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Effect of potassium sorbate and gamma irradiation on the shelf-life of Hilsa shad, *Tenualosa ilisha* (Hamilton, 1822) at low temperature

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Abstract
Shelf-life of potassium sorbate (2%) and gamma irradiation (2kGy) treated Hilsa shad (*Tenualosa ilisha*) were determined after storing at 0^oC and 4^oC for a period of upto 35 days. Organoleptic evaluation showed that control, potassium sorbate treated and irradiated samples remained acceptable upto 21, 28 and 35 days of storage periods respectively at 0^0 C. Whereas, the same samples remained acceptable upto 7, 14 and 21 days of storage respectively at 4^0 C. Tyrosine and pH of fish flesh increased with the increasing of storage periods in all samples but, this increasing trend was somewhat lower in irradiated sample compare to other samples. Microbial assessment showed the best results in case of irradiated sample and remained within the acceptable limit.

Kew words: Potassium sorbate, gamma irradiation, low temperature and Hilsa shad (*Tenualosa ilisha*)

INTRODUCTION

Bangladesh is blessed with Hilsa shad (*Tenualosa ilisha*) which is very much popular for its unique test and nutritive value and regarded as national fish. This fish is important socially and culturally to Bengali people living both in Bangladesh and abroad and it has great contribution to economy of the country. During the 2001-02 the production of Hilsa was 284.60 thousand MT whereas, in 2013-14 this production increased to 385.14 MT (Shamsuzzaman, 2017). Due to some restrictions this fish are not available throughout the year. It is well known that fishes are considered as one of the most perishable of all food stuffs. As soon as a captured fish dies, it begins to deteriorate. Huge amount of fishes were spoiled in peak catching period due to lack of preservation techniques. So, to prevent spoilage of fish, some forms of preservation techniques are necessary. Among the various techniques of preservation, freezing is considered to be the only long-term method which can preserve a fish with a minimum change in its quality. Storage time and temperature are the major factors affecting the rate of loss of quality and shelf-life of fish (Whittle, 1997). Preservation techniques often use chemical preservatives like sorbic acid or potassium sorbate which has been approved in the United States as a GRAS (generally regarded as safe) substance (Liewen & Marth, 1985). Sorbic acid has been proved as an effective preservative of chilled fish by inhibiting the spoilage bacteria (Robach, 1979).

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On the other hand, Food irradiation is a preservation technique that uses ionizing radiation and has minimum influence on food properties. Gamma irradiation has been considered as an effective method of preservation to extend the shelf-life of chilled, stored fish and also to reduce qualitatively and quantitatively the microbial population in fish and fish products (Laycock & Regier, 1970 and Abu-Tarboush *et al.*, 1996). Considering the above facts in mind, a study was carried out to examine the shelf-life of potassium sorbate and gamma irradiation treated Hilsa shad (*Tenualosa ilisha*) after storing at low temperature.

MATERIALS AND METHODS

Fresh Hilsa Shad, *Tenualosa ilisha* were collected from the local fish market of Savar and immediately brought to the laboratoryof Food Technology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka in a presterilized polythene bag with ice. The fish samples were then beheaded, degutted, descaled, sliced and finally washed with tap water. Total fish sample were then divided into 3 lots. The fishes of lot 1 was kept as control, 2^{nd} lot was subjected to dip 1 minute in 2% potassium sorbate solution and 3^{rd} lot was subjected to 2 kGy gamma irradiation. Thereafter, each lot was further divided into equal two parts, packaged separately and one part was preserve at 0^0C and another part at 4^oC temperature upto 35 days. Shelf-life was analyzed after each 7 days of interval.

Organoleptic analysis i.e., appearance, color, odorand texture were assessed according to Peryam & Pilgram (1957) developed nine points hedonic scales which were used for sensory evaluation by 3-6 judges. Tyrosine value was determined by following the method as described by Wood *et al*. (1942). To determine the pH of fish about 5gm macerated fish sample was taken in a homogenizer and 25 ml of water was added in the homogenized sample. Then the sample was taken in a beaker. The pH of the sample was determined by the help of a pH meter (JENWAY 3510).

The total bacterial count (TBC), total mould count (TMC), and total coliform count (TCC) were estimated and determined after Burgey's manual by applying determinative dilution technique, followed by standard spread plate count (Sharp & Lyles, 1969).

RESULTS AND DISCUSSION

Organoleptic evaluation: Organoleptic and sensory evaluation is a universally accepted technique for estimating the quality of fish.At the initial period of storage all the samples showed the same value (8±0) after that the value decreasedsignificantly ($p<0.05$) with increasing of storage period in both the storage temperature (Table 1). According to Miyauchi *et al*. (1964) the acceptable limits of sensory score is 5. So, control, potassium sorbate treated (2%) and irradiated (2kGy) samples remained acceptable for upto 21, 28 and 35 days of storage periods respectively at 0^0 C and they were significantly different ($p<0.05$). Whereas, the same samples remained acceptable upto 7, 14 and 21 days of storage respectively at 4^0 C (Table 1). So, organoleptic evaluation revealed that the irradiated sample has more shelf-life than other samples. Hossain *et al.* (2001) stated low dose of ionizing radiation to reduce the spoilage causing factor in food andthereby extended the shelf-life of irradiated products. Same declined pattern were reported by Alam *et al.* (2009); Ali *et al.* (2009) and Kamrujjaman *et al.* (2003) in Hilsa, Catla and Cichlid respectively. The appearance, odor, color and texture of fishes deteriorated during the storage due to microbial spoilage as a consequent the organoleptic scores are decreased.

Tyrosine value: It was observed that the rate of increase of tyrosine value for 4^0 C were comparatively higher than 0^0 C (Table 1). Again, the range of tyrosine value during the 35 days of storage were 9.40-14.26mg%, 8.89-12.40mg% and 8.5-12.20mg% for control, potassium sorbate treated and irradiated sample respectively (Table 1). So, the degree of autolytic and bacterial proteolysis was lowest in irradiated sample and statistically significant ($p<0.05$). Pearson (1968) found that tyrosine value must increase as a result of spoilage.Similar results have been reported by Bose (1969), Ozden (2005) and Sultana *et al.* (2010).

Table 1. Quality of potassium sorbate and gamma irradiated Hilsa shad (*Tenualosa ilisha*) during the storage at 0°C and 4°C ("-" indicate data were not possible to take due to spoilage of fish samples)

Quality tests	Storage Period (Days)	Samples					
		Control		Potassium sorbate treated (2%)		Irradiated (2kGy)	
		0°C	4°C	0°C	4°C	0°C	4°C
Organoleptic evaluation	0	$8\pm0_{d}^{c}$	$8\pm0_{c}^{a}$	$8\pm0_{d}^{a}$	$8\pm0_{c}^{b}$	$8\pm0_{d}^{b}$	$8\pm0_{c}^{c}$
	7	$7.2\pm0.26_{d}^{c}$	$5.1\pm0.51_{b}^{a}$	$7.8\pm0.14_{d}^{a}$	$7.1\pm0.13_{b}^{b}$	$7.6\pm0.16_{d}^{b}$	$7.6\pm0.3_{b}^{c}$
	14	$6.4\pm0.17_{d}^{c}$	$2.5\pm0.25_{a}^{a}$	$7.4\pm0.17_{d}^{a}$	$5.2\pm0.3_{a}^{b}$	$7.5\pm0.25_{d}^{b}$	$7.5\pm0.25_{a}^{c}$
	21	$5\pm0.25_{b}^{c}$	-	$6.2\pm0.02_{b}^{a}$	-	$6.6\pm0.15_{b}^{b}$	$5.1\pm0.26_{d}^{c}$
	28	$3.6\pm0.17_{c}^{c}$	-	$5.1\pm0.30_{c}^{a}$	-	$5.8\pm1_{c}^{b}$	-
	35	$2.4\pm0.14_{a}^{c}$	-	$4.4\pm0.14_{a}^{a}$	-	$5.1\pm1.02_{a}^{b}$	-
Tyrosine	0	$9.40\pm0.3_{a}^{b}$	$9.40\pm0.52_{b}^{b}$	$8.89\pm0.84_{a}^{a}$	$8.89\pm0.50_{b}^{a}$	$8.5\pm0.46_{a}^{a}$	$8.8\pm0.6_{b}^{c}$
	7	$10.06\pm0.03_{a}^{b}$	$12.32\pm0.37_{c}^{b}$	$9.08\pm0.04_{a}^{a}$	$9.97\pm0.40_{c}^{a}$	$8.85\pm0.40_{a}^{a}$	$10.42\pm0.26_{c}^{c}$
	14	$10.88\pm0.07_{b}^{b}$	$19.42\pm0.40_{d}^{b}$	$10.36\pm0.30_{b}^{a}$	$12.62\pm0.34_{d}^{a}$	$9.88\pm0.61_{b}^{a}$	$13.06\pm0.2_{d}^{c}$
	21	$12.18\pm0.56_{c}^{b}$	-	$11.04\pm0.33_{c}^{a}$	-	$11.05\pm0.34_{c}^{a}$	$17.26\pm0.20_{a}^{c}$
	28	$13.24\pm0.48_{d}^{b}$	-	$11.68\pm0.34_{d}^{a}$	-	$11.72\pm0.55_{d}^{a}$	-
	35	$14.26\pm0.51_{e}^{b}$	-	$12.40\pm0.45_{e}^{a}$	-	$12.20\pm0.72_{e}^{a}$	-
pH	0	$6.74\pm0.35_{a}^{b}$	$6.74\pm0.21_{b}^{a}$	$6.69\pm0.27_{a}^{a}$	$6.69\pm0.24_{b}^{a}$	$6.62\pm0.23_{a}^{a}$	$6.62\pm0.32_{b}^{b}$
	7	$6.85\pm0.47_{ab}^{b}$	$7.55\pm0.52_{c}^{a}$	$6.67\pm0.29_{ab}^{a}$	$6.82\pm0.49_{c}^{a}$	$6.65\pm0.23_{ab}^{a}$	$7.09\pm0.39_{c}^{b}$
	14	$6.8\pm0.52_{ab}^{b}$	$8.16\pm0.42_{d}^{a}$	$6.69\pm0.17_{ab}^{a}$	$8.04\pm0.23_{d}^{a}$	$6.67\pm0.24_{ab}^{a}$	$7.6\pm0.53_{d}^{b}$
	21	$7.43\pm0.20_{ab}^{b}$	-	$6.68\pm0.24_{ab}^{a}$	-	$6.69\pm0.23_{ab}^{a}$	$7.78\pm0.45_{a}^{b}$
	28	$7.45\pm0.23_{ab}^{b}$	-	$6.72\pm0.22_{ab}^{a}$	-	$6.71\pm0.23_{ab}^{a}$	-
	35	$7.5\pm0.26_{b}^{b}$	-	$6.75\pm0.14_{b}^{a}$	-	$6.73\pm0.22_{b}^{a}$	-

* Values are means ± standard deviation (SD) of three replicates. Columns with different subscript (days) and rows with different superscript (samples) letter indicate significant difference ($P < 0.05$(for relevant temperature of each).

pH value : The pH parameter is a good indicator for the determination of freshness of fish. The pH of the fresh fishes is 6.4 or around 7 and during the spoilage the pH falls or rises from that. In the present study, though the variation among the days was insignificant ($p>0.05$) but, there were a significant differences among the samples ($p<0.05$). It was evident that pH value of all samples increased over the time and these increasing trends was significantly higher at 4^oC temperature compared to 0^oC (Table 1). Again, control sample showed acidic to alkaline and both potassium sorbate treated and irradiated sample showed acidic to near neutral at 0^oC (Table 1). On the other hand, irradiated sample showed acidic to slightly alkaline whereas, both control and potassium sorbate treated sample showed acidic to alkaline at 4^oC temperature (Table 1). During the storage period decomposition products such as volatile bases could lead to a pH rise (Debbarma & Majumdar, 2013). Licciradello *et al*. (1986), Reddy & Rao (1996); Papadima & Bloukas (1999); Singh & Verma (2000) and Nayak & Tanwar (2004) and Gandotra *et al.* (2012b) also reported similar increasing trend of pH during refrigerated storage of different fish and meatproducts.

Microbiological quality: Total bacterial counts (TBC) were significantly different between storage temperatures. During the storage at 0^oC, bacterial growth increased gradually upto 28 days and thereafter slightly declined both in control and potassium sorbate treated samples (Table 2). Whereas, in irradiated sample the bacterial growth increased upto 21 days and thereafter slowly declined. Same increasing trend was observed in samples kept in 4^oC and the count was higher than the sample kept in 0^oC (Table 2). Again, the irradiated sample comparatively contained lower bacteria than other two samples (Table 2). According to Laycock & Reigier (1970), the acceptable limit of TBC is 1.0×10^7 cfu/g in fish sample. According to the above statement all the samples showed acceptable limit of TBC both at 0^0 and 4^0C. Same increasing trend of bacterial count was also reported by Gandotra *et al.* (2012a), Mustafa *et al.* (2014), Nur-A-Sayed *et al*. (2012), Ahmed *et al.* (2009) and Rashid *et al*. (1998) in case of different fishes. But, Sultana *et al.* (2010) stated declining trends of salted Hilsa during storage at $0\text{-}4^oC$ temperature.

The total mould count (TMC) was significantly lower than total bacterial count (TBC). The numbers of mould colonies were found only upto 7 days both at 0^0 and 4^0 C. It was found that total mould count gradually declined in all samples and this count was lowest in irradiated sample compare to other two samples (Table 2). Mould showed very sensitive to temperature as the low temperature adversely effects the mould growth and multiplication. On the other hand, Nur-A-Sayed *et al.* (2012) reported that TMC increased in case of irradiated stinging catfish storage at -20^oC temperature.

Total coliform counts (TCC) were observed upto the 35 and 14 days at 0^0 C and 4^0 C storage temperature respectively (Table 2). In case of control sample TCC gradually increased upto 14 days of storage and thereafter gradually decreased at both storage temperatures whereas, potassium sorbate treated and irradiated samples the TCC increased upto 7 days of storage and thereafter gradually decreased at both storage temperature But, the count was significantly lower ($P < 0.05$) in irradiated sample followed by potassium sorbate treated and controls (Table 2). According to ICMSF

(1986) guideline, acceptable total coliform count for fish is less than 500cfu/g. So the potassium sorbate treated and irradiated sample was acceptable during whole investigation period except control sample which remained acceptable upto 7 days of storage period (Table 2). Similar trend of TCC was reported by Hossain *et al*. (1991), Gandotra *et al*. (2012b), Uzeh *et al*. (2006) and Bhat *et al*. (2010).

Table 2. Microbiological quality of potassium sorbate and irradiated Hilsa shad (*Tenualosa ilisha*) during storage at 0°C and 4°C("-" indicate data were not possible to take due to spoilage of fish samples)

Quality tests	Storage Period (Days)	Samples					
		Control		Potassium sorbate treated (2%)		Irradiated (2KGy)	
		0°C	4°C	0°C	4°C	0°C	4°C
TBC (cfu/gm)	0	$2.6\pm0.91\times10^{3}{}_{d}^{b}$	$2.6\pm0.3\times10^{3}{}_{d}^{a}$	$7.5\pm0.60\times10^{2}{}_{d}^{a}$	$7.5\pm0.52\times10^{2}{}_{d}^{b}$	$7\pm1\times10^{2}{}_{d}^{a}$	$7\pm1\times10^{2}{}_{d}^{c}$
	7	$7.9\pm0.85\times10^{4}{}_{b}^{b}$	$1.24\pm0.09\times10^{6}{}_{a}^{a}$	$1.0\pm0.5\times10^{3}{}_{b}^{a}$	$1.1\pm0.5\times10^{4}{}_{a}^{b}$	$1.2\pm0.4\times10^{3}{}_{b}^{a}$	$1.4\pm0.45\times10^{4}{}_{a}^{c}$
	14	$9\pm1\times10^{5}{}_{c}^{b}$	$1.6\pm0.26\times10^{6}{}_{c}^{a}$	$1.4\pm0.45\times10^{4}{}_{c}^{a}$	$3.8\pm0.52\times10^{4}{}_{c}^{b}$	$1.6\pm0.3\times10^{4}{}_{c}^{a}$	$3.2\pm0.45\times10^{4}{}_{c}^{c}$
	21	$7.1\pm0.36\times10^{5}{}_{bc}^{b}$		$1.8\pm0.4\times10^{4}{}_{bc}^{a}$	-	$2.7\pm0.2\times10^{4}{}_{bc}^{a}$	$5.8\pm0.52\text{x}10^{4}{}_{b}^{c}$
	28	$6.2\pm0.45\times10^{6}{}_{bc}^{b}$		$2.6\pm0.3\times10^{4}{}_{bc}^{a}$	-	$2.3\pm0.36\times10^{4}{}_{bc}^{a}$	-
	35	$2.0\pm0.52\times10^{5}{}_{a}^{b}$		$1.0\pm0.5\times10^{3}{}_{a}^{a}$	-	$1.0\pm0.5\times10^{3}{}_{a}^{a}$	
TMC (cfu/gm)	0	$2\pm0.45\times10^{1}{}_{b}^{ab}$	$2\pm0.5\times10^{1}{}_{b}^{a}$	$3\pm1\times10^{1}{}_{b}^{b}$	$3\pm1\times10^{1}{}_{b}^{b}$	$1.5\pm0.5\times10^{1}{}_{b}^{a}$	$1.5\pm0.5\times10^{1}{}_{b}^{a}$
	7	$1.8\pm0.45\times10^{1}{}_{a}^{ab}$	$1.1\pm0.45\times10^{1}{}_{a}^{a}$	$1.3\pm0.45\times10^{2}{}_{a}^{b}$	$1.5\pm0.5\times10^{2}{}_{a}^{b}$	$1.1\pm0.4\times10^{1}{}_{a}^{a}$	$1.3\pm0.4\times10^{1}{}_{a}^{a}$
	14	Nil	Nil	Nil	Nil	Nil	Nil
	21	Nil	-	Nil	-	Nil	-
	28	Nil	-	Nil	-	Nil	-
	35	Nil	-	Nil	-	Nil	-
TCC (cfu/gm)	0	$3.7\pm0.36\times10^{2}{}_{d}^{c}$	$3.7\pm0.45\times10^{2}{}_{b}^{c}$	$1.2\pm0.5\times10^{2}{}_{d}^{a}$	$1.2\pm0.4\times10^{2}{}_{b}^{a}$	$8.6\pm0.26\times10^{1}{}_{d}^{b}$	$8.6\pm0.55\times10^{1}{}_{b}^{b}$
	7	$7.1\pm0.7\times10^{2}{}_{d}^{c}$	$5.4\pm0.5\times10^{2}{}_{a}^{c}$	$3.3\pm0.3\times10^{2}{}_{d}^{a}$	$3.6\pm0.7\times10^{2}{}_{a}^{a}$	$2.9\pm0.7\times10^{2}{}_{d}^{b}$	$2.7\pm0.5\times10^{2}{}_{a}^{b}$
	14	$9.2\pm0.5\times10^{2}{}_{d}^{c}$	$6.7\pm0.55\times10^{2}{}_{a}^{c}$	$2.4\pm0.45\times10^{2}{}_{d}^{a}$	$2.8\pm0.72\times10^{2}{}_{a}^{a}$	$1.8\pm0.6\times10^{2}{}_{d}^{b}$	$1.6\pm0.36\times10^{2}{}_{a}^{b}$
	21	$3.2\pm0.36\times10^{2}{}_{b}^{c}$	-	$2.1\pm0.6\times10^{2}{}_{b}^{a}$	-	$1.2\pm0.36\times10^{2}{}_{b}^{b}$	-
	28	$1.5\pm0.5\times10^{2}{}_{c}^{c}$	-	$5\pm0.5\times10^{1}{}_{c}^{a}$	-	$3\pm0.5\times10^{1}{}_{c}^{b}$	-
	35	$2\pm1\times10^{1}{}_{a}^{c}$	-	$1\pm1\times10^{1}{}_{a}^{a}$	-	$1\pm0.5\times10^{1}{}_{a}^{b}$	-

* Values are means ± standard deviation (SD) of three replicates. Columns with different subscript (days) and rows with different superscript (samples) letter indicate significant difference ($P < 0.05$(for relevant temperature of each).

The present studies demonstratethat irradiation (2kGy) has good effects on the preservation of Hilsa shad (*Tenualosa ilisha*) at 0°C temperature. So, it might be an effective preservation means with maintaining all the international standards needed for consumer satisfaction and safety and thereby to make it available throughout the year. Though irradiation process is unavailable and costly, potassium sorbate treatment could be an alternative method.

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