Combined effect of gamma irradiation and low temperature storage on the microbial and bio-chemical quality of sutchi cat fish (*Pangasius hypophthalmus*)

Abstract

Gamma irradiation is known as an effective method to reduce microbial contamination in food products. Its application in seafood food not only enhances safety and quality but also extends shelf life, thereby minimizing post-harvest losses. This study focuses on evaluating the combined effect of low dose gamma irradiation and low-temperature storage on the quality and shelf life of fresh Sutchi Catfish (*Pangasius hypophthalmus*). The fish samples were subjected to different gamma irradiation doses (0.0 kGy, 1.0 kGy, 3.0 kGy, and 5.0 kGy) and then stored under refrigerated conditions (4°C) and frozen conditions (-18°C). Both non-irradiated and irradiated samples were periodically analyzed for microbial counts, including coliforms (total and fecal), total mesophilic bacteria, *Salmonella*, *E. coli*, and *Staphylococcus aureus*. The biochemical quality indices such as total volatile base nitrogen (TVB-N), pH, peroxide value (PV), and thiobarbituric acid reactive substances (TBARS) were also assessed at regular intervals.

The findings revealed that the gamma irradiation and storage at low temperatures significantly ($p < 0.05$) reduced microbial populations, with higher irradiation doses resulting in greater reductions. Chemical quality indicators in irradiated samples were notably better maintained compared to the control samples under both storage conditions. The study concluded that combining gamma irradiation with refrigeration or freezing effectively minimized microbial loads and preserved biochemical quality. This approach extended the shelf life of the fish up to 21 days in refrigeration and 90 days under frozen storage conditions.

Key words: *Gamma irradiation, frozen storage, microbial quality, PV, TBARS, Sutchi Cat fish*

I. Introduction

The freshness of fish is a crucial factor in determining its overall quality. Compared to other animal meat, fish is more susceptible to spoilage due to its higher protein and poly unsaturated fatty acidscontent, elevated water activity, and favorable pH levels, all of which limit its shelf life. “In India, inland aquaculture primarily revolves around major carps such as Catla, Rohu, and Mrigal, alongside exotic carps scuch as Common carp, Silver carp, and Grass carp. In recent years, catfish farming has emerged as a promising alternative for Indian farmers, with Sutchi catfish (*Pangasius hypophthalmus*)—a species native to Thailand and Vietnam—being the most widely cultivated. During the 2020–22 period, catfish production in India reached 1.29 million tonnes” (Handbook on Fisheries Statistics, 2022). Aquaculture industry of Andhra Pradesh state of India has witnessed the fastest development in single-species farming, especially for Pangasius.

“Catfish is highly sought after due to its boneless structure, soft texture, mild flavor, and appealing taste, making it a preferred option for consumers” (Viji et al., 2014). These characteristics, are coupled with its rich nutritional profile and sensory attributes, contribute to its popularity. “However, fish and shellfish can serve as reservoirs for specific spoilage organisms and pathogenic bacteria capable of causing foodborne illnesses” (Hocaoglu et al., 2012; Manjanaik et al., 2018). To address this issue, many preservation methods have been introduced to enhance the shelf life of fish and fishery products while mitigating health risks to consumers. One such method is irradiation, which uses electromagnetic energy to preserve food. “This technique effectively reduces the risk of foodborne illnesses, delays spoilage, and extends the shelf life of the food products without leaving harmful residues” (ICGFI, 2002; Nagar and Bandekar, 2011). Furthermore, irradiation has minimal impact on the sensory and nutritional properties of food, such as taste, colour, and aroma.

“The application of multiple preservation techniques often produces synergistic effects, creating robust microbiological barriers” (Leistner and Gorris, 1995). “For example, an integration of food irradiation with proper refrigeration can significantly enhance the shelf life of fish and shellfish. Gamma irradiation, particularly at low doses (below 10 kGy), has been proven to eliminate most microorganisms without compromising the superior quality of the food product” (Javanmard et al., 2006; Hocaoglu et al., 2012). This process is effective in virtually eradicating foodborne pathogens. “Currently, more than 26 countries employ irradiation on a commercial scale for preservation of food” (Ouattara, 2001). The purpose of this study is to investigate the combined effect of low-dose gamma irradiation and low temperature storage on the shelf life of fresh catfish, highlighting its potential to improve product longevity and quality.

2. Material and Methods

2.1. Materials

Fresh Sutchi catfish (*Pangasius hypophthalmus*) were sourced from fish farms in Northern Karnataka, India. The fish were promptly packed in polyethylene bags with ice under aseptic conditions and transported to the laboratory within 6 hours. After arrival, they were kept at -18 ºC until irradiation.

2.2. Preparation of sample

The fresh fish were cleaned and dressed. The prepared samples were rinsed properly and sliced into steaks 2-3 cm thick, each weighing 100-200 g and packed into polyethylene bags. Then samples were sepereted into 2 groups, each subjected to four levels of the gamma irradiation exposure: 0.0 (control), 1.0, 3.0, and 5.0 kGy. One group was stored at 4°C following irradiation, while the other was stored at -18°C under the same radiation treatment conditions.

2.3. Irradiation

The fish samples were subjected for irradiation at the Centre for Application of Radioisotope and Radiation Technology (CARRT), Mangalore University, utilizing a cobalt-60 radiation source supplied by BRIT, Mumbai. The study employed doses of radiation at 1.0, 3.0, and 5.0 kGy, with corresponding exposure times of 6.38, 32, and 45 minutes, respectively, based on a dose rate of 6.94 kGy/hour. During irradiation, the samples were maintained at a temperature of 4 ± 1°C using airtight ice covers. The absorbed doses were measured using Fricke dosimeter. Post-irradiation, both the irradiated as well as non-irradiated (control) samples were transported to the laboratory within one hour, packed in polystyrene ice boxes with ice. They were then stored at refrigeration (4°C) or freezing (-18°C) temperatures until further study.

2.4. Chemical Analysis

The proximate biochemical analysis of fresh fish was estimated using standard methods. Moisture content was measured using the hot air oven method as per AOAC (2010). The crude protein content was analysed by estimating the total nitrogen content using the Kjeldahl method (AOAC, 2010). Crude lipid content was measured through Soxhlet extraction (AOAC, 2010), and ash content was analysed following the procedure outlined in AOAC (2010). Total volatile base nitrogen (TVBN) levels in the fish meat were assessed using Conway's micro-diffusion technique (Beatty and Gibbon, 1937) and expressed in mg N/100 g of meat. Lipid oxidation was evaluated by measuring

thiobarbituric acid reactive substances (TBARS) using the method described by Raghavan and Hultin (2005), with the results expressed as mg malonaldehyde per kg of meat. The pH of the samples was determined following the method of Vyncke (1981), using a Systronix 361 pH meter (India).

2.5. Microbial analysis

Total plate counts (TPC) and bacterial pathogen levels were monitored during the 90-day experimental period. A 25 g sample was aseptically transferred into a sterile blender containing 225 ml of sterilized physiological saline solution (0.85 % NaCl) and homogenized at low speed for 3 minutes. Decimal dilutions of 0.1 ml were plated onto plate count agar and incubated at 35°C for 24-48 hours. The resulting total plate counts were converted into logarithms to express the number of colony-forming units per gram of sample (CFU/g). For total coliform enumeration, the three-tube most probable number (MPN) method was used. Samples were inoculated into LSTB and EC broth (Himedia, Mumbai) at appropriate dilutions and incubated at 37°C and 44.5°C, respectively. Specific pathogens, including *Escherichia coli* (on EMB agar), *Staphylococcus aureus* (on Baird-Parker agar), and *Salmonella* (on BSA agar), were analysed following guidelines from APHA (1998) and ICMSF (1986). Microbial counts were expressed as log CFU/g.

2.6. Sensory analysis

The sensory attributes of both irradiated and control samples were evaluated using a 9-point hedonic scale as outlined by Meilgaard et al. (1999). The overall impression of the product was assessed based on its overall acceptability, determined by summing the scores of all attributes. A high score (7–9) indicated fish with no off-odours, while scores below 6 denoted unacceptable quality.

2.7. Statistical analysis

The data gathered from microbiological, biochemical, and sensory analyses were statistically evaluated using the Statistical Package for Social Sciences (SPSS, version 23.0 for Windows). One-way analysis of variance (ANOVA) was applied to identify variations across the storage periods. Duncan's Multiple Range Test was used to assess significant differences between means, with statistical significance considered at a threshold of $p < 0.05$.

3. Results and Discussion

The fish used for this study had an average length of 46.83 ± 4.19 cm and an average weight of 1.31 ± 0.36 kg. The proximate composition of the fish meat revealed moisture content of 77.80%, protein at 16.50%, crude lipid at 4.50%, and ash content of 0.97%. These findings closely align with those reported by Viji et al. (2014).

3.1. Bio chemical analysis of fish (*Pangasius hypophthalmus*) stored under refrigeration (4°C) and frozen (-18 °C) storage

3.1.1. pH

On the first day of refrigerated storage, the value of pH for non-irradiated (control) samples was 6.35, while the values for fish irradiated at 1, 3, and 5 kGy were 6.35, 6.31, and 6.30, respectively (Fig. 1). A rise in the applied irradiation dose was related with a decrease in the pH value. Throughout refrigerated storage, pH values decreased in all fish samples. The pH values for both non-irradiated and irradiated fish samples ranged from 6.35 to 6.56. At the end of the 21-days storage period, no clear trend was found in the pH of the fish stored under refrigeration. However, non-irradiated samples exhibited higher pH values compared to irradiated samples, likely due to the accumulation of nitrogenous compounds resulting from chemical and biological decomposition. Despite these, there was no statistically significant difference obsereved in pH ($p < 0.05$) between treatments. “Similar results have been reported for farmed sea bass and turbot stored using ice” (Papadopoulos et al., 2004; Rodriguez et al., 2006).

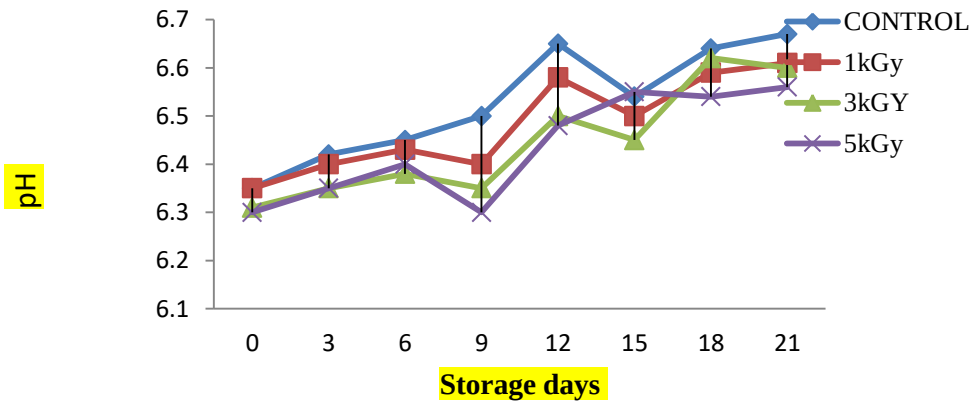

Fig. 1. Changes in pH of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4°C)

Fish samples stored at -18°C after irradiation exhibited a significant decrease in pH values for both irradiated and control fish between 0 and 1 days, with irradiated samples showing a greater decrease than the controls (Fig. 2). At the end of 90-days of storage period, a significant difference observed between both irradiated and control samples at doses of 1, 3, and 5 kGy. The pH of the control sample began at 6.40 and increased to 6.82 by the end of the 90-day storage period. During frozen storage, no consistent trend was observed in the pH of fish steaks. However, irradiated samples consistently exhibited lower pH values compared to the control samples due to the accumulation of nitrogenous compounds in control samples resulting from chemical and biological decomposition. These findings suggest that pH may be a poor indicator of catfish freshness under frozen storage conditions.

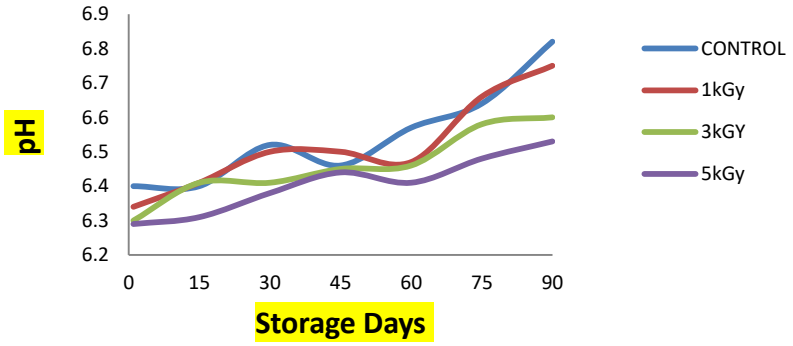

Fig. 2. Changes in pH of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18ºC)

3.1.2. Total volatile base nitrogen (TVB-N)

During refrigerated storage (4°C), TVB-N value of fish was significantly ($p<0.05$) higher in non-irradiated control samples compared to irradiated samples, as shown in Fig. 3. The starting TVB-N value in control fish was 6.87 mg N/100 g, which increased to 12.13 mg N/100 g by the end of the 21-day storage period. In contrast, irradiation at 1, 3, and 5 kGy efficiently reduced the formation of TVB-N during storage, with values reaching 9.80, 7.47, and 6.53 mg N/100 g, respectively, after 21 days. “Irradiated fish samples consistently exhibited significantly lower TVB-N concentrations as compared to control samples, likely due to the reduced microbial load” (Venugopal et al., 1999). In this study, the TVB-N value found within acceptable limit of 35 mg N/100 g of fish throughout the 21-day storage period. These findings were consistent with Castro et al. (2006), who observed similar results in European sea bass stored in ice for 20-22 days. The lower TVB-N content in catfish steaks is primarily attributed to the absence of trimethylamine (TMA), the major component of volatile bases in fish meat. Changes in TVB-N are largely attributed to the TMA component, which is a significant constituent of volatile bases.

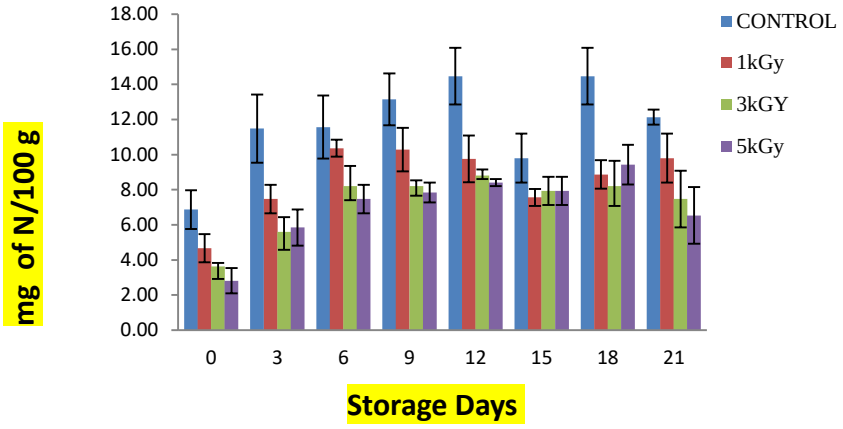

Fig.3. Changes in TVBN of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC). Vertical bars indicate error bars

At the starting of frozen storage (-18^{o}C), the TVB-N value was found 6.87 mg N/100g for the control sample and 6.40, 6.41 and 5.80 mg N/100g respectively for irradiated samples at 1,3 and 5 kGy **(Fig 4).** However, TVB-N values followed a different trend throughout the storage period, increasing at the end of 90 days at -18^{o}C. The TVB-N values were 14.40, 11.60, 10.60 mg N/100g for non- irradiated and 1, 3 and 5 kGy irradiated fish samples respectively at the storage period of 90 days at -18^{o}C.

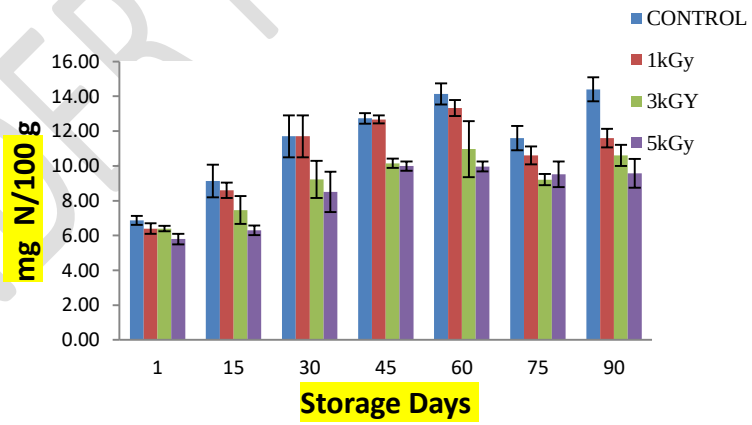

Fig.4. Changes in TVBN of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18ºC). Vertical bars indicate error bars

The TVB-N data revealed a significant difference between control and irradiated fish samples stored at -18°C after 90 days of storage ($p < 0.05$). "TVB-N levels in both non-irradiated and irradiated fish samples did not exceed 35 mg/100g at both temperatures, which is considered the maximum acceptable level for fish" (Huss, 1988). This finding

aligns with the TVB-N levels reported for irradiated Chinese pomfret (*Pampus chinensis*) stored in ice, as stated by Ahmed et al., (2009). However, Cakli et al., (2006a; 2006b) found TVB-N levels of 39.89 and 35 mg/100g, respectively, for seabass after 18 and 14 days of ice storage. As per findings Chouliara et al. (2005), the initial TVB-N levels of vacuum-packed irradiated (1-3 kGy) sea bream samples stored in refrigeration were 27.5, 27.3, and 25.1 mg/100g, reaching acceptable limits at day 10 for control samples, and on day 21 and 28 for 1 and 3 kGy irradiated samples. Similarly, Jo et al. (2004) observed lower TVB-N levels for irradiated samples as compared to the control samples. Mendes et al. (2005) found an initial TVB-N level of 15.6 mg/100g in chilled horse mackerel, which touched the limit levels of 30-35 mg/100g at 12th day for non-irradiated control samples, while 1 and 3 kGy irradiated samples showed 13.6 and 12.7 mg/100g TVB-N levels, respectively, after 20 days. These results revels that the combination of gamma irradiation and low-temperature storage significantly reduced TVB-N levels even after extended periods of 90 days at -18°C and 21 days at 4°C.

3.1.3. Peroxide value (PV)

The PV of irradiated fish (Fig. 5) was significantly higher ($p < 0.05$) than the control on the 1st day of storage, indicating the promotion of lipid oxidation due to irradiation. Quattara et al. (2002) reported that gamma irradiation increased lipid oxidation in ground beef samples, consistent with the results of Lambert et al. (1992), who observed rapid fat oxidation in beef irradiated at 0.25–1 kGy under O2-permeable conditions. Lipid oxidation is attributed to the combination of free radicals with oxygen, leading to the formation of hydroperoxides. On the 12th day of storage, PV values increased for all treatments, followed by a decreasing trend. Higher levels of hydroperoxides as primary oxidation products may decompose into secondary oxidation products. “The reduction in primary oxidation products correlates with hydroperoxide degradation, generating secondary lipid peroxidation products” (Boselli et al., 2005). “Similarly, reduced lipid deterioration was reported in black skipjack tuna (*Euthynnus linteus*) stored in ice for 24 days” (Mazorra-Manzano, 2000). Overall, the fish samples (control and irradiated) stored at refrigerated temperatures remained in good condition throughout the storage period, with PV values consistently within the acceptable range of 10-20 meq/kg of oil, as recommended by Connell (1995). Therefore, peroxide value may not be the most reliable indicator of freshness in this study, as values remained within acceptable limits throughout the storage period.

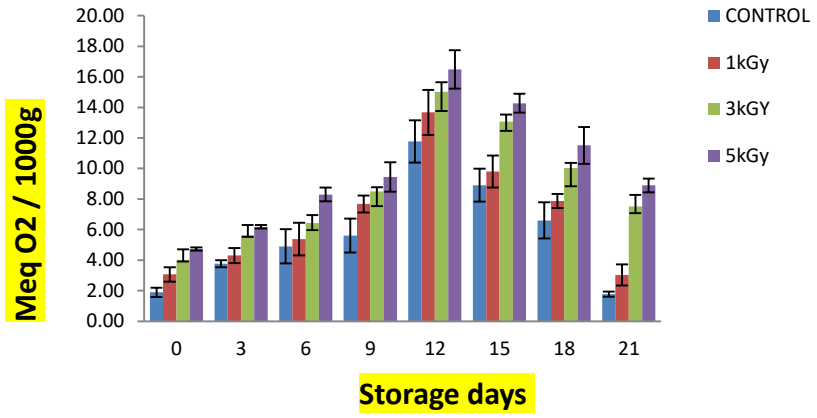

Fig. 5. Changes in peroxide value (PV) of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC)

A significant ($p<0.05$) increase in PV was observed from the initial day of storage to the 75th day for all samples. "Irradiated fish steaks exhibited significantly higher PV values ($p<0.05$) compared to the control throughout the frozen storage period (Fig. 6), indicating that lipid oxidation was initiated by gamma irradiation and that peroxide values increased in direct proportion to the irradiation dose. Similar findings have been reported for ground beef samples" (Quattara et al., 2002). A significant ($p<0.05$) decrease in peroxide value of irradiated steaks was observed after the 75th day of storage. "Throughout the study period, PV values for all samples remained below the acceptance limit of 10-20 meqO2/kg oil" (Connell, 1995). "The decrease in primary oxidation products is associated with the breakdown of hydroperoxides, leading to the formation of secondary lipid peroxidation products" (Boselli et al., 2005).

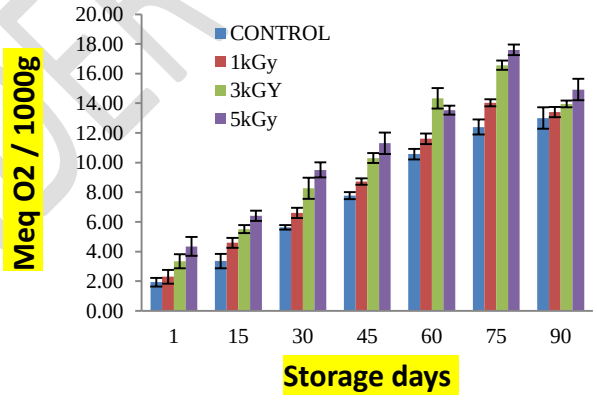

Fig. 6. Changes in peroxide value (PV) of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18ºC)

3.1.4. Thiobarbeturic acid reactive substances (TBARS)

During refrigeration storage, an increase in TBARS values were observed across all samples (Fig. 7). The initial TBARS value for the non-irradiated control sample was 0.083 mg MDA/kg, which rose to 1.17 mg MDA/kg by the 21st day of storage. It was noted that the TBARS values of irradiated samples (1, 3, and 5 kGy) were higher than the control group throughout the 21-day storage period, indicating that gamma irradiation-initiated lipid oxidation. Additionally, the TBA values of the fish increased in indirect proportion to the irradiation dose. Statistical analysis revealed significant differences among the groups (control, 1, 3, and 5 kGy irradiation doses), with p-values <0.05. Furthermore, the increase in TBARS values after 21 days of storage at 4°C was also statistically significant ($p<0.05$). According to Connell (1990), the ideal TBARS value should be less than 3 mg malonaldehyde/kg.

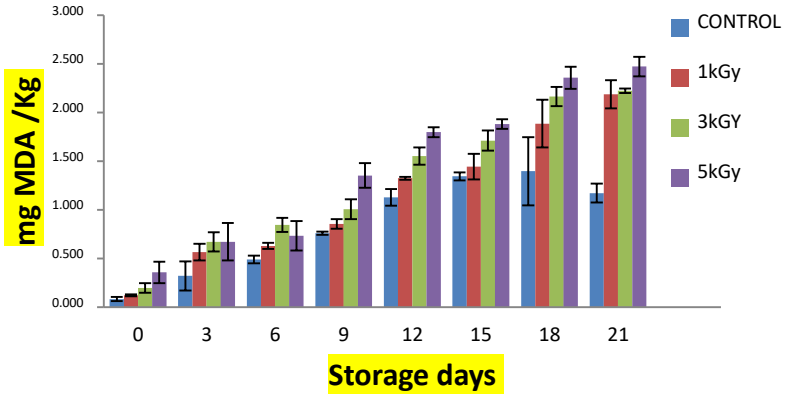

Fig. 7. Changes in TBARS of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC)

The proposed TBA limit of 3 mg malonaldehyde/kg was not exceeded in both irradiated and non-irradiated fish samples after 21 days of refrigerated storage. Fig. 8 shows the degree of lipid oxidation in fish meat during storage at -18°C. After 90 days of storage at -18°C, the fish samples irradiated with a 5 kGy dosage had the highest TBA value of 2.05 mg malonaldehyde/kg. TBA levels in all fish samples held at -18°C were within permissible limits. For both the control and irradiation groups, the development of thiobarbituric acid reactive substances (TBARS) did not show a consistent pattern throughout frozen storage. “These differences can be due to the various stages of peroxide decomposition, production of carbonyl compounds, and interactions with nucleophilic chemicals found in shrimp” (Aubourg et al., 2004). “Likely similar findings have been reported for irradiated seabass, anchovyan threadfin bream” (Lakshmanan et al., 1999; Jeevanandam et al., 2001; Chouliara et al., 2004; Hocaglu et al., 2012). For non-irradiated fish, the TBA value raised to a maximum of 1.13 mg malonaldehyde/kg during storage up to 90 days.

A similar trend was also detected in irradiated fish meat. TBA levels showed significant changes ($p<0.05$) between control and irradiated fish samples throughout storage. This suggests that gamma irradiation enhanced lipid oxidation in fish meat. TBA levels in this investigation stayed well within permissible limits for all treatments during the storage period.

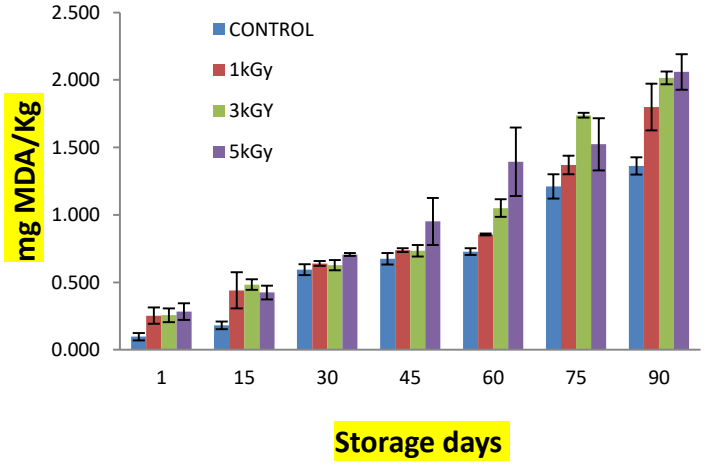

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Fig. 8. Changes in TBARS of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18ºC)

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3.2. Microbiological analysis of fish (*Pangasius hypophthalmus*) stored under refrigeration (4°C) and frozen (-18 °C) storage

The goal of the current study was to monitor a variety of microorganisms, such as Salmonella, Staphylococcus aureus, Escherichia coli, total aerobic bacteria, and total coliforms. Initial counts for arobic bacteria and coliforms in non-irradiated fish were 4.80 log cfu/g and 1.40 log cfu/g, respectively (Day 0). Fig 9 depicts the impact of gamma irradiation and refrigeration on bacteria counts in fish samples. As the irradiation dose increased, the number of aerobic plate counts, coliforms, and E. coli decreased. The number of viable bacteria decreased quickly following irradiation, depending on the dose absorbed. Coliforms and *E. coli* were not detected in fish irradiated at 3 and 5 kGy immediately after irradiation or during storage. Pathogens such as Salmonella and *S. aureus* were absent in all fish samples. Irradiation doses of 3 and 5 kGy resulted in immediate reductions of 2 and 3 log units of aerobic plate counts, respectively. Studies by Venugopal et al. (1999), Molins et al. (2001), and Jo et al. (2004) suggest that irradiation doses ranging from 1 to 5 kGy are effective in extending the shelf life of fresh fish. Additionally, research by Chen et al. (1996) and Mendes et al. (2005) found that mesophilic bacterial counts in irradiated shrimp, crab, and fish were

consistently lower than those in non-irradiated samples during storage at 4°C. In this investigation, gamma irradiation combined cold storage reduced total coliforms and E. coli counts more effectively than either treatment alone.

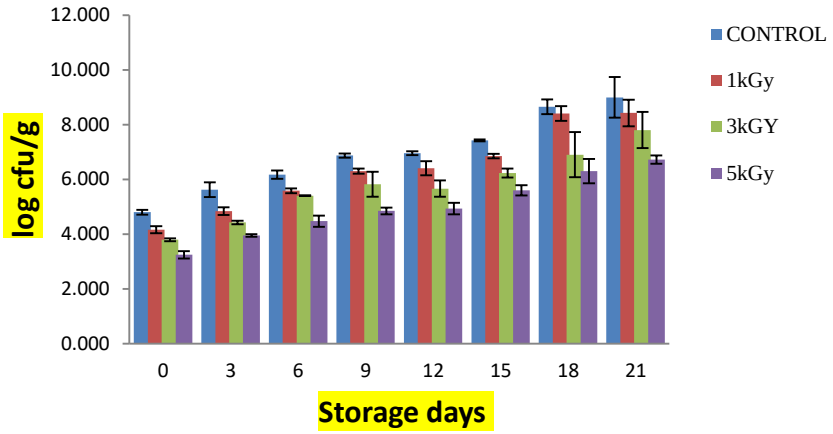

Fig. 9. Changes in total mesophilic count (log cfu/g) of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC). Vertical bars indicate error bars

Total aerobic plate count in fishery products is an important metric for determining shelf life and post-processing contamination. “Psychrotrophic bacteria are the main type of microbe responsible for deterioration in freshly caught seafood” (Huss, 1994). The overall bacterial count shown in **Fig 9 and 10** demonstrates that irradiation influenced bacterial growth at the start of the storage period. The number of viable bacteria reduced soon after irradiation, dependent on the dosage that was absorbed. The control fish sample had an initial bacterial load of 4.63 log cfu/g, whereas the values for fish samples irradiated at 1, 3, and 5 kGy were 3.77, 2.69, and 2.40 log cfu/g. After a 90-day storage period at -18°C (Figure 7), these values increased to 5.30 log cfu/g in the control sample, 4.35 log cfu/g in 1 kGy, 3.82 log cfu/g in 3 kGy, and 3.10 log cfu/g in 5 kGy samples. The International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommends a total microbial count of 5.70 to 6.00 log cfu/g for frozen shellfish.

The findings from this study suggest that irradiated fish samples remain within acceptable microbial limits after 90 days of storage at -18°C. Coliforms and E. coli were found in the control sample at the beginning; however, after irradiation at 3 and 5 kGy, counts were reduced to zero in all samples. Pathogens such as Salmonella and S. aureus were not detected in control or irradiated samples throughout the study period.

Gamma irradiation efficiently suppresses the proliferation of microorganisms in fish and seafood (Radomyski et al., 1994). In a similar study, Cozzo-Siqueira et al. (2003) found no evidence of S. aureus in samples of Tilapia (Oreochromis niloticus) fish that were exposed to different doses (1.0, 2.2, and 5 kGy) and kept for 20–30 days at 0.5°C and -2°C. Dose-dependent declines in viable cells were seen immediately after irradiation. The results showed that irradiation with 3 kGy or higher efficiently ensured the fish's microbiological safety. Irradiation at dosages of 1, 3, and 5 kGy resulted in a significant decrease in microbial load ($p<0.05$) and improved microbial quality. Salmonella and Staphylococcus aureus were not present during storage. These findings align with Hesham's (2012) study on the irradiation of cold-smoked salmon at a dose of 3 kGy.

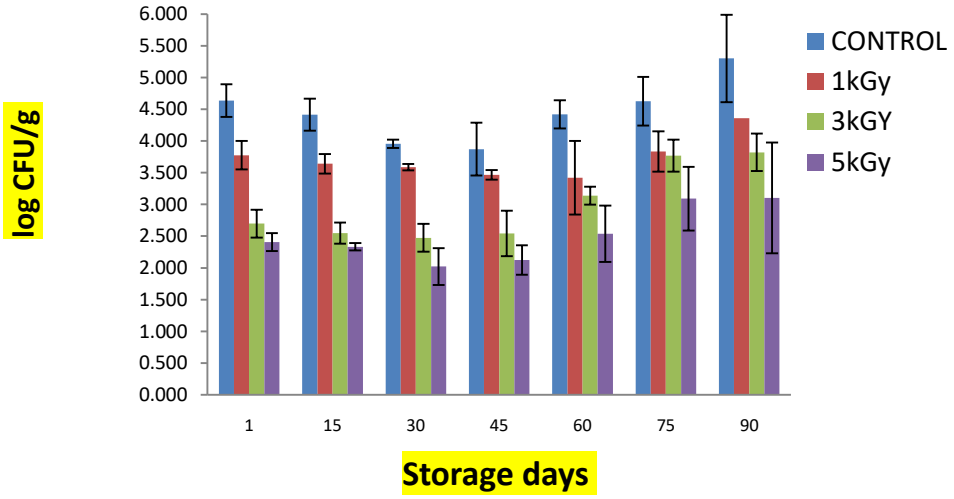

Fig. 10. Changes in total mesophilic count (log cfu/g) of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC). Vertical bars indicate error bars

3.3. Sensory analysis

The initial sensory scores for non-irradiated and irradiated samples were 9.00 (control), 9.00 (1 kGy), 8.75 (3 kGy), and 8.75 (5 kGy) **(Fig 11 and 12**). Irradiation of pangasius steak did not lead to significant changes in most sensory properties, with the exception of odor at a dose of 5 kGy. Hesham (2012) observed that none of the sensory properties showed significant changes due to irradiation of cold-smoked salmon at doses up to 3 kGy. Only a noticeable degradation in the characteristic cherry red color was observed in samples exposed to 4 kGy, although they remained acceptable. Similarly, Venugopal et al. (1991) noted that irradiated samples only exhibited a detectable rancid odor when irradiated at 5 kGy. On the other hand, Alessandra et al. (2008) found no significant sensory changes up to a 5 kGy irradiation dose in tilpia.

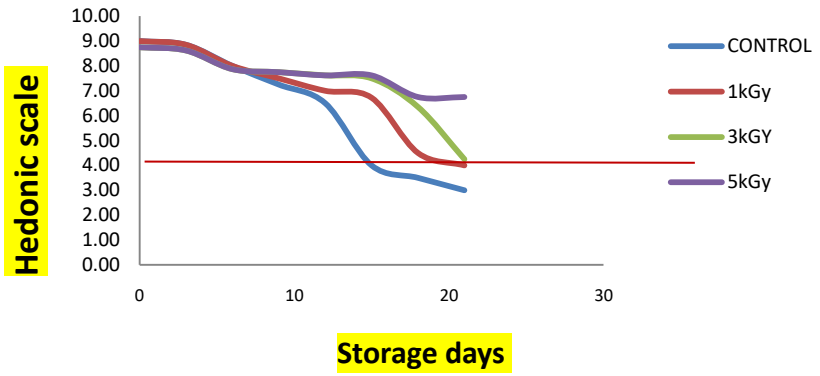

Fig. 11. Changes in sensory attributes of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4ºC)

The rate of sensorial property deterioration during the storage period was higher in unirradiated samples compared to irradiated samples. By the end of the frozen storage study period, the sensory scores were 6.25, 7.00, 7.25, and 7.62 for control, 1kGy, 3kGy, and 5kGy treated samples, respectively. However, panellists were unable to reject the samples until the end of the storage period, and irradiated samples were more acceptable than the control. Similar findings were reported by Nur-A-Sayed et al. (2012) in their study on stinging catfish (*Heteropneustes fossilis*) stored at -20°C for 60 days following irradiation. Javanmard M. et al. (2006) also concluded that irradiation doses of 5 kGy or below and frozen storage conditions did not significantly affect the organoleptic quality of chicken meat.

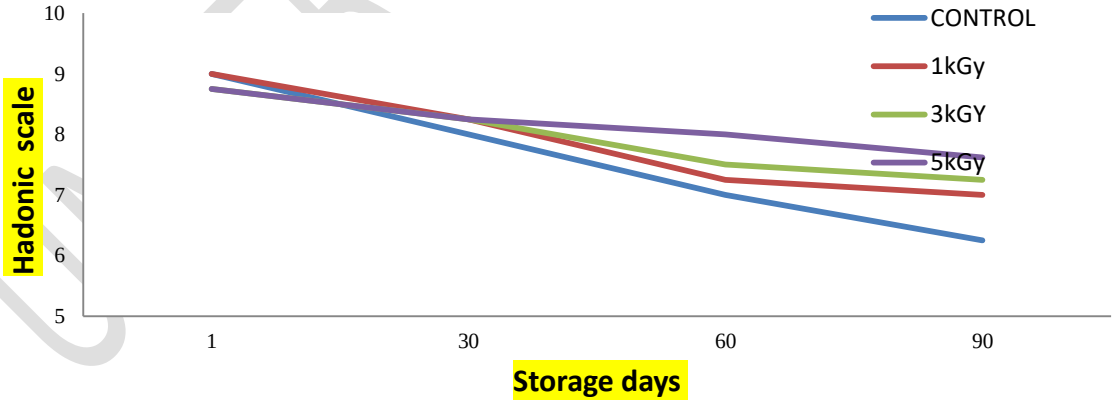

Fig. 12. Changes in sensory attributes of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18ºC)

Conclusion

The results of this study demonstrated that the combination of gamma irradiation and low-temperature storage (both refrigeration and frozen conditions) significantly reduced bacterial growth. Gamma irradiation at doses of 3 and 5 kGy, along with storage at -18°C (frozen) or 4°C (refrigerated), effectively inhibited E. coli completely. Additionally, the study revealed that the radiation doses ranging from 1-5 kGy, in conjunction with frozen storage, extended the fish's shelf life by approximately 90 days.

Chemical quality parameters such as pH, TVB-N, PV, and TBARS were analyzed in both irradiated and non-irradiated fish samples. Irradiated fish exhibited significantly lower levels of TVB-N and TBARS compared to control samples during both refrigerated and frozen storage, attributed to the reduction in microbial populations. These quality parameters remained within acceptable limits throughout both storage conditions in both irradiated and control samples. However, gamma irradiation at higher doses (5 kGy) was found to slightly enhance lipid oxidation, though microbial growth was effectively suppressed.

In conclusion, the combination of gamma irradiation and low-temperature storage demonstrated a substantial reduction in bacterial growth and maintained the biochemical quality of fish. An irradiation dose of 5 kGy, coupled with subsequent storage in ice, proved to be the most effective. The combined use of low-dose gamma irradiation and subsequent icing and frozen storage efficiently eliminates pathogens such as Salmonella, Vibrios, Listeria, and E. coli without compromising product quality. This method reduces spoilage microorganisms, enabling longer storage durations and ensuring compliance with international safety standards for fish trade and post-harvest management. For consumers, this approach ensures pathogen-free seafood, minimizes the risk of foodborne illnesses, retains nutrients, and boosts confidence in food safety.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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