, M. (2012). Effectiveness of gamma irradiation in the inactivation of histamine-producing bacteria. *Food Control*, 28(1): 143–146. https://doi.org/10.1016/j.foodcont. 2012.05.006.

Nei, D.; Uchino, T.; Sakai, N. and Tanaka, S. (2005). Effect of high temperature on the apparent activation energy of respiration of fresh produce. *Postharvest Biology and Technology*, 37(3): 277–285. https://doi.org/10.1016/j.postharvbio.2005.05.001.

Nishihira, J. (2020). Safety of irradiated food. In Genetically Modified and Irradiated Food. Academic Press, (pp. 259-267). https://doi.org/10.1016/B978-0-12-817240-7.00016-4.

Niven, C. F.; Jeffrey, M. B. and Corlett, D. A. (1981). Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology*, 41(1): 321–322. https://doi.org/10.1128/aem.41.1.321-322.1981.

Novoslavskij, A.; Terentjeva, M.; Eizenberga, I.; Valciņa, O.; Bartkevičs, V. and Bērziņš, A. (2016). Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, 66(1): 1–15. https://doi.org/10.1007/s13213-015-1102-5.

Olanya, O. M.; Niemira, B. A. and Phillips, J. G. (2015). Effects of gamma irradiation on the survival of *Pseudomonas fluorescens* inoculated on romaine lettuce and baby spinach. *LWT-Food Science and Technology*, 62(1): 55–61. https://doi.org/ 10. 1016/j.lwt.2014.12.031.

Osaili, T. M.; Al-Nabulsi, A. A.; Aljaafreh, T. F. and Olaimat, A. N. (2018). Use of gamma radiation to inactivate stressed *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in tahini halva. *LWT*, *98*: 438–443. https://doi.org/10.1016/j.lwt.2018.09.017.

Özogul, Y. and Özogul, F. (2020). Biogenic amines formation, toxicity, regulations in food. In *Biogenic Amines in Food: Analysis, Occurrence and Toxicit. The Royal Society of Chemistry*, (pp. 1–17). https://doi.org/10.1039/9781788015813-00001.

Ramakrishna Reddy, P.; Kumar, S. H.; Layana, P. and Nayak, B. B. (2020). Survival and histamine production by histamine-forming bacteria exposed to low doses of gamma irradiation. *Journal of Food Protection*, 83(7): 1163–1166. https://doi.org/10.4315/JFP-19-511.

Russo, P.; Fragasso, M.; Berbegal, C.; Grieco, F.; Spano, G and Capozzi, V. (2020). Microorganisms able to produce biogenic amines and factors affecting their activity.

In *Biogenic Amines in Food: Analysis, Occurrence and Toxicity*. *The Royal Society of Chemistry*, (pp. 18–40). https://doi.org/10.1039/9781788015813-00018.

Sommers, C. H. and Boyd, G. (2006). Variations in the radiation sensitivity of foodborne pathogens associated with complex ready-to-eat food products. *Radiation Physics and Chemistry*, 75(7): 773–778. https://doi.org/10.1016/j.radphyschem.2005.12.036.

Sommers, C. H. and Rajkowski, K. T. (2011). Radiation inactivation of foodborne pathogens on frozen seafood products. *Journal of Food Protection*, 74(4): 641–644. https://doi.org/10.4315/0362-028X.JFP-10-419.

Thayer, D. W.; Boyd, G.; Fox Jr., J. B.; Lakritz, L. and Hampson, J. W. (1995). Variations in radiation sensitivity of foodborne pathogens associated with the suspending meat. *Journal of Food Science*, 60(1): 63–67. https://doi.org/10.1111/j.1365-2621.1995.tb05607.x.

Tsai, Y. H.; Lin, C. Y.; Chang, S. C.; Chen, H. C.; Kung, T.; Wei, C. I. and Hwang, D. F. (2005). Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology*, 22(5): 461–467. https://doi.org/10.1016/j.fm.2004.11.003.

Tsai, Y. H.; Lin, C. Y.; Chien, L. T.; Lee, T. M.; Wei, C. I. and Hwang, D. F. (2006). Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. Food Chemistry, 98(1): 64–70. https://doi.org/10.1016/j.foodchem.2005.04.036.