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GAMMA RADIATION EXPOSURE TOWARDS SHELF-LIFE EXTENSION OF BRINE-TREATED INDIGENOUS CLIMBING PERCH, *ANABAS TESTUDINEUS*

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ABSTRACT: The study was conducted to evaluate the effects of gamma radiation (1.5 and 3.5 kGy) on quality and shelf-life of brine-treated (18% NaCL) cultured and indigenous Climbing perch, *Anabas testudineus* during storage at low temperature (4°C) through sensory (Organoleptic Score, OS), chemical (Tyrosine Value, TV) and microbial analyses (Total Bacterial Count, TBC; Total *Salmonella* and *Shigella* Count, TSS; and Total *Staphylococcus* Count, TSC) at weekly intervals for a period of 35 days. The quality of both cultured and indigenous perch deteriorated with the increase of time. The values of TV (23.69 – 86.20 mg/100 g) and TSS (0 – 4.43 cfu/g) were tended to be high in non-irradiated perch while the values of TBC (3.00 – 11.63 cfu/g) and TSC (2.00 – 6.52 cfu/g) showed increasing trends in irradiated samples for both stains. The findings demonstrated that the synergistic effects of brine and gamma radiation in combination with low temperature could be the most effective treatment for the shelf-life extension of perch and this technique might be applied for large scale preservation of any other local fishes of Bangladesh towards food security.

Key words: Climbing perch, gamma radiation, low temperature, shelf-life extension.

INTRODUCTION

Owing to low elevation and deltaic nature of Bangladesh, the country forms a wide range of geographically and ecologically distinct aquatic habitats which harbor 250 to 266 freshwater fish species (Rahman 2005, Siddiqui *et al.* 2007 and IUCN 2015). Fish is considered as the second most valuable agricultural crop in Bangladesh (DoF 2019) and the main source of animal protein as fish itself supplements about 60% of animal protein for the country people (Chowdhury 2001 and DoF 2019). Fisheries sector plays a crucial role in

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economic development by ensuring food security and stimulating the growth of a number of subsidiary industries with contribution of 3.5% to the country's GDP and 25.7% to the agricultural GDP in Bangladesh (DoF 2019).

However, fisheries sector undergoes huge post-harvest loss every year due to ignorance and negligence in handling and processing of harvested or landed catch during passing different stages of marketing channels until reach the retail distribution (Alam 2010, Kamrunnahar *et al.* 2019). Over this period, fish takes very little attention to extend the normal shelf-life; therefore fish quality reduced and this low quality fish becomes an important issue of food security and public health with negative impacts on economy (Alam 2010, Chakraborty *et al.* 2012 and Mou *et al.* 2020).

Fishes are classified as highly perishable commodity because of its high nutrient containing biochemical composition (Mahin *et al.* 2011). They are the most susceptible animals to autolysis, oxidation and hydrolysis of fats and microbial spoilage (Fraziar and Westhoff 1988). Soon after the fishes die, rigor mortis started and thereafter the deterioration is caused by the microbial activity which brings about very noticeable changes in their sensory characteristics (Alam *et al.* 2009 and Mahin *et al.* 2011) and huge amount of fish were spoiled due to lack of proper preservation method in Bangladesh (Alam *et al.* 2009; Sheuty *et al.* 2017). To assure the fish safety and quality through shelf-life extension, there is a crucial need for the development of new techniques and efficient fish preservation methods (Moini *et al.* 2009, Chakraborty *et al.* 2012 and Mou *et al.* 2020).

The goals of any food preservation techniques are to increase the shelf-life of food and ensure the safety for human consumption. Among the techniques, low temperature is used to retard the chemical and enzymatic actions which are responsible for fish spoilage through lowering the microbial growth and activity in fish (Bakermans and Skidmore 2011 and Mahin *et al.* 2011). Gamma radiation is also used as an effective method of preservation to increase the shelf-life of fish and fishery products by reducing microbial loads without any significant losses of food values (Mahin *et al.* 2011 and Haque *et al.* 2013). Moreover, brine salting is one of the oldest preservation methods of fish (Yanar *et al.* 2006 and Gallart-Jornet *et al.* 2007); the synergistic effects of brining in conjunction with irradiation might be an effective means for shelf-life extension of fish and fishery products. Thus, the combination method (i.e., brining and irradiation) would offers a promising approach to increase shelf-life of fish under 4°C storage conditions as the knowledge about the shelf-life extension using the said combination method of fish preservation is almost absent in Bangladesh.

Therefore, the present study was carried out to observe the quality and the synergistic effects of brining in combination with different doses of gamma (γ) radiation (1.5 kGy and 3.5 kGy) followed by refrigeration at 4°C on degutted fresh of cultured and indigenous Climbing perch, *Anabas testudineus.* Both cultured and indigenous Climbing perch, *A. testudineus* were selected for the study as the stains are easily found in Bangladesh and provide good source of nutrients for the country people (Ara, 2013 and Ara and Nabi, 2018). The present study will provide information to select the best preservation technique for their shelf-life extension towards food security.

MATERIAL AND METHODS

Place of study: All investigations were carried out at the laboratory of Food Safety and Quality Analysis Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka.

Sample collection: Fresh cultured and indigenous Climbing perch, *Anabas testudineus* were purchased from the Mirpur fish market, Dhaka, and immediately brought to the laboratory in a presterilized insulated ice-box. These fish samples were washed finely with clean tap water and then were taken for sample preparation and processing.

Sample preparation: Samples of both cultured and indigenous perch were beheaded, degutted, descaled and sliced to separate the flesh (edible parts) from the non-edible parts. Only fish fleshes were cleaned with tap water and used in this experiment. Then, the sliced fleshes were randomly divided into 2 lots for each type of samples (i.e., culture and indigenous perch). First lot was delivered for analysis of proximate composition and mineral contents to obtain the information about nutritional qualities and second lot was used to evaluate the effect of gamma radiation on the shelf-life of brine-treated *A. testudineus*. Samples of lot 2 were randomly divided into 2 sub-lots; samples of sub-lot 1 was kept as control, and those of sub-lot 2 were divided again into two parts for irradiation by the doses of 1.5 kGy and 3.5 kGy using 50,000 curie Co^{60} source (Gamma Beam, 650, AECL, Canada) with 267 Kr/hr situated at the Gamma Source Unit, IFRB, AERE. Before irradiation, samples were immersed in NaCl brine (18%, wt/vol; fish:brine ratio 1:2) for 17 h at 4^0C to achieve a concentration of 19.1 ± 1.8 g kg^{-1} in their flesh. Thereafter, fish samples were packed and marked according to the treatments along with control samples into pre-sterilized and sealed polythene bags separately and were stored at 4°C for 28 days for further investigations. Sensory evaluation (organoleptic score), chemical

(tyrosine value) and microbiological analyses (Total Bacterial Count, TBC; Total *Salmonella* and *Shigella* Count, TSS and Total *Staphylococcus* Count, TSC) were carried out at weekly interval.

Determination of proximate composition and mineral contents: Moisture of fish samples was determined by drying a sample at certain elevated temperature and reporting the loss in weight (AOAC 1975). Total nitrogen of crude protein in perch was measured by using universally accepted Micro-Kjeldahl method (AOAC 1975). Lipid is estimated as crude ether extractives by Soxhlet apparatus using acetone as solvent (Jahan *et al.* 2021). Ash in fish was determined by incineration either raw or dried sample at about 600^0C for 5-6 hours (AOAC 1975). The iron in fish was determined by converting the iron to ferric form using oxidizing agents the potassium per sulphate or hydrogen peroxide and treated thereafter with potassium thiocyanate which is measured colorimetrically at 450 nm (Ranganna 1986). Calcium was being determined by the titration method using standard $KMnO_4$ (Jahan *et al.* 2021) as calcium precipitate as oxalate. Phosphorus was measured using colorimetric procedure (Ranganna 1986).

Sensory evaluation: The freshness of the fish samples was judged entirely on the appearance, odor and texture using nine point hedonic scales by 3-6 judges for sensory evaluation following the method of Miyauchi *et al.* (1964).

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

Microbial analysis: TBC, TSS and TSC were determined according to the Burgey's manual by applying determinative dilution technique, followed by standard spread plate count (Sharp and Lyles 1969). Microbiological counts were expressed in $\log_{10}$ value of cfu/g.

Statistical analysis: Least significant differences (LSD) were used to determine the significant differences in the values of all parameters among control and irradiated samples of both perch at a confidence level of $p<0.05$. All of the analyses were conducted using SPSS ver. 24 (SPSS Chicago, IL).

RESULTS AND DISSCUSSION

Proximate composition and mineral contents: Table 1 represents the proximate composition of the cultured and indigenous perch used in the present study. Among the proximate composition, cultured perch were found significantly ($p<0.05$) enriched with moisture (mean ± SD: 78.67 ± 0.83) and lipid (3.93 ± 0.06) than those of indigenous perch; whereas the protein content (19.38

± 2.63) in indigenous perch were not significantly (p>0.05) higher than those (18.82 ± 0.39) of cultured perch (Table 1). Moreover, ash (1.50 ± 0.17) and calcium contents (9.60 ± 0.27) were estimated significantly (p<0.05) higher in indigenous perch than those of cultured fish samples; whereas iron (0.38 ± 0.01) and phosphorus (462 ± 1.00) were determined significantly (p<0.05) higher in cultured perch than those of indigenous perch (Table 1). These findings are more or less similar with the previous researches (Nargis 2006, Ara and Nabi 2018, Munshi *et al*. 2018 and Akter *et al*. 2020). For example, Ara and Nabi (2018) found higher levels of protein, ash and calcium in local perch compare to Thai perch. In general moisture content varied from 60-80% in fish species (Murra and Burt 2001). Hossain *et al*. (2015) reported as 72.60 ± 0.17% moisture in *A. testudineus* collected from fish market. Variations in proximate composition of fish flesh may differ with age, size, species, fat content, sex, spawning, starvation, physical activities, food items, growth and developmental stages of life cycle, metabolism systems and environmental conditions, etc. (Satnsby 1954, Jacquot 1961, Jana *et al*. 2018, Ara and Nabi 2018 and Islam *et al*. 2019).

Table 1: Proximate composition of cultured and indigenous Climbing perch, *Anabas testudineus*

Proximate composition and mineral contents		Climbing perch	
		Cultured	Indigenous
Proximate composition	Moisture (%)	78.67 ± 0.83	74.67 ± 0.58
	Protein (%)	18.82 ± 0.39	19.38 ± 2.63
	Lipid (%)	3.93 ± 0.06	1.78 ± 0.03
	Ash (%)	0.70 ± 0.10	1.50 ± 0.17
Mineral contents	Iron (mg/100 g)	0.38 ± 0.01	0.16 ± 0.02
	Calcium (mg/100 g)	6.37 ± 0.30	9.6 ± 0.27
	Phosphorous (mg/100 g)	462 ± 1.00	448.33 ± 0.34

Values are expressed as mean ± SD of three replicates.

However, the comparison of proximate composition and mineral contents of the studied fish samples revealed that perch are a good source of essential nutrients, and this study also broadened our knowledge on the differences of nutritional qualities between cultured and wild perch. Since cultured fishes are very popular in rural and urban areas, there are still some confusions about their nutritional values to the consumers. Findings may be useful as one can easily choose fishes through maintaining nutrient-balanced and cost-effective values in their diets (Zaman *et al*. 2014 and Jahan *et al*. 2021).

Sensory evaluation: Organoleptic scores (OS) of control and irradiated cultured and indigenous perch samples were investigated based on hedonic scores during storage at 4^0C from 1-5 weeks. On the basis of spoilage, the

sensory qualities of both cultured and indigenous fish samples can be arranged as 'Control < Irradiated (1.5 kGy) < Irradiated (3.5 kGy)'. All the samples showed higher OS initially, and after that the values decreased with the progress of storage periods at 4°C (Fig. 1). The highest OS were found in irradiated (3.5 kGy)

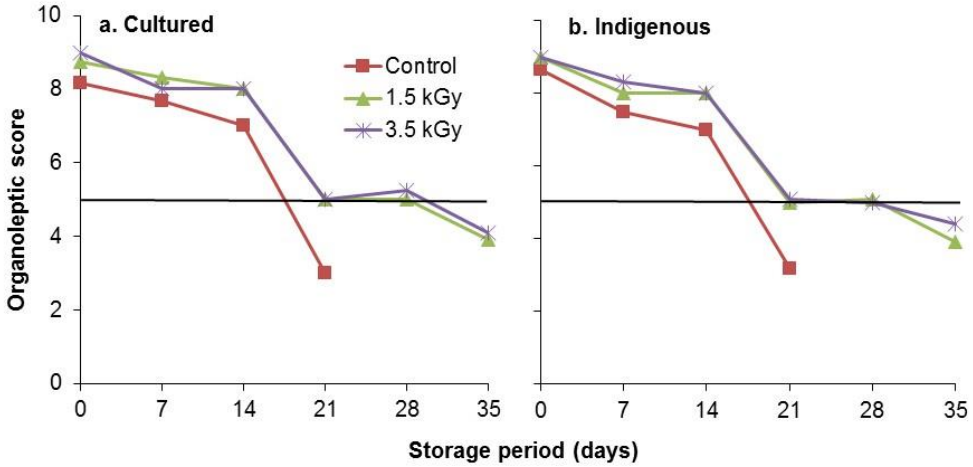

Fig. 1. Organoleptic scores of control and irradiated Climbing perch, *Anabas testudineus* during storage at low temperature; a. cultured and b. indigenous

samples whereas lowest values were observed in control samples. The irradiated samples with dose of 3.5 kGy were organoleptically better than those of 1.5 kGy treated samples. Among durations and treatments, scores were significantly (p<0.05) different. The acceptable limit of sensory score is 5 in case of organoleptic test (Miyauchi *et al.* 1964). However, the control and irradiated samples remained acceptable upto 14, 28, and nearly 35 days of storage at 4°C, respectively. Organoleptic evaluation revealed that the irradiated samples were much more acceptable than the samples without treatments as low dose γ-radiation exposure slows down the deterioration level (Ahmed 2009 and Akter *et al.* 2011). Similar trends of OS were also reported by Mou *et al.* (2020), Kamrunnahar *et al.* (2019) in Spotted snakehead, *Channa punctata* and Bata, *Labeo bata*; respectively. Same declined patterns were also found in case of Hilsha shad, *Tenualosa ilisha* (Sheuty *et al.* 2017) and Calta, *Gibelion catla* (Ali *et al.* 2009).

Though the values of OS decreased during storage at low temperature, but the values remained within the acceptable limits (Tokur 2006). Moreover, the sub-tropical environment possibly be one of the crucial reasons for different microbial abundance in fishes which might be responsible for spoilage as well as

acceptability (Nilla *et al.* 2012a and Nilla *et al.* 2012b). Due to microbial spoilage with the progress of storage periods; the appearance, odor, color and texture of the fishes also be deteriorated (Sayed *et al.* 2013 and Mustafa *et al.* 2013), therefore the OS were down-scaled (Islam *et al.* 2019).

Tyrosine value (TV): TV is used as an indicator of a degree of autolysis and bacterial proteolysis in fish, and the value increase with the increase of fish spoilage (Pearson 1968). Being an indicator of protein degradation, TV were found to increase with the progress of storage periods preserved at low temperature (4°C) for both cultured and indigenous climbing perch (Fig. 2). Since the OS of control samples in 2nd week of storage indicated the unacceptability, therefore the TV values of those samples were not measured from 2nd to 5th weeks. The findings revealed that the increasing rates of TV were significantly lower (p<0.05) in irradiated samples compared to the control samples for both stains of perch. Furthermore, TV increasing trends of both stains of perch with doses of 1.5 and 3.5 kGy irradiation indicated that protein degradation was lowest in higher dose irradiated (3.5 kGy) samples which also suggest γ-radiation exposure have preventive potentiality against autolytic and

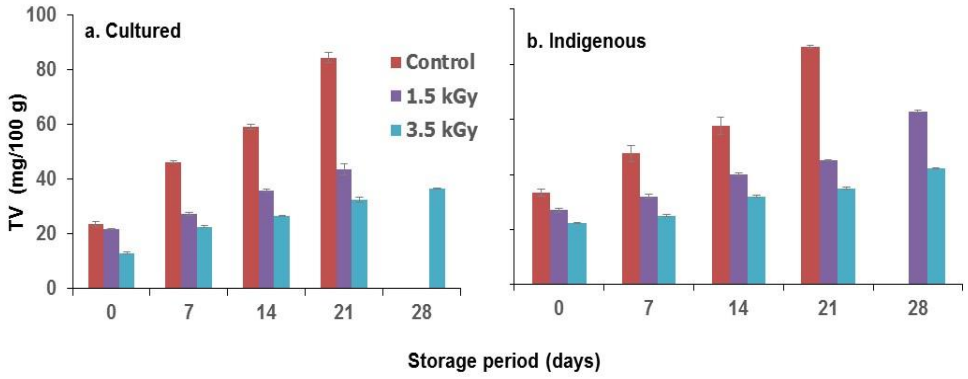

Fig. 2. Tyrosine values (TV) of control and irradiated Climbing perch, *Anabas testudineus* during storage at low temperature; a. cultured and b. indigenous.

bacterial proteolysis of fish during storage at low temperature (Islam *et al.* 2019 and Mou *et al.* 2020). Similar effects of storage periods on TV were also reported by Sheuty *et al.* (2017), Islam *et al.* (2019), Kamrunnahar *et al.* (2019) and Mou *et al.* (2020) in their fish preservation studies of Hilsha shad (*Tenualosa ilisha)*, Poa (*Pama pama*), Bata (*Labeo bata*) and Spotted snakehead (*Channa punctata*), respectively.

Microbiological analysis: Though the bacteria growths were observed at 7 days of interval, but those values were not counted from 3rd weeks to onwards in control samples of both cultured and indigenous climbing perch due the unacceptability as like earlier OS. TBC in both fish samples increased gradually upto 28 days (Table 2). TBC were significantly (p<0.05) different between initial and final storage periods. The highest bacterial load was observed in control samples whereas those were lowest in irradiated samples (1.5 kGy) for both strains of perch. The bacterial load in irradiated samples with dose of 3.5 kGy tended to be higher compared to the 1.5 kGy irradiated samples from 2nd to 4th weeks.

Table 2: Microbial counts of control and irradiated (1.5 and 3.5 kGy) cultured and indigenous Climbing perch, *Anabas testudineus*

Microbial counts (cfu/g)	Storage period (days)	Climbing perch					
		Cultured			Indigenous		
		Control	Irradiation		Control	Irradiation	
			1.5 kGy	3.5 kGy		1.5 kGy	3.5 kGy
TBC	0	6.74 ± 6.32	5.48 ± 0.00	4.00 ± 0.00	7.30 ± 7.15	5.35 ± 4.32	3.00 ± 0.00
	7	7.00 ± 0.00	5.60 ± 5.15	4.60 ± 5.15	8.48 ± 0.00	5.69 ± 0.00	5.30 ± 0.00
	14	7.16 ± 0.00	5.85 ± 0.00	5.39 ± 4.84	10.74 ± 9.84	6.60 ± 0.00	8.39 ± 0.00
	21	0	6.00 ± 0.00	6.06 ± 6.07	0	6.83 ± 5.54	10.00 ± 0.00
	28	0	7.18 ± 6.84	6.74 ± 5.84	0	7.18 ± 6.84	11.63 ± 0.00
TSS	0	3.16 ± 2.08	0	0	2.48 ± 0.00	2.00 ± 0.00	0
	7	3.35 ± 2.32	0	0	3.00 ± 0.00	2.48 ± 0.00	0
	14	4.41 ± 3.45	0	0	4.71 ± 4.53	2.90 ± 0.00	0
	21	0	0	0	0	2.95 ± 0.00	0
	28	0	0	0	0	3.04 ± 0.00	0
TSC	0	3.26 ± 1.86	3.60 ± 3.15	2.51 ± 1.54	2.89 ± 0.54	3.81 ± 3.32	2.00 ± 0.00
	7	3.53 ± 2.54	3.93 ± 2.84	2.76 ± 0.45	3.98 ± 2.15	4.74 ± 4.54	3.69 ± 0.00
	14	3.67 ± 2.99	4.06 ± 3.32	2.99 ± 0.00	4.05 ± 2.54	5.54 ± 4.84	4.57 ± 3.75
	24	0	4.62 ± 4.12	3.78 ± 0.00	0	7.29 ± 7.36	4.69 ± 0.00
	28	0	4.88 ± 3.84	3.97 ± 2.75	0	6.61 ± 5.75	5.04 ± 3.32

Microbial counts are expresed in $\log_{10}$ values as mean ± SD.

According to the guideline of International Commission on the Microbiological Specification of Foods (ICMSF 1986), acceptable limit of TBC for fish is 10^6 cfu/g. Laycock and Reigier (1970) and Shewan (1975) recommended 1.0×10^7 cfu/g and $1\text{-}10^6$ cfu/g, respectively as acceptable limit in fish samples. Hence, according to the above statements only irradiated samples remained acceptable upto 28 days during storage at 4°C though TBC in irradiated (3.5 kGy) non-cultured climbing perch showed a little variation possibly due to contamination. Despite this variation, similar increasing trends of TBC were reported by Chakraborty *et al.* (2012), Sheuty *et al.* (2017), Islam *et al.* (2019), Kamrunnahar *et al.* (2019) and Mou *et al.* (2020) in *A. testidunius*, *T. ilisha*, *P.*

poa, *L. bata*, and *C. punctata*, respectively using low dose of radiation towards shelf-life extension during storage at low temperature.

Though indigenous perch samples with radiation dose 1.5 kGy showed the increasing trends, but TSS counts were completely eliminated with radiation dose of 3.5 kGy in both strains of perch throughout the storage period at 4°C (Fig. 2). The results suggest that irradiated (1.5 kGy) indigenous perch have a change of fecal contamination. *Salmonella* are generally predominant in spoiled fish flora (Moini *et al*. 2009) and radiation sensitivity of *Salmonella* in fish and fishery products were well documented (Fallah *et al*. 2008 and Moini *et al*. 2009). Badr (2004) reported that 1.5 kGy was not enough for complete elimination of *Salmonella* of rabbit meat, while no *Salmonella* were detected in irradiated samples with 3 kGy. Moreover, Sedeh *et al*. (2007) reported 3.0 kGy as an optimum dose of gamma radiation for complete elimination of *Salmonella*. *Salmonella* have a very low resistance to radiation; therefore elimination of these bacteria by gamma radiation could be effective for shelf-life extension of fish as these bacteria play a major role in fish spoilage (Moini *et al*. 2009).

TSC showed gradual increase with progress of storage periods (Fig. 2). During the entire storage periods, TSC in cultured climbing perch tended to be high than those of indigenous perch. TSC in irradiated samples of both strains showed the lowest values with good response to the doses of γ-radiation. Similar types of results were also reported by Ito *et al*. (1993) and Haque *et al*. (2014). Haque *et al*. (2013) reported that TSC were reduced considerably at 2.5 and 5.0 kGy and eliminated completely at irradiation with 7.5 kGy. TSC was also reduced in *Otolithoides pama* exposure with 3.0 kGy (Acharjee *et al*. 2014). Presence of *Staphylococcus* spp. indicated higher level of environmental contamination with and possible risks of food poisoning (Nanu and Narayan 1992, Brady *et al*. 2007).

CONCLUSION

To ensure the consumer satisfaction and safety, it is essential to develop any proper preservation methods for fish and fishery products through maintaining all the international standards to make them available throughout year (Sheuty *et al*. 2017). Most of the previous studies were based on the single treatments either radiation or low temperature or combination treatments, no one consider brine treatment followed by gamma radiation exposure. The present study showed the synergistic effect of brine and gamma radiation during storage at refrigerated temperature (4°C) on *A. testudineus* to extend its shelf-life. Our results revealed that low-dose gamma irradiation (1.5 kGy) can be applied for shelf-life extension, but slightly higher dose (3.5 kGy) with refrigerated temperature (4°C), fish can be maintained with better quality up to a

considerable long period to enhance food security through reducing the microbial load of both stains of *A. testudineus* which lower the risk of food borne illness caused by microorganisms. Indeed, more researches to justify the economic efficiency of these preservation methods in large scale are essential.

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