

Shelf life Extension of Whole and Steaks of Rohu (*Labeo rohita*) by Gamma Irradiation

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Fresh rohu were procured from the local market and packed in polyethylene pouches and were subjected to gamma irradiation as whole or steaks at a dose of 0, 1 or 2 kGy. The treated fish and steaks were stored under ice. At periodic intervals, quality of the fish was determined by sensory, microbiological and chemical parameters. The unirradiated whole fish and steaks were acceptable up to 14 and 20 days in comparison to a storage life of 28 and 32 days for the respective samples irradiated at 1 kGy. Irradiation of whole fish at 2 kGy gave a shelf life of 35 days. The extent of lipid oxidation due to irradiation was not significant and had no effect on sensory quality of the ice-stored fish.

Key words: Rohu, gamma irradiation, shelf life extension, storage life, fish steaks.

Consumer demand for fish and fishery products is increasing as a result of awareness on the health benefits of fish consumption. However, since the global marine fisheries has started showing signs of stagnation, culture of freshwater fish species is becoming important to meet the requirements of fish and fishery products. Aquaculture is world's largest fish producing system, growing at an annual rate of 10%. Asia produces about 91% of global aquaculture products, with China, India, Japan, the Republic of Korea, Philippines, Indonesia and Thailand, as the top producers within Asia (Dey *et al*; 2001).

India is the second largest producer of farmed fish in the world, carp species contributing most of the countries aquaculture production (Murthy, 2002). Aquaculture in our country is targeted towards higher domestic consumption of fish as well as exports (Balla, 2001). The three major species indigenous to the rivers of Indian sub-continent, namely, catla, rohu and mrigal, are widely cultivated in India and also parts of

Southeast Asia. Current annual production of these species is two million metric tons (Murthy, 2002). Among the above mentioned three species, rohu (*Labeo rohita*) is the main preferred one, due to its faster growth and consumer preference. Annual production of rohu is about 60,000 metric tons (MPEDA, 2000). With rising demand for these freshwater fish species, it is necessary to develop processes for better distribution, particularly in the interior parts of the country. The present work was carried out to study the feasibility of low dose gamma radiation for shelf life extension of whole or steaks of rohu under chilled conditions.

Materials and Methods

Chemicals used were of analytical grade and procured from local suppliers.

Fresh whole rohu (*Labeo rohita*) were brought to the laboratory under ice from the local market. The average weight of the fish was taken (800 g). The fish were washed in cold water. The fish were descaled and eviscerated using a stainless steel knife.

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Some fish were further beheaded, eviscerated, deskinning and cut into steaks of 2.5 cm thickness, each weighing approximately 42 to 45 g.

For each treatment six to eight fish samples (whole or steaks) were taken. Whole fish samples were individually packaged, while two steaks were packaged in polyethylene pouches (500 gauge) for irradiation treatment. The packages were held under flake ice. The packages of whole fish and steaks were irradiated at doses of 1 and 2 kGy using a package irradiator (Atomic energy of Canada Ltd., Ottawa) with ^{60}Co as source, having a dose rate of 0.022 kGy/min. The unirradiated fish samples served as control.

Both irradiated and unirradiated whole fish, and steaks were stored under ice in 5 cm thick polystyrene insulated boxes (52.2x34x24 cm). An outlet at the bottom of the insulated box facilitated removal of water from the melting ice. The insulated box was maintained at 0-2°C. Ice was replenished periodically.

The fish samples were withdrawn at periodic intervals for quality assessment. Meat from the fish was picked, minced and the meat homogenate was used for biochemical and microbiological analysis.

A panel of 6 scientists from the Department conducted sensory evaluation of the samples during the course of storage study. The panel members evaluated odour of the fish before and after subjecting the whole and steaks of rohu to cooking in a microwave oven for one min. The cooked samples were immediately served to the panel members. The scale used for odour scores of the fish on a 10-point hedonic scale (Bilinski, 1983) was as follows: 10, fresh odour, characteristic of fresh rohu; 9, marginal loss of fresh odour; 8, light loss of fresh odour; 7, definite loss of fresh odour; 6, almost no odour; 5, most detectable ammoniacal and rancid odour; 4-2, increasing

off odour; and 1, putrid odour. Fish that scored between 10-5 were considered acceptable and those scoring less than 5 were considered as spoiled.

Samples (10g) each were aseptically homogenized for 1-2 min with 90 ml of sterile saline in a pre-sterilized Sorvall Omni mixer cup. Appropriate dilutions were pour-plated in duplicates. The mesophilic counts (colony forming units, CFU) were determined using plate count agar (Difco, Detroit, MI, USA). The plates were incubated at 30°C for 48 h before counting. Plates with 30-300 colonies were taken for counting.

Total volatile basic nitrogen (TVBN), total volatile acid number (TVA), and 2-thiobarbutaric acid (TBA) values were analyzed. Fish mince (10g) was homogenized with 90 ml of 10% trichloro acetic acid (TCA) in a Sorvall omni mixer homogenizer cup for 1 min, the homogenate was allowed to stand for 15 min and then filtered through Whatman filter paper (No.1). Filtered extract (1 ml) was used for the estimation of TVBN by convey micro diffusion method of Farber and Ferro (1956). TVBN values were expressed as mg N/100 g of fish muscle. The test was carried out in triplicates and mean value was noted.

The total volatile acid (TVA) content was determined by the method described by Venugopal, *et al.* (1981). An amount of 30 g minced fish muscle was homogenized with 90 ml of distilled water using Sorvall omni mixer cup for 1 min. To the sample, 15 ml of 1N H_2SO_4 and 24 ml of 15% (w/v) phosphotungstic acid in water were added and further homogenized. The precipitated proteins were removed by filtering the homogenate through a Whatman filter paper (No.1). Fifteen ml of the filtrate was steam distilled for approximately 10 min, and 40 ml of steam distillate was collected. TVA content of distillate was determined by titrating against 0.01M NaOH, using phenolphthalein as indicator. TVA number was expressed as ml of 0.01M alkali required to

neutralize the acids from 100 g fish muscle. Mean of triplicates was recorded.

TBA was estimated according to Witte *et al.*, (1970). A weighed sample (5 g) of fish mince was homogenized with 20 ml of chilled 5% aqueous TCA containing 0.5M ortho-phosphoric acid in a Sorvall omni mixer cup for 1 min. The homogenate was filtered through Whatman filter paper (No.1). Five ml of the filtrate was added to 5 ml of aqueous solution of TBA reagent (0.005M 2-thiobarbituric acid in 1M phosphoric acid). The sample was incubated in a boiling water bath for 45 min. After cooling the sample to room temperature; 5 ml of butanol were added to extract the colour. The extract was then centrifuged at 3000 rpm for 10 min; the colour of the butanol extract was read at 532 nm in UV-Visible recording spectrophotometer UV-2500 (Chemito, Japan). The TBA value was calibrated using malonaldehyde (prepared by treating 1,1,3,3 tetra-ethoxy propane (TEP) with phosphoric acid). TBA values were calculated by multiplying a factor of 5.8. The TBA values were expressed as μg malonaldehyde/g sample.

Protein and moisture of the fish were determined according to AOAC method (1990). The protein content was determined by measuring nitrogen by Kjeldahl method using Kjelpus digestion and distillation system (Pelican Instruments, Madras, India)

and was expressed as $\text{N} \times 6.25$. Crude lipid was determined by the procedure of Bligh & Dyer (1959).

Statistical analysis of the data was done by the standard methods. Analysis of variance (ANOVA) was employed to find out significance between different treatments and days of storage. Significant level was determined at 95% ($p < 0.05$).

Results and Discussion

The proximate composition of fresh fish used for the study was $77.94 \pm 0.1\%$ moisture, $17.42 \pm 1.59\%$ proteins and $2.19 \pm 0.4\%$ crude lipid. The values are comparable to those reported in the literature (Devadasan *et al.*, 1978; Mukundan *et al.*, 1986).

Fig.1 shows the microbial profile during ice storage of whole rohu and rohu steaks subjected to irradiation. The whole unirradiated rohu had an estimated initial microbial count of 3.1×10^5 cfu/g. This range of counts has also been reported by several workers (Ehlermann and Munzner, 1970; Nair *et al.*, 1974; Devadasan *et al.*, 1978; Bandyopadhyay *et al.*, 1986). The initial counts increased to a value of 5.2×10^7 after 14 days of storage (Fig 1A). Immediately after irradiation at 1 kGy, the initial count of the whole fish was 7.9×10^4 cfu/g. Upon storage, the value reached 1.1×10^8 cfu/g within 28 days. Treatment at 2 kGy decreased

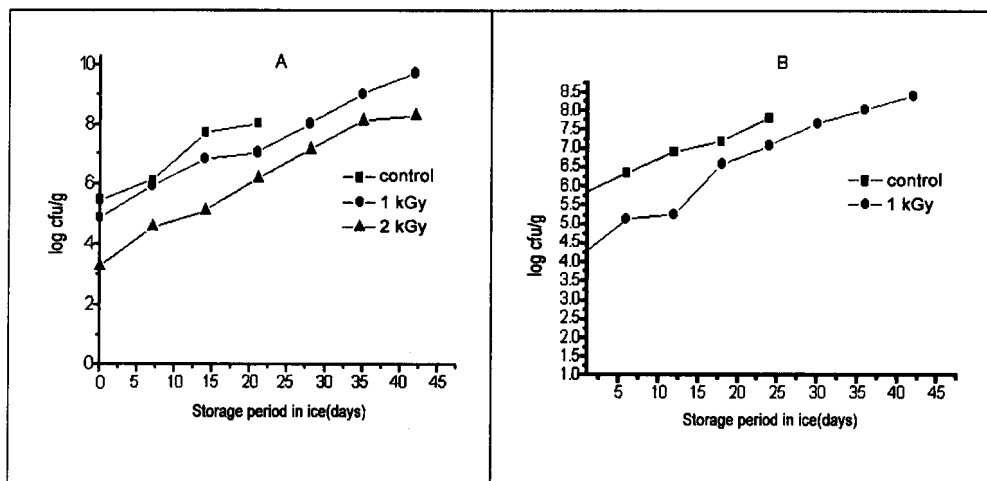


Fig. 1. Influence of gamma irradiation on total plate count of whole rohu (A) and rohu steaks (B) during ice storage.

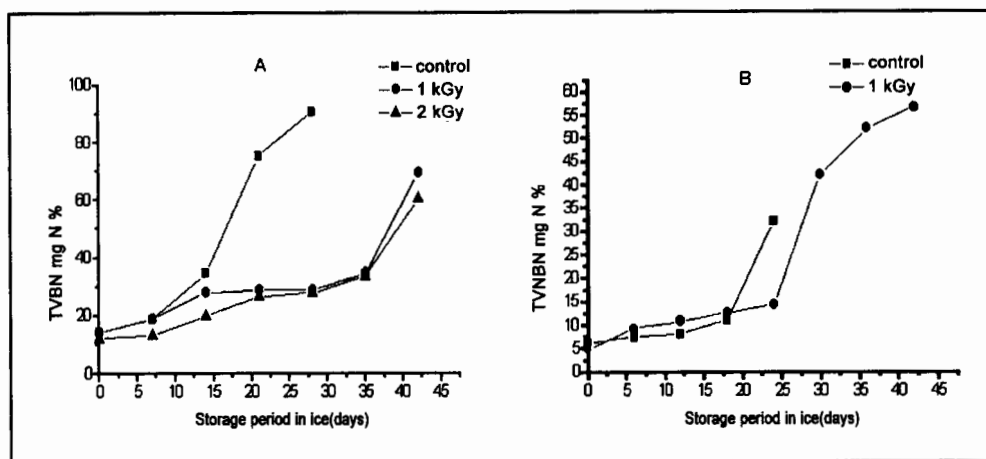


Fig. 2. Influence of gamma irradiation on TVBN values of whole rohu (A) and rohu steaks (B) during ice storage.

the initial counts to 1.8×10^3 cfu/g and after 35 days the counts were 1.3×10^8 cfu/g (Fig 1A). In the case of control steaks the initial value was 5.8×10^5 cfu/g, which reached 6.2×10^7 cfu/g within 24 days. Irradiation at 1 kGy reduced the initial value to 1.3×10^4 cfu/g. This value reached 2.5×10^8 cfu/g during a storage period of 42 days (Fig 1B).

Fig. 2 shows the TVBN values of whole rohu and rohu steaks during the course of ice storage. As shown in Fig. 2A, the initial value of TVBN in untreated whole rohu was 14.40 mg N%; the value increased to 90.90 mg N% after 28 days of storage. On the other hand, irradiation of 1 and 2 kGy of whole rohu suppressed the formation of TVBN during storage; the values reached 69.53 and 60.53 mg N% respectively, after 42 days. The

initial TVBN value for unirradiated rohu steak was 6.08 mg N%; the value increased to 32.21 mg N% within 24 days. While the TVBN values of rohu steaks irradiated at 1 kGy reached 56.70 mg N% after 42 days of storage (Fig. 2B). Through out storage period, the TVBN value of irradiated fish were significantly ($p < 0.05$) less as compared with control.

The effect of low dose irradiation on shelf life of fishery products including freshwater fish has been well documented (Venugopal *et al.*, 1999). Low dose gamma radiation have been shown to suppress spoilage and formation of TVBN of fishery products during chilled storage (Lakshmanan *et al.*, 1999; Venugopal *et al.*, 1999; Jeevanandam *et al.*, 2001). The suppression

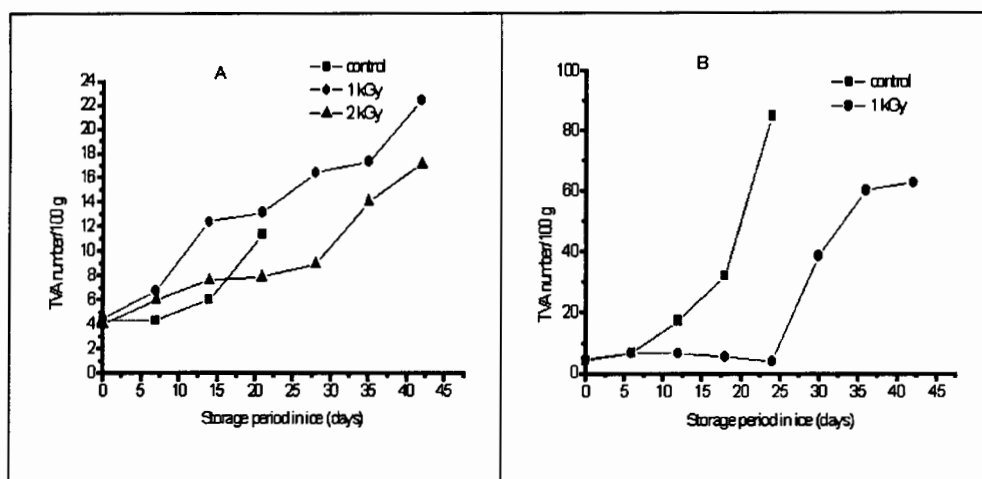


Fig. 3. Influence of gamma irradiation on TVA number of whole rohu (A) and rohu steaks (B) during ice storage.

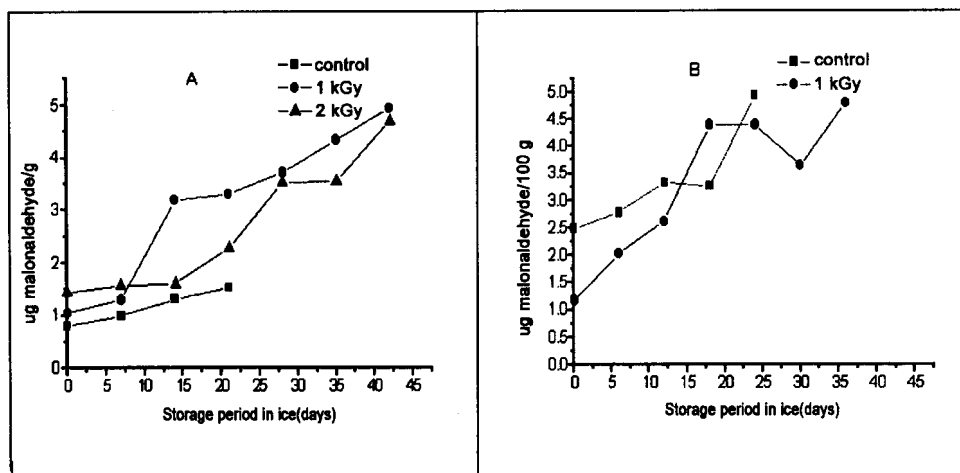


Fig. 4. Influence of gamma irradiation on TBA values of whole rohu (A) and rohu steaks (B) during ice storage.

in TVBN formation in radurized fish was probably due to elimination of spoilage causing gram-negative bacteria, survival of micrococci and other less active bacteria (Kumta *et al.*, 1973).

TVA number of control and irradiated fish during ice storage is given in Fig 3. Initial TVA number of untreated whole rohu was 4.3, which increased to 11.3 within 21 days of iced storage. Immediately after irradiation (1 and 2 kGy) the TVA values of the fish were 4.4 and 4 respectively. These values were increased to 22.4 and 17.1 after 42 days storage (Fig. 3A). In the case of untreated steaks the initial TVA value was 4, which reached 84.8 after 24 days whereas in irradiated steaks the value was increased to only 62.7 after 42 days. (Fig. 3B).

Lipid oxidation in the fish in terms of TBA value during the course of ice storage of the fish are shown in Fig. 4. The TBA values of both whole and steaks of rohu increased during ice storage. In the unirradiated whole rohu, the initial TBA value was $0.79\mu\text{g}$ malonaldehyde/g, which increased marginally after 21 days. Fish subjected to irradiation at 1 and 2 kGy showed a gradual increase in the TBA value and reached a maximum of 4.9 and 4.7, respectively (Fig. 4A). In the case of steaks, an initial value of $2.5\mu\text{g}$ malonaldehyde/g in control increased to 4.9 after 24 days, whereas in irradiated steaks the value was increased to 4.8 after 42 days storage at $0-2^\circ\text{C}$ (Fig. 4B). The observations by Alkahtani *et al.*, (1996) (in tilapia and Spanish mackerel) Quaranta *et al.*, (1984) (in tuna),

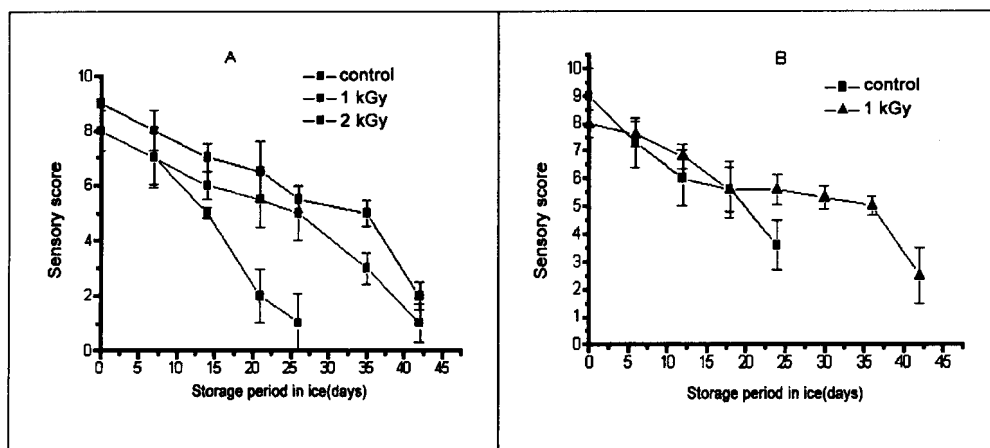


Fig. 5. Influence of gamma irradiation on sensory score values of whole rohu (A) and rohu steaks (B) during ice storage.

and Kamat and Kumta (1972) (in pomfret) are comparable with present results. In the present study, the TBA values increased during storage in both whole fish and steaks. However, there was no detectable rancid odour and off flavour in the treated fish. Similar observations were made by Spinelli *et al.*, (1969), Venugopal *et al.*, (1973) and Ghadi and Venugopal (1991) in the case of marine fish.

The appearance of the fresh fish was bright, and flesh was firm. There was no irradiation odour in samples treated even at 2 kGy. Fig. 5 depicts the sensory scores of the whole fish and steaks. The odour scores of both uncooked and cooked samples decreased rapidly in the case of whole fish and steaks during the course of ice storage. Based on a border line score of '5' the unirradiated control whole fish and steaks had a shelf life of 14 and 20 days, respectively (Fig. A). As per the sensory score, 1 and 2 kGy irradiated fish showed a shelf life 28 and 35 days respectively. In the case of 1 kGy irradiated steaks the shelf life was 32 days (Fig. 5).

In comparison with unirradiated whole fish, steaks exhibited longer storage life. Gutting, evisceration and thorough washing of the steaks could be the reason of extended shelf life, which remove contaminating bacteria. Similarly evisceration and gutting are known to enhance the refrigerated shelf life of fish. Further irradiation suppressed formation of TVBN in carp during six week storage period regardless of the radiation dose, whereas in control samples strong putrid odour developed after two weeks of storage (Ehlermann and Munzner, 1970).

The present study demonstrates the feasibility of low dose gamma irradiation on shelf life extension of rohu. The enhanced shelf-life of rohu will help in better distribution of the fish in the interior parts of the country. Radiation processing of pre-packed steaks helps to increase the storage life and convenient handling by the consumers.

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