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Effect of Gamma Radiation in Combination with Low Temperature Refrigeration on the Chemical, Microbiological and Organoleptic Changes in *Pampus chinensis* (Euphrasen, 1788)

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Abstract: The present study was carried out to evaluate the efficiency of gamma radiation (3, 5 and 8 kGy) in combination with low temperature (-20°C) for extending the quality and shelf-life of degutted fresh Chinese pomfret, *Pampus chinensis*. Quality assessment was studied by monitoring the chemical (TVN, TMA), microbiological (TBC, TMC) and organoleptic changes in irradiated and non-irradiated (control) samples. Among chemical indicators of spoilage, total volatile nitrogen (TVN) values increased to 55 mg/100 g for nonirradiated samples whereas for irradiated fish lower values of 45 mg/100 g, 27.5 mg/100 g and 18.7 mg/100g were recorded at 3, 5 and 8 kGy respectively (day 90). Significantly lower values were obtained for irradiated samples reaching a final trimethylamine (TMA) value of 7.1, 5.3 and 4.9 mg N/100 g at 3, 5 and 8 kGy respectively whereas for non-irradiated samples it increased slowly attaining a value of 32.5 mg N/100 g after 90 days. Total bacterial counts as well as total mould counts for non-irradiated pomfret were higher than that of irradiated fish. The organoleptic scores of control sample were gradually decreased with the progress of storage period whereas irradiated samples showed the acceptable score upto 90 days. This study concludes that gamma radiation in combination with low temperature showed maximum shelf-life extension (90 days) in each dose of radiation used but in 8 kGy radiation, *Pampus chinensis* showed best quality.

Key words: Gamma radiation · Low temperature refrigeration · Chemical and sensory properties · *Pampus chinensis*

INTRODUCTION

Pomfrets or Chandas contribute a significant portion of the total fisheries resources collected from the waters between 50 and 60 m depth contour of the Bay of Bengal [1] Chinese pomfret (*Pampus chinensis*) is one of the common species found in the Bay of Bengal, is quite tasteful having rich nutritional properties and high demand in the global markets. After landing the catch passes through different marketing chains and the time required to reach the destination varies widely according to location but it often takes a considerable portion of the normal shelf life of a tropical fish species [2]. Two major problems with respect to marketing and distribution of seafood are their high perishability and poor hygienic quality; this is essentially due to contamination of the commodity by spoilage and pathogenic microorganisms. [3, 4]. Along with increasing demand of this high quality fish, there is an obvious need for development of new technologies and efficient fish preservation methods which permit shelf life extension of these products.

Besides traditional methods used to extend the shelf life of fish and fishery products, rapid chilling and ice storage [5], freezing in low temperature, use of organic acids, antimicrobials [6, 7], modified atmosphere packaging [8] and ionizing radiation [9, 10] have been proposed. Irradiation at a dose of up to 10 kGy has been used in both animal and vegetable foods as an effective, safe and economical method of food preservation posing no nutritional, toxicological or microbiological problems [11]. Food irradiation has been used for the purposes of inhibition of sprouting, destruction of food borne insect's

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and parasites, delay of physiological ripening and extension of shelf life or improvement of food qualities. [12].

Combination of treatments for food preservation may result in synergistic or cumulative effects of microbiological barriers or hurdles, leading to a reduced level of one or all the treatments [13]. Food irradiation, in combination with good refrigeration and handling practices, might provide a means to increase fish product shelf life. Hence this study was designed to observe the effect of gamma-radiation (1, 3 and 8 kGy) in combination of refrigeration (-20°C) on degutted fresh Chinese pomfret, *Pampus chinensis*.

MATERIALS AND METHODS

Fish Samples: Fresh pomfret used in this experiment were collected from Malibagh bazaar, Dhaka metropolis early in the morning and immediately transferred to icebox and carried to the laboratory of Food Processing and Preservation Division, Institute of Food and Radiation Biology, Atomic Energy and Research Establishment (AERE), Savar, Dhaka, Bnagladesh. Length and weight of the samples were recorded. Then the samples were degutted, deheaded and detailed and divided into four lots: non-irradiated (control) and irradiated (3, 5 and 8 KGy).

Irradiation: Samples were irradiated using a ^{60}Cobalt radiation source supplied by the Atomic Energy of Canada Limited. The applied doses in this study were 3, 5 and 8 KGy. Fish samples were maintained at 2±2°C during irradiation by using sealed ice covering. Non-irradiated (control) fish samples were kept in polystyrene boxes with sealed ice at the ambient temperature of 18-20°C.

Storage Conditions: After irradiation, the non-irradiated and irradiated samples were transported to the laboratory in packed ice via insulated polystyrene boxes. Four groups of samples were subsequently stored at -20°C. The storage of the fish lasted 90 days and samples (3 fish) from each four lots were taken at intervals of 15 days for sensory, chemical and microbiological analysis. Two samples for microbiological analysis and one sample for sensory analysis were separated. After sensory and microbiological analysis, samples were homogenized and subjected to chemical analysis.

Chemical Analysis: Total volatile nitrogen (TVN) was determined by the Conway Micro-diffusion method. About 5-10 g sample was grounded with 50ml of 10% Trichloroacetic acid (TCA) solution and stored overnight in a conical flask adding another 30ml of 10% TCA solution. The next day the solution was filtered through a filter paper and volumed up to 50ml in a volumetric flask. Then 2ml of 2% of boric acid was transferred to the inner chamber and 2ml of sample extract and finally 2ml of saturated potassium carbonate (K_2CO_3) were transferred to the outer chamber of the Conway dish. The Conway dish was left overnight at room temperature for micro-diffusion. The residual boric acid solution was titrated against standard N/70 H_2SO_4 solution. Finally the TVN values were calculated.

Same procedure of TVN estimation was followed to determine the TMA value, except 1ml of 40% formaldehyde solution was added to the outer chamber of the conway dish. The TVN and TMA values were expressed as mg N/100 g fish.

Microbiological Analysis: The bacteriological changes were estimated by total bacterial count (TBC) technique following Withfogel [14]. Total mould count (TMC) was estimated by pour plate technique in potato dextrose agar (PDA) media at the ratio of 3.1gm per 100ml of sterile water. 1gm of fish muscle from each lot was taken as sample and homogenized in 50ml sterilized distilled water. The sample was diluted in a series of 5 serial dilutions in test tubes with distilled water and from each dilution 1ml of the sample was poured with nutrient agar media and then incubated at 37°C for 24 hours for TBC count. Another 1ml from the dilution was poured with PDA media and incubated at 30°C for 72 hours for total mould count. The viable count of TBC and TMC were expressed as cfu/gm.

Sensory Assessment: Five experienced panelists analyzed fish at 15days intervals of 90 days storage according to a method developed by Peryam and Pilgrim [15] based on nine point hedonic scale in taste, color, texture, slime, mucus and smell etc. The mean points of each panelist were calculated and considered to be the borderline of acceptability.

Statistical Analysis: Analysis of variance (ANOVA) was employed to find out the level of significance between different treatments and days of storage.

RESULTS AND DISCUSSION

Chemical Analysis: TVN content of non-irradiated and 3, 5 and 8 kGy irradiated pomfret stored at-20°C is shown in Table 1. At the beginning of storage, the TVN value was 7.5 mg/100g for control sample and 6, 5 and 4 mg/100g for irradiated 3kGy, 5kGy and 8kGy samples, respectively. However, TVN values increased with the progress of storage period. At the end of the storage period of 90 days this value was 55, 45, 27.5 and 18.7 mg/100g for non-irradiated and 3, 5 and 8 kGy irradiated fish sample, respectively. The statistical analysis of the TVN data showed that significant differences were found between control and each irradiated samples stored at-20°C after 90 days of storage (p=0.000146, df=5 and 10, a=0.05).

TVN levels for non-irradiated and irradiated sea bass exceeded 35 mg/100g [16], which is considered the maximum level for acceptability for marine fish after 9 and 15 days respectively, in agreement with TVN levels of sea bass stored in ice studied by Kyrana and Lougovois [17] Chouliara *et al.* [18] reported that initial TVN levels of vacuum packed-irradiated (1-3 kGy) sample stored under refrigeration sea bream were 27.5 mg/100 g, 27.3 mg/100 g and 25.1 mg/100 g reaching the limits of acceptable at day 10 in control, at day 21 and 28 for 1 and 3 kGy irradiated sea bream. Mendes *et al.* [19] reported that initial TVN level of chilled horse mackerel was 15.6 mg/100g, reaching the limit levels of 30-35 mg/100g at day 12, in 1 and 3 kGy irradiated samples at day 20, 13.6 mg/100g and 12.7 mg/100g, respectively. In agreement of these results it can be said that the combination of irradiation and low temperature preservation used in this study results in very low level of TVN production after a considerable period (90 days) of storage.

Initial average value of TMA-N was found to be 4.5mgN/100g in control and 3, 2.5 and 1.5 mgN/100g were observed in 3, 5 and 8 kGy irradiated pomfrets, respectively. The final values of TMA-N were 32.5 mg/100g (control), 7.1mg/100g (3 kGy), 5.3 mg/100g muscle (5 kGy) and 4.9 mg/100 g muscle (8 kGy). TMA-N values showed significant increase for all groups after 90 days of storage at-20°C (p=0.000146, df =5 and 10, a=0.05). Connel [20] and Huss [21] suggested that the TMA value ranged from 10-15 mg N/100 g of fish muscle was the upper limit of acceptability while according to Yamamura [22], in case of marine fish acceptable limit of TMA is 30 mg N/100 g of fish.

Chouliara *et al.* [18] reported that TMA-N production was significantly (p<0.05) reduced by 1 and 3 kGy irradiation. The concentration of TMA-N was found to be 3.92 mg/100 g and 2.96 mg/100 g in sea bream kept vacuum packed for 35 days at 4°C. Mendes *et al.* [19] found the highest concentration of TMA-N nonirradiated horse mackerel, followed by horse mackerels stored in refrigeration and the lowest in irradiated samples (1 and 3 kGy) for 24 days at 3-5°C. Chouliara *et al.* [18] also refer that TMA-N formation is lower in irradiated and cold stored sea bream than in unirradiated fish. From the present investigation it was found that the irradiated fishes when stored at -20°C could be acceptable after 90 days which is due to the synergistic effect of two preservation methods.

Table 1: Total Volatile Nitrogen (TVN) in control and irradiated Chinese pomfret, *P. chinensis* during storage period at-20°C

Storage period (Days)	Control (mgN /100g)	Irradiated 3 kGy(m gN/100g)	Irradiated 5 kGy(m gN/100g)	Irradiated 8kGy(mg N/100g)
0	4.5	3	2.5	1.5
15	5.6	3.2	2.9	2.0
30	7.5	4.1	3.5	2.7
45	10.6	5.2	3.9	3.1
60	16.8	5.9	4.3	3.5
75	24.6	6.3	4.8	4.2
90	32.5	7.1	5.3	4.9

Table 2: Trimethylamine (TMA) in control and irradiated Chinese pomfret, *P. chinensis* during storage period at-20°C

Storage period (Days)	Control (mgN /100g)	Irradiated 3 kGy(m gN/100g)	Irradiated 5 kGy(m gN/100g)	Irradiated 8kGy(mg N/100g)
0	4.5	3	2.5	1.5
15	5.6	3.2	2.9	2.0
30	7.5	4.1	3.5	2.7
45	10.6	5.2	3.9	3.1
60	16.8	5.9	4.3	3.5
75	24.6	6.3	4.8	4.2
90	32.5	7.1	5.3	4.9

Table 3: Total Bacterial count (TBC) in control and irradiated Chinese pomfret, *P. chinensis* during storage period at-20°C

Storage period (Days)	Control (cfu/g)	Irradiated 3 kGy (cfu/g)	Irradiated 5 kGy (cfu/g)	Irradiated 8 kGy (cfu/g)
0	1.3×10^4	2.1×10^2	00	00
15	2.5×10^4	2.7×10^3	00	00
30	3.2×10^4	4.1×10^3	1×10^3	00
45	2.9×10^4	3.7×10^3	2.3×10^2	1.5×10^2
60	3.5×10^4	1.4×10^4	5.4×10^3	1.3×10^3
75	3.8×10^4	1.8×10^4	6.1×10^3	2.8×10^3
90	2.1×10^4	2.3×10^4	6.7×10^3	3.5×10^3

Microbiological Analysis: Total Bacterial Count (TBC) presented in Table 3 shows that at the beginning of the storage period bacterial growths were affected by the radiation. The initial bacterial load of control was maximum (1.3×10^4 cfu/g) followed by 3 kGy irradiated fishes (2×10^2 cfu/g).At 5 and 8 kGy radiation the samples were completely sterilized resulting no bacterial growth. At days 90, this value increased as 2.1×10^5cfu/g in control sample stored at -20°C, 2.3×10^4cfu/g in 3KGy, 6.7×10^3cfu/g

Table 4: Total Mould Count (TBC) in control and irradiated Chinese pomfret, *P. chinensis* during storage period at -20°C

Storage period (Days)	Control (cfu/g)	Irradiated 3 kGy (cfu/g)	Irradiated 5 kGy (cfu/g)	Irradiated 8 kGy (cfu/g)
0	1.1×10^3	2.1×10^2	2×10^2	1.2×10^2
15	1.5×10^3	2.7×10^2	2.5×10^2	1.5×10^2
30	1.5×10^4	4.1×10^3	3.1×10^2	2×10^2
45	1.9×10^4	3.7×10^3	3.5×10^3	2.5×10^3
60	2.3×10^4	1.4×10^3	4×10^3	3.2×10^3
75	2.8×10^4	1.8×10^3	3.5×10^4	2.6×10^4
90	3.1×10^5	2.3×10^3	3.8×10^4	3.5×10^4

Table 5: Organoleptic scores during storage period at-20°C

Storage period	Control	Irradiated 3 kGy	Irradiated 5 kGy	Irradiated 8 kGy
0	8.49	8.74	8.74	9.0
15	8.24	8.41	8.66	8.5
30	7.33	7.66	7.83	8.0
45	6.49	7.08	7.49	8.0
60	4.91	5.91	6.66	7.5
75	3.66	5.00	5.24	7.0
90	3.20	4.00	5.50	6.5

in 5 kGy and 3.5×10^3cfu/g in 8 kGy sample stored at -20°C. Shewan [23] recommended that the microbial limit as 1-10^6 cfu/g of fish flesh for tropical fishes. Hence, TBC values in the present investigation suggest that the irradiated samples remain acceptable after 90 days at -20°C.

In case of total mould count (TMC) it was found that the population increased with the increase of storage period. Table 4 shows that at the end of 90 days observation TMC values were 3.1×10^5, 5.3×10^3, 3.8×10^4 and 3.5×10^4 cfu/g in contol, 3, 5 and 8 kGy treated samples respectively.

Sensory Assessment: The sensory assessment of irradiated and non-irradiated samples was investigated respect of sensory variables such as external appearance, odor, color, texture of the fishes. Average score of initial sensory investigation was 8.48 (Table 5) and it decreased slowly with the progress of storage period. After 90 days of observation at-20°C, the sensory score for control was 3.2 and for irradiated sample it was 4.00 (3 kGy), 5.5 (5 kGy) and 6.5 (8 kGy). The acceptable limit of sensory score being fixed at 5.0, the irradiated pomfret remain acceptable at 5 and 8 kGy after 90 days storage at -20°C.

The current study showed the synergistic effect of two preservation method, food irradiation and freezing in low temperature on *Pampus chinensis* to extend its shelf life. Irradiation at low dose is effective to extend the shelf life of fish and fishery product for a few days but at high dose with low temperature fish can be maintained at highest quality up to a considerable long period.

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