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Effect of Gamma Radiation at -20°C on Microbiological Changes in Wild and Cultured Stinging Catfish, *Heteropneustes fossilis*

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Abstract: Quality and shelf life of non-irradiated (control) and irradiated (1.5 and 3 KGy) stinging catfish (*Heteropneustes fossilis)* stored at -20°C were investigated by microbiological analysis. The main concern was to evaluate the efficiency of gamma radiation as a preservation method in combination with low temperature (-20°C) refrigeration for keeping the quality and shelf-life extension of two types (wild and culture) of degutted stinging catfish for 60 days storage period. Total bacterial count (TBC), total mould count (TMC) and total yeast count (TYC) were obtained higher in wild fish than those of cultured one. Significant differences ($p<0.05$) were observed among treated groups (0, 1.5 and 3 KGy) for total bacterial, mould and yeast counts in both wild and cultured fish meanwhile within storage duration (1, 7, 15, 30, 45 and 60 days), the trend was converse. Significantly higher values ($p<0.05$) for TBC, TMC and TYC were obtained for non-irradiated wild and cultured samples, whereas at 1.5 and 3 KGy the values were significantly lower for both fish sources after 60 days. But during the storage period, all the values were found at slow increasing trend in all treated groups for both wild and cultured fish. On the contrary, total coliform count (TCC) and *Salmonella* were absent in both of them for the entire storage period. This study reveals that gamma radiation in combination with low temperature (-20°C) refrigeration showed maximum shelf-life extension (60 days) in each dose of radiation used but 3 KGy irradiated both wild and cultured *H. fossilis* were found with the best quality.

Key words: Gamma Radiation % Microbiological Changes % Frozen Storage % Wild % Culture % *Heteropneustes Fossilis*

INTRODUCTION

In all seasons and levels of technological progress, fishes have usually played an essential part of human diet. Most people of developing countries are still depending almost entirely on fish as a source of animal protein. Fishes supply more than 58% of the animal protein in the diet of Bangladesh [1]. Bacteria are one of the major organisms contributing to the rapid deterioration of fish quality [2]. When the fish dies, bacteria present on the surface and in the guts multiply rapidly and invade the flesh, which provides an ideal medium for growth and multiplication [3]. However, during handling, fish are likely to pick up more bacteria, from being washed in polluted water, careless gutting, dirty boxes etc. and with careful handling; the numbers can be controlled [4].

The microorganisms present in fish foods are closely connected to the micro flora of the environment [5]. In aquaculture, the micro flora of various fish and invertebrate hosts share ecosystem and as a result becomes easily colonized by bacteria. The duration of bacterial viability in frozen fish tissues or the length of time tissues can remain frozen and still yield viable bacteria upon partial thawing is an area warranting further study. Fish is one of the most perishable and difficult to handle of all foods. Bacterial activity is by far the most important factor influencing fish quality [6]. Microbiological criteria, including analysis methods, are laid down when there is a need to protect public health [7]. Therefore, it is logical to use bacteria numbers as an index of quality [8].

Spoilage due to microbial activity is the main limitation of the shelf life of refrigerated fish. The spoilage

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development in fish is due to a combination of chemical, autolytic and microbiological changes, but the spoilage rate can be reduced by taking different preservation measures, the use of refrigeration to extend the storage life of the fish [9]. Refrigeration inhibits the activity of food spoilage organisms and the low storage temperature greatly slows down the enzymatic and biochemical reactions that normally occur in unfrozen foods [10]. Moreover, refrigeration is characterized with easy application and storage conditions, neither adding nor removing any ingredients for fish meat [11].

Besides refrigeration, ionizing radiation is the only alternative to heat processing for food preservation that has a lethal effect on micro-organisms. Further, it is the only novel method of food preservation suggested for many countries [12]. It has been considered that ionization radiation or energy exerts its lethal effects on bacteria mainly through direct action. With the progress in the radiological research, it become obvious that free radicals and inactive molecules indirectly informed by reaction of the radiation with medium, could also contribute much to the rate of destruction of microbes or bacteria. Inactivation of bacterial cells or loss of their capacity for indefinite reproduction is due to the energy deposition in the critical cell components [13].

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 [14]. So, estimation of bacterial numbers in fish is being used to retrospectively assess microbiological quality as well as to assess the presumptive safety of the fish product for human consumption [15]. The safety of irradiated foods for human consumption has been questioned because ionizing radiation can lead to chemical changes. The wholesomeness of irradiated foods has, therefore, been the subject of considerable national and international researches, which has been reviewed and evaluated by joint expert committees of the International Atomic Energy Agency (IAEA), the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations. These expert groups have uniformly concluded that the food irradiation process does not present any enhanced toxicological, microbiological, or nutritional hazard beyond those brought about by conventional food processing techniques [16]. Hence the objective of this study was to observe the effect of gamma radiation (1.5 and 3.0 KGy) in combination with low temperature refrigeration (-20°C) on microbiological changes in two types (wild and culture) of stinging catfish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Samples Collection and Preparation: In this study, wild and cultured stinging catfish were collected from the local markets and fish hatcheries. The wild fishes are brought to the market from different natural sources (ponds, ditches, swamps and marshes) while different fish hatcheries supply cultured fishes. The wild and cultured catfish were differentiated by their general appearance as the wild fish is usually dark in color, possess elongated and compressed body and small in size whereas the cultured one is usually characterized with light body color, slightly deep body and relatively large in size [17]. Collected fresh fish were collected from the local market and immediately transferred to pre-sterilize polythene bag with ice and carried to the laboratory of Food Processing and Preservation Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy and Research Establishment (AERE), Savar, Dhaka, Bangladesh for microbiological analysis. The entire samples were randomly taken for preservation purposes under different techniques. To do this, total numbers of fish from the lot were beheaded and degutted and detailed. Then the dressed samples were washed with tap water and drained. Therefore, the remained whole body of the fishes were taken into the following 6 sample groups containing 10 fishes in each group for the wild (A, C, E) and cultured (B, D, F) sing fish.

Sample A: Control Sample C: 1.5 KGy Sample E: 3.0 KGy
Sample B: Control Sample D: 1.5 KGy Sample F: 3.0 KGy

Gamma Irradiation: Except the samples A and B, the other samples were subjected to irradiate in a 50,000 curie Co-60 source. In this experiment, the applied doses were 1.5 and 3.0 KGy and maintained at 2±1°C during irradiation.

Storage Conditions: After irradiation, the non-irradiated and irradiated samples were stored at -20°C in polypropylene bags. The storage duration for all samples (non-irradiated and irradiated) lasted 60 days and samples from each six lots were taken at 1, 7, 15, 30, 45 and 60 days of storage periods for organoleptic and chemical analysis.

Microbiological Analysis: The microbial changes were estimated by total bacteriological count technique following Wittfogel [18]. In present investigation, the changes in total bacteria and mould count were estimated by the pour plate technique in petri dishes.

Selection of Suitable Media: Dehydrated nutrient agar (Difco) was used as the media for the bacterial growth at the ratio of 2.3 g per 100 ml of water, potato dextrose agar media at the ratio of 3.1 g per 100 ml of water for the mould growth and yeast extract media at the ratio of 4.6 per 100 ml of water for yeast growth.

Sterilization of Media and Glassware: The cleaned petri dishes were sterilized in the thermal oven (D-78532, Germany) at 160°C temperature for 3 hours. All the glassware and media were sterilized by autoclave (OSK-8870, Japan) at 15Ibs pressure for 20 minutes at 121°C temperature before the experiment started.

Preparation of Homogenized Fish Sample: 2 g of raw fish was taken, homogenized and then transferred to 50 ml distilled water contained in a conical flask and made uniform by shaking. Then 1 ml sample was diluted stepwise through test tube containing 9 ml of distilled water and shacked with vortex mixture for uniform solution.

Plating Procedures: 1 ml of homogenized samples was poured into petri dishes with micropipette. Sterilization media of conical flask was then poured in petri dishes and shacked horizontally to spread out the sample uniformly over the media. The lids of the petri dishes kept few minutes partially closed for solidification of the media. The petri dishes were placed in inverted position at 37°C in an incubator.

Counting Method: Colonies that developed on the plates after incubation for 24 and 48 hours were counted with the help of Stuart colony counter. The number of bacterial colony per gram of the sample was obtained by multiplying the number of colonies on the dish dilution factor. The count was expressed as colony forming unit (cfu) per gram.

Statistical Analysis: Statistical analysis was executed with the SPSS software package (verson11.5, SAS Institute Inc, USA). Multiple comparisons was taken by Tukey's HSD post hoc to present the data as mean±SEM considering the level of significance ($p<0.05$) between different treatments and days of storage.

RESULTS AND DISCUSSION

Total bacterial count (TBC) during different storage period showed in Figs. 1a & b for wild and cultured *H. fossilis*, respectively illustrate that at the beginning of 60 days storage period bacterial growth were affected by the radiation. TBC for non-irradiated wild samples showed significant differences (Tables 1 and 2) within all storage duration whereas within treatment, all counts were significantly different ($p<0.05$). Cultured sing also exhibited similar trends.

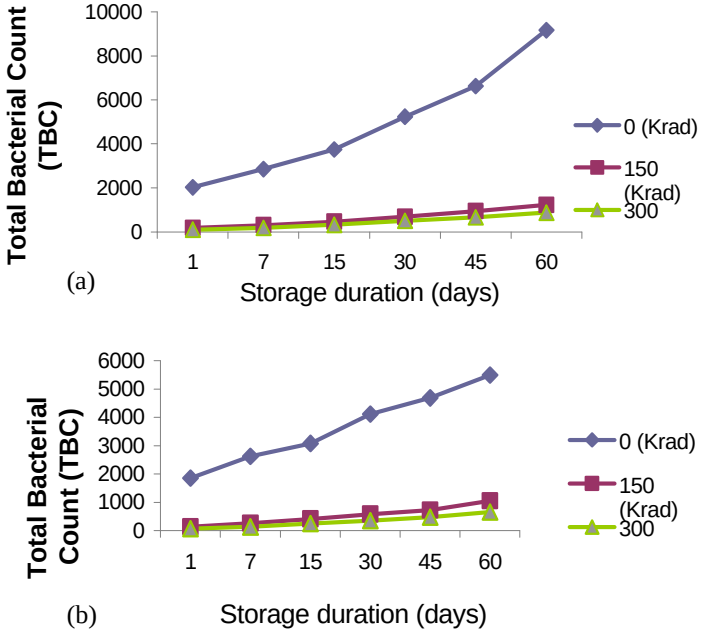

Fig. 1: Total bacterial load (cfugG[1]) in control and irradiated (150 and 300 Krad) (a) wild (b) cultured *H. fossilis* during different storage periods at-20°C.

Table 1: Microbial load with different radiation doses at different storage duration in wild stinging catfish.

Microbial Flora	Storage Duration (Days)	Radiation Dose (kGy) 0	1.5	3
Total Bacterial Count (TBC)	1	$2.03\pm27.51 \times 10^{3f}$	$1.84\pm25.46 \times 10^{2e}$	$1.00\pm13.92 \times 10^{2e}$
	7	$2.86\pm44.74 \times 10^{3e}$	$3.07\pm31.54 \times 10^{2d}$	$1.92\pm17.71 \times 10^{2d}$
	15	$3.74\pm41.63 \times 10^{3d}$	$4.73\pm22.62 \times 10^{2c}$	$3.23\pm33.27 \times 10^{2c}$
	30	$5.23\pm57.41 \times 10^{3c}$	$6.85\pm43.10 \times 10^{2bc}$	$5.08\pm22.53 \times 10^{2b}$
	45	$6.62\pm41.15 \times 10^{3b}$	$9.43\pm86.44 \times 10^{2ab}$	$6.62\pm14.47 \times 10^{2ab}$
	60	$9.17\pm63.53 \times 10^{3a}$	$1.23\pm26.24 \times 10^{3a}$	$6.62\pm14.47 \times 10^{2a}$
Total Mould Count (TMC)	1	$1.51\pm14.57 \times 10^{2f}$	$1.05\pm6.44 \times 10^{2f}$	$6.83\pm8.95 \times 10^{1d}$
	7	$2.25\pm9.29 \times 10^{2e}$	$1.56\pm6.81 \times 10^{2e}$	$1.06\pm13.42 \times 10^{2cd}$
	15	$3.52\pm14.11 \times 10^{2d}$	$2.46\pm20.31 \times 10^{2d}$	$1.59\pm19.50 \times 10^{2c}$
	30	$5.01\pm6.93 \times 10^{2c}$	$3.96\pm5.79 \times 10^{2c}$	$2.56\pm14.72 \times 10^{2b}$
	45	$6.52\pm10.73 \times 10^{2b}$	$5.00\pm6.39 \times 10^{2b}$	$4.07\pm13.23 \times 10^{2a}$
	60	$8.17\pm13.80 \times 10^{2a}$	$6.76\pm9.39 \times 10^{2a}$	$5.33\pm10.10 \times 10^{2a}$
Total Yeast Count (TYC)	1	$8.23\pm7.62 \times 10^{1e}$	$6.40\pm9.87 \times 10^{1d}$	$3.33\pm5.21 \times 10^{1d}$
	7	$1.72\pm10.58 \times 10^{2d}$	$1.27\pm9.24 \times 10^{2c}$	$8.56\pm7.22 \times 10^{1c}$
	15	$2.67\pm10.98 \times 10^{2c}$	$1.72\pm13.61 \times 10^{2c}$	$1.06\pm5.29 \times 10^{2c}$
	30	$3.87\pm8.67 \times 10^{2b}$	$2.72\pm13.69 \times 10^{2b}$	$1.87\pm14.13 \times 10^{2b}$
	45	$4.80\pm23.86 \times 10^{2b}$	$3.70\pm9.87 \times 10^{2ab}$	$2.50\pm9.26 \times 10^{2ab}$
	60	$6.84\pm12.24 \times 10^{2a}$	$5.06\pm15.76 \times 10^{2a}$	$3.29\pm8.63 \times 10^{2a}$
Total Coliform Count (TCC)	1	ND*	ND	ND
	7	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	ND	ND	ND
Salmonella Count	1	ND	ND	ND
	7	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	ND	ND	ND

Different superscript letters within the same row show significant difference ($p<0.05$) within the treatments. *ND = Not Defined

Table 2: Microbial load with different radiation doses at different storage duration in cultured stinging catfish

Microbial Flora	Storage duration (Days)	Radiation Dose (kGy) 0	1.5	3
Total Bacterial Count (TBC)	1	$1.85\pm19.00 \times 10^{3f}$	$1.43\pm15.72 \times 10^{2e}$	$7.00\pm8.72 \times 10^{1e}$
	7	$2.61\pm54.12 \times 10^{3e}$	$2.67\pm17.44 \times 10^{2d}$	$1.36\pm15.88 \times 10^{2d}$
	15	$3.08\pm34.26 \times 10^{3d}$	$4.13\pm13.78 \times 10^{2c}$	$2.57\pm13.96 \times 10^{2c}$
	30	$4.11\pm38.93 \times 10^{3c}$	$5.86\pm11.39 \times 10^{2b}$	$3.48\pm34.61 \times 10^{2bc}$
	45	$4.68\pm26.91 \times 10^{3b}$	$7.22\pm18.59 \times 10^{2b}$	$4.82\pm32.22 \times 10^{2ab}$
	60	$5.49\pm44.82 \times 10^{3a}$	$1.05\pm13.30 \times 10^{3a}$	$6.56\pm17.44 \times 10^{2a}$
Total Mould Count (TMC)	1	$7.17\pm4.10 \times 10^{1e}$	$4.47\pm4.81 \times 10^{1e}$	$2.83\pm2.90 \times 10^{1e}$
	7	$1.58\pm8.15 \times 10^{2d}$	$9.77\pm7.22 \times 10^{1d}$	$6.60\pm5.57 \times 10^{1d}$
	15	$2.71\pm15.81 \times 10^{2c}$	$1.58\pm6.81 \times 10^{2c}$	$1.10\pm11.89 \times 10^{2c}$
	30	$3.52\pm10.04 \times 10^{2b}$	$2.52\pm8.21 \times 10^{2b}$	$1.87\pm6.77 \times 10^{2b}$
	45	$4.02\pm7.00 \times 10^{2b}$	$3.15\pm9.82 \times 10^{2ab}$	$2.42\pm11.57 \times 10^{2ab}$
	60	$4.63\pm11.59 \times 10^{2a}$	$3.68\pm10.31 \times 10^{2a}$	$2.90\pm11.72 \times 10^{2a}$
Total Yeast Count (TYC)	1	$5.47\pm7.62 \times 10^{2e}$	$2.83\pm3.18 \times 10^{2e}$	$2.67\pm4.49 \times 10^{2e}$
	7	$1.23\pm11.73 \times 10^{2d}$	$7.47\pm4.98 \times 10^{2d}$	$5.20\pm3.60 \times 10^{2d}$
	15	$1.67\pm16.82 \times 10^{2d}$	$1.30\pm14.01 \times 10^{2c}$	$9.47\pm7.06 \times 10^{2c}$
	30	$2.52\pm11.14 \times 10^{2c}$	$1.92\pm7.23 \times 10^{2b}$	$1.43\pm6.64 \times 10^{2bc}$
	45	$3.68\pm14.75 \times 10^{2b}$	$2.79\pm7.79 \times 10^{2a}$	$1.93\pm13.23 \times 10^{2ab}$
	60	$5.17\pm21.49 \times 10^{2a}$	$3.45\pm13.35 \times 10^{2a}$	$2.63\pm14.44 \times 10^{2a}$
Total Coliform Count (TCC)	1	ND	ND	ND
	7	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	ND	ND	ND
Salmonella Count	1	ND	ND	ND
	7	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	ND	ND	ND

Different superscript letters within the same row show significant difference ($p<0.05$) within the treatments. *ND = Not Defined

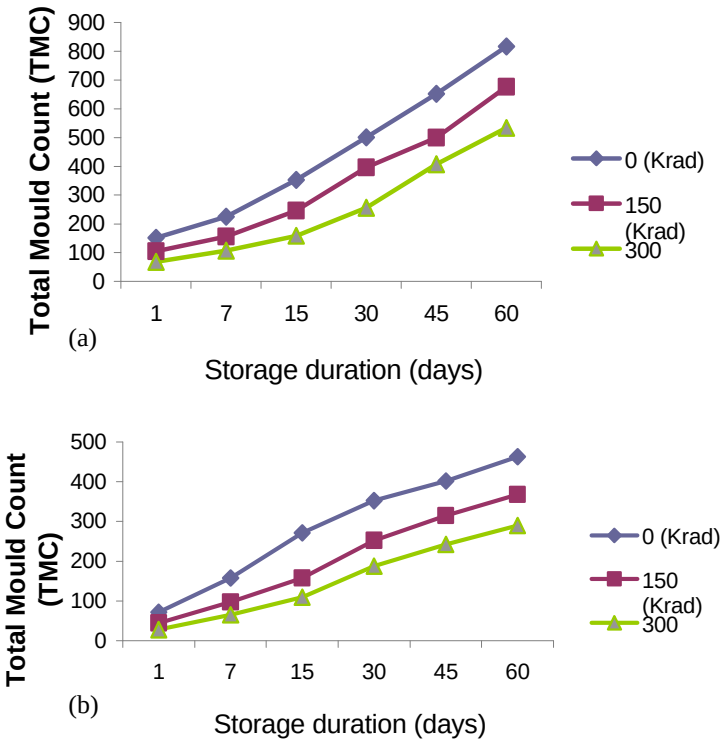

Fig. 2: Total mould level (cfug^{-1}) in control and irradiated (150 and 300 Krad) (a) wild (b) cultured sing during 60 days storage periods at-20°C.

Ahmed *et al*. [19] observed that the bacterial load increased gradually to 2.1×10^4, 2.3×10^4, 6.7×10^3 and 3.5×10^3 cfu/g from an initial count of 1.3×10^4, 2.1×10^2, 0 and 0 cfu/g in control, 3, 5 and 8 kGy treated Chinese pomfret (*Pampus chinensis*) under -20°C frozen storage of 90 days. Rashid *et al*. [20] reported that the TBC of rita (*Rita rita*) fish kebab was initially 4.6×10^3, 7.5×10^1 and < 10 cfu/g for control, 2.5 and 5.0 KGy samples, respectively and after 60 days of storage at ambient temperature, the counts reached to 5.2×10^8 cfu/g (Control), 1.0×10^6 cfu/g (2.5 KGy) and 1.9×10^4 cfu/g (5.0 Kgy).

Besides, Mahin *et al*. [21] stated that both of high radiation dose (2.5 and 5.0 KGy) reduced TBC by 3 logarithmic cycles for mola (*Amblypharyngodon mola*) at -20°C for 6 months. Khan *et al*. [22] reported that total bacterial count went up gradually from 3.30×10^4 to 7.18×10^6 cfu/g and 3.54×10^4 to 9.91×10^6 cfu/g for unirradiated *Pangasius Pangasius* and *Pangasius sutchi* and 3.30×10^4 to 4.99×10^5 cfu/g and 3.54×10^4 to 5.50×10^5 cfu/g for irradiated (150 Krad) *P. Pangasius* and *P. sutchi* within the storage period of 35 days at 0°C. Shewan [23] recommended the microbial limit as $1\text{-}10^6$ cfu/g for tropical fishes. Therefore, TBC values in the present study confirmed that irradiated samples remained acceptable up to 60 days of storage period. It was also revealed in the study that bacterial count was far higher in wild variety than that of culture variety (Figs. 1a & b).

Figs. 2a & b demonstrate the initial total mould count (TMC) of wild and cultured sing showing the reduction of mould population with the increase of radiation dose. Significant differences ($p<0.05$) were observed among duration of preservations for control and 150 Krad treated wild samples whereas no similarities were demonstrated by TMC among treatment groups. Cultured sing followed the trend only among treatment groups.

Total yeast count (TYC) for wild and cultured sing are presented in Figs. 3a & b, respectively it was evident that the population increased with the increase of storage period. TYC demonstrated similarities in some storage duration counts for both types of sing all treatment groups were significantly different ($p<0.05$) in wild and cultured sing.

Ahmed *et al*. [19] determined increasing trend of TMC from 1.1×10^3 to 3.1×10^5 cfu/g in control, 2.1×10^2 to 2.3×10^3 cfu/g in 3 KGy, 2×10^2 to 3.8×10^4 cfu/g in 5 KGy and 1.2×10^2 to 3.5×10^4 cfu/g in 8 KGy treated *P. chinensis*, respectively. Kazanas and Emerson [24] reported that yeasts and moulds were reduced by 0.1 Megarad irradiation from approximately 3.3×10^3 to 8×10^2 organisms per g but increased to 8×10^4 after 15 days and

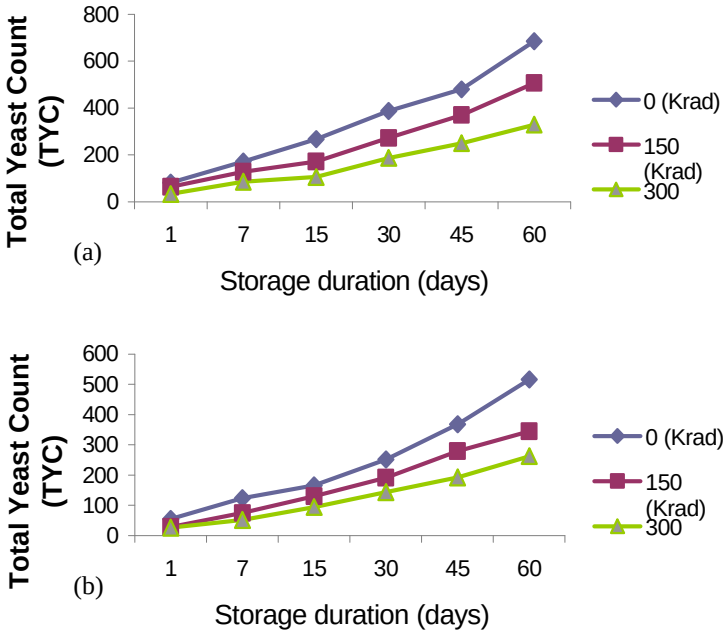

Fig. 3: Variation in TYC (cfug^{-1}) in control and irradiated (150 and 300 Krad) (a) wild (b) cultured *H. fossilis* during different storage periods at-20°C.

subsequently decreased to approximately the original count after 23 days of storage at 1°C in yellow perch (*Perca flavescens*) fillet. Prachasitthisak *et al*. [25] did a study on the improvement of microbiological quality of Pla-ra (Fermented Fish) by gamma radiation and found total yeast and moulds count reduced to <10 cfu/g in 10 KGy treated samples from an initial count of 3.0×10^3 cfu/g in control samples.

During 60 days of storage period, total coliform count (TCC) was absent in controlled and all treated samples of both types of sing (Tables 1 & 2). Oraei *et al*. [26] recorded that TCC were not detected in all irradiated (1, 3 and 5 kGy) and control rainbow trout (*Oncorhynchus mykiss*) fillet throughout the storage period of 5 months at -20°C. Mahin *et al*. [21] stated that no viable coliform was found when mola (*Amblypharyngodon mola*) fish samples were treated with 2.5 KGy or more even after 6 month's storage at -20°C. Hossain *et al*. [27] stated that coliform count increased from 30×10^3 to 21×10^6 cfu/g during 9 weeks of 5°C temperature storage while the count was absent in 150 Krad treated hilsha (*Hilsha ilisha*) samples during the whole storage period. The current investigation revealed that the entire sample was acceptable during whole investigation period as consistent with International Commission on Microbiological Specifications for Foods (ICMSF) [28] guideline; acceptable total coliform count for fish is less than 10^5 cfu/g.

Salmonella was absent in all samples including one control and two irradiated samples during the whole investigation period (Tables 1 & 2). Oraei *et al*. [26] recorded that *Salmonella* were not detected in all irradiated (1, 3 and 5 kGy) and control rainbow trout (*O. mykiss*) fillet throughout the storage period of 5 months at -20°C. Hossain *et al*. [27] stated that *salmonella* was absent in all (control and 150 Krad) hilsha (*H. ilisha*) fish samples during 9 weeks of 5°C temperature storage. According to ICMSF [28] guideline, acceptable *Salmonella* for fish is absent per 25g of sample. So it is unveiled that all the samples were acceptable during whole storage period.

Fishes having highly perishable protein content in body are very much susceptible to be contaminated with different bacteria. Besides, the sub-tropical environment might also be the crucial reason for bacterial contamination and the presence of different types of bacteria in fishes might be the reasons to lose their exportability as well as acceptability [29]. Moreover, virtually aquatic habitats of Bangladesh are heavily contaminated with fecal coliform bacteria. So fishes living in these polluted habitats thus can easily intake these bacteria during feeding along with contaminated aquatic foods [30].

The present investigation ascertained that gamma radiation in combination with low temperature refrigeration effectively extended the self-life of wild and

cultured stinging catfish due to the synergistic effect of the combination of two preservation methods. Present investigation revealed that 3 KGy irradiated wild and cultured fish samples gave the best result. Finally, the effect of gamma radiation during frozen storage was significantly effective in reducing TBC than total mould and yeast count, which proves the necessity of gamma radiation in addition to frozen preservation. So, this combined treatment can be applied for large scale preservation of sing and any other catfish in Bangladesh for long time preservation without any significant loss of texture and nutritive loss.

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