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

Kamrunnahar, Md. Enamul Haque*, Arzina Hossain, Mahfuza Islam, Md. Kamruzzaman Munshi, Khandoker Asaduzzaman and Roksana Huque

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 up to 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 foodstuffs 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 turn 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.
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 (Frazier & 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 up to 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 assessed 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 considered 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
Citric acid, gamma radiation, shelf life, *Labeo bata*

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.

![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](image1)

![Tyrosine values of control, citric acid (2%) treated and irradiated (1.0 kGy and 1.5 kGy) *Labeo bata* stored at 4°C for 28 days](image2)
Microbiological quality: Total bacterial counts (TBC) ranged from $4.55 \times 10^6$ to $4.45 \times 10^{10}$ cfu/g in control samples and those in citric acid (2%) treated samples ranged from $3.00 \times 10^5$ to $2.50 \times 10^9$ cfu/g during storage at 4°C (Fig. 3a). The TBC also varied from $3.50 \times 10^5$ to $3.30 \times 10^7$ cfu/g in samples with 1.0 kGy irradiation and from $1.50 \times 10^5$ to $1.10 \times 10^6$ cfu/g in irradiated samples with 1.5 kGy preserved at 4°C. The highest TBC ($4.45 \times 10^{10}$ cfu/g) was observed in control samples whereas lowest ($1.50 \times 10^3$ 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 $1.0 \times 10^7$ 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](image_url)

Total coliform counts (TCC) ranged from $6.00 \times 10^2$ to $4.00 \times 10^6$ cfu/g in control samples. In citric acid (2%) treated samples ranged from $1.00 \times 10^5$ to $1.35 \times 10^3$ cfu/g and in irradiated (1.0 and 1.5 kGy) samples from $4.50 \times 10^2$ to $1.00 \times 10^3$ cfu/g and $1.53 \times 10^4$ $1.00 \times 10^2$ cfu/g, respectively (Fig. 3b). The highest count ($4.00 \times 10^6$ 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.

Acknowledgements: The authors are grateful to the Director of the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka for providing laboratory facilities and other necessary technical supports to carry out this research work. This research work was partly supported by the University Grants Commission research fund in 2018-2019 which is also gratefully acknowledged.

REFERENCES

Abu-Tarboush, H.M., Al-Kahtani, H.A., Atia, M., Abou-Arab, A.A., Bajaber, A.S. and El-Mojaddidi, M.A. 1996. Irradiation and post irradiation storage at 2 ± 2°C of Tilapia (Tilapia nilotica x T. aurea) and Spanish Mackerel (Scomberomorus commerson). Sensory and Microbial Assorbate. J. Food Protect. 59(10): 1041-1048.
Abu-Ghazaleh, B.M. 2013. Effects of ascorbic acid, citric acid, lactic acid, NaCl, potassium sorbate and Thymus vulgaris extract on Staphylococcus aureus and Escherichia coli. Afr. J. Microbiol. Res. 7(1): 7-12.
Ahmed, M.K., Hasan, M., Alam, M.J., Ahsan, N., Islam, M.M. and Akter, M.S. 2009. Effect of gamma radiation in combination with low temperature refrigeration on the chemical, microbiological and organoleptic changes in Pampus chinensis (Euphrasen, 1788). World J. Zool. 4(1): 9-13.
Akter, R., Alam, Z.M., Billah, M.B. and Salam, M.A. 2011. Biochemical composition and effect of gamma radiation on shelf life of Kalbaush, Labeo calbas (Hamilton- Buchanan, 1822) preserved at low temperature. Bangladesh J. Life. Sci. 23(2): 17-24.
Anthony, O., Richard, J. and Lucky, E. 2016. Biochemical composition of five fish species (C. laticeps; D. rostratus; S. schall; S. mystus and H. bebe) from river Niger in Edo State, Nigeria. Int. J. Fish. Aquat. 4(3): 507-512.
AOAC (Association of Official Analytical Chemists). 1975. Official Method of Analysis, 12th edn., Association of Analytical Chemists, Washington DC, pp. 832.

Chakrabarty, S.C., Uddin, M.B. and Islam, M.N. 2003. A study on the composition of common freshwater fishes of Bangladesh. Bangla. J. Fish. 26: 23-26.

Chakraborty, S., Mustafa, M.G., Alam, M.Z. and Jannat, M. 2012. Effect of gamma radiation on the sensory, chemical and microbiological changes in two strains of climbing perch (Anabas testudineus, Bloch 1792). J. Asiatic. Soc. Bangladesh Sci. 38(2): 183-188.

Debnath, C., Sahoo, L., Singha, A., Yadav, G.S., Datta, M. and Ngachan, S.V. 2014. Protein and Mineral Compositions of Some Local Fishes of Tripura, India. Indian J. Hill Farming. 27: 210-218.

Frazier, C.W. and Westhoff, C.D. 1988. Food Microbiology, 4th edn., McGraw-Hill Book Inc., New York, pp. 19-245.

Ghose, B. 2014. Food Security and food self-sufficiency in China: from past to 2050. Food Energy Secur. 3(2): 86-95.

Haque, M.G. 1997. Analysis of faecal originated bacteria and their decontamination by different treatments of drinking water of water vehicles (Launce). M.Sc. thesis. Jahangirnagar University, Savar, Dhaka, Bangladesh.

Hamilton, F. 1822. An account of the fishes found in the river Ganges and its branches. Printed for A. Constable and company, Edinburgh & London, pp. 405.

ICMSF. 1986. Microorganisms in Foods. 2. Sampling For Microbiological Analysis: Principles and Specific Applications, 2nd edn., University of Toronto Press, Buffalo, NY.

Islam, M.D., Munshi, M.K., Huque, R., Hossain, A., Khatun, M.A., Islam, M., Rahman, M.M. and Khan, M.S.I. 2019. Effect of gamma radiation and potassium sorbate on sensory evaluation, chemical and microbial analysis of poa (Pama pama) preserved at low temperature. Int. J. Biosci. 15(2): 78-91.

James, S. 1982. The Future for Fish in Nutrition, info. Fish Int. Mar. DI 4: 41-44.

Jana, P., Paul, M., Kumar, P.P., Sahu, S. and Chowdhury, A. 2018. Nutrient profile study on locally available small indigenous species (SIS) of paschim Medinipur district of West Bengal, India. Int. J. Curr. Microbiol. App. Sci. 7(10): 634-640.

Khan, M.N., Banu, N., Hossain, M.M. and Hossain, M.A. 1997. Effects of gamma irradiation in low temperatures on the shelf-life extension of Pangasius pangasius (Hamilton-Buchanan, 1822) and Pangasius butchi (Fowler). Bangladesh J. Life. Sci. 9(2): 17-24.

Laycock, R.A. and Reigier, L.N. 1970. Pseudomonas and Achromobacter in the spoilage to irradiated hadhock of different pre-irradiation quality. Appl. Microb. 12: 65-69.

Julio, C., Karina, P., Giselda, B. and Leda, G. 2014. The use of chlorine, citric and ascorbic acid and packed film to extend the shelf life of Pejerrey (Odontesthes boenerensis) during storage at different temperature. Food Nutr. Sci. 5: 1506-1520.

Kim, J.H., Ahn, H.J., Lee, J.W., Park, H.J. and Ryu, G.H. 2005. Effects of gamma irradiation on the biogenic amines in pepperoni with different packaging conditions. Food Chem. 89: 199-205.

Mustafa, M.G., Hossain, M.A., Alam, M.J., Khan, M.M.R., Nilla, S.S. and Alam, M.Z. 2014. Effects of gamma irradiation on the shelf-life of frozen tiger shrimp, Peneaus monodon (Fabricius, 1798). Int. J. Nat. Sci. 4(1): 10-15.

Miyauchi, D.T., Eklund, M.W., Spinelli, J. and Stoll, N.V. 1964. Irradiation preservation of pacific coast shellfish, storage life of icing crab meats at 35°F and 42°F. Food Tech. 18: 99-103.

Nickerson, J.T.R., Lokart, E., Proctor, B.E. and Licciardello, J.J. 1954. Ionizing radiation for control of fish spoilage. Food Tech. 8: 32.

Omojowo, F.S., Omojasola, P.F., Libata, J.G. and Adoga, IJ. 2009. Evaluation of citric acid and potassium sorbate as preservatives on the safety and shelf-life of smoked catfish. Nat. Sci. 7(11): 1-8.
Pearson, D. 1968. Application of chemical methods for the assessment of beef quality. II. Methods related to protein breakdown. *J. Sci. Food Agri.* 19: 366-369.

Peryam, D.R. and Pilgram, F.I. 1957. Hadonic assessment and food technology. Food Technology. 11: 9-14.

Proctor, B.E., Goldblith, S.A., Nickerson, J.T.R. and Farkas, F.R. 1960. Evaluation of the technical economic and practical feasibility of radiation preservation of fish. USAEC. Rpt. No. NYO-9182.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control for fruits and vegetable products*. 2nd edn., Tata McGraw-Hill Pub. Com. Ltd., New Delhi, India, pp. 126-127.

Sarower, M., Hossain, M.B. and Minar, M.H. 2012. Biochemical composition and endangered fish, *Labeo bata* (Hamilton, 1822) from Bangladesh fresh waters. *Am. J. Food Technol.* 7: 633-641.

Sharp, M.S. and Lyles, S.T. 1969. *Laboratory instructions in Biology of Microorganisms*, C.V. Masby Company, pp. 23-25.

Sheuty, T.F., Kamrujjaman, M., Islam, M., Hossain, M.A. and Huque, R. 2017. Effect of potassium sorbate and gamma irradiation on the shelf-life of Hilsa shad, *Tenualosa ilisha* (Hamilton, 1822) at low temperature. *Jahangirnagar University J. Biol. Sci.* 6(2): 67-73.

Talwar, P. K. and Jhingran, A.G. 1991. *Inland fishes of India and adjacent countries*. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp. 1158.

WHO, 1994. *Wholesomeness of Irradiated Food*. WHO, Geneva, Switzerland.

Whittle, K.J. 1997. Opportunities for improving the quality of fisheries products. In: Luten, J.B., Borrensen, T. & Oehlenschlager, J. (eds.). *Seafood from producer to consumer, integrated approach to quality*, Proceedings of the International Seafood Conference on the 25th anniversary of WEFTA, Netherlands, 13-16th November 1995.

Wood, A.J., Sigurdsson, G. J. and Dyer, W.J.D. 1942. The surface concept in measurement of fish spoilage, *J. Fish. Res. Bd. Canada.* 6(1): 53-62.