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Quality and shelf-life extension of refrigerated (4°C) Spotted snakehead (*Channa punctata*) using γ-radiation and food-grade preservative

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Abstract

Investigations were carried out to evaluate the effect of gamma radiation (1.0 and 1.5 kGy) and food-grade preservative (2% potassium sorbate) on the shelf-life of refrigerated (4°C) Spotted snakehead, *Channa punctata* (Bloch, 1793). Quality assessments were evaluated by sensory (OS), chemical (TV), microbial (TBC and TCC) analyses. Based on control panel, OS was shown to be gradually decreased with the storage time. Irradiated and potassium sorbate treated samples were found acceptable (edible) up to 28 and 21 days, respectively of storage period. TV were lower in irradiated samples with dose of 1.5 kGy compared to other samples. Though microbial load of each of the irradiated samples were within the acceptable limits up to 28 days but 1.5 kGy dose was found more effective. Gamma radiation in combination with good refrigeration could be most effective treatment for the shelf-life extension and microbiological quality improvement for snakehead preservation. Moreover, this preservation technique may be applicable for other fish species to enhance food safety for public health issues.

Key words: Gamma radiation, Potassium sorbate, Refrigeration, Shelf-life, Taki.

INTRODUCTION

Being for tropical climate, Bangladesh is one of the suitable regions for warm water fisheries in the world as it harbors the huge flooded wetlands, like lakes, ponds, rivers, canals, etc., coastal water and mangroves. The diverse aquatic habitats in Bangladesh support a wide variety of fish in which 264 species are from freshwater habitats (Rahman, 2005). Fish and fisheries sector have a significant role in nutrition, culture, employment and economy by contributing 3.6% to the national GDP and 24.4% to the agricultural GDP in Bangladesh (DoF, 2017). Moreover, fish are one of the richest sources of nutrients; moisture, protein, lipid, vitamins and minerals are the important constituents of the fish and body parts (Roos *et al*., 2003; Moghaddam *et al*., 2007). They contribute about 60% to the nation's total animal protein as a supplementary source in Bangladesh (DoF, 2018).

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About 85% fish produced in the inland waters (DoF, 2018); and the quality of landed fish after harvesting is reduced before reaching to the consumers due to ignorance and/or negligence of the engaged people during harvesting, post-harvest handling, distribution, processing, preservation, transportation, marketing and trade, etc. (Alam, 2004; Hossain & Barman, 2016). Furthermore, almost 28% fish lost over 60% of freshness before it reached the retail trader's shop (Alam, 2010); therefore, fish become progressively unacceptable for human consumption (Ali *et al*., 2014). It has been assumed that the inclination of post-harvest loss of fish is almost similar throughout the country, though the actual loss with respect to economy might be very high. Previous studies also agreed with the high level of post-harvest loss during handling, processing, transportation and storage of fish and fishery products (Alam, 2005, 2006). Thus, the low quality fish are not only a great concern of food security and public health but also have a serious negative impact on economy (Alam, 2010).

Fish are very much susceptible to spoilage (Farid *et al.*, 2014a) and can't be kept for long time for consumption (Bank, 1962) as they start to spoil soon after harvesting (Farid *et al.*, 2014a). According to Fraziar & Wetshoff (1988), fish are the most susceptible to autolysis, oxidation and hydrolysis of fat and microbial spoilage. The deterioration is believed to cause mainly by bacterial activity which brings a very noticeable changes in the texture, flavor, odor and the general appearance of the product (Sheuty *et al*., 2017). To prevent the spoilage, increase the availability, minimize the market price and to increase the consumer safety and demands of fish, the preservation techniques which is to be helpful for the extension of shelf-life of fish should be followed.

Low temperature is used to retard chemical reactions through the reduction of food enzymes and by reducing the growth and activity of microorganisms in food (Frazier & Wetshoff, 1988; Khan *et al*., 2012). Delay or prevention of microbial spoilage of fish may also be achieved by using different food-grade preservatives. Among the food-grade preservatives, potassium sorbate is the safest, most effective and versatile against a wide spectrum of food spoilage microorganisms (Sheuty *et al*., 2017). This food-grade preservative has been approved in the United States as a GRAS (generally regarded as safe) substance (Liewen & Marth, 1985), and used as an effective preservative of chilled fish (Robach, 1979). Furthermore, gamma radiation offers a potential for improving the microbial, chemical and sensory qualities and also for extending storage life of fish and fishery products (Chakraborty *et al.*, 2012; Haque *et al*., 2013). Gamma radiation in a combination with good refrigeration might provide a means to increase shelf-life while storage time and temperature are the major factors for controlling the rate of quality loss and shelf-life of fish and fishery products (Whittle, 1997; Akter *et al*., 2011).

The knowledge about shelf-life extension of refrigerated (4°C) commercial fish by using gamma radiation and potassium sorbate is not available in Bangladesh. The spotted snakehead, *C. punctata* is air-breathing fish and popularly known as Taki. Farid *et al*., (2014b) mentioned that the fish has great economic importance because of its good nutritional and commercial values in Bangladesh. Therefore, the present study was aimed to assess the extension of shelf-life of the spotted snakehead, *C. punctata* in relation to sensory, chemical and microbial qualities using gamma radiation and food-grade preservative (potassium sorbate) in combination with good refrigeration through laboratory analyses to enhance food security.

MATERIALS AND METHODS

Experiments were carried out in the laboratory of Food Technology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka.

Sample collection: Taki, *Channa punctata* (Bloch, 1793) used in this study were collected from the Tanguar haor, Sunamgonj in 2018. Collection was made early in the morning and the sample were carried out in a polythene bag with ice and brought in the laboratory of Food Technology Division, IFRB, AERE, Ganakbari, Savar, Dhaka. First the samples were washed with clean water and morphometric studies were carried out. Thereafter the samples were processed for the successive experiments.

Experimental: Fish samples were beheaded and degutted and then the samples were sliced into small pieces. The sliced samples were washed four times in clean water and kept in trays to fallen over the water from the sliced samples. Then, the entire sliced samples were randomly divided into two lots. Samples of first lot were used for biochemical composition analysis to obtain the information on the nutritional qualities, and those of lot 2 were used to evaluate the effect of gamma radiation and food-grade preservative (i.e., potassium sorbate) on shelf-life of Taki. The samples of second lot were divided into 3 sub-lots. Samples of sub-lot 1 were used as control, and those of sub-lots 2 and 3 were used for irradiation and potassium sorbate treatment, respectively on preservation. The fish samples of sub-lot 2 were irradiated by the dose of 1.0 kGy and 1.5 kGy using 50,000 curie Co^{60} source (Gamma beam, 650, AECL, Canada) with 267 Kr/hr irradiation dose rate. The samples of sub-lot 3 were dipped in 2% potassium sorbate solution for 60 seconds. Thereafter, according to the radiation doses and potassium sorbate treatment fish samples were packed into pre-sterilized and sealed polythene bags separately and were stored at refrigerated temperature (4°C) for 28 days. Sensory (organoleptic score), chemical (tyrosine value) and microbiological analyses (TBC and TCC) were carried out at weekly interval.

Biochemical composition: Moisture of fish was determined by drying a sample at some elevated temperature and reporting the loss in weight (AOAC, 1975). 5 g of fairly minced sample was taken in pre-weighted crucible and crucible with sample was kept at oven at 105°C for 5-6 hours. After heating, it was cooled in desiccator and weighted. Thus, the moisture was calculated by using the formula (Farid *et al*., 2014b).

$$Moisture(\%) - \frac{Weight\ loss}{Original\ weight\ of\ taken\ sample} \times 100$$

The universally accepted "Micro-Kjeldahl" method was used for determining crude protein in fish samples. At first 2-4 g sample was taken for each experiment and was poured in a cleaned and dried “Micro-kjedahl” flask (100 ml) to which 2 g of digestion mixture and 25 ml concentrated H_2SO_4 were added and the mixture was digested by heating at 315˚C for 5-6 hours until the mixture becomes clear. The digested products were cooled and volume up to 100 ml in volumetric flask. Then 5 ml from dilute digested mixture was transferred in kjedahl dilution apparatus and distilled with 10 ml of 30% NaOH. The distilled was collected in excess of 2% boric acid solution with indicator and was titrated by 0.01N HCL until a faint pink color appears. A similar digestion and distillation were carried out without samples (blank).

$$Nitrogen\ content\ (\%) = \frac{(S-B) \times N \times 14.007 \times C}{A \times W} \times 100$$

Where, S = Titration reading for sample, B = Titration reading for blank, N = Strength of HCL (0.01 N), C = Volume made up of the digest (100 ML), A = Aliquots of digest taken, W = Weight in g of the sample. The protein content is obtained by multiplying the nitrogen value by 6.25 (AOAC, 1975).

Ash in fish and fish products were readily determined by incineration either raw or dried sample at about 600˚C for 5-6 hours, depending on the method used (AOAC, 1975). About 5 g macerated samples were taken in a pre-weighed crucible and kept in a electric muffle-furnace at about 600°C for about 3-5 hours till the ash is almost white or grayish white in color. Then the crucible was cool in a desiccator and re-weighted. The difference of the weight of the crucibles before and after the combustion was reported as ash.

$$Ash\ (\%) = \frac{A-B}{C} \times 100$$

Where, A = Weight of the crucible with raw sample, B = Weight of the crucible with combusted sample, C = Weight of the sample

Determination of minerals: The phosphorus content in fish was measured by using colorimetric procedure (Rangana, 1986). First, 1 ml of pre-prepared mineral solution was taken and mixed up thoroughly with 1 ml ammonium molybdate, 1 ml hydroquinone and 1 ml of Na_2SO_4 solution. The volume was then made up to 15 ml with distilled water and after 30 minutes, the optical density of the solution was measured in a photoelectric colorimeter, against 660 nm. A blank sample runs off side by side. The phosphorous content of the sample was calculated from a standard curve prepared with standard phosphate solution (range 0.01 - 0.1 mgP).

The iron in fish was determined by converting the iron to ferric form using oxidizing agents the potassium persulphate or hydrogen peroxide and treating thereafter with potassium thiocyanate which was measured colorimetrically at 450 nm (Rangana, 1986).

$$Iron\ (mg/100g) = \frac{Optical density\ of\ sample \times 0.1 \times Total\ volume\ of\ ash\ solution \times 100}{Optical\ density\ of\ standard \times 5 \times Weight\ of\ sample\ taken\ for\ ashing}$$

Sensory evaluation of fish quality: Sensory evaluation for the freshness or shelf-life and consumer acceptance of the fish samples was determined with high degree of reliability by organoleptic evaluation. Peryam & Pilgram (1957) had developed a useful method for assessing the overall acceptability of fish products. Nine points' hedonic scales were used for sensory evaluation by 3 - 6 judges followed by Miyauchi *et al*. (1964). The hedonic scales were: 9 – like extremely; 8 – like very much; 7 – like moderately; 6 – like slightly; 5 – neither like nor dislike; 4 – dislike slightly; 3 – dislike moderately; 2 – dislike very much; and 1 – dislike extremely. In case of organoleptic evaluation, fish samples were judged into 4 scales (appearance, color, odor and texture).

Chemical and biological evaluation of fish quality: The degree of autolytic and bacterial proteolysis has been assessed in fish by means of tyrosine value (TV). Pearson (1968) reported that TV increase with the progress of spoilage. TV was determined following the method as described by Wood *et al*. (1994).

Total Bacterial Count (TBC) and Total Coliform Count (TCC) were determined according to the Burgey's manual of determinative dilution techniques followed by standard spread plate count described by Sharp & Lyles (1969).

Statistical analysis: Least significant differences (LSD) at level of significance at 5% ($p < 0.05$) were conducted to test the differences in the values of tyrosine content and microbial counts among the control, irradiated and potassium sorbate dipped fish samples. All of the analyses were conducted using SPSS ver. 24 (SPSS Chicago, IL).

RESULTS AND DISCUSSION

Biochemical composition: The results of biochemical composition of Taki (*C. punctata*) have been presented in Table 1. The moisture content (mean ± SD) of Taki was found as 77.8 ± 0.72 %. Previous studies were agreed with our findings. For example, Farid *et al*. (2014) reported 77.03 % and Hossain *et al*. (1999) as 75.75 % moisture in Taki. High level of moisture content would increase the deterioration level of fish as high moisture content provides favorable environment for rapid growth and multiplication of associated bacteria which increased the rate of microbial decomposition of fish (Islam *et al*., 2019). Protein was determined as 17.7 ± 2.60 % in Taki. Similar results were observed by several researchers. Such as, Farid *et al*. (2014) found protein as 17.32 %, Bogard *et al*. (2015) as 18.3 % and Jana *et al*. (2018) as 16.32 % in Taki. Ash was recorded as 1.9 ± 0.10 % in Taki. Similarly, Farid *et al*. (2014) found ash as 1.44 % and Jana *et al*. (2018) as 1.55 % in Taki samples. Mineral contents such as iron and phosphorus contents were 3.9 ± 0.02 and 130.0 ± 0.19 mg/100 g of fish flesh, respectively. The values of biochemical composition in fish depend on size, age, species, sex, season, food items, physical activities, growth and developmental stages of life cycle etc. of the species (Jana *et al*., 2018; Islam *et al*., 2019).

However, biochemical compositions were determined using three replicas of control samples to get basic nutritional information of the fish sample as preservation treatment (i.e., low dose radiation, potassium sorbate, etc.) for short-term storage have no significant effects on the food qualities (Leistner *et al*., 1995). Moreover, these analyses suggest Taki (*C. punctata*) as an excellent source of animal protein and can able to meet the demand of animal protein consumption which urges to develop and/or justify any preservation techniques for extending shelf-life towards food security and public safety.

Table 1. Biochemical composition of Taki, *C. punctata*

Moisture (%)	Protein (%)	Ash (%)	Iron (mg/100 g)	Phosphorus (mg/100 g)
77.8 ± 0.72	17.7 ± 2.60	1.9 ± 0.10	3.9 ± 0.02	130.0 ± 0.19

Values are the mean ± SD of three replicates.

Organoleptic Score (OS): Organoleptic Score (OS) of control and treated samples of Taki were investigated during storage at 4°C on the basis of hedonic scores. In control samples, the OS were 8.0, 7.1, 6.2, 3.2, and 3.0 during 0, 7, 14, 21 and 28 days of storage, respectively. Similarly, for 2% potassium sorbate treated samples, the values were 8.0, 7.1, 6.2, 5.0 and 4.0 in similar observation periods. The OS were 8.0, 7.5, 7.0, 5.7 and 4.5 for 1.0 kGy irradiated samples during 0, 7, 14, 21 and 28 days of storage; and those of 1.5 kGy irradiated samples were 8.0 7.5, 7.2, 5.7 and 4.5 in similar observation periods of storage at refrigerated temperature. The OC were gradually decreased with the increase of storage periods (Fig. 1). The irradiated samples with the dose of 1.5 kGy were organoleptically better than those with 1.0 kGy than those with 2% potassium sorbate dipped samples. Based on the acceptable limit of OS (i.e. 5.0) suggested by Miyagauchi *et al*. (1964), our findings revealed that irradiated samples have much more acceptability than other samples.

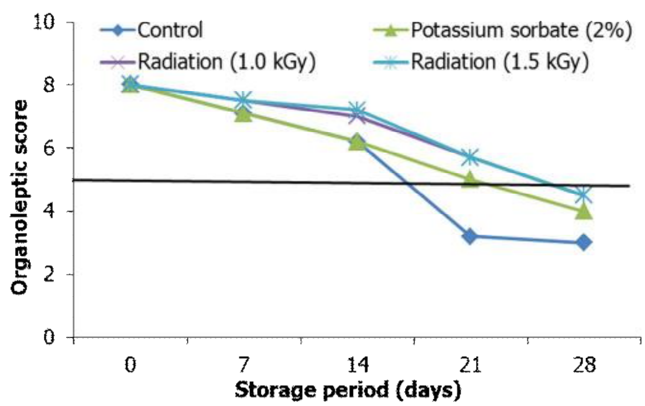

Fig. 1. Organoleptic scores of control, 2% potassium sorbate (60 seconds dip) and radiation (1.0 and 1.5 kGy) treated samples of Taki, *C. punctata* during storage at 4°C

Hossain *et al*. (2001) found that low doses of ionizing radiation were effective to extend the shelf-life of food through the reduction of spoilage causing factor within food items. Similar trends of OS were also observed by Sayed *et al*. (2013) and Ahamed *et al*. (2009) in fish during irradiation at low temperature. Moreover, the OC of potassium sorbate dipped samples was greater than irradiated samples possibly due to the chemical reactions associated with biochemical compositions resulted deleterious changes in nutritional and sensory properties (Erickson, 1997; Islam *et al*., 2019). The appearance, odor, color and texture deteriorated due to microbial spoilage with the increase of storage period (Sayed *et al*., 2013; Mustafa *et al*., 2013; Sheuty *et al*., 2017); therefore OS were decreased (Islam *et al*., 2019).

Tyrosine Value (TV): A gradual change of tyrosine content of Taki was found during storage periods. The TV were found to increase with the increase of storage periods (Table 2). The values (mean ± SD) ranged from 5.3 ± 0.15 to 62.9 ± 0.59, 4.3 ± 0.23 to 46.5 ± 0.80, 4.8 ± 0.15 to 26.8 ± 1.24, 4.2 ± 0.15 to 22.6 ± 0.34 for control, potassium sorbate dipped and irradiated samples (1.0 and 1.5 kGy), respectively during the entire period of observations at refrigerated temperature (Table 2). The rate of increase of TV of irradiated samples were significantly ($p < 0.05$) lower than the control and potassium sorbate dipped samples which indicated that the degree of autolytic and bacterial proteolysis was lowest in irradiated samples than others as TV increased with the increase of spoilage until deamination of amino acid during storage periods (Pearson, 1968). These findings also suggest the preventive potential of irradiation against protein deterioration to storage period (Islam *et al*., 2019). Similar effects of storage period on TV were observed in other studies of fish preservation (Das *et al*., 2014; Islam *et al*., 2019).

Table 2. Tyrosine values of control and other treated samples of Taki, *C. punctata* during different storage periods at 4°C

Storage period (days)	Control	Potassium sorbate (2%)	Irradiated	
			(1.0 kGy)	(1.5 kGy)
0	5.3 ± 0.15	4.3 ± 0.23	4.8 ± 0.15	4.2 ± 0.15
7	13.5 ± 0.98	12.5 ± 0.18	12.3 ± 0.07	10.7 ± 0.36
14	19.9 ± 0.74	16.2 ± 0.35	17.4 ± 0.25	11.8 ± 0.10
21	38.8 ± 0.49	35.1 ± 0.75	25.7 ± 0.53	20.1 ± 0.15
28	62.9 ± 0.59	46.5 ± 0.80	26.8 ± 1.24	22.6 ± 0.34

Values are the mean ± SD of three replicates.

Microbiological changes: Microbial counts were observed at 7 days of interval (Table 3). Bacterial growth increased gradually up to 28 days of storage at 4°C. During the entire storage period, total bacterial count (TBC) ranged from 1.8×10^5 to 4×10^9 cfu/g in control samples while those of 2% potassium sorbate (60 sec dip) dipped samples varied from 5.7×10^4 to 2.4×10^9 cfu/g. The values varied from 2.8×10^3 to 1.5×10^7 cfu/g for 1.0 kGy irradiated samples and those in samples of 1.5 kGy irradiation ranged from 1.3×10^3 to 3.5×10^6 cfu/g during the entire storage period (Table 3). TBC were significantly ($p < 0.05$) different between 0 and 28 days. According to Laycock & Reigier (1970), the acceptable limit of TBC is 1.0×10^7 cfu/g in fish sample. According to this statement the samples showed acceptable limit of TBC at 7 days in control, 14 days in potassium sorbate dipped and 28 days in irradiated samples. This result also indicated that TBC was increased slowly in both potassium sorbate and radiation treated samples than others. Same increasing trend of TBC was also reported by Ahmed *et al*. (2009), Mustafa *et al*. (2014) and Isalm *et al*. (2019) in case of Rup chanda, Tiger shrimp and Poa fishes. Previous researches suggest low dose irradiation (i.e., 1.0 - 3.0 kGy) for shelf-life extension of fresh fish (Molins *et al*., 2001; Jo *et al*., 2004). Antimicrobial action of potassium sorbate through the inhibition of bacterial spore formation might be responsible for the reduction of TBC in potassium sorbate dipped samples (Laxmareddy & Benarjee, 2013; Islam *et al*., 2019).

Table 3. Qualitative assessment of microorganisms in control and other treated samples of Taki, *C. punctata* during different storage periods at 4°C

Sample	Count of viable microorganisms (cfu/g)	Storage periods (days)				
		0	7	14	21	28
Control	TBC	1.8×10^5	2.8×10^6	5.3×10^7	1.0×10^9	4.0×10^9
	TCC	4.5×10^2	1.0×10^3	6.2×10^3	6.7×10^4	6.6×10^5
2% Potassium sorbate	TBC	5.7×10^4	7.0×10^5	1.2×10^7	3.1×10^8	2.4×10^9
(60 sec dip)	TCC	1.3×10^2	7.5×10^2	2.8×10^3	3.5×10^4	2.5×10^4
Irradiated	TBC	2.8×10^3	2.8×10^4	2.7×10^5	3.2×10^6	1.5×10^7
(1.0 kGy)	TCC	2.0×10^1	6.7×10^2	2.5×10^3	5.0×10^3	3.0×10^3
Irradiated	TBC	1.3×10^3	2.0×10^3	3.2×10^3	2.0×10^5	3.5×10^6
(1.5 kGy)	TCC	0	4.8×10^2	1.5×10^3	2.0×10^2	1.0×10^2

TBC and TCC indicate Total Bacterial Count and Total Coliform Count, respectively.

Total coliform count (TCC) varied from 4.5×10^2 to 6.6×10^5 cfu/g in control, 1.3×10^2 to 2.5×10^4 cfu/g in potassium sorbate dipped samples during the entire storage period. Similarly, those in irradiated samples of 1.0 kGy ranged from 2.0×10^1 to 3.0×10^3 cfu/g and from nil to 1.0×10^2 cfu/g in irradiated sample of 1.5 kGy (Table 3). These results showed that TCC gradually increased up to 28 days in control samples but in case of 2% potassium sorbate (60 second dip) and irradiated samples the count were increased for a period of time but slowly in 1.5 kGy than 1.0 kGy and 2% potassium sorbate dipped samples and reduced gradually. TCC also significantly ($p < 0.05$) different between initial and final storage periods. Our results revealed that irradiation has good impact on the elimination of coliform and TCC were increased slowly in both potassium sorbate dipped and irradiated samples than control samples. Treatment with potassium sorbate alone or in a combination with citric acid is effective for shelf-life extension through the reduction of growth of coliform bacteria (Abu-Ghazaleh, 2012). The presence of coliform is fish possibly has been linked with the practice of inadequate hygienic measure and all unhygienic condition of the shops. According to ICMSF (1986) guideline, the acceptable TCC for fish is < 500 cfu/g. Therefore, the potassium sorbate dipped and irradiated samples were acceptable during whole investigation period except control sample which remained acceptable upto 7 days of storage period. Similar trend of TCC was reported by Hossain *et al*. (1991) and Islam *et al*. (2019) in Hisha and Poa fishes.

Conclusions: Freshwater fishes are very popular in Bangladesh and our country secured 5th in world aquaculture production from natural resources (DoF, 2016). Though the fish productions are increasing day by day in our country but it will be quite impossible to gain benefits from the fisheries sector if we can't preserve fishes properly. However, to get benefits from the fisheries sector, priorities need be given on proper preservation methods to prevent the spoilage of the fish, how to increase the availability and minimize the market price. Food borne infection and intoxication has been an important public health hazards in recent times and are expected to continue in future. This study depicted that the combination of food grade preservative with irradiation (1.5 kGy) and refrigeration (4°C) could be most effective tool for shelf-life extension and overall reduction in microbial load of Taki (*C. punctata)*. Further researches are essential to justify the economic efficiency of these preservation methods in large scale.

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