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Effect of gamma radiation and potassium sorbate on sensory evaluation, chemical and microbial analysis of poa *(Pama pama)* preserved at low temperature

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Key words: Gamma radiation, Potassium sorbate, Poa *(Pama pama)*.

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Abstract

Poa is an economical important fish species in southern regionof Bangladesh. The study was carried out to evaluate the effectiveness of gamma radiation (1.0 and 1.5 kGy) and 2% potassium sorbate (dipped in 30 and 60 seconds) at low temperature (±4°C) on sensory quality, biochemical composition, microbial load and shelf-life of poa *(Pama pama)*. According to control panel, the organoleptic scores gradually decreased with the progress of storage time. Irradiated and potassium sorbate (2%) treated samples showed the highest acceptable score (5) up to 28 and 21days respectively. Tyrosine value was lower in irradiated (1.5 KGy) sample than potassium sorbate (2%) treated sample and significantly lower than control. Irradiation has led to a significant reduction in total number of microbes. The highest total bacterial count was observed 9.4×10^7 cfu/g (14^{th} day) in control sample. The highest total coliform and *staphylococcus* species count was 3.2×10^4 cfu/g (14^{th} day) and 4.15×10^4 cfu/g (0^{th} day) respectively in control sample. Bacteria were identified based on their cultural and biochemical characters. *Lactococcus lactis, Klebsiella, E. coli* and *Enterobacter* were sensitive to all the tested 10 antibiotics except erhromycin and penicillin. All were highly sensitive to ciprofloxacin and chloramphenicol and resistant to erhromycin and penicillin. *B.cereus, Stephylococcus, Pseudomonas, Micrococcus* and *P. aeroginosa*were resistant except *Stephylococcus* to gentamicin and imipenem. Study of antibiogram revealed multi-drug resistance of some of the isolates. Combination with irradiation (1.5 KGy) and low temperature (±4°C) could be most effective treatment in extending shelf-life and overall reductions in microbial load of poa.

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Introduction

Bangladesh abounds in large varieties of fish species in which 475 species of marine. The fisheries sector contributes 3.69% to the national GDP and 23.12% to the agricultural GDP in Bangladesh (DoF, 2016). The demand for fresh sea fish in whole world markets has increased significantly over the past decade. *Pama pama* is a popular fish species in southern region of Bangladesh. It contains 18.6% crude protein, 1.7% fats and 75% water (Basu and Gupta, 1939). However, fish and fishery products declines rapidly due to microbial cross contamination during the harvesting, transportation, storage, and poor hygienic maintenance is a global problem regarding food safety and economic impact (Rostamzad *et al.,* 2010; Eze *et al.,* 2011; Noor *et al.,* 2013). Therefore, considering thepopularity, nutritional and economic importance, it is necessary to maintain the microbiological quality of these sea fish as well as to establish the preventive mechanism, which can eliminate the microbial growth and hence may ensure the public health safety. Traditionally, fish preservation rely on the ice storage, rapid chilling, freezing, smoking, heating, organic acids, use of antimicrobials and antioxidants, edible coating, modifiedatmosphere packaging and ionizing radiation (Himelbloom *et al.,* 1994; Gelman *et al.,* 2001; Masniyom *et al.,* 2002; Savvaidiset *et al.,* 2002;Haghparast *et al.,* 2010; Motalebi *et al.,* 2010;Noor *et al.,* 2013), which can inhibit or eliminate microbial growth but the chance of cross contamination is left. Irradiation at appropriatedoses, eliminates insects, molds, bacteria and other potentially harmful micro-organisms that cause spoilage in foods (Aly *et al.,* 2014). It is a safe and effective method of food preservation used in many countries around the world. Food irradiation, in combination with good refrigeration and handling practices, might provide a means to increase fish product shelf life.

Various chemicals and preservatives such as the nitrate, benzoates, sorbic acid, potassium and others have been tested for their efficiency in preserving fresh fish (Tomisayu and Zenitani, 1957) but we are not aware of any being used commercially in this country. A more recent generation preservative, sorbic acid or potassium sorbate has been reported effective in extending the shelf-life of fresh whole broilers (Robach, 1979a) and it has also been found to be effective with certain sea foods (Debevere and Voets,1972a; Chung and Lee, 1981a; and Ampola and Keller,1985a).

Therefore, a comparison of the efficiency of these two seafood preservation methods (irradiation vs. sorbate treatment) may be useful for those fish processors who may not have access to an irradiation source or service, when the process is sanctioned for use. Refrigeration inhibits the food spoilage organism's activity and the low storage temperature greatly slows down the enzymatic and biochemical reactions (Zarei *et al.,* 2011). Freezing kills some but not all of the microorganisms present and growth will take place after thawing if time permits. Combination of treatments for food preservation may result in synergistic or cumulative effects of microbiological barriers or hurdles, leading to a reduced level of one or all the treatments (Leistner and Gorris, 1995).

Therefore, the aim of this study is to find a possible mean for shelf-life extension of selected Poa sea fish by applying gamma radiation and potassium sorbate treatment, in combination with good refrigeration and handling practices. The results might be helpful preventing poa fish spoilage, increases its availability and minimizes the price in local and international market.

Materials and methods

Sample collection and preparation

The Fish sample was purchased from local market of Patuakhali, located at 22.3542°N, 90.3181°E in the southern part of Bangladesh (Fig. 1).

The fish samples were allocated into two parts: control and treatment with gamma radiation (1.0 and 1.5 KGy) using 50,000 curie Co^{60}source (Gamma beam, 650, AECL, Canada) and 2% potassium sorbate (30 seconds dip and 60 seconds dipping).

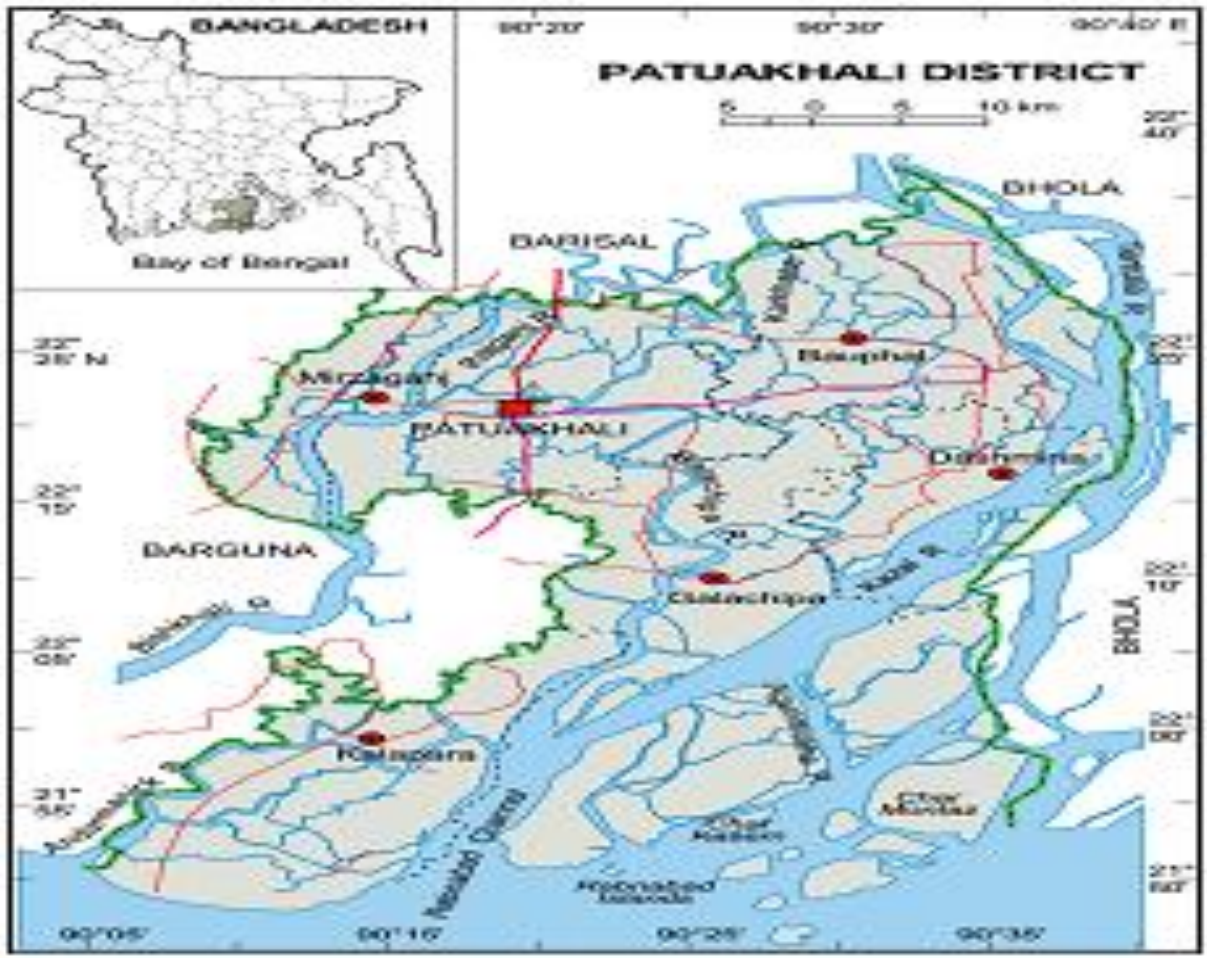

Fig. 1. Map of sampling location.

Then samples were stored at 4**º**C. Chemical analysis (tyrosine value), and microbiological analysis (TBC, TCC and TSC) and organoleptic analysis were carried out in weekly interval.

Proximate composition and Organolepticanalysis

Proximate components (protein, moisture, lipid, ash) were determined as per the Association of Official Analytical Chemists (AOAC) method (AOAC, 1990). The Micro-Kjeldahl methodwas used to measure the protein content. Calcium and phosphorus determined by the method of Ranganna (1986a) and Ranganna (1986a).Sensory evaluation for the detection of freshness or shelf life of the stored poa was performed with high degree of reliability by organoleptic evaluation. Nine points hedonic scale were used for sensory evaluation by 4-6 judges according to Peryam and Pilgram (1957). The hedonic scale was as follows: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; 1-Dislike extremely. In case of organoleptic analysis, the fish was judged into 4 scales (Appearance,Color, Odor, and Texture).

Determination of tyrosine and microbial count

The degree of autolysis and bacterial proteolysis has been assessed by means of tyrosine value. Tyrosine value was determined following the method as described by Wood *et al.* (1942).The total bacterial count (TBC), total coliform count (TCC) and total *Staphylococcus* species count (TSC) were determined by decimal dilution technique followed by standard spread plate count described by Sharp and Lyles (1969).

Identification of bacterial isolates and Antibiotic sensitivity test

Different biochemical tests such as catalase, methyl red, Vogs-Proskauer, citrate utilization, indole,

carbohydrate fermentation, nitrate reduction, starch hydrolysis, and hydrogen Sulfide (H_2S) production (Cappuccino and Sherman, 2014)were performed to identify the bacterial isolates. Bacterial isolates were tested for antimicrobial susceptibility by a standard disc diffusion method (Bauer *et al.,* 1966) on Mueller–Hinton agar.

The protocol was performed as follows: a 6h-broth bacterial cultures were swabbed over the entire surface of Mueller–Hinton agar with a sterile cotton swab (CLSI, 2006) and then antibiotic disks were placed in a sterile environment. The plates were incubated at 37°Cfor 18–20h. The diameters (in millimeters) of clear zones of growth inhibition around each antimicrobial agent disks were measured using a standard scale (Ferraro, 2001; Munshi *et al.,* 2012). Each bacterial species was classified as Sensitive (S) and Resistant (R). The organisms were investigated using 10 commercially available antimicrobial sensitivity discs including ampicillin (10µg), amoxicillin (10µg), amikacin (30µg), chloramphenicol (20µg), erythromycin (30µg), ciprofloxacin (10µg), Penicillin (6µg), Tetracycline (30µg), Gentamycin (30µg), Imipenem (10µg).

Statistical analysis

The data represent the figures and tables are average ±standard deviation. The raw data of tyrosine content and microbial count were analyzed using SPSS for Microsoft version 24.0 software package (SPSS Chicago, IL) and least significant difference (LSD) at $p<0.05$ was used to determine significant difference among the control, irradiated and potassium sorbet-dipped samples.

Results and discussion

Proximate composition of Poa fish

Table1 shows the approximate composition of Poa fish used in the present study. The moisture content was recorded as 82.66±.02 %. Similar results observed by Mithun *et al.* (2016) as range from 79.87% to 84.67% in *Pama pama*. Other results observed by Bogard *et al.* (2015) as 75.2% in lalpoa *(Johnius argentatus)*, Zaman *et al.* (2014) as 76.51± 5.24% in *Otolithoides pama.*

Table 1. Proximate composition of Poa fish.

Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Iron (Fe) (mg/100 gm)	Calcium (mg/100 gm)	Phosphorus (mg/100 gm)
82.66±.02	14.10±.16	0.73±.23	0.84±.23	2.36±0.09	457.33±62.22	535±10

* Values are the mean±SD of three replicates.

The high moisture content of the fish sample would increase the deterioration level of fish when kept for a long time. High moisture favors the growth and multiplication of bacteria which leads to higher microbial decomposition of fish. Moisture content also found to vary considerably within the same species of fish depending on the age, fat content, feeding condition, spawning etc. Protein was determined as 14.10±.16%. Similar results observed by Mithun *et al.* (2016) that range of protein content was 14.26% to 15.4% of fresh *Pama pama*. In another study, Bogard *et al.* (2015) found pama fish contains crude protein 18.1% in lalpoa, Zaman *et al.* (2014) as 15.37± 1% in *Otolithoides pama*. Lipid content was found as 1.73±0.23%. Basu and Gupta (1939) reported pama fish contains 1.7% fat. Mithun *et al.* (2016) found the range of lipid content of fresh pama fish from 3.00% to 5.00%. Bogard *et al.* (2015) as 2.4% in lalpoa, Zaman *et al.* (2014) as 3.01±1.12% in *Otolithoides pama*. Based on the fat content, pama fish could be considered as low to medium fatty fish but not fatty fish. The chemical composition, especially the lipids in marine fish can vary between species and within the same species, being influenced by several factors including diet, overcrowding, growth stage, quality and salinity of the water, and variations attributed to the time of year (Saldanha *et al.,* 2008).

In the present study, ash content was 0.84±.23%. Similar results observed by Mithun *et al.* (2016) that range of ash content of fresh *Pama pama* were varied from 0.99% to 1.99 %. In another study, Bogard *et al.* (2015) as 5.3% in lalpoa, Zaman *et al.* (2014) as 4.78±.92% in *Otolithoides pama* which were higher than the present study. Ash content ranged from 0.7 to 5.3 g/100 g. The large variation in ash content is likely related to inclusion of bones as edible parts in some species, which would lead to higher ash content in these Bogar d*et al.* (2015). Iron content was 2.36mg/100 gm. Average iron content was 2.36 mg/100g in poa. Iron content varied considerably with a range from 0.34 to 19 mg/100 g. (Bogard *et al.,* 2015). Average calcium (Ca) content was 457.0mg/100g in poa muscle. Other results observed by Zaman *et al.* (2014) as 786.72 ± 4.1% in *Otolithoides pama,* Bogard *et al.* (2015) as Calcium 1900 mg/100g which were higher than the present study. As would be expected, calcium content is much higher in bones which are commonly consumed and included in the edible parts. As we estimated calcium without bones, so the calcium content was lower than others. Calcium content ranged considerably from 8.6 to 1900 mg/100 g with a mean content of 600 mg/100 g. These results are within the range of fish and seafood reported (Bogard *et al.,* 2015).

Table 2. Quantitative assessment microorganisms during different storage periods.

Sample	Count of viable microorganism (cfu/g)	Storage period (Days)					
		0	7	14	21	28	35
Control	TBC	2.0×10^{4}	4.7×10^{5}	1.4×10^{6}	2.8×10^{7}		
	TCC	1.4×10^{2}	3.2×10^{2}	4.8×10^{3}	5.7×10^{3}		
	TSC	4.0×10^{2}	8.0×10^{2}	1.0×10^{3}	3.0×10^{3}		
Rad 1.0 KGy	TBC	1.2×10^{3}	1.6×10^{4}	1.8×10^{5}	1.9×10^{6}	2.5×10^{7}	4.9×10^{8}
	TCC	4.5×10^{1}	9.0×10^{1}	1.8×10^{2}	2.2×10^{2}	3.5×10^{3}	2.5×10^{4}
	TSC	1.5×10^{1}	7.8×10^{2}	8.0×10^{2}	1.0×10^{3}	1.5×10^{3}	2.0×10^{4}
Rad 1.5 KGy	TBC	1.0×10^{3}	1.1×10^{4}	1.2×10^{5}	1.1×10^{6}	1.5×10^{7}	4.0×10^{8}
	TCC	3.5×10^{1}	6.0×10^{1}	1.6×10^{2}	2.0×10^{3}	2.7×10^{3}	2.2×10^{4}
	TSC	1.0×10^{1}	2.5×10^{2}	Nill	Nill	Nill	Nill
PS 30 sec	TBC	3.5×10^{3}	5.5×10^{4}	4.5×10^{5}	6.9×10^{6}	3.1×10^{7}	6.5×10^{8}
	TCC	1.0×10^{2}	1.7×10^{2}	3.1×10^{3}	8.1×10^{3}	2.9×10^{4}	8.5×10^{4}
	TSC	3.5×10^{1}	4.7×10^{2}	9.0×10^{2}	1.4×10^{3}	3.2×10^{3}	2.9×10^{4}
PS 60 sec	TBC	2.3×10^{3}	4.4×10^{4}	4.1×10^{5}	3.2×10^{6}	2.4×10^{7}	5.3×10^{8}
	TCC	8.9×10^{1}	1.5×10^{2}	3.0×10^{3}	7.0×10^{3}	1.3×10^{4}	6.0×10^{4}
	TSC	3.0×10^{1}	4.1×10^{2}	7.5×10^{2}	1.2×10^{3}	2.0×10^{3}	2.7×10^{4}

TBC = Total bacterial count; TCC = Total coliform count; TSC = Total staphylococcal count (TSC); Ps 30= 30 secondsdip in 2% potassium sorbate; Ps 60=60 seconds dip in 2% potassium sorbate; Rad 1.0= Radiation 1 KGy; Rad 1.5= Radiation 1.5KGy.

Average phosphorus content was 535.0mg/100g in poa muscle. Other results observed by Bogard *et al.*(2015) as phosphorus 1000 mg/100g which were higher than the present study. Phosphorus content ranged from 110 to 1000 mg/100 g, with higher composition in fish species with bones included in edible parts, also consistent with values reported elsewhere (FAO/INFOODS, 2013).However, the quantities of these constituents may vary considerably within the species and between the species, size, sex, sexual condition, feeding season, athletic activity, Molting stage, Reproductive stage of the life cycle etc. (Rosa and Nunes, 2003; Nargis, 2006).

Organoleptic score (OS)

Organoleptic score of control (without treatment), radiation (1 KGy and 1.5 KGy) and 2%potassium sorbate (30 seconds and 60 seconds dip) treated samples were stored at ±4° C and investigated during the storage periods on the basis of hedonic scores. At the initial, all the samples showed the higher value, after that the value decreased significantly with increasing of storage period stored at ±4°C(Fig.2). The highest organoleptic score (OS) was found in the 1.5 KGy treated sample, whereas the lowest score was measured in the control group. Among durations and treatments scores were significantly different. Under the investigation, best result of OS was found in the treatment of 1.5 KGy. The treated samples with 1.5 KGy were better organoleptically than those with 1.0 KGy than those with 2%Potassium sorbate 60 seconds dip and then 30 seconds dip.

Table 3. The biochemical characteristics of the microorganisms isolated from Poa.

Organisms	Biochemical tests												
	Fermentation tests					Cat	MR	VP	Cit	Ind	Nit	Sta	Hyd
	Glu	Suc	Man	Mtl	Xy								
Shigella	-	-	+	-	-	+	+	-	-	-	+	+	-
B.cereus	+	-	-	-	-	+	-	+	-	-	+	+	-
P.aeruginosa	-	-	-	+	-	+	-	-	+	-	+	-	-
E.coli	+	+	+	+	+	+	+	-	-	+	+	-	-
Stephylococcus	+	+	+	+	-	+	+	-	-	-	+	-	-
Proteus vulgaris	+G	-	-	-	+	+	+	-	+	+	+	+	+
Alcaligenes	-	-	-	-	-	+	-	-	+	-	-	-	-
Klebsiella	+	+	+	+	+	+	+	+	+	-	+	-	-
Micrococcus	-	-	-	-	-	+	+	-	-	-	-	-	-

Glu= Glucose, Suc= Sucrose, Man= Mannose, Mtl= Mannitoll, Xy= Xylose, Cat= Catalase Activity, MR= Methyl Red,VP= VogesProskauer, Cit= Citrate Use, Ind= Indole Production, Nit= Nitrate Reduction, Sta= Starch Hydrolysis, shHyd= Hydrogen Sulfide. G= Positive test with gas production; + = Positive test; - = Negative test.

According to Miyauchi *et al.* (1964) the acceptable limits of sensory score is 5. However,the control (without treatment), Radiation (1 KGy and 1.5 KGy) and 2%Potassium sorbate (30 seconds and 60 seconds dip) samples remained acceptable for up to 21, 28, 28,21 and 21 days of storage periods respectively. So, organoleptic evaluation revealed that the irradiated sample has more acceptability than other samples. Similar result observed by Sheuty *et al.* (2017) in Hilsa shad. Hossain *et al.*(2001) stated low dose of ionizing radiation to reduce the spoilage causing factor in food and there by extended the shelf-life of irradiated products. Same declined pattern were reported by Alam *et al.* (2009) and Ali *et al.* (2009) in Hilsa and Catla respectively. Similar trend was found by Sayed *et al.* (2013) and Ahmed *et al.* (2009) in fish during irradiation at low temperature. Organoleptic score of potassium sorbate treated poa was less than irradiated sample. This may be due to chemical reactions associated with lipids and proteins occurred that leads to detrimental changes in nutritional and sensory properties (Erickson, 1997).

Tokur (2006) reported that values of sensory parameters decreased during the frozen storage period but they were still within acceptable limits. Numerous researchers have reported that protein solubility decreased during frozen storage due to denaturation and aggregation of myofibrillar proteins in fish mince (Benjakul *et al.*, 2005; Leelapongwattana *et al.*, 2005).

It was clear that with the increase of storage period the OS were rapidly decreased in the controlpoa than 2%Potassium sorbate 30 seconds dip and then 60 seconds dip, than those irradiated with 1.0 KGy than those with 1.5 KGy. Besides, the sub-tropical

environment might also be the crucial reason for the presence of different types of bacteria in fishes might be the reason to spoilage as well as acceptability (Nilla *et al.,* 2012a; Nilla *et al.,* 2012b). Due to microbial spoilage with the increase of storage period, the appearance, odor, color and texture will also be deteriorated (Sayed *et al.,* 2013, Mustafa *et al.*, 2013), therefore the OS were decreased.

Tyrosine value

Fish is an important source of quality animal proteins containing essential amino acids. Being an indicator of protein degradation, a gradual change in the tyrosine content has been found during storage periods in this study. The tyrosine contents were found to increase with the increasing of storage periods (Fig.3). From the organoleptic score we have found that the control sample of 28 days was unacceptable, so we didn't measure the tyrosine content on that day. It was observed that the rate of increase of tyrosine value was significantly lower (p=0.000) in irradiated sample (1.5KGy) compared to control and potassium sorbate treated fish sample.

This pattern indicates the preventive potential of gamma radiation process against protein degradation leading to storage time. Similar effect of storage period on tyrosine value was observed in some other studies on fish preservation (Eyas, 2001; Das *et al.,* 2014). Tyrosine value increased with storage period until deamination of amino acid limits the formation of free amino acid (Pearson, 1968).

Table 4. Antibiotic resistance pattern of bacteria (mm).

Bacteria	Ampicillin (10µg)	Amoxycillin (10µg)	Amikacin (30µg)	Chloramphenicol (20µg)	Erhromycin (30µg)	Penicillin (6µg)	Ciprofloxacin (10µg)	Tetracycline (30µg)	Gentamicin (30µg)	Imipenem (10µg)
Shigella	21	22	19	25	-	-	26	-	19	25
B. cereus	-	-	-	-	-	-	-	-	-	-
P. aeroginosa	-	-	-	-	-	-	-	-	-	-
E. coli	-	13	20	31	-	-	35	27	16	29
Stephylococcus	-	-	-	-	-	-	-	-	35	20
Proteus vulgaris	14	21	18	35	-	-	32	17	15	27
Alcaligenes	-	-	-	-	-	-	-	-	-	-
Klebsiella	16	20	19	25	-	-	38	27	19	24
Micrococcus	-	-	-	-	-	-	-	-	-	-

Microbiological analysis

The bacterial growth was observed at 7 days of interval (Table 2).During entire storage period, lowest bacterial load was observed ($1.0x10^3$cfu/g) in radiation (1.5 KGy) treated sample among four treatments. At 21 day, control sample had significantly higher bacterial load compared to other samples. The results showed that bacterial count wasincreasedslowly in both irradiated and potassium sorbate treated sample than that of control. The same trend of results was confirmed by Michele *et al.* (2013). TBC was found to be reduced by 3 logs in blend *Otolithoides pama* at 3 KGy (Acharjee *et al.,* 2014). Irradiation doses ranging from 1 to3 KGy have been suggested for shelf life extension of fresh fish (Molins *et al.,* 2001; Jo *et al.,* 2004). Mendes *et al.* (2005) reported that bacterial count of irradiated sea fish were lower than those in non-irradiated samples during the storage at 4°C which is in agreement with the result of this study. Irradiation treatments induced ionization for the cell of bacteria and directly effects on DNA of nucleus cells (Temur and Tiryaki,

2013). The lower count in potassium sorbate dip treated samples might be due to the antimicrobial action of potassium sorbate. The decrease in microbial count may be attributed to the fact that potassium sorbate inhibits bacterial spore formation in fish fillets (Laxmareddy and Benarjee, 2013).

The initial TCC level was 1.4x10² cfu/g (0 day) in control (untreated) fishes that has been increased to 5.7x10³ on21day. The irradiated and dipped fish sample had lower bacterial count (Table 2). The results depicted that irradiation has good impact on elimination of coliform and coliform count were increased slowly in both irradiated and potassium sorbate treated sample than control. Abu-Ghazaleh (2012) reported that potassium sorbate alone or in combination with citric acid is effective in reducing the growth of coliform. Omojowo *et al.* (2009) found 3-5% potassium sorbate inhabit the growth of TCC in smoked Tilapia fillets.

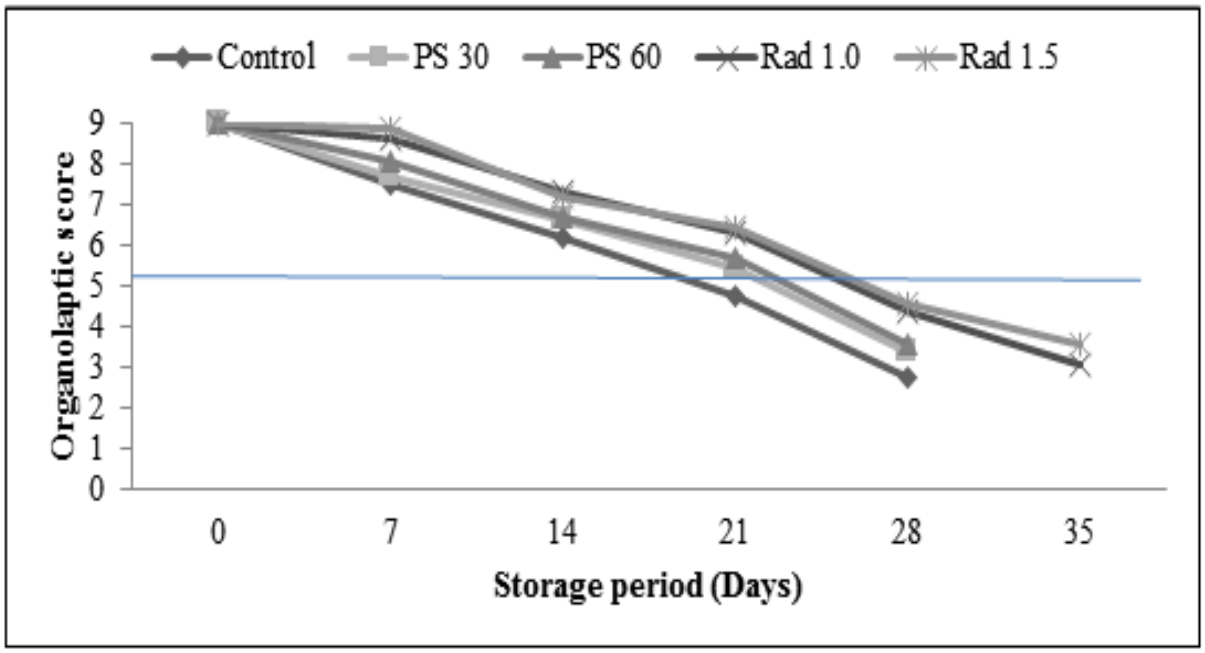

Fig. 2. Organoleptic score of control, radiation (1.0 KGy and 1.5 KGy) and 2% potassium sorbate (30 seconds and 60 seconds dip) treated sample during the storage period at4˚C. The legends notation: Ps 30= 30 seconds dip in 2% potassium sorbate; Ps 60=60 seconds dip in 2% potassium sorbate; Rad 1.0= Radiation 1 KGy; Rad 1.5= Radiation 1.5KGy.

TSC ranged from4.0x10² (0 day) to 3.0x10³ (21 day) cfu/g in control samples. During 35 days of storage period, lowest *Staphylococcus* count was observed in radiation 1.5 KGy samples. Nouchpramool *et al.* (1985) and Research (1978) observed that the dose of radiation near about 2.5 kGy was able to eliminate *Staphylococcus aureus* in frozen and smoked sea fish. Haque *et al.* (2013) found that TSC became reduced at 2.5 and 5.0 kGy of irradiation doses and became nil at the radiation doses of 7.5 kGy.Acharjee *et al.* (2014) found that *Staphylococcus* spp. was reduced by 3 log in *Otolithoides pama* at 3 KGy. Presence of *Staphylococcus* sp. suggests that there was higher level of environmental contamination and its presence indicates the possible risks of food poisoning as found by (Nanu and Narayan, 1992).Abu-Ghazaleh (2012) reported that potassium sorbate alone or in combination with citric acid is effective in reducing the growth of *Staphylococcus aureus*.

Biochemical identification of microorganisms

To identify specific grown microbes, different bacterial isolates were selected from different media on the basis of agar colony morphology and biochemical characteristics. A total of nine different

organisms were identified from the samples including gram-positive (*B. cereus, Stephylococcus, Micrococcus)* andgram-negative (*Shigella, P.aeruginosa, E. coli, Proteus vulgaris, Alcaligenes, Klebsiella)* (Table 3) microorganisms are associated with fish spoilage. Acharjee *et al.* (2014) found *Salmonella spp*., *Vibrio spp*., *Pseudomonas spp*., *Staphylococcus spp*. in *Otolithoides pama*.

Antibiotic sensitivity test

Nine selected isolates were carried out to antibiotic susceptibility test against pre-selected antibiotics (Table 4). Antibiotics used in this experiment were: Ampicillin, Amoxycillin, Amikacin, Chloramphenicol, Erythromycin, Penicillin, Ciprofloxacin, Tetracycline, Gentamicin and Imipenem. The results were prepared according to the zone of inhibition produced on plates.Several studies worldwide and in Bangladesh reported antimicrobial activity in different food samples (Dubey *et al.,* 2010, Hussain *et al.,* 2010). We found that, *B. cereus,P. aeroginosa, Alcaligenes* and *Micrococcus* were resistant to all the tested 10 antibiotics. All were highly sensitive to Ciprofloxacin and Chloramphenicol and resistant to Erhromycin and Penicillin.

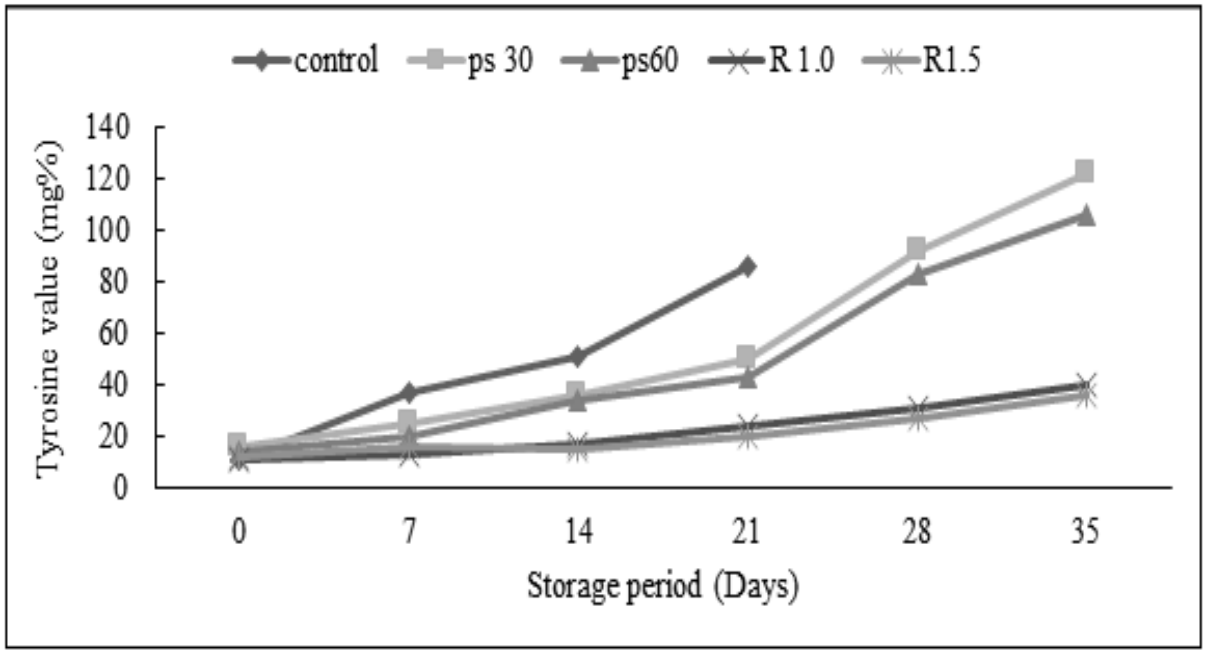

Fig. 3. Tyrosine value of control, radiation (1.0 KGy and 1.5 KGy) and 2% potassium sorbate (30 seconds and 60 seconds dip) treated sample during the storage period at4˚C. Ps 30= 30 seconds dip in 2% potassium sorbate; Ps 60=60 seconds dip in 2% potassium sorbate; Rad 1.0= Radiation 1 KGy; Rad 1.5= Radiation 1.5KGy.

The uncontrolled and irresponsible use of antibiotics and other chemotherapeutic agents are responsible for the occurrence of the antibiotic resistant trait among the pathogens and the majority of the drug resistant bacteria carry drug resistant (R) factor. It was interesting to observe some antibiotic sensitive strains in this study.

Conclusion

Poa*(Pama pama)*is very popular and economical to poor people. If we cannot preserve properly, it will be quite impossible to gain benefits from fisheries sector. Various preservation methods are used to preserve the fishes like low temperature, irradiation, and chemical preservatives. This study found that irradiation is more effective, although costly and is not available everywhere in Bangladesh. However, preservation by using potassium sorbate has more or less same effective during preservation of fish at low temperature. So, we can use minimum doses of potassium sorbate as preservative in our local market channel as well as for export purpose. This study

depicted that the combination with irradiation (1.5 KGy) and low temperature (±4°C) could be most effective treatment in extending shelf-life and overall reductions in microbial load of poa. Further research could be done to evaluate the economical efficacy of these preservation techniques in large scale.

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