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

Abstract

Irradiation is considered one of the most efficient novel methods for reducing microorganisms in food. It can be used to enhance the safety and quality of aquatic food products and extend their shelf life, thereby reducing post-harvest losses. This investigation aims to study the combined effect of gamma irradiation and low-temperature storage on the quality and shelf-life extension of fresh Sutchi Catfish (*Pangasius hypophthalmus*). The samples were exposed to varying doses of gamma irradiation (0.0 kGy [non-irradiated], 1.0 kGy, 3.0 kGy, and 5.0 kGy) and stored under refrigeration (4°C) and frozen storage (-18°C) conditions. The control (non-irradiated) and irradiated fish samples underwent periodic microbial analysis such as total mesophilic counts, total coliforms, faecal coliforms, *Salmonella*, *E. coli*, and *Staphylococcus aureus*. Biochemical characteristics, such as total volatile base nitrogen, pH, peroxide value, and thiobarbituric acid reactive substances, were also evaluated at different intervals. The microbial analysis indicated that irradiation and frozen storage had a significant effect ($p < 0.05$) on reducing microbial loads, with the reduction being more pronounced at higher irradiation doses ($p < 0.05$). The chemical parameters of the irradiated fish samples were significantly lower than those of the non-irradiated samples at both storage conditions. The results demonstrated that the combination of irradiation and refrigeration or frozen storage effectively reduced microbial loads and stabilized the biochemical characteristics of fish meat for up to 21 days and 90 days during refrigeration and frozen storage respectively.

UNDER PEER REVIEW

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

I. Introduction

The freshness of fish is the most important and fundamental criterion for judging the quality of the product. Fish is more vulnerable to deterioration than red meat due to its relatively high content of free amino acids, higher water activity, and pH, which limit the shelf life of the final product. In India, inland aquaculture is predominantly focused on the farming of major carps (Catla, Rohu, and Mrigal) and exotic carps (Common carp, Silver carp, and Grass carp). Recently, catfish farming has gained importance among Indian farmers as an alternative to major carps. The primary catfish species currently adopted in India is the Sutchi catfish (*Pangasius hypophthalmus*), which is native to Thailand and Vietnam. Catfish production in India reached 1.29 million tonnes in 2020–22 (DAHDF, 2022). Pangasius farming in India, particularly in the state of Andhra Pradesh, represents the fastest growth of single-species farming recorded in the aquaculture sector in the country.

Catfish is in high demand due to the absence of intramuscular bones, tender flesh, sweet taste, and lack of fishy odor (Viji et al., 2014). These qualities, along with its high nutritional value and excellent sensory properties, have made it a preferred choice among consumers. However, fish and shellfish are known carriers of several specific spoilage organisms and pathogenic microorganisms that can cause foodborne illnesses (Hocaoglu et al., 2012).

In recent years, reliable methods of fish preservation have been developed to extend the shelf life of fish and shellfish and reduce health risks to consumers. Irradiation has emerged as an effective and widely applicable food preservation technique. This process exposes food to a carefully controlled amount of energy in the form of electromagnetic waves, reducing the risk of food poisoning, preventing spoilage, and extending shelf life. Irradiation has minimal effects on the nutritional and sensory qualities of food, such as taste, color, and smell, and it does not leave radioactive residues (ICGFI, 2002).

The combination of preservation techniques may result in synergistic effects that enhance microbiological barriers (Leistner and Gorris, 1995). For instance, food irradiation, when combined with good refrigeration practices, can significantly extend the shelf life of fish and shellfish. Gamma irradiation at low doses (below 10 kGy) effectively kills most microorganisms without compromising food quality (Javanmard et al., 2006; Hocaoglu et al., 2012). This process is known to virtually eliminate all foodborne pathogens. Globally, more than 26 countries use irradiation on a commercial scale for food preservation (Ouattara, 2001). The present study aims to evaluate the combined effect of low dose gamma irradiation and low-temperature storage on the shelf life of fresh catfish and its potential for enhancing shelf life.

UNDER PEER REVIEW

2. Material and Methods

2.1. Materials

Fresh farm raised sutchi cat Fish (*Pangasius hypophthalmus*) used in the study were procured from Fish farms located at Northern Karnataka state, India and immediately packed in polyethylene bags with ice and brought to the laboratory (within 6 hr) in an aseptic condition and stored at -18ºC until irradiation.

2.2. Preparation of sample

The fresh fish was washed and degutted. The dressed samples were thoroughly washed and cut into steaks of 2-3 cm thickness weighing 100-200 gm each and packed in polyethylene bags. The samples were divided into two groups of four levels of exposure to gamma irradiation. One group was exposed to doses of 0.0 (control) 1.0, 3.0, and 5.0 kGy and subsequently stored at 4^{o}C, while the second group was exposed to the same doses of radiation and stored at-18 ^{o}C.

2.3. Irradiation

The samples were irradiated at the Centre for Application of Radioisotope and Radiation Technology (CARRT), Mangalore University using ^{60}cobalt radiation source (BRIT, Mumbai). The doses applied in the study were 1.0, 3.0 and 5.0 kGy with the exposure time of 6.38, 32 and 45 minutes respectively (dose rate of 6.94 kGy/hr). The samples were maintained 4$\pm$ 1^{o}C using sealed ice covering during irradiation. The absorbed dose was monitored by Freaky dosimeter. The samples After irradiation both the irradiated and non-irradiated (control) samples were transported within 1 hr to the laboratory with packed ice in isolated polystyrene ice boxes and held for refrigeration (4^{o}C) and frozen temperatures (-18^{o}C) until the last day of experiment.

2.4. Chemical Analysis

The proximate analyses of fresh fish were carried out. The moisture content was determined by hot air oven method (AOAC, 2010). The crude protein content of the fish meat was determined by estimating its total nitrogen content by Kjeldahl method (AOAC 2010). Crude lipid content of the fish meat was determined by Soxhlet extraction method (AOAC, 2010). Ash content of the meat was determined by the method described in AOAC, (2010). Total volatile base nitrogen (TVBN) content of fish meat was determined by Conway's micro-diffusion technique (Beatty and Gibbon 1937) and expressed as mg N/100 g meat. Thiobarbituric acid reactive substances (TBARS) were determined using method described by Raghavan and Hultin (2005) to determine the degree of oxidation in fish meat. Lipid oxidation was measured using thiobarbituric acid (TBA values), which are expressed as mg malonaldehyde/kg meat. pH was determined at room temperature on homogenates of samples in distilled water (1/10 w/w) (Vyncke, 1981). pH was monitored using pH meter (Systronix 361, India).

UNDER PEER REVIEW

2.5. Microbial analysis

Aerobic mesophilic counts and pathogens were analyzed throughout the experiment for a period of 90 days. Twenty five grams of sample were taken aseptically into a sterile blender containing 225 ml of sterilized physiological saline (0.85% NaCl) and blended for 3 min at low speed. 0.1 ml of decimal dilutions were placed on to plate count agar and incubated at 35^{o}C for 24-48hrs. Total plate counts were transformed into logarithms of the number of colony forming units per gram of sample (CFU/g). For the enumeration of total coliforms three tube MPN method was followed and appropriate dilutions were inoculated into LSTB, EC broth (Himedia, Mumbai) and incubated at 37^{o}C and 44.5^{o}C respectively. Pathogens such as *Escherichia coli* (EMB agar); *Staphylococcus aureus (*Baird parker agar) and *Salmonella* (BSA agar) (APHA, 1998 and ICMSF, 1986). Microbial counts were expressed as the number of viable bacterial colonies per gram (log CFU/g).

2.6. Sensory analysis

The sensorial attributes of irradiated and control samples were monitored with 9-point hedonic scale prescribed by Meilgaard *et al*. (1999). The overall impression of the product was estimated by overall acceptability, by adding score of all the attributes. A high score (9-7) was given to fish with no off odours and score below 6 corresponded to unacceptable quality.

2.7. Statistical analysis

The data obtained from microbiological, biochemical and sensory analysis were further analyzed by using Statistical Package for Social Sciences (SPSS, version 23.00 for Windows). Analysis of variance (one way - ANOVA) was performed to determine the differences between storage days. The tests for differences were done by using Duncan's Multiple Comparison Test. Significance of differences was defined at $p < 0.05$.

UNDER PEER REVIEW

3. Results and Discussion

The Fish used in this study was an average length of 46.83 (±4.19) cm, and average weight of 1.31 kg (± 0.36). The moisture, protein, crude lipid and ash contents of fish meat was 77.80 %, 16.50 %, 4.50 % and 0.97 % respectively. These values are comparable to those reported in other study (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 pH value was 6.35 for the non-irradiated (control) samples, while the values were 6.35, 6.31, and 6.30 for the fish meat irradiated at 1, 3, and 5 kGy, respectively **(Fig.1).** An increase in the applied irradiation dose corresponded to a decrease in the pH value. During refrigerated storage, the pH values decreased across all fish samples. The pH values for both non-irradiated and irradiated fish samples ranged from 6.35 to 6.56. By the end of the 21-day storage period, no definite trend was observed in the pH values of the fish stored under refrigeration. However, the non-irradiated samples exhibited higher pH values than the irradiated ones, likely due to the accumulation of nitrogenous compounds resulting from chemical and biological decomposition. Despite these differences, there was no statistically significant difference in pH ($p < 0.05$) between the treatments. A similar trend was reported for farmed sea bass and turbot stored on ice (Papadopoulos et al., 2004; Rodriguez et al., 2006).

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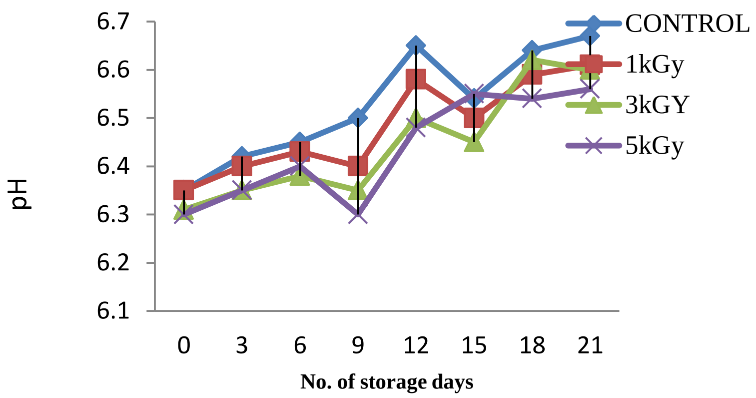

Fig. 1. Changes in pH of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4°C)

For fish samples stored at -18°C, the pH values of both the control and irradiated fish meat decreased significantly between days 0 and 1, with the irradiated samples showing a greater decrease than the controls **(Fig. 2).** By the end of the 90-day storage period, a statistically significant difference was observed between the control and irradiated samples (1, 3, and 5 kGy). The pH value of the control sample was 6.40 at the beginning and increased to 6.82 at the end of the 90-day storage period. No consistent trend was observed in the pH values of the fish steaks during frozen storage. However, the pH values of irradiated samples were consistently lower than those of the controls, likely due to the accumulation of nitrogenous compounds in the control samples caused by chemical and biological decomposition. The results of this study indicate that pH is a poor-quality indicator of catfish freshness under frozen storage conditions.

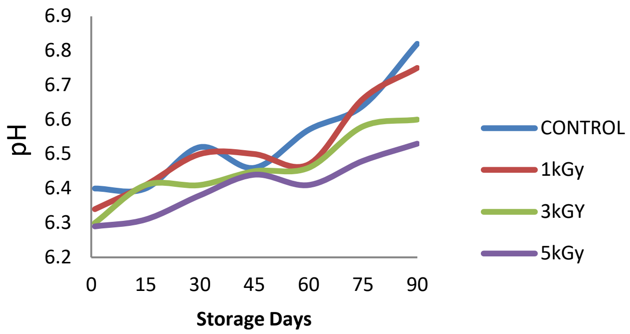

Fig. 2. Changes in pH of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18^{o}C)

3.1.2. Total volatile base nitrogen (TVB-N)

During the period of refrigerated storage (4^{o}C), the TVB-N value of fish meat was significantly ($p<0.05$) higher in the non- irradiated control samples than in the irradiated samples as shown in **Fig. 3.** The initial TVB-N value in control fish was 6.87mg N/100g the value increased to 12.13 mg N/100g at the end of 21 days of storage period. On the other hand irradiation at 1,3 and 5 kGy suppressed the formation of TVB-N during storage and value reached 9.80, 7.47 and 6.53 mg N/100g respectively, after 21 days.The irradiated samples of fish had significantly much lower concentrations of TVBN during their refrigerated storage as compared with the controls, which may be attributed to the reduction of microbial populations (Venugopal *et al*., 1999). In the present study, TVB-N value didn't cross the acceptable limit of 35 mg N/ 100 g of fish during the entire storage period (21 days). The results are quite comparable to the study carried out by Castro *et al.*, (2006) even after 20-22 days of storage European sea bass in ice storage. The lower TVB-N content in the cat fish steaks may be due to the absence of trimethyl amine which is the major component of volatile bases in fish meat, virtually all changes in TVB-N are due to TMA component, which is a major constituent of volatile bases.

UNDER PEER REVIEW

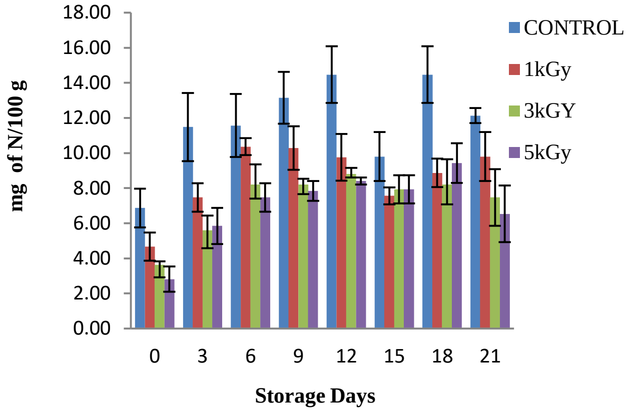

Fig.3. Changes in TVBN of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4°C). Vertical bars indicate error bars

At the beginning of frozen storage (-18°C), the TVB-N value was 6.87 mg N/100g for the control sample and 6.40, 6.41 and 5.80 mg N/100g respectively for the samples irradiated at 1,3 and 5 kGy **(Fig 4).** However, the TVB-N values showed a different trend within the progression of the storage period and increased at end of storage for 90 days at -18°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°C.

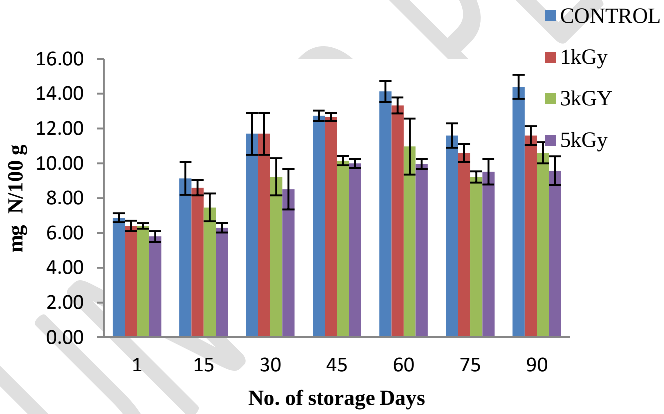

Fig.4. Changes in TVBN of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18°C). Vertical bars indicate error bars

The statistical analysis of the TVB-N data showed that significant difference was found between the control and irradiated fish samples stored at -18°C after 90 days of storage ($p<0.05$). TVB-N levels for non- irradiated and irradiated fish samples did not exceed 35mg/100g at both storage temperatures which is considered to be the maximum acceptable level for fish (Huss, 1988). This result is in agreement with TVB-N levels of irradiated chines

UNDER PEER REVIEW

pomfret (*Pampus chinensis*) stored in ice as reported by Ahmed *et al*., (2009). However, Cakli *et al*., (2006a; 2006b) reported TVB-N levels of 39.89 and 35 mg/100g respectively, for seabass after 18 day and 14 days of storage on ice. Chouliara *et al*., (2005) reported that the initial TVB-N levels of vacuum packed irradiated (1-3 kGy) sea bream samples stored under refrigeration were 27.5, 27.3 and 25.1 mg/100g reaching the acceptable limits at day 10 in control samples, and at day 21 and 28 for 1 and 3 kGy irradiated samples. Similarly, lower TVB-N levels in the irradiated samples compared to control samples were reported by Jo *et al*., (2004). Mendes *et al*., (2005) reported that the initial TVB-N level of 15.6 mg/100g in chilled horse mackerel reached the limit levels of 30-35 mg/100g at day 12 in the control non –irradiated samples where as the 1 and 3 kGy irradiated samples TVB-N levels of 13.6 and 12.7 mg/100g respectively at day 20. Form this results it can be said that the combination of gamma irradiation and low temperature storage resulted in very low levels TVB-N even after considerable period of 90 days storage at -18^oC and after 21days of refrigerated storage at 4^oC.

3.1.3. Peroxide value (PV)

Peroxide value of irradiated fish were **(Fig 5)** significantly higher ($p < 0.05$) than the control on the 1st day of storage indicating that the onset of lipid oxidation is getting promoted by the irradiation. Quattara *et al.* (2002) reported that gamma irradiation increased lipid oxidation in ground beef samples. That is consistent with the report of Lambert *et al.* (1992) who found a rapid fat oxidation in beef samples irradiated at 0.25–1 kGy under O_2-permeable conditions. The lipid oxidation was attributed to the combination of free radicals with O_2 to form hydro peroxides. On the 12th day of storage period again the PV values were increased for all the treatments thereafter showing a decreasing trend. Hydro peroxide formed as a primary oxidation products at higher levels might undergo the decomposition into secondary oxidation products. A decrease in the level of primary oxidation products is related to hydroperoxide degradation, producing secondary lipid peroxidation products (Boselli *et al.*, 2005). Reduced lipid deterioration was also reported in black skipjack tuna (*Euthynnus linaetus*) stored in ice for 24 days (Mazorra-Manzano, 2000). Results revealed that the fish sample (control and irradiated) stored at refrigerated temperature were in good condition throughout the storage period based on values of 10-20 meq/kg of oil as recommended by Connell (1995). Peroxide value might not be considered a good indicator of freshness in this study as values were within the range of acceptability throughout the storage period.

UNDER PEER REVIEW

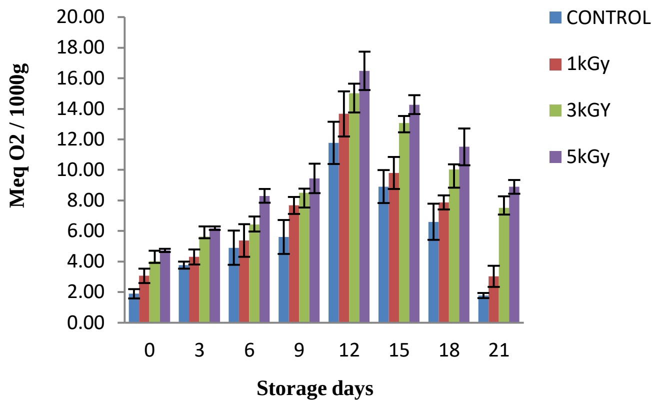

Fig. 5. Changes in peroxide value (PV) of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4^{o}C)

Significant (p< 0.05) increase in PV values occurred from the initial day of storage period to the 75 days of storage for all the samples. PV of irradiated steaks were significantly higher (p< 0.05) than the control sample throughout the frozen storage period **(Fig 6)** suggesting that lipid oxidation was initiated by gamma irradiation and that the peroxide value of the steaks increased in direct proportion to the irradiation dose. Similar results have been reported for ground beef samples (Quattara *et al*., 2002). Significant (p< 0.05) decrease in peroxide value of irradiated steaks was observed after 75th day of frozen storage period. The PV values for all the samples never crossed the acceptance limit of 10-20 meqO_2/kg oil (Connell, 1995) throughout the period of study. A decrease in the level of primary oxidation products is related to hydroperoxide degradation, producing secondary lipid peroxidation products (Boselli *et al*., 2005).

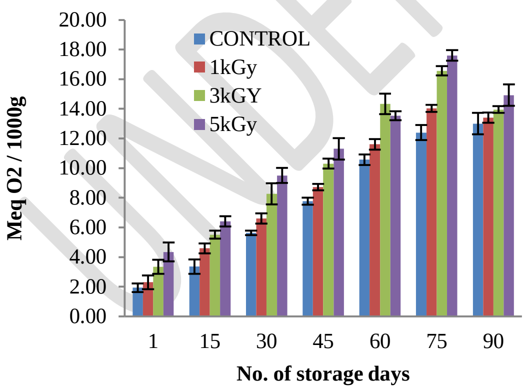

Fig. 6. Changes in peroxide value (PV) of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18^{o}C)

UNDER PEER REVIEW

3.1.4. Thiobarbeturic acid reactive substances (TBARS)

An increase in the TBARS value was noticed in all the samples during refrigeration storage **(Fig 7).** The initial TBARS value of the non- irradiated control sample was 0.083 mg MDA/ Kg with the value increasing to 1.17 mg MDA/ Kg on the 21 days of storage. It was observed that the TBARS values of the irradiated samples (1, 3 and 5 kGy) were higher than the control group during 21 days of storage periods suggest that lipid oxidation was initiated by gamma irradiation and that the TBA values of the fish increased indirect proportion to the irradiation dose. According to the statistical analysis it was found that the difference among the groups (control, 1,3 and 5 kGy irradiation dose) was statistically significant ($p<0.05$). In addition the increase in TBA values at the end of 21 days storage at 4^{o}C was also found to be statistically significant ($p<0.05$). According to Connell (1990) the ideal TBA value should be less than 3 mg malonaldehyde/kg.

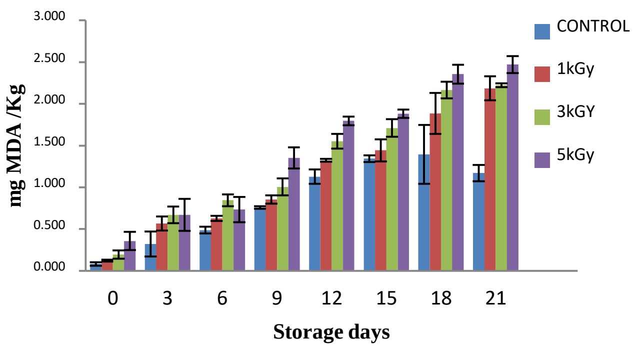

Fig. 7. Changes in TBARS of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4^{o}C)

The proposed TBA limit of 3 mg malonaldehyde/kg were not exceeded at the 21 days of refrigerated storage for both irradiated and non- irradiated fish samples. The extent of lipid oxidation in the fish meat during storage at -18^{o}C is shown in the **Fig. 8**. The highest TBA value was 2.05 mg malonaldehyde/kg after 90 days of storage at -18^{o}C for fish sample irradiated at 5 kGy dose. TBA values for all the fish samples stored at -18^{o}C were within acceptable limits. The formation of thiobarbituric acid reactive substances (TBARS) didnot show consistant trend during frozen storage for both control and irradiated groups. The variations can be explained as a result of the different phases of decomposition of peroxides, formation of carbonyls and the interaction compounds with nucleophilic molecules present in the shrimp (Aubourg et al, 2004). Similar results have been reported for irradiated seabass and anchovyan threadfin bream (Lakshmanan et al., 1999; Jeevanandam et al.,2001; Chouliara et al.,2004, Hocaglu et al., 2012). In the case of non – irradiated fish, the TBA value increase to maximum of 1.13 mg malonaldehyde/kg during storage up to the 90 days. A similar trend was observed in the irradiated fish meat as well. Significant differences ($p<0.05$) in the

TBA values were found between the control and each irradiated fish sample during the storage period. This indicates that lipid oxidation in the fish meat increased due to gamma irradiation. In the present study the TBA values were well within the acceptable limit throughout the storage period for all the treatments.

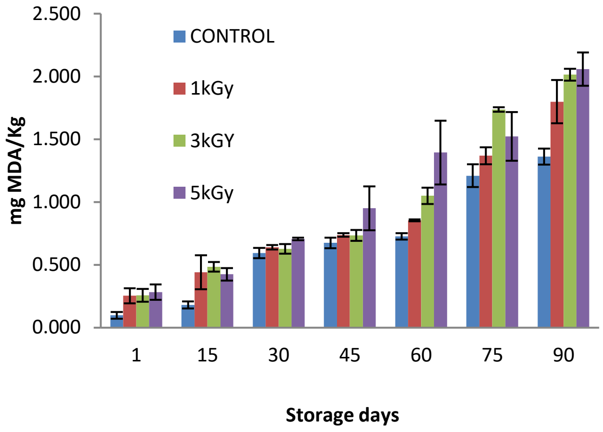

Fig. 8. Changes in TBARS of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18°C)

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

The present study focused on the monitoring of the following microorganisms; mesophilic aerobic bacteria, total coliforms, *Escherichia coli, Staphylococcus aureus*, *Salmonella* (The initial counts of mesophilic bacteria and cloiforms in non- irradiated fish were 4.80 log cfu/g and 1.40 log cfu/g respectively (Day 0). The effect of gamma irradiation and refrigerated storage on microbial counts in the fish samples are presented in **Fig 9**. The number of aerobic plate counts, coiliforms, *E coli* decreased with increase of irradiation dose. The level of viable microorganisms decreased immediately after irradiation, depending on the absorbed dose. Coliforms, *E coli* were not observed in 3 and 5 kGy irradiated fish immediate after irradiation as well as during storage. Pathogens such as *Salmonella, S. aureus* were not detected in any of the fish samples. Irradiation doses of 3 and 5 kGy produced immediate reduction of 2 and 3 log units of aerobic plate counts respectively in fish. In fish irradiation doses ranging from 1to 5 kGy have been suggested for the shelf life extension of fresh fish (Venugopal et al., 1999; Molins et al., 2001 ; Jo et al., 2004). Chen et al., (1996), Mendes et al., (2005) reported that mesophilic bacterial counts of irradiated shrimp, crab and fish were lower than those in non- irradiated samples during storage at 4°C. In this study gamma irradiation and frozen storage were more effective than either treatment alone in decreasing total coliforms and E coli counts. Irradiation reduced the bacterial population in a dose dependent manner.

UNDER PEER REVIEW

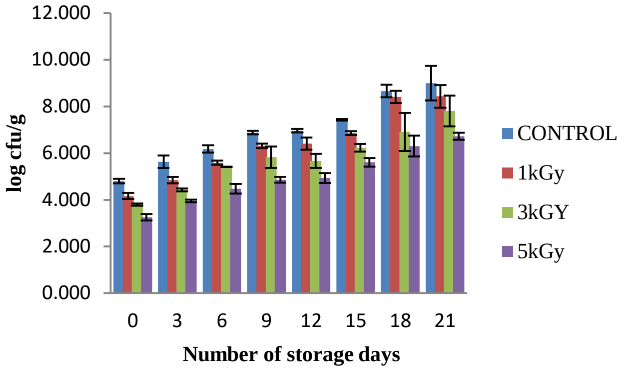


Fig. 9. Changes in total mesophilic count (log cfu/g) of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4°C). Vertical bars indicate error bars

Total aerobic plate count (APC) in fishery products is a useful tool for quality evaluation of shelf life and post processing contamination. Psychrotropic bacteria are especially major group of microorganisms responsible for spoilage of fresh seafood (Huss, 1994). The total bacterial count presented in **Fig 9 & 10.** shows that the beginning of the storage period, bacterial growth was affected by the irradiation. The level of viable microorganisms decreased immediately after irradiation depending upon the absorbed dose. The initial bacterial load of the control fish sample was 4.63 log cfu/g whereas the values for 1, 3 and 5 kGy irradiated fish were 3.77, 2.69 and 2.40 log cfu/g respectively. After storage period of 90 days this value increased 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 fish samples stored at – 18°C (Fig 7). The total microbial count limit recommended by the international commission of microbiological specification for foods bulletin (ICMSF, 1986) is 5.70- 6.00 log cfu/g for frozen shell fishes.

Hence the TBC values reported in the present investigation suggest that the irradiated fish samples remain within acceptable limits after storage for 90 days at -18°C. The coliforms and *E. coli* were detected in the control sample in the beginning, however after irradiation at 3 and 5 kGy the counts were reduced to zero in all the samples. Pathogens such as *Salmonella*, *S. aureus* were not detected in control as well as irradiated samples throughout the study period.

Gamma irradiation was found to inhibit microbial proliferation in fish and seafood **(**Radomyski et al.,1994). Similar results were reported by Cozzo-Siqueira et al., (2003) did not find *S. aureus* in the irradiated and non – irradiated samples of the Tilapia (Oreochromis niloticus) fish which were irradiated at different doses (1.0, 2.2 and 5 kGy) and stored for 20-30 days at 0.5°C and -2°C. In the irradiated samples there was dose dependent reduction in the viable cells immediately after irradiation. The results indicated that irradiation at 3 kGy or above was effective in securing

UNDER PEER REVIEW

the microbial safety of the fish. Generally, just after irradiation at doses of 1,3 and 5 kGy, the microbial load was significantly reduced ($p<0.05$) and irradiated samples showed a good microbial quality and *Salmonella* and *S. aureus* were absent during storage period. The results of the sensory attributes of fish in an agreement with study reported by Hesham, (2012) on irradiation of cold smoked salmon at the dose of 3 k Gy.

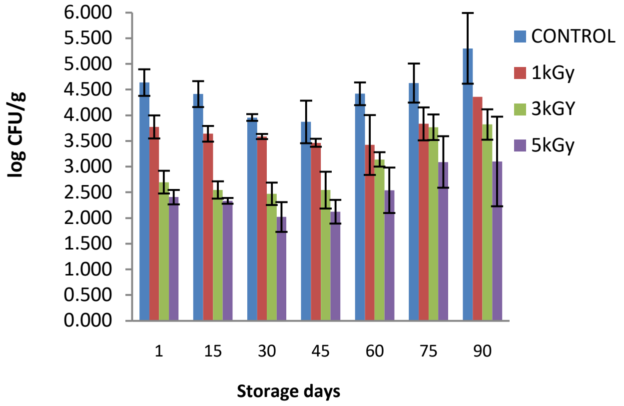
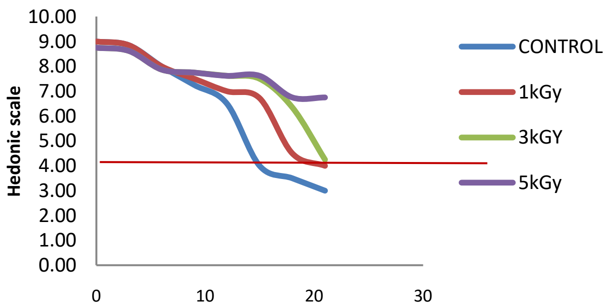


Fig. 10. Changes in total mesophilic count (log cfu/g) of irradiated and nonirradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (4^{o}C). Vertical bars indicate error bars

3.3. Sensory analysis

The initial sensory score of 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**). None of the evaluated initial sensory properties showed a significant change due to irradiation of pangasius steak except odour at doses 5 kGy. Hesham, (2012) observed that None of the sensory properties showed a significant change due to irradiation of cold-smoked salmon at doses up to 3 kGy. Only significant degradation of the normal cherry red color was observed in samples exposed to 4 kGy dose, but samples were still acceptable. Venugopal *et al.*, (1991) also stated that irradiated rancid smell could be detected only when irradiated at 5kGy however Alessandra *et al.* (2008) found no irradiation induced sensory changes up to 5kGy irradiation dose in tilapia.



Number of Storage days

UNDER PEER REVIEW

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

Rate of decrease in sensory properties during storage period was higher in unirradiated sample then irradiated samples. Finally, the sensory score reached to 6.25, 7.00, 7.25 and 7.62 for control, 1kGy, 3kGy and 5kGy treated samples respectively on the last day of frozen storage study period. However, the panellist were unable to reject the samples till the end of storage period, irradiated samples were more acceptable then control. Similar results obtained by Nur-A-Sayed *et al*. (2012) when stinging catfish (*Heteropneustes fossilis*) stored at -20°C after irradiation for 60 days. Javanmard M. *et al*. (2006) also found that irradiation of 5 kGy or below and storage in frozen condition did not affect the organoleptic quality of the chicken meat.

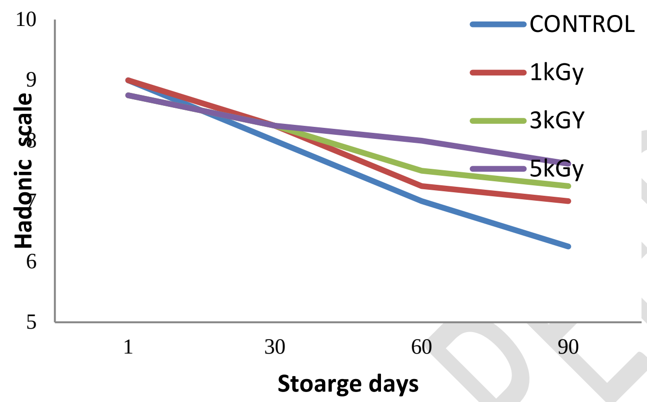


Fig. 12. Changes in sensory attributes of irradiated and non-irradiated fish (*Pangasius hypopthalmus*) sample during refrigerated storage (-18°C)

Conclusion

The results obtained from this study showed that the combination of irradiation and low temperature (Refrigeration and frozen) storage resulted in a significant reduction of bacterial growth and irradiation at 3 and 5 kGy dose with frozen (-18°C) or refrigerated (4°C) storage could inhibit *E. coli* completely. Results showed that the employed radiation dose (1-5 kGy) in conjunction with frozen storage extended the shelf life of fish about 90 days. As chemical quality parameters, levels of pH, TVB-N, PV and TBARS in irradiated and non irradiated fish samples were also examined. Irradiated fish had significantly lower concentrations of TVB-N and TBARS during refrigerated and frozen storage as compared with the controls which may attributed to the reduction of microbial populations. These parameters were within an acceptable limit until the end of both refrigeration and frozen storage in irradiated and control samples. The results revealed that gamma irradiation at high dose (5 kGy) might enhance lipid oxidation,

UNDER PEER REVIEW

although the growth of microorganisms was inhibited. In conclusion the results of the study demonstrated that the combination of gamma irradiation and low temperature storage resulted in a significant reduction of bacterial growth and stabilized the bio chemical attributes of fish.

Disclaimer

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

Ethical approval

Not applicable

UNDER PEER REVIEW

References

Ahmed MK, Hasan M, Alam ML, Ahsan Islam MM, Akter MS, 2009. Effect of gamma radiation in combination with low temperature refrigeration on chemical, microbiological and organoleptic changes in *Pampus chinensis*. *World Journal of Zoology*.,**4**(1): 9-13. https://www.researchgate.net/publication/315739575

AOAC, 2010. Official method of analysis of AOAC International, Vol II, 18th edn. Association of Official and Analytical Chemists International, Virginia, p 39.

APHA, 2000. Compendium of Methods for the Microbiological Examination of Food (3rd Ed). Washington: American Public Health Association.

Aubourg SP, Pérez-alonso F and Gallardo JM, 2004. Studies on rancidity inhibition in frozen horse mackerel (*Trachurus trachurus*) by citric and ascorbic acids. *European Journal of Lipid Science and Technology*, **106**(4): 232-240. http://dx.doi.org/10.1002/ejlt.200400937

Beatty SA, and Gibbons NE, 1936. The Measurement of Fish Spoilage in Fish. *J. Biol. Bd. Can*., **3**: 77-91.

Benjakul S, Visessanguan W, Thongkaew C, and Tanaka M, 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Res. Intl*., **36:** 787-795. http://dx.doi.org/10.1016/S0963-9969(03)00073-5

Boselli E, Caboni MF, Redriguez-estrada MT, Toschi TG, Daniel M and Lercker G, 2005. Photoxidation of cholesterol and lipids of turkey meat during storage under commercial retail conditions. *Food Chem*., **91**: 705–713. http://dx.doi.org/10.1016/j.foodchem.2004.06.043

Castro P, Penedo padron JC, cansino MJ, Sanjuan Velazquez E, and Millán De larrivar, 2006. Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. *Food Control*, **17**: 245–248. http://dx.doi.org/10.1016/j.foodcont.2004.10.015

Chouliara I, Savvaidis LN, Riganakos K, Kontaminas MG, 2005. Shelf-life extension of vacuum-packaged sea bream (*Sparus aurata*) fillets by combined γ-irradiation and refrigeration: microbiological, chemical and sensory changes. *J.Sci. Food Agric.*, **85**: 779-784. http://dx.doi.org/10.1002/jsfa.2021

Connell JJ, (1990). Methods of assessing and selecting for quality. In: CONNELL JJ, (Ed), Control of fish quality. *Fishing News Books*. Oxford, UK, pp. 122-150.

Connell JJ, (1995). Control of fish quality, Fishing News (Books). Blackwell Science Ltd., pp 256.

Cozzo-siqueira A, Oetterer M, and Gallo CR, (2003). Effects of irradiation and refrigeration on the nutrients and shelf-life of tilapia (*Oreochromis niloticus*). *Journal of Aquatic Food Product Technology*, **12**(1): 85 – 101. http://dx.doi.org/10.1300/J030v12n01_06

Hesham MB, (2012). Control of the potential health hazards of smoked fish by gamma irradiation. *International Journal of Food Microbiology*. **154:**177–186. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.037

Hocaoglu AD, Gumus T, and Demirci M, (2012). Effects of gamma irradiation on chemical, microbial quality and shelf

life of shrimp. *Radiation Physics and Chemistry*., **81**:1923-1929. http://dx.doi.org/10.1016/j.radphyschem.2012.07.017

Huss HH, (1995). Quality and quality changes in fresh fish. *In*: Fisheries Technical Paper No 348. Food and Agricultural Organization of the United Nations, Rome, Italy, pp. 195-202

ICGFI, 2002. International consultative group on food irradiation. *Food irradiation: A Global Food Safety Tool. Official publication.IAEA, Vienna, Austria.*

UNDER PEER REVIEW

Joclee, NY, Hon SP, Kim YH, Byun MW, (2004). Microbial contamination of the food materials for manufacturing Korean laver rolls (Kimbab) and the effect of gamma irradiation. *J. Food Sci. Nutr*.,**9:** 236-239. http://dx.doi.org/10.3746/jfn.2004.9.3.236

Lambert AD, Smith JP, and Doods KL, (1992). Physical, chemical and sensory changes in fresh pork packaged in modified atmosphere. *J. of Food Sci*., **57**:1294–1299. https://doi.org/10.1111/j.1365-2621.1992.tb06840.x

Leistner L, and Gorris LGM, (1995). Food preservation by hurdle technology. *Trends in Food Science and Technology*., **6**: 41-46.

Meilgaard M, Civille GV and Caar BT, (1999). Quality evaluation of seafood products. *In* : Sensory evaluation techniques *Edt*. CARR, B.T. *Edn.* 8th, CRC Press, *pp* 387-397.

Mendes R, Silva HA, Nunes ML, Empis JMA, (2005). Effect of low-dose irradiation and refrigeration on the microflora, sensory characteristic and biogenic amines of Atlantic horse mackerel (*Trachurus trachurus*). *Eur. Food Res. Technol*, **221**: 329-335. http://dx.doi.org/10.1007/s00217-005-1172-x

Molins RA, Motarjemi Y and Käferstein FK, (2001). Irradiation: a critical control point in ensuring the microbiological safety of raw foods. *Food Control*,**12**(6): 347-356. http://dx.doi.org/10.1016/S0956-7135(01)00035-4

Papadopoulos V, Chouliara I, Badeka A, Savvaidid IN, and Kontominas MG, (2003). Effect of gutting on microbiological, chemical and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*). *Food Microbiol*., **20**: 411–420. http://dx.doi.org/10.1016/S0740-0020(02)00148-X

Rodriguez O, Barros-velazquez J, Gallardo JM and Aubourg SP, (2006). Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (*Psetta maxima*). *Food Chem*., **95**: 270–278. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.11.039

Vyncke W, (1981). 12TH Western European Fish Technologists Association (WEFTA) Meeting. Copenhagen, Denmark.

UNDER PEER REVIEW