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Quality and Shelf Life Extension of Freshwater Endangered Kalibaush, *Labeo calbasu* (Hamilton-Buchanan, 1822) by Combined Treatment of Gamma Radiation and Low Temperature

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Abstract: With the purpose of determining the effect of gamma radiation in combination with low temperature (-20°C) on quality and shelf life extension of *Labeo calbasu* fish, microbiological changes considering Total Bacterial Count (TBC), Total Yeast Count (TYC), Total Mould Count (TMC), Coliform and Salmonella in irradiated and non-irradiated (control) samples were observed at regular 15 days interval for up to 120 days. The total bacterial count increased to 3.1×10^3 cfu/g for control sample whereas lower count of 1.9×10^3 cfu/g and 6×10^2 cfu/g were observed at a dose of 5 kGy and 10 kGy respectively, after 120 days of storage. The total yeast count was initially higher which reached to a value of 1×10^2 cfu/g after ongoing decrease of 120 days storage. No yeast colony was found after 30 days at 5 kGy. However, a negligible amount of yeast colony was noticed only at the first observation of 10 kGy. The mould colony in control sample dropped from 4×10^2 cfu/g to 0.5×10^2 cfu/g after 120 days storage. However, the mould colony was completely disappeared after 15 days at 5 kGy and similar result was reported less than 15 days at 10 kGy. Overall, total bacterial count, total yeast count and total mould count for irradiated samples were significantly lower ($p<0.05$) compared to non-irradiated fish samples. No Coli form or Salmonella were noticed throughout the study period. This research concluded that combined treatment of gamma radiation with low temperature can aid in extending shelf-life in both doses of radiation. However, in 10 kGy dose, *Labeo calbasu* showed the best quality of shelf life.

Key words: Shelf life · Gamma radiation · Low temperature · *Labeo calbasu* · Microbiological properties

INTRODUCTION

Labeo calbasu, a fish of Cyprinidae family is an important source of animal protein. It has also a great commercial importance because of consumer preference, tastiness and its adaptability to a wide range of environments [1]. It is distributed in many countries namely Bangladesh, Pakistan, India, Myanmar, Thailand and China [2]. However, the number of its population has seriously been decreased in nature due to overfishing, degradation of fish habitat, aquatic pollution, dam construction and natural causes [1]. *L. calbasu* is now considered as the endangered species from the biodiversity point of view [3]. Moreover, there has been a decline in the quantity of fish reached to market due to spoilage by bacterial contamination, enzymatic action or by combination of these during handling, processing and transportation. Spoilage of fish occurs due to the action of bacteria, moulds and enzymes present in the fish [4]. About 4 % of the catch equivalent 4.25 million tons is wasted and never reached the market [5-7].

The spoilage of Kalibaus has effects on country's economy as mass people depend on fish to meet their protein demands. In Bangladesh, a very toxic chemical, formaldehyde, is commonly used for preserving fish without knowing about the health hazards of

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formaldehyde consumption with fish [8]. Continuous ingestion of fish with formaldehyde, can lead to cancer and a variety of unknown pathology [9, 10]. Therefore, it is undoubtedly important to supply the quality fish to the market to prevent health and economic loss. As a result, there is an obvious need for development of new technologies and efficient fish preservation methods which permit shelf life extension of these products.

Preservation methods are designed to inhibit the growth of organisms. It kills the organisms and minimizes the changes in texture, taste and appearance in fish and fishery products [11]. Many researchers utilized traditional methods for fish preservation such as ice storage, freezing, rapid chilling, smoking and heating [12-14]. Beside traditional methods, other methods involving the use of organic acids, antimicrobials [15, 16] edible coating [17], antioxidants [18], modified atmosphere packaging [19] and ionizing radiation [20-22] have been proposed to extend the shelf life of fish and fisheries products.

Refrigeration has inhibitory effect for microbial activity. It greatly slows down the enzymatic and biochemical reactions [23]. Moreover, application and storage conditions of refrigeration are easy to follow [24]. However, preservation at -20°C does not kill the microorganisms but reduces microbial metabolism which is responsible for spoilage [25].

Irradiation is an effective and widely accepted food processing technique. This process has no effect on food taste, color and smell. It does not leave any radioactive residue [26]. Irradiation at a dose of up to 10 kGy has been used as an effective, safe and economical method of food preservation without posing nutritional, toxicological or microbiological problems [27].

Among all methods of food preservation, only rarely is a single method effective and usually several or combined [28]. Combination of treatments for food preservation may result in a synergistic or cumulative effect of microbiological barriers or hurdles, leading to a reduced level of one or all the treatments [29]. The effects of combination of low temperature and gamma radiation (5 kGy and 10 kGy) in extending the shelf life of *Labeo calbasu* had been practiced in the present investigation.

MATERIALS AND METHODS

Sample Collection: Fresh *Labeo calbasu* fish was collected early in the morning from a local market, Islampur Bazar, Dhaka, Bangladesh. Collected fish were taken in a polythene bag with ice. It was then transferred to the laboratory of Food Technology Division, IFRB, AERE, Savar, Bangladesh. Figure 1 shows the collected sample of a *Labeo calbasu*. The fish was washed by tap water, skinned, beheaded, be tailed and degutted. The sample was cut into small pieces and washed with tap water again.

Fig.1: Experimental Sample (Labeo calbasu)

Fig. 2(a): Sample treated with 5 kGy

Fig. 2(b): Sample treated with 10 kGy

Gamma Irradiation: Collected fish samples was divided into three lots and packaged into three different polypropylene polythene bags. Two packets were then brought under radiation at a dose of 5 kGy and 10 kGy in a 50,000-curie ^{60}Co (Cobalt ^{60}) source with a dose rate of 3 kGy/hour supplied by the Atomic Energy of Canada Ltd. The third packet remained untreated and named control. Both control and irradiated samples were stored at -20°C temperature and microbiological analysis was carried out at 15 days of interval up to 120 days. Figure 2 (a and b) shows the sample specimens.

Microbiological Analysis: Microbiological analysis was conducted by pour plating technique [30]. Total Bacterial Count (TBC), Total Yeast Count (TYC), Total Mould Count (TMC), Total Coliform Count (TCC) and Total Salmonella Count were completed by pour plate technique in Petri dishes.

Selection of Suitable Media: Dehydrated nutrient agar (Difco) was used as the media for the bacterial growth at 2.3 g per 100 ml of water. Difco is a compound of peptone, yeast and agar. Potato dextrose agar media was applied for the mould growth at the ratio of 3.1 g per 100 ml of water and for yeast growth at the ratio of 4.6 g per 100 ml of water. For Coliform and *Salmonella*, violet red bile agar (VRBA) and Lactose broth were utilized deferentially.

Sterilization of Media and Glassware: The cleaned Petri dishes (two Petri dishes for each count) were sterilized in the oven (Grieve, Eocene Thermal oven) at 160°C temperature for 3 hours. All glassware such as conical flask containing media, conical flask with distilled water; micropipettes were sterilized by autoclave (OSK-8870, OWAGA SEIKI Co. Ltd, Japan) at 15 lbs pressure for 20 minutes at 121°C temperature.

Preparation of Homogenized Fish Sample: One gram of raw fish was taken from irradiated (5 kGy and 10 kGy) and control fish samples respectively to prepare homogenized fish samples. Each sample was separately grinded in a mortar and pastel. Grinded sample was then dissolved with 100 ml of distilled water in a conical flask and prepared a uniform solution by shaking.

Plating Procedure: Two Petri dishes were used for preparing experimental plate for each microbiological count. Plate for each sample was prepared by using 1ml of homogenized fish sample with a small amount of sterilized media. Plates were shaken horizontally to disperse the sample uniformly over the media. For solidification of media, lids of the Petri dishes were kept partially closed for few minutes. After cooling of media, Petri dishes were covered by lids. The Petri dishes were then placed in inverted position in an incubator. All the operations were carried out aseptically in a laminar air cabinet.

Counting Method: Colonies were formed on the plates after incubation. Colonies were counted by using a colony counter (Stuart Scientific Counter- S.S Co. Ltd.). Bacterial counts were done after 24 to 48 hours of incubation whereas other plates were counted after 72 to 96 hours of incubation.

Statistical Analysis: Standard methods were followed to carry out the statistical analysis. Analysis of variance (ANOVA) was performed to evaluate significance between different treatments and days of storage at 95% level of significance (*$p < 0.05$).

RESULTS AND DISCUSSION

Total Bacterial Count (TBC): Figure 3 shows that the control sample had initial bacterial count of 2.25×10^2 cfu/g which gradually increased to 3.1×10^3 cfu/g after 120 days of storage on ice while the counts at 5 kGy and 10 kGy raised from 1×10^2 cfu/g to 1.9×10^3 cfu/g and 1×10^2 cfu/g to 6.0×10^2 cfu/g respectively. This represents the bacterial count in irradiated samples (5 kGy and 10 kGy) were significantly ($p<0.05$) lower than that of control sample and the count decreased with the increase of radiation dose. The combined treatment of radiation and frozen storage had significant effect on the reduction of bacterial load and shelf life extension of *Labeo calbasu.*

A study on Mola (*Amblypharyngodon mola*) was conducted by Mahin *et al*. [31]. They reported that non-irradiated samples stored at -20°C showed a negligible change ($9.7x10^7$ to $5.6\ x10^6$). Their investigation also reported that for both the radiation dose at 2.5 kGy and 5.0 kGy reduced TVBC count by 3 logarithmic cycles without low temperature treatment. However, when irradiated samples were stored at low temperature (-20°C), TVBC counts were further reduced by 1 ($1.3x10^4$ to $1.3x10^3$) and 3 ($1.1x10^4$ to $6.7x10^1$) logarithm cycle respectively after 6 months. Interestingly, radiation dose at 7.5 kGy or more was completely eliminated TVBC in the samples without low temperature treatment. Moreover, when these samples were kept at low temperature, no bacterial growth was found until the end of the study period [31]. Irradiation of the fillets to 0.1 Mrad reduced the initial total plate count from an average of $1.1x10^6$ to approximately $4.2x10^4$ bacteria per g and irradiation to 0.2 Mrad produced a reduction from an average of $1.1x10^6$ to approximately $9.6x10^2$ [32].

The effect of gamma radiation in combination with low temperature (-20°C) on *Pampus chinensis* was evaluated by Ahmed *et al.* [33]. This study revealed that after 90 days of storage the bacterial load of control and 3 kGy increased (1.3×10^4 cfu/g to 2.1×10^5cfu/g) and (2×10^2 cfu/g to 2.3×10^4 cfu/g). With 5 kGy and 8 kGy radiations, the samples were completely sterilized and finally reached to 6.7×10^3 cfu/g and 3.5×10^3 cfu/g respectively. It was investigated that the TBC of Rita (*Rita rita*) fish kebab was initially 4.6×10^3cfu/g, 7.5×10^1 cfu/g and <10 cfu/g for control, 2.5 kGy and 5.0 kGy samples respectively [34].

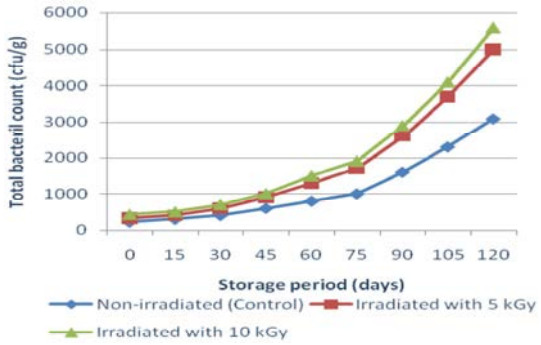

Fig. 3: Changes of total bacterial count (cfug^{-1}) in control and irradiated (5 kGy and 10 kGy) Labeo calbasu stored at -20°C temperature

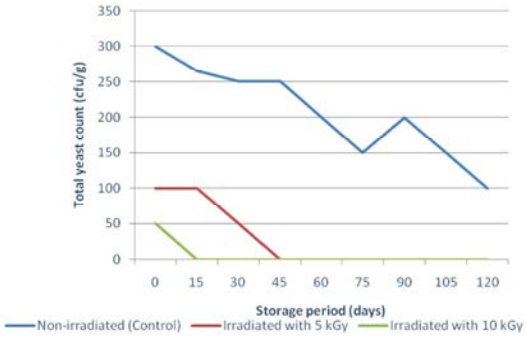

Fig. 4: Changes of total yeast count (cfug^{-1}) in control and irradiated (5 kGy and 10 kGy) Labeo calbasu stored at -20°C temperature

The counts reached to a value of 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) after 60 days of storage at ambient temperature [34].

Irradiation has a significant effect on bacterial count [35]. Total bacterial count 1.0×10^6 cfu/g was considered as maximum allowable limit [36]. Based on this limit, all the samples stored at -20°C were of acceptable up to 120 days.

Total Yeast Count (TYC): Figure 4 depicts the changes of total yeast count in control, 5 kGy and 10 kGy during 120 days of storage of *Labeo calbasu* at -20°C. The maximum yeast colony was found in control sample followed by 5 kGy and 10 kGy samples. The initial colony-forming unit in control sample was 3×10^2 per g which reached to a value of 1×10^2 cfu/g after ongoing decrease of 120 days storage.

The initial yeast colony in 5 kGy was 1×10^2 which decreased into half at 30 days of storage and from the days onward no yeasts colony was observed. Total yeast count decreased with the increase of storage period as well as with the increase of radiation dose. No Yeast colony was found at 10 kGy throughout the storage period with the exception of zero (0) days' observation. The yeast colony was 0.5×10^2 cfu/g during the zero days' observation.

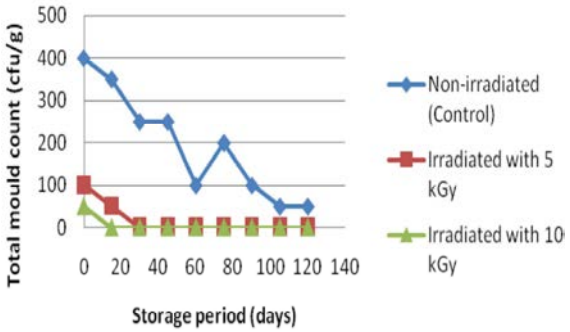

Fig. 5: Changes of total mould count (cfug^{-1}) in control and irradiated (5 kGy and 10 kGy) Labeo calbasu stored at -20°C temperature

The total yeast and moulds were reduced at the samples irradiated to 0.1 Mrad irradiation from approximately 3.3×10^3 to 8×10^2 per g but increased to 8×10^4 after 15 days and subsequently decreased to approximately the original count after 23 days of storage at 1°C in Yellow perch (*Perca flavescens*) [32]. Yeast and moulds were not recovered from the fillets until the 6th day of storage and remained at low levels throughout the entire storage of 23 days in the samples irradiated to 0.2 Mrad.

Wild and cultured stinging catfish was studied for investigating the effect of irradiation at 1.5 kGy and 3 kGy by Nur-A-Sayed *et al*. [37]. This study reported that total yeast count increased with the increase of storage period and the counts went up to 6.85×10^2 cfu/g (control), 5.06×10^2 cfu/g (1.5 kGy) and 3.29×10^2 cfu/g (3 kGy) in wild catfish whereas in cultured the counts climbed up to 5.17×10^2 cfu/g (control), 3.45×10^2 cfu/g (1.5 kGy) and 2.63×10^2 cfu/g (3 kGy) after 60 days storage.

Total Mould Count (TMC): The mould colony in control sample dropped from 4×10^2 cfu/g to 1×10 cfu/g after 60 days of storage at -20°C temperature (Figure 5).

After that, with a sharp increase to 2×10^2 cfu/g at 75 days. However, the moulds colony declined in growth and reached to 0.5×10^2 cfu/g after 120 days storage. No mould colony was revealed after 15 days in samples treated with 5 kGy and 10 kGy. Control sample showed irregular growth whereas in irradiated samples the moulds colony completely disappeared 15 days early with the

increase of radiation dose. The initial mould count in the present study was identical with the findings of Vinh *et al*. [38]. They found the initial mould count ranged from 27-1500 cfu/100 g in several varieties of semi-dried non-irradiated and irradiated (1 kGy and 3 kGy) fish.

A study by Kamrujjaman *et al*. [39], reported that 2.0×10^2 cfu/100 g TMC in initial condition of cichlid which range was less than the present findings in control sample and higher than that the sample of 5 kGy and 10 kGy. The total mould count (TMC) increased with the increase of storage period and TMC values were 3.1×10^5, 5.3×10^3, 3.8×10^4 and 3.5×10^4 cfu/g in control, 3, 5 and 8 kGy treated samples respectively, at the end of 90 days observation [33]. Moulds are sensitive to radiation process because of their large genomic structure [40]. Therefore, it is obvious from the above findings that irradiation in combination with low temperature (-20°C) is effective in reducing the total mould count and higher dose had more inhibitory effect. The findings confirm that the treatment of radiation (5 kGy and 10 kGy) was statistically significant at 5% level of significance on the elimination of mould count.

Total Coliform Count (TCC) And Salmonella Count: The total Coliform and Salmonella were absent in all irradiated (5 kGy and 10 kGy) and control samples throughout the storage at -20°C temperature. This result is in agreement with the findings of Motalebi *et al*. [41] and Nur-A-Sayed *et al*. [37]. The count of Coliform at irradiated samples corresponded to the findings of Mahin *et al.* [31] who 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. However, they found a very negligible change (6.40×10^5 to 5.2 $\times$105 cfu g^{-1}) in Total Coliform Count (TCC) in non-irradiated samples stored for 6 months.

A study on the shelf life extension of Hilsha fish (*Hilsha ilisha*) by combined treatment of radiation and salt was performed by Hossain *et al*. [42]. They found that Coliform count increased from 30×10^3 cfu g^{-1} to 21×10^6 cfu g^{-1} while the count was absent in 150 Krad treated samples during 9 weeks of 5°C temperature storage and Salmonella was absent in all Hilsha fish samples during the whole storage period.

Salmonella and Coliform, these gram-negative bacteria have a very low resistance to radiation [41]. The lowest temperature for growth of Salmonella in foods is 6.7°C [43] whereas the minimum temperature of growth of Coliform on meat may be taken as 8°C [44]. According to ICMSF [45] guideline, acceptable total Coliform count for fish is less than 10^5 cfu g^{-1} and acceptable Salmonella for fish is absent per 25 g of sample. Therefore, it is undisputed that the entire samples were acceptable during whole investigation period. The present study indicated that combined treatment was much more effective than individual treatment of either irradiation or low temperature and increased the shelf life of *Labeo calbasu* due to synergistic effect of two preservation methods. This method can be used in a large scale for long-term preservation of Kalibaush fish as well as other local fish in Bangladesh.

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