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Effects of citric acid and gamma radiation on the shelf life of *Labeo bata* (Hamilton, 1822)

Kamrunnahar, Md. Enamul Haque*, Arzina Hossain[1], Mahfuza Islam[1], Md. Kamruzzaman Munshi[1], Khandoker Asaduzzaman[2] and Roksana Huque[1]
Department of Zoology, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

The effects of citric acid (2%) and gamma radiation (1.0 and 1.5 kGy) on the quality and shelf life extension of Bata, *Labeo bata* (Hamilton, 1822) stored at refrigerated temperature (4°C) for 28 days were examined. Quality assessment were made by sensory (organoleptic scores), chemical (TV) and microbiological (TBC and TCC) evaluations. Organoleptic evaluation showed that irradiated samples were more acceptable than control and citric acid treated samples which also remained acceptable upto 28 days. TV of fish muscles increased with the increase of storage periods in all the samples. The rate of increase was lower in citric acid treated samples and the lowest was observed in samples with 1.5 kGy irradiation. Irradiated samples showed the best results in microbiological assessment. Gamma radiation in combination with low temperature (4°C) could increase the shelf life, and this technique may be applied for other fish species preservation.

Key words: *Labeo bata*, citric acid, gamma radiation, shelf life.

INTRODUCTION

Fishes are one of the cheapest sources of animal protein and other essential nutrients required in human diets (Ghose, 2014). However, after harvesting and/or landing the catches pass through different marketing channels, and it often takes a little attention to extend the normal shelf life of a tropical fish species (Chakraborty *et al*., 2012) because of ignorance and negligence of the people involved in different stages from the harvest to retail distribution. Moreover, fishes are extremely perishable foodstuff and easily damaged due to its high water content. Spoilage begins as soon as the fish dies. A huge amount of captured fishes are spoiled due to the lack of proper preservation techniques which in turns affects our national economy and adequate nutritional supply (Sheuty *et al*., 2017). Therefore, some forms of preservation techniques are necessary to prevent spoilage and for extension of shelf life of fishes.

[1]Food Technology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Ganakbari, Savar, Dhaka-1349, Bangladesh
[2]Health Physics and Radioactive Management Unit, Institute of Nuclear Science and Technology, Atomic Energy Research Establishment, Ganakbari, Savar, Dhaka-1349, Bangladesh
*Corresponding author. E-mail address: enamul@juniv.edu (Md. Enamul Haque)

Bata, *Labeo bata* (Hamilton, 1822) is one of the commercial carp species for aquaculture in Bangladesh (Talwar & Jhingran, 1991). Like other cyprinids, Bata can also help to meet the nutritional demand of the people (Sarower *et al*., 2012). Fish is most susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage (Fraziar & Westhoff, 1988), and about 4% of total catch never reached to the market and ultimately wasted (James, 1982; Akter *et al*., 2011). Thus, to minimize the economic loss and maintain the nutritional quality; it is essential to develop efficient fish preservation techniques which permit shelf life extension of fish and fishery products (Akter *et al*., 2011; Chakraborty *et al*., 2012).

Low temperature is a very important factor with regard to quality and shelf life of fish by lowering microbial activity through the reduction of microbial enzymes (Khan *et al*., 1997). Furthermore, rate of loss of quality and shelf life of fish strongly depend on the storage time and temperature (Whittle, 1997; Akter *et al*., 2011). Some food additives like citric acid, ascorbic acid or potassium sorbate those naturally appear in many foods has been used as chemical preservatives for their antimicrobial effects in preserving fresh fish and have been generally recognized as safe (Julio *et al*., 2014). Citric acid preserves food longer than the average shelf life (Omojowo *et al*., 2009).

Food irradiation has been used as a preservation technique that uses ionizing radiation for the purposes of inhibition of sprouting, destruction of food borne pathogens, insects and parasites, delay of physiological ripening and extension of shelf life or improvement of food properties (Kim *et al*. 2005; Chakraborty *et al*., 2012). Nevertheless, food irradiation plant establishment is costly, but the operational cost is lower than most of other preservation techniques (Chakraborty *et al*., 2012). Several researches were carried out by considering gamma irradiation as one of the effective method of preservation to extend shelf life of fish and fish products (Nickerson *et al*., 1954; Proctor *et al*., 1960; Laycock & Regier, 1970; Abu-Tarboush *et al*., 1996). Irradiation dose upto 10 kGy considered as an effective, safe and economical as the said dose don't pose any nutritional, toxicological or microbiological problems in fish and fishery products (WHO, 1994). Gamma irradiation in combination with low temperature might provide a means to increase shelf life of fish and fishery products. Therefore, the present study was aimed to assess the proximate composition to get basic nutritional information and examine the shelf life of citric acid and gamma radiation treated Bata (*L. bata*) during storage in refrigerated (4°C) temperature.

MATERIALS AND METHODS

Collection of fish: Fresh Bata (*L. bata*) were collected from the local markets of Rooppur, Ishwardi, Pabna and immediately brought to the laboratory of Food Technology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka in presterilized insulated ice box.

Sample processing: Before experiment, the samples were beheaded, degutted, descaled, sliced and finally washed with tap water. The entire fish samples were randomly divided at first into two lots. The first lot was used for proximate composition analysis, and the second lot was used to evaluate the spoilage after citric acid and gamma radiation treatments on preservation. The lot for preservation was divided into three sub-lots; fish samples of sub-lot 1 was kept as control, sub-lot 2 was subjected for food additive preservation (dip 1 minute in 2% citric acid solution) and sub-lot 3 was subjected for irradiation preservation. The fish of sub-lot 2 were dipped into 2% citric acid solution for 60 seconds. Fish samples of sub-lot 3 were divided into two parts for irradiation with two different doses and thereafter irradiated by radiation dose of 1.0 kGy and 1.5 kGy. After that, all samples were stored at refrigerated (4°C) temperature for further investigations. First the proximate compositions of fish samples were determined using the first lot of fish samples and then sensory (organoleptic scores), chemical (TV) and microbial analyses (TBC and TCC) were carried out at weekly intervals upto 28 days of storage period.

Proximate composition: Moisture of fish is commonly determined by drying a sample at some elevated temperature and reporting the loss in weight (AOAC, 1975). The total nitrogen of crude protein in fish was determined by using universally accepted "Micro-Kjeldahl" method (AOAC, 1975). Ash in fish was determined by incineration of sample at about 600˚C for 5-6 hours (AOAC, 1975).

Determination of mineral contents: Determination of phosphorus was carried out by measuring colorimetric procedure (Ranganna, 1986). The iron in fish was determined by converting the iron to ferric form using oxidizing agents the potassium per sulphate or hydrogen peroxide and treating thereafter with potassium thiocyanate which is measured colorimetrically at 450 nm (Ranganna, 1986).

Organoleptic analysis: Organoleptic evaluations were assessed for the detection of freshness or shelf life of stored fish and consumers' acceptance by a useful method developed by Peryam & Pilgrim (1957). Nine points' hedonic scales were used for sensory evaluation by 3-6 judges Miyauchi *et al*., 1964.

Chemical assessment: The degree of autolytic and bacterial proteolysis has been assessment in fish by means of tyrosine value (TV). TV was determined according to the method described by Wood *et al*. (1942).

Microbial evaluation: To determine the microbial changes, TBC and TCC were estimated and determined after Burgey's manual by determinative dilution technique followed standard spread plate count (Sharp & Lyles, 1969). Organoleptic, chemical (TV) and microbial analyses (TBC and TCC) were carried out at weekly intervals upto 28 days of storage period.

Statistical analysis: The data of tyrosine values and microbial counts were analyzed and the least significant difference (LSD) at $p < 0.05$ was employed to test the significant differences among control, citric acid treated and irradiated samples. All the statistical analyses were performed using SPSS ver. 24 (SPSS Chicago, IL).

RESULTS AND DISCUSSION

Proximate composition: Moisture, protein and ash contents of fresh Bata (n = 3) ranged from 82.3 – 82.6% (mean ± SD = 82.4 ± 0.1%), 19.1 – 20.8% (19.7 ± 0.9%) and 0.9 – 1.1% (0.9 ± 0.1%), respectively. Mineral contents such as phosphorus and iron of fresh Bata ranged from 171.2 – 210.0 mg/100 g (184.6 ± 21.9 mg/100 g) and from 2.6 – 4.0 mg/100 g (3.4 ± 0.2 mg/100 g), respectively. Similar results were reported by previous researches (Chakrabarty *et al*., 2003; Sarower *et al*., 2012; Debnath *et al*., 2014). Variations in proximate compositions also occurred in relation to age, size, species, fat content, sex, spawning, starvation, environmental conditions, etc. (Jana *et al*., 2018; Islam *et al*., 2019). However, proximate composition is considerated as a good indicator of the physiological condition and nutritional qualities of a fish (Anthony *et al*., 2016). Though only the studies on the proximate composition of Bata have not really caught attention of researchers in fisheries; however, these results were presented to get basic information about the nutritional qualities of the fish samples as an important dietary sources of protein for human consumption.

Organoleptic evaluation: Organoleptic scores of control (without treatment), 2% citric acid (60 seconds dip) treated and irradiated (1.0 and 1.5 kGy) fish samples were evaluated during the storage periods on the basis of nine point hedonic scales for sensory and overall acceptability evaluation. At the beginning of storage periods, organoleptic scores were same (8.1 ± 0.1) in control, 2% citric acid treated and irradiated samples (Fig. 1). The sensory scores were gradually decreased for all samples with the increase of storage period. Miyauchi *et al*. (1964) suggested that the average sensory score of 5 might be acceptable in case of organoleptic test. On the basis of acceptable limit, the samples without any treatments (control) started to spoil after 7 days of storage while treated samples were remained acceptable nearly one month. Citric acid treated samples remained acceptable upto 21 days at low temperature (4°C). Irradiated samples of 1.5 kGy remained acceptable upto 28 days while samples with 1.0 kGy irradiation showed good result upto 21 days during storage at same refrigerated temperature (4°C) which suggested that irradiation with low dose is effective to extend shelf life of fish and fishery products as gamma radiation limits the deterioration considerably (Ahmed, 2009; Akter *et al*., 2011). These results also revealed that spoilage of control fish samples were rapid than any other treated samples while stored at 4°C. Moreover, organoleptic scores indicated that irradiated samples have longer shelf life than control and citric acid treated samples. Our findings were also supported by previous researches (Khan *et al*., 1997; Akter *et al*., 2011; Sheuty *et al*., 2017). Due to microbial spoilage with the increase of storage period; the appearance, odor, color and texture of fish would be deteriorated that developed declined trends of organoleptic scores (Sheuty *et al*., 2017; Islam *et al*., 2019).

Tyrosine Value (TV): The tyrosine contents were found to increase with the progress of storage time (Fig. 2). The TV ranged from 10.4 5– 39.87, 10.05 – 38.37, 6.62 – 33.79 and 5.68 – 23.27 mg% during 28 days of storage for control, citric acid (2%) treated, 1.0 irradiated and 1.5 irradiated samples, respectively (Fig. 2). The degree of autolytic and bacterial proteolysis has been evaluated in fish by means of TV, and the value increase with the increase of fish spoilage (Pearson, 1968). The rates of increase of TV was significantly lower (p < 0.05) in irradiated samples than control samples. Moreover, between the irradiated samples TV increasing rate was higher in samples with 1.5 kGy irradiation with respect to the low dose irradiated (1.0 kGy) samples which indicated that autolytic and enzymatic proteolysis was lowest in higher dose (1.5 kGy) irradiated samples. Sheuty *et al*. (2017) and Islam *et al*. (2019) supported this type of phenomenon in their studies on shelf life extension of Hilsha shad and Poa, respectively.

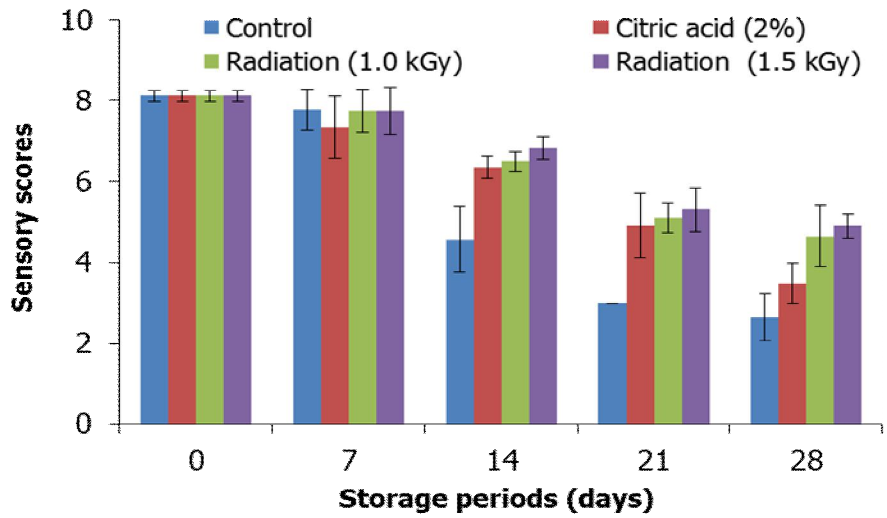

Fig. 1. Sensory scores of control, citric acid (2%) treated and irradiated (1.0 kGy and 1.5 kGy) *Labeo bata* stored at 4°C for 28 days

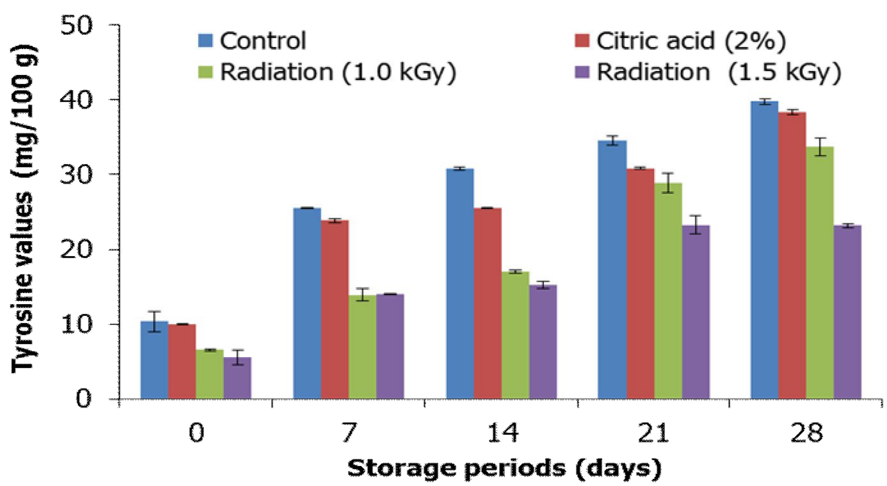

Fig. 2. Tyrosine value of control, citric acid (2%) treated and irradiated (1.0 kGy and 1.5 kGy) *Labeo bata* stored at 4°C for 28 days

Microbiological quality: Total bacterial counts (TBC) ranged from 4.55×10^6 to 4.45×10^{10} cfu/g in control samples and those in citric acid (2%) treated samples ranged from 3.00×10^3 to 2.50×10^8 cfu/g during storage at 4°C (Fig. 3a). The TBC also varied from 3.50×10^3 to 3.30×10^7 cfu/g in samples with 1.0 kGy irradiation and from 1.50×10^3 to 1.10×10^6 cfu/g in irradiated samples with 1.5 kGy preserved at 4°C. The highest TBC (4.45×10^{10} cfu/g) was observed in control samples whereas lowest (1.50×10^3 cfu/g) was in 1.5 kGy irradiated samples stored at 4°C. TBC were significantly ($p < 0.05$) different between initial and final storage periods. Present results also indicated that TBC in irradiated samples comparatively lower than any other samples. Moreover, between irradiated samples, samples with 1.5 kGy irradiation have good effects in reducing bacterial contamination (Fig. 3a). Similar increasing trends of TBC were reported by several researches in different fishes (Khan *et al*., 1997; Ahmed *et al*., 2009; Mustafa *et al*., 2014; Sheuty *et al*., 2017; Islam *et al*., 2019). However, the acceptable limit of TBC is 1.0×10^7 cfu/g in fish samples (Laycock & Reigier, 1970), and the ICMSF (1986) recommended that TBC $< 10^6$ cfu/g in raw fish products as good quality while those $> 10^7$ cfu/g as unacceptable. According to the above statements, only irradiated samples showed acceptable limit of TBC on 28th days of storage at 4°C.

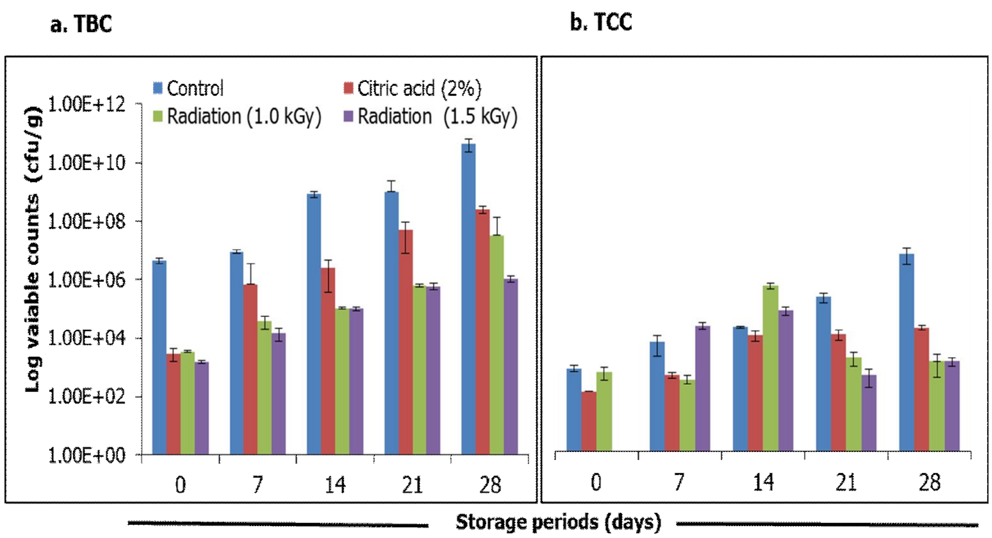

Fig. 3. Microbiological quality of control, citric acid (2%) treated and irradiated (1.0 kGy and 1.5 kGy) *Labeo bata* stored at 4°C for 28 days; a) TBC and b) TCC

Total coliform counts (TCC) ranged from 6.00×10^2 to 4.00×10^6 cfu/g in control samples. In citric acid (2%) treated samples ranged from 1.00×10^2 to 1.35×10^4 cfu/g and in irradiated (1.0 and 1.5 kGy) samples from 4.50×10^2 to 1.00×10^3 cfu/g and 1.53×10^4 1.00×10^3 cfu/g, respectively (Fig. 3b). The highest count (4.00×10^6 cfu/g) was observed at 28 days in control samples. TCC also showed significant ($p < 0.05$) differences between 0 and 28 days. TCC gradually increased in control and citric treated samples while those in 1.0 kGy irradiated samples gradually increased upto 14 days thereafter decreased during 28 days of storage period. TCC in 1.5 kGy irradiated samples showed almost decreasing trends than others for unknown reasons. According to ICMSF (1986) guideline, acceptable TCC for fish is less than 500 cfu/g. Citric acid treatment alone or combination with potassium sorbate is effective to reduce coliform bacteria (Abu-Ghazaleh, 2013). Poor water quality used for washing the samples as well lacks of proper hygienic condition in the fish market channels were responsible for the presence of coliform in food (Haque, 1997).

Considering all of the parameters used in this study, proximate analysis of Bata is of paramount importance not only from academic point of view, but also for its nutritional values. However, to increase the shelf life of Bata, combination of gamma irradiation with low temperature (4°C) was better than any other treatments though the irradiation plant is not commonly available yet in Bangladesh. Between two doses of gamma radiation (i.e., 1.0 and 1.5 kGy) in combination with refrigerated temperature (4°C), 1.5 kGy irradiation are more effective to enhance food security through the extension of shelf-life and inhibition in microbial load of *L. bata* which lower the risk of food borne illness caused by microorganisms. Further research need to be conducted on fish preservation techniques to minimize the economic loss and strengthen safety for storage, human consumption and proper utilization of our fisheries resources.

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