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RESEARCH PAPER

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Effect of low dose gamma irradiation and refrigeration on the chemical and microbial quality of shrimp (*Penaeus monodon*)

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SUMMARY :

The present investigation is aimed at studying the effect of gamma irradiation (1, 3 and 5 kGy) and subsequent storage at refrigeration temperature (4°C) on the chemical, microbial quality and extended shelf-life of shrimp (*Penaeus monodon*). The total volatile base nitrogen (TVB-N) and trimethyl amine nitrogen values (TMA-N) of the irradiated shrimp samples significantly decreased in comparison with the control (non-irradiated) stored at 4°C. The thiobarbituric acid values for the irradiated shrimp was significantly lower than of the non-irradiated samples stored at 4°C ($p<0.05$). The pH value of the shrimp was affected significantly by both, irradiation dose and storage temperature ($p<0.05$). The total microbial load for the non-irradiated shrimp samples was higher than those of irradiated samples at 4°C temperature. The results revealed that the combination of low dose gamma irradiation and refrigeration storage resulted in overall reductions of microbial loads and stabilized the biochemical characteristics of shrimp.

KEY **W**ORDS : Gamma irradiation, Refrigeration storage, TBARS, TMA-N, *P. monodon*

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Fisheries sector in India occupies a very important place in the socio-economic development of the country. It is a source of livelihood for over 14 million people and contributed foreign exchange revenue to about US$ 3511.67 million in the year 2014.-15 (MPEDA, 2015). The production of fish and shrimp by aquaculture is increasing steadily and is becoming a major source of raw material for processing and export. Among all the shellfishes consumed shrimp, being a very lucrative commodity and rich in proteins, free amino acids, minerals and other soluble non-nitrogenous substances demonstrates an exceptional nutritional value in the diet of human beings (Hocaoglu *et al*., 2012).

The tiger shrimp (*Penaeus monodon*) is the major species exported and are important foreign exchange earner for the country. Cross contamination with bacterial pathogens is an important barrier for shrimp world trade (Mahto *et al*., 2015). There have been incidences of rejection of seafood consignments exported from India by the importing countries due to detection of antibiotics residues, presence of potential bacterial pathogens and unhygienic condition of the products (WFT, 2009). With increasing demand of high quality seafood, there is an obvious need for the development of new technologies and efficient seafood preservation methods which permits the extension of shelf-life for a longer period.

The shelf-life of seafood during cold storage and shipping is greatly influenced by the enzyme and microbial activities. A number of studies have been carried out on alternative methods of food preservation for the effective extension of shelf-life in seafood such as use of antimicrobials (Gelman *et al.*, 2001), modified atmosphere packaging (Kalleda *et al.*, 2013), etc. There is an increasing consumer demand for high quality, minimally processed, additive-free sea foods. Low dose gamma irradiation (upto10 kGy) is an effective against wide range of spoilage as well as pathogenic micro-organisms without significant rise in product temperature, thereby improving the food safety without significant loss of food quality. Fresh shrimp have been approved for irradiation with pasteurizing doses of 1-3 kGy while frozen seafoods have been approved for irradiation with doses of 4-6 kGy (Venugopal, 2002 and DAE, 2012).

Irradiation is recognized as an effective, widely applicable food preservation technique. The process exposes foods to a carefully controlled amount of energy in the form of high speed particles or rays that reduce the risk of food poisoning, control food spoilage and extend the shelf-life of food without detrimental effects to health and with minimal effect on nutritional or sensory quality (Hocaoglu *et al.*, 2012). This process has no effect on food taste, colour and smell, and it does not leave radioactive residues (ICGFI, 2002). Food irradiation, in combination with good refrigeration and handling practices, might provide a means to increase the shelf- life of fish products. Many researchers have recognized and reported that gamma irradiation at low doses (below 10 kGy) kill most organisms without any deterioration of food quality (Javanmard *et al.*, 2006; Locroix *et al.*, 1991; Mulder,1982; Olson,1998 and Thayer *et al.*,1995). Against this background the present work aims to study the biochemical and microbial changes in this shrimp samples upon irradiation and subsequent storage at refrigeration temperature.

EXPERIMENTAL METHODS

Materials :

Freshly harvested cultured shrimp (*Penaeus monodon*) was procured from the shrimp farms located at Mangalore, India. The sample was immediately packed in polyethylene bags with ice and brought to the laboratory and stored at 4^{o}C until irradiation.

Irradiation :

The samples were divided into two groups and subjected to irradiation at the centre for application of radioisotope and radiation technology (CARRT), Mangalore University, India using ^{60}cobalt radiation source (BRIT, Mumbai). The two sample groups were irradiated with 1.0 kGy, 3.0 kGy and 5.0 kGy at a dose rate of 6.94 kGy/h. The radiation dose was monitored using the Freaky dosimeter. The samples were maintained at 4+1^{o}C using sealed ice covering during irradiation. After irradiation, the samples were iced and transported within 1 h to the laboratory and held at refrigeration (4^{o}C) temperature until the last day of the experiment. The experiment was carried out in triplicate.

Bio-chemical analysis :

A proximate composition of the fresh shrimp meat was carried out. The moisture content was determined by the hot air oven method. The crude protein content of the shrimp meat was analysed for total nitrogen content by the Kjeldahl method. The crude lipid content of the shrimp meat was determined by the Soxhlet extraction method (AOAC, 2010). The ash content of the meat was determined by the method described in AOAC (2010). The total volatile base nitrogen (TVB-N) and Trimethyl amine nitrogen (TMA-N) content was determined by the Conway microdiffusion method (Beatty and Gibbon, 1937) and expressed as mg N/100 g meat. The thiobarbituric acid reactive substances (TBARS) (Raghavan and Hultin, 2005) was expressed as mg malonaldehyde/kg meat. The pH was measured at room temperature on the homogenates of the samples in distilled water (1/10 w/w) (Vyncke, 1981) and monitored using the pH meter (Systronix 361, India).

Microbial analysis :

The aerobic mesophilic counts and bacterial pathogens were analysed throughout the experiment. Twenty- five grams of the sample was placed aseptically into a sterile blender containing 225 ml of sterilized physiological saline (0.85% NaCl) and blended for 3 min at low speed. About 0.1 ml of serially diluted homogenates were plated onto a plate count agar (Himedia, Mumbai, India) incubated at 35°C for 24-48 h and the total plate counts were reported as CFU/g. The coliforms were enumerated by the MPN method. Pathogens such as the *Escherichia coli* (EMB agar, Himedia, Mumbai), *Staphylococcus aureus* (Baird parker agar, Himedia, Mumbai), *Salmonella* (BSA agar, Himedia, Mumbai) and Vibrios (TCBS, Himedia, Mumbai) were analysed (APHA, 2000 and ICMSF, 1986). The microbial counts were expressed as the number of viable bacterial colonies per gram of meat (log CFU/g).

Statistical analysis :

The data obtained from the microbiological and bio-chemical analyses were subjected to statistical analysis using the standard statistical package (SPSS, version 21.00). Analysis of variance (one way - ANOVA) was performed to examine whether any significant difference existed for the values obtained with respect to the number of storage days. The significance of differences was calculated at 5 per cent level ($p < 0.05$).

EXPERIMENTAL FINDINGS AND ANALYSIS

Among the seafood consumed, shrimp being rich in proteins, free amino acids, minerals and other soluble nitrogenous substances, demonstrates an exceptional nutritional value in the human diet (Konosu and Yamaguchi, 1982 and Hocaglu *et al.*, 2012). The shrimp used in the study had 74.90 per cent moisture; 22.43 per cent protein; 1.609 per cent crude lipids and 1.06 per cent ash which were comparable to the values reported in earlier studies by Hocaglu *et al*. (2012) and Viji *et al.* (2014).

Microbial analysis of shrimp samples held at refrigeration temperature (4°C):

The present study was focused on the monitoring of the following micro-organisms: Mesophilic aerobic bacteria, Coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and Vibrios. The initial counts of mesophilic bacteria in the non- irradiated shrimp was 5.62 log CFU/g and 7.41 log CFU/g for 0 and 12 days, respectively. The effect of gamma irradiation and refrigerated storage on the microbial counts in the shrimp samples is presented in Fig. 1. The number of aerobic plate counts and coliforms decreased with the increase in irradiation dose. Depending on the absorbed dose, the level of viable micro-organisms also decreased immediately after irradiation. Pathogens such as *Salmonella*, *S. aureus* and *E. coli* were not detected in any of the shrimp samples. Irradiation doses of 1, 3 and 5 kGy produced an immediate reduction of 1, 2 and 3 log units of aerobic plate counts, respectively, in the shrimp. Further, a fish irradiation dose ranging from 1 to 5 kGy has been suggested for shelf-life extension (Venugopal *et al.*, 1999 and Jo *et al.*, 2004). Chen *et al*. (1996) and Mendes *et al*. (2005) reported that the mesophilic bacterial counts of irradiated shrimp, crab and fish were lower than those in the non- irradiated samples during storage at 4°C. In this study, gamma irradiation and refrigerated storage were more effective than either treatment alone in decreasing the mesophilic counts and total coliforms. The 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 a major group of micro-organisms, especially responsible for spoilage of fresh seafood (Huss, 1994). It was observed that irradiation affected bacterial growth during the initial period (Fig. 1).

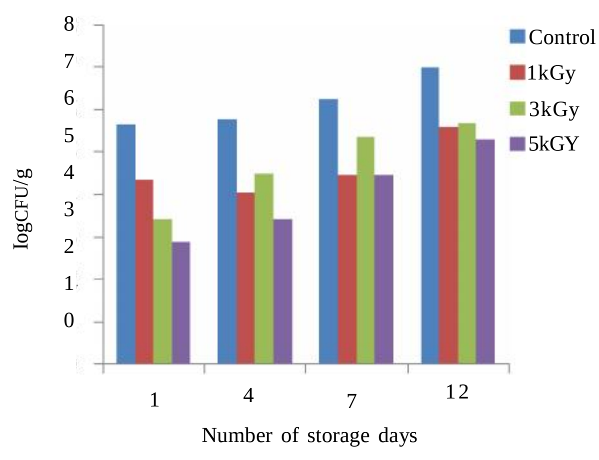

Fig. 1: Total mesophilic counts (log CFU/g) in irradiated and non- irradiated shrimp samples on different days of refrigeration (4°C)

The total bacterial count presented in Fig. 1 shows that at the beginning of the storage period, the bacterial growth was affected by irradiation. A decrease in the viable micro-organism count was observed immediately after irradiation depending upon the absorbed dose. 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 total mesophilic counts reported in the present investigation suggest that the irradiated shrimp samples remained within the acceptable limits after storage for 12 days at 4°C. The coliforms were detected in the control sample in the beginning; however, after irradiation at 1, 3 and 5 kGy, the counts reduced to zero in all the samples.

Gamma irradiation was found to inhibit microbial proliferation in fish and seafood (Radomyski *et al.*, 1994). Similar results were obtained by Cozzo-Siqueira *et al.* (2003), wherein they did not find *S. aureus* in the irradiated and non – irradiated tilapia (*Oreochromis niloticus*) fish irradiated with 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 indicate that irradiation at 3 kGy or above are effective in securing the microbial safety of the shrimp. Generally, just after irradiation with doses of 3 and 5 kGy, the microbial load significantly reduced ($p<0.05$) and the irradiated samples showed a good microbial quality. *Salmonella, E. coli, and S. aureus* were absent during the entire storage period.

Chemical analysis of shrimp at refrigeration temperature (4°C) :

Total volatile base nitrogen (TVB-N):

During the period of refrigerated storage (4°C), the TVB-N value of the shrimp meat was significantly ($p<0.05$) higher in the non-irradiated (control) samples than in the irradiated samples (Fig. 2). The initial TVB-N value in the control sample was 5.27 mg N/100 g, which increased to 56.83 mg N/100 g at the end of 12 days of the storage period. On the other hand, irradiation at 1, 3 and 5 kGy suppressed the formation of TVB-N during storage and the value reached 10.33 and 11.27 and 9.23 mg N/100 g, respectively, after 12 days (Fig. 2). The irradiated samples of the shrimp had significantly much lower concentrations of TVB-N during its refrigerated storage as compared to the control, which may be attributed to the reduction of the microbial population (Venugopal *et al.*, 1999).

The acceptable limit of the TVB-N in fish is a

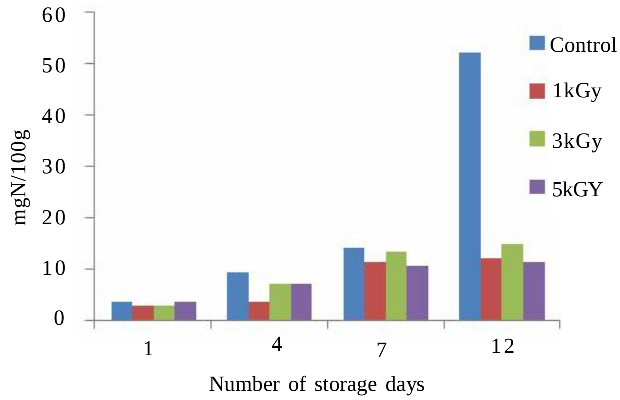

Fig. 2: TVB-N of irradiated and non-irradiated shrimp samples on different days of refrigeration (4°C)

maximum of 35 mg N/ 100 g (Huss, 1988). In the present study, the TVB-N value did not cross the acceptable limit of 35 mg N/ 100 g in the irradiated squid meat even after 12 days of storage. Our results are comparable to an earlier study, wherein the TVB-N in iced European sea bream did not increase even after 20-22 days of storage (Castro *et al.*, 2006). Lower TVB-N levels in the irradiated samples as compared with the control was also reported for fish (Jo *et al*., 2004). Mendes *et al*. (2005) reported that the initial TVB-N level of 15.6 mg N/100 g in chilled horse mackerel reached a maximum limit of 30-35 mg N/100 g on day 12 in the non –irradiated samples, while in the 1 and 3 kGy irradiated samples, the TVB-N values recorded were 13.6 and 12.7 mg N/100 g, respectively, on days 20.

Trimethyl amine nitrogen (TMA-N):

TMA-N is considered as a valuable tool to evaluate the quality of fish and shellfish because of its rapid accumulation in the muscle under refrigerated conditions (Gokodlu *et al*., 1998). In the present investigation, the initial average values were <5 mg N/100 g indicating the freshness of the initial product. The TMA-N levels increased in all the samples ($p<0.05$), but the values in the irradiated samples remained lower than those of the non-irradiated samples during the refrigeration storage of 12 days. The initial TMA-N level of the shrimp meat was as low as 5.87 mg N/100 mg and subsequently, the TMA-N levels rose significantly in the control but the values in the irradiated group remained lower than the rejection limit (Fig. 3). Ahmed *et al*. (1997) reported that the rate of formation of TMA-N and TVB-N was reduced in the irradiated fish compared with the non-irradiated

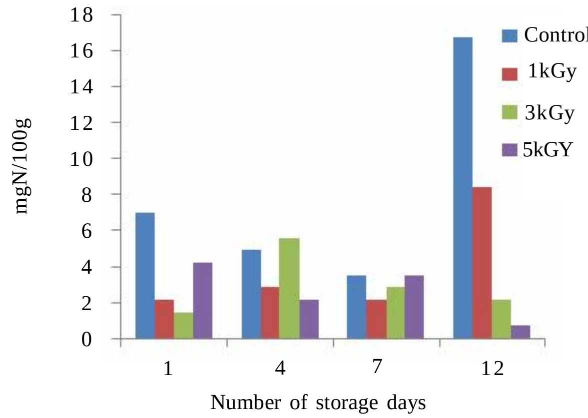

Fig. 3: TMA-N of irradiated and non-irradiated shrimp samples on different days of refrigeration (4°C)

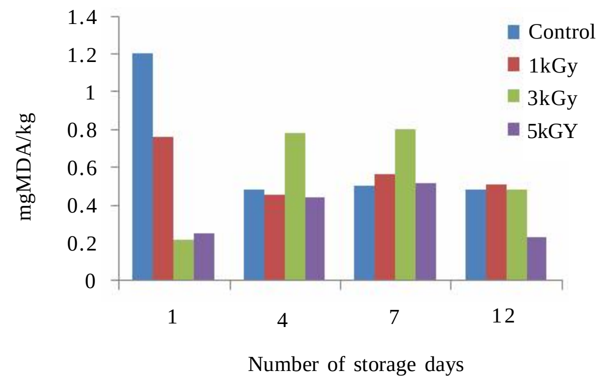

Fig. 4: TBARS of irradiated and non-irradiated shrimp samples on different days of refrigeration (4°C)

samples because of the radiation sensitivity of *Pseudomonas* and *Shewenella*, which are the micro-organisms responsible for the decomposition of tri-methylamine oxide (Spinelli and Pelroy, 1969). Connell (1975) suggested that TMA-N values between 10–15 mg N/100 g of fish muscle were at the upper limit of acceptability, while according to Yanamura (1938), the acceptable limit of TMA-N was 30 mg N/100 g for marine fish. Microbial activities are also implicated in the formation of chemical compounds such as TVB-N, TMA-N, etc., which are suggested as indicators of fish quality (Gram and Huss, 1996).

Thiobarbeturic acid reactive substances (*TBARS*):

An increase in the TBARS value was noticed in all the samples during refrigeration storage (Fig. 4). The initial TBARS value of the non- irradiated control sample was 1.23 mg MDA/ kg with the value decreasing to 0.54 mg MDA/ kg on the 12th day of storage. It was observed that the TBARS values of the irradiated samples (1 and 3 kGy) were higher than the control group during the 7th days of storage, however, the trend was further decreased (5 kGy) during 12th day of storage. In addition, the decrease in TBA values at the end of 12 days storage at 4°C was also found to be statistically significant ($p<0.05$). According to Connell (1990), the ideal TBA value should be < 3 mg malonaldehyde/kg. However, this limit was observed to increase during subsequent storage in the control as well as in irradiated shrimp samples (Fig. 4). The highest TBA value recorded for 5 kGy was 0.50 mg malonaldehyde/kg on the 12th day of storage.

The formation of thiobarbituric acid reactive substances did not show a consistent trend during refrigerated storage for both the control and irradiated groups. The variations can be explained as a result of the different phases of decomposition of the 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 sea bass and anchovyn threadfin bream (Lakshmanan *et al*., 1999; Jeevanandam *et al*., 2001; Chouliara *et al*., 2005 and Hocaglu *et al*., 2012). Significant differences ($p<0.05$) in the TBA values were found between the control and the irradiated shrimp samples during the storage period. This indicates that the lipid oxidation in the shrimp meat increased due to gamma irradiation.

pH:

The initial pH value of the fresh non – irradiated shrimp was 6.82, while for 1, 3 and 5 kGy irradiated shrimp meat, the pH was 7.02, 7.86 and 7.02, respectively (Fig. 5). Increasing the applied irradiation dose resulted in a decrease in the pH value. During the course of refrigerated storage, the pH values showed a decrease in all the shrimp samples. On further storage, the pH values of both non – irradiated and irradiated shrimp samples were in the range of 7.02- 7.86 and on the 12th day of storage no definite trend was observed. However, the non- irradiated samples showed a higher pH than the irradiated ones. This is probably due to the accumulation of nitrogenous compounds, resulting from chemical and biological decomposition, but there was no significant difference in pH ($p< 0.05$) between the samples. A similar trend was observed for farmed sea bass and turbot on ice (Papadopoulos *et al.*, 2000). From the present study,

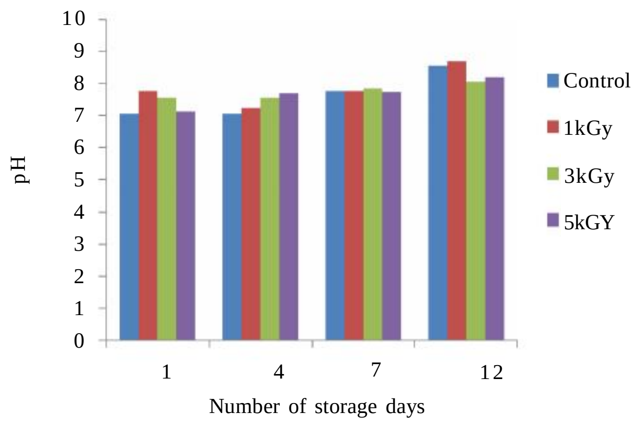

Fig. 5: pH of irradiated and non-irradiated shrimp samples on different days of refrigeration (4°C)

it was found that the irradiated squids when stored at refrigeration temperature (4°C) for 12 days showed acceptable bio- chemical quality indices, which may be due to the combined effect of the two preservation methods.

Conclusion :

The results obtained from this investigation show that the combination of low dose irradiation and refrigerated storage resulted in a significant reduction of bacterial growth and irradiation of 3.0 and 5.0 kGy doses with refrigerated (4°C) storage could inhibit coliforms completely. The results also show that the employed radiation dose of 3.0 and 5.0 kGy in conjunction with refrigeration storage extended the shelf-life of squid to about 12 days. The levels of pH, TVB-N, TMA- N, PV, and TBARS in irradiated and non- irradiated squid samples were also examined. The irradiated samples had significantly lower concentrations of TVB-N and TBARS during refrigerated storage as compared to the control, which may be attributed to the reduction of the microbial population. These parameters are within the acceptable limits until the last day (12th day) of refrigeration storage in the irradiated samples. In conclusion, the results of the study demonstrated that the combination of low dose gamma irradiation and refrigerated storage resulted in a significant reduction of bacterial growth and stabilised the biochemical attributes of the shrimp meat.

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