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## Effects of ascorbic acid and gamma radiation on shelf life extension of freshwater hilsa, *Tenuulosa ilisha* (Hamilton, 1822) at refrigerated temperature

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### Abstract

This research work has been conducted to evaluate the effect of gamma radiation and ascorbic acid on organoleptic, biochemical and microbial quality to extend shelf life of freshwater hilsa. Organoleptic evaluation showed that ascorbic acid (1% and 2%) treated and irradiated (1.5 and 3 kGy) samples remained acceptable up to 19 and 21 days, respectively of storage periods at 4 °C. Tyrosine and phenolic content of fish flesh increased with the increase of storage periods in all the samples but increment of tyrosine value was somewhat lower in irradiated sample compare to control. However, the increasing trend of thiobarbituric acid reactive substance (TBARS) value of fish flesh was higher in both treated samples compared than control. Microbial assessment showed the better result in irradiated (3 kGy) sample compared to other and remained within the safe limit. The present study demonstrates that irradiation is effective for preservation of hilsa at 4°C temperature. Though, radiation process is expensive and inconvenient, ascorbic acid could be a substitute process for preservation.

**Keywords:** Ascorbic acid, microbial assessment, organoleptic evaluation, radiation, shelf-life

### Introduction

*Tenuulosa ilisha* belonging to the family Clupeidae, is locally known as 'ilish' or 'ilsha' in Bangladesh. Hilsa got official registration as a geographical indication product of the country and is now globally identified as a fish of Bangladesh origin. Hilsa has a wide range of distribution and occurs throughout the entire coastal waters, estuaries and tidal rivers and also in the further upstream rivers in Bangladesh [1].

Ice storage is the conventional method to preserve fresh fish. In spite of the availability of modern cold-chain and transport facilities, distribution of fresh fish remains a problem, especially in temperate and tropical climate countries [2]. Food irradiation is a process exposing food to ionizing such as gamma rays emitted from radioisotopes <sup>60</sup>Co27 and <sup>137</sup>Cs55 or high energy electrons and X-rays produced by machine sources. The radiation doses required to inactivate 90% of the colony forming units (cfu) of the common food-borne pathogens associated with meat and meat products are in the range of 1-4 kGy. Depending on the absorbed radiation dose, various effects can be achieved resulting in reduced storage losses [3]. Spoilage due to microbial activity is the main limitation of the shelf life of refrigerated fish. Spoilage microflora produces enzymes that cause proteolysis, deamination, and decarboxylation resulting in accumulation of unpleasant metabolites and loss of taste substances. Changes in the sensory characteristics related to appearance, odour, taste and texture on fresh fish are mainly due to the activity of spoilage organisms [4]. Refrigeration inhibits the activity of food spoilage organisms and the low storage temperature greatly slows down the enzymatic and biochemical reactions that normally occur in unfrozen foods [5]. The antimicrobial effect of ascorbic acid is an attractive alternative because these acids appear naturally in many foods; they are an essential nutrient and have been generally recognized as safe. The concentration of ascorbic acid, treatment time, temperature and the type of organism plays an important role in reducing the number of microorganisms [6]. However, there are limited research works carried out on shelf life extension by using gamma radiation and chemical [7, 8]. Therefore, this study was undertaken to evaluation of gamma radiation (1.5 and 3.0 kGy)

and ascorbic acid (1 and 2 %) induced changes on organoleptic, tyrosine, phenol content, thiobarbituric acid value and microbial status during storage at refrigerated temperature for extended shelf-life of freshwater hilsa.

## Materials and Methods

### Sample collection

Freshwater hilsa were collected from the Sandhya river, Babuganj, Barisal. All samples are immediately brought to the laboratory of food technology division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka in a sterilized polythene bag with ice.

### Preparation of sample

The fish samples were then deheaded, 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, 2nd lot was subjected to dip 1 minute in 1% and 2% ascorbic acid solution and 3rd lot was subjected to 1.5 and 3 kGy gamma radiation. Thereafter, each lot was further divided into equal two parts, packaged separately and one part was preserved at 4 °C temperature up to 21 days. Shelf-life was analyzed after each 7 days of interval.

### Organoleptic evaluation

Organoleptic analysis i.e., appearance, color, odor and texture were assessed according to Peryam and Pilgram<sup>[9]</sup> developed nine points hedonic scales which were used for sensory evaluation by 3-6 judges.

### Tyrosine, thiobarbituric acid and phenol content

Tyrosine value was determined by following the method as described by Wood *et al.*<sup>[10]</sup>. Thiobarbituric acid was determined by conducting TBA mixture with extraction in a water bath to produce red color which was then estimated spectrophotometrically. Total phenolic content of the fish extract was determined by using Folin-ciocalteu reagent following a slightly modified method of Ahmed *et al.*<sup>[11]</sup>.

### Microbiological evaluations

The total bacterial count (TBC), total salmonella-shigella count (TSSC), and total coliform count (TCC) were estimated and determined after Burgey's manual by applying determinative dilution technique, followed by standard spread plate count<sup>[12]</sup>.

## Results and Discussion

### Organoleptic evaluation

Organoleptic evaluation is a universally accepted technique for estimating the quality of fish. Organoleptic score was comparatively higher in control and irradiated (1.5 and 3 KGy) samples at 7 days of storage than ascorbic acid treated fishes. After day 7 the organoleptic score of control sample decreased sharply and became unacceptable at the 14<sup>th</sup> days of storage. Whereas ascorbic acid (1 and 2%) and irradiated (1.5 and 3 kGy) samples were found acceptable up to day 14 and 21 respectively during storage (Fig. 1).

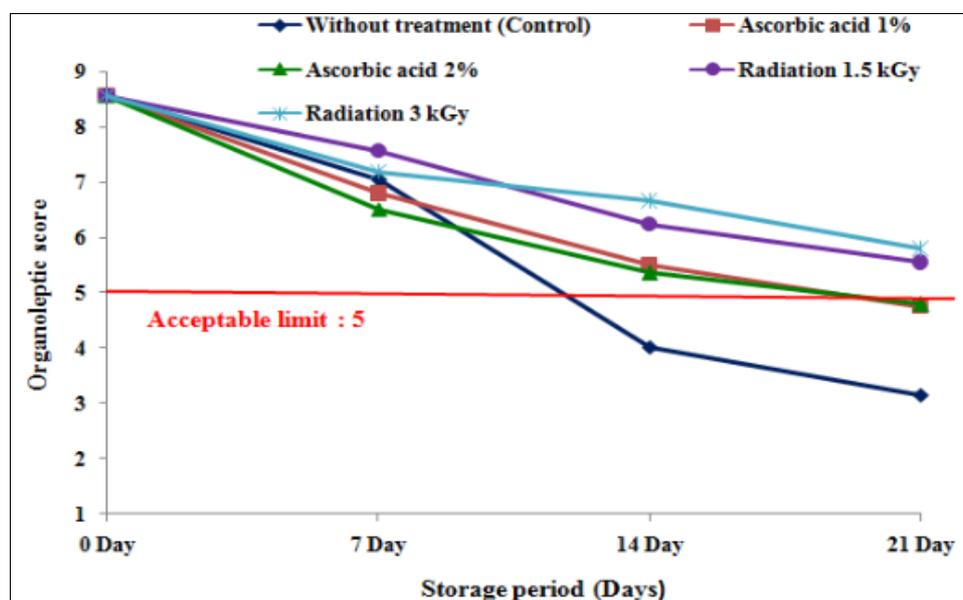


Fig 1: Organoleptic scores of freshwater hilsa at 4 °C temperature

The present result indicated that irradiation could be an effective technique for hilsa fish preservation from the organoleptic point of view. Sheuty *et al.*<sup>[7]</sup> found that irradiated (2 kGy) samples remained acceptable upto 21 days of storage respectively at 4°C. There is no study found regarding ascorbic acid treatment on fish preservation.

### Tyrosine value

Results on the tyrosine value in gamma radiation and ascorbic acid treated and stored at refrigerated temperature of freshwater hilsa fish have presented in Fig. 2. The tyrosine

values were found to increase with the increase of storage periods. Tyrosine values increased slowly in radiation (1.5 and 3 kGy) treated fishes whereas increased sharply in control and ascorbic acid (1 and 2 %) treated samples from 0 day to 21 days of storage period. Reduced rate of increment in tyrosine values when fish treated with gamma radiation indicated lower rate of autolytic and bacterial proteolysis during storage. Sheuty *et al.*<sup>[7]</sup> reported that gamma radiation (2 kGy) treated hilsa showed reduced rate of increment in tyrosine values than control and potassium sorbate treated samples stored at 4 °C.

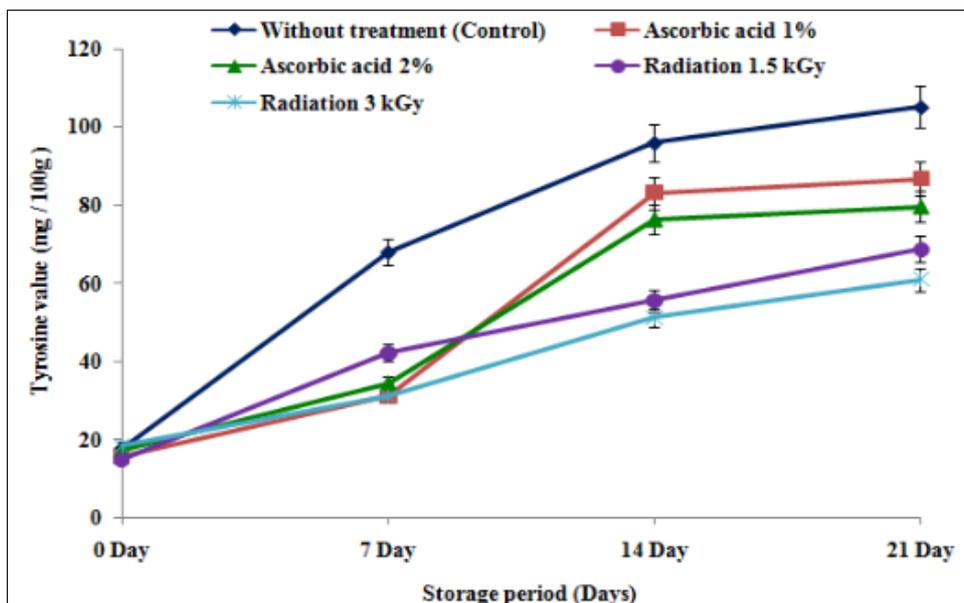


Fig 2: Tyrosine values of freshwater hilsa in ascorbic acid and radiation treated samples at 4 °C temperature

**Thiobarbituric acid reactive substance value**

The Thiobarbituric acid reactive substance (TBARS) value found to be decreased in control and irradiated (3 kGy) samples upto 14 days and increased on day 21. On the other hand, the TBARS values in ascorbic acid (1 and 2 %) treated fishes increased on day 7 then decreased at day 14 but increased again on day 21. Radiation (1.5 and 3 kGy) treated fishes showed a significantly higher TBARS values compared to other treatments. Present TBARS values for irradiated at

1.5 and 3kGy were higher than those for the control and ascorbic acid treated samples throughout the entire storage period (Fig. 3). This may be attributed to a higher concentration of free radicals formed in the substrate upon irradiation [13]. TBARS value used to indicate the good quality of the fish frozen, chilled or stored with ice is 5 mg malonaldehyde/kg while the fish may be consumed up to level of 8 mg malonaldehyde/kg.

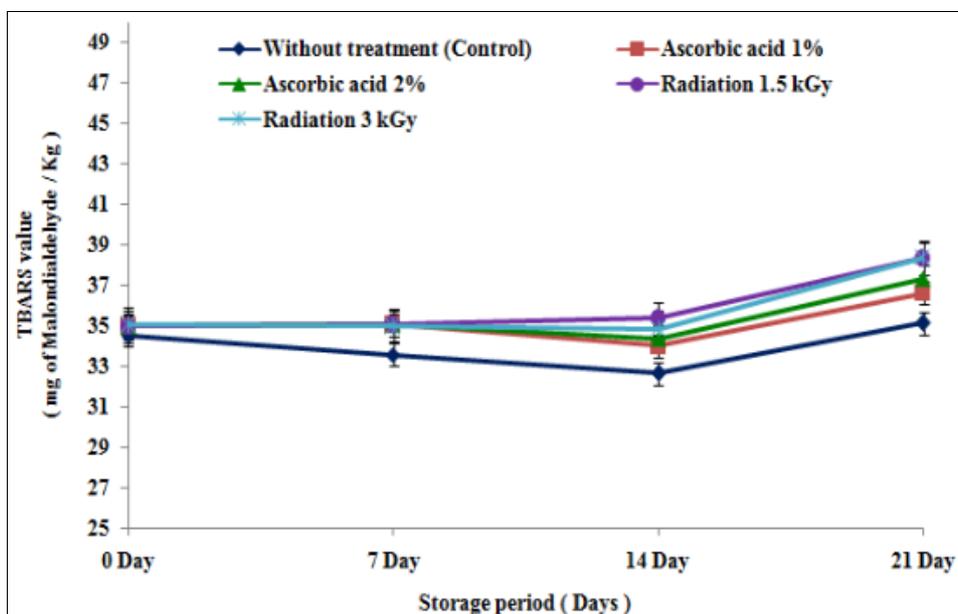


Fig 3: TBARS values of freshwater hilsa in ascorbic acid and radiation treated samples at 4 °C temperature

**Total phenolic content**

The present result showed that the total phenol content in control and irradiated (1.5) fish increased with increasing storage time. But total phenol content sharply decreased in ascorbic acid (1and 2 %) and radiation (3 kGy) treated fishes on day 7 and increased again upto day 21 of storage period. It was found that ascorbic acid (2%) treated sample showed

higher phenol content whereas irradiated sample (1.5 and 3 kGy) showed significantly lower phenol content compared to the other treatments. Although the radiation has affected the phenolic content, radiation treatment showed interesting results by extending shelf life of hilsa fishes in the present study.

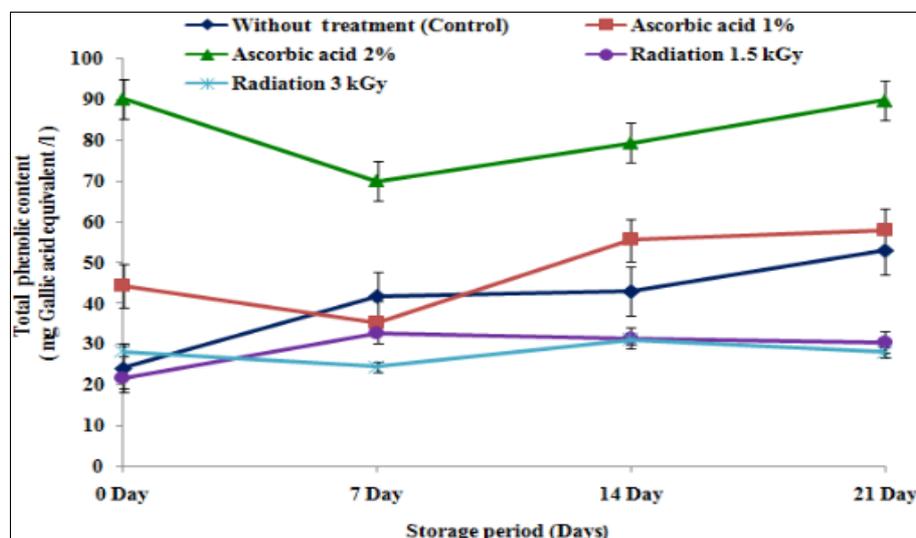


Fig 4: Total phenolic contents of freshwater hilsa in ascorbic acid and radiation treated samples at 4 °C temperature

### Microbiological quality

Total bacterial counts (TBC) were significantly different between storage temperatures. The present investigations reveal that control samples have the highest bacterial count,  $1.9 \times 10^9$  log cfu/gm observed in 21 days. Ascorbic acid (2%), gamma radiation (1.5 and 3 kGy) treated fishes showed significantly lower bacterial count compared to the other treatments and remained within the acceptable limit upto 21 days during storage. So, irradiation and ascorbic acid were found to be most effective treatment for maintaining microbial safety. Sheuty *et al.* [7] reported that microbial assessment showed the best results in case of irradiated hilsa and remained within the acceptable limit.

Total salmonella-shigella counts (TSSC) were observed up to 21 days at 4 °C storage temperature respectively. The present results indicated that TSSC in control samples increased with increasing the storage period. TSSC in ascorbic acid (1 and 2 %) and 1.5 kGy radiation treated samples gradually decreased up to day 14 but increased on day 21 and remained

unacceptable during whole storage period. 3 kGy irradiated sample had a low count of TSSC compare to the all other treatments at day 0 and counts were not observed from 7 to 21 days of storage. The present investigation revealed that radiation (3 kGy) can eliminate salmonella-shigella but ascorbic acid showed no positive effect on TSSC.

Total coliform counts (TCC) were observed upto 21 days at 4°C storage temperature respectively. In the present study, coliform count in 3 kGy irradiated fishes remained acceptable ( $10^2$  cfu/gm) recommended by ICMSF, [14] from 0 to 21 days during storage period at 4°C. But coliform counts of control, ascorbic acid (1 and 2%) and 1.5 kGy irradiated fishes were above the acceptable limits throughout the whole storage period. Sheuty *et al.* [7] also found that total coliform count in potassium sorbate (2%) and radiation (2kGy) treated hilsa were acceptable during whole investigation period. Similar trend of TCC was reported by Hossain *et al.* [15], Gandotra *et al.* [16].

Table 1: Microbial quality of freshwater hilsa in ascorbic acid and radiation treated samples at 4°C temperature

Microbial analysis	Storage Period (Days)	Samples				
		Control	Ascorbic acid 1%	Ascorbic acid 2%	Irradiated (1.5 kGy)	Irradiated (3 kGy)
		4 °C	4 °C	4 °C	4 °C	4 °C
TBC	0	$1.9 \times 10^8$	$3.2 \times 10^5$	$5.9 \times 10^4$	$3.5 \times 10^5$	$3.5 \times 10^4$
	7	$3.7 \times 10^8$	$2.7 \times 10^7$	$7.6 \times 10^5$	$3.5 \times 10^4$	$7.4 \times 10^3$
	14	$6 \times 10^8$	$1.5 \times 10^7$	$4.2 \times 10^5$	$5.2 \times 10^4$	$8 \times 10^3$
	21	$1.9 \times 10^9$	$4.2 \times 10^7$	$2.8 \times 10^6$	$3.2 \times 10^5$	$3.3 \times 10^4$
TSSC	0	$1.5 \times 10^5$	$8.5 \times 10^4$	$5.9 \times 10^4$	$9.9 \times 10^4$	$3.5 \times 10^4$
	7	$8.6 \times 10^4$	$1.5 \times 10^4$	$3.3 \times 10^3$	$1.4 \times 10^3$	Nil
	14	$4.84 \times 10^5$	$1.5 \times 10^5$	$1.2 \times 10^3$	$6.5 \times 10^2$	Nil
	21	$5.3 \times 10^6$	$2.9 \times 10^6$	$3.9 \times 10^4$	$4.3 \times 10^3$	Nil
TCC	0	$1.02 \times 10^4$	$2.5 \times 10^3$	$5 \times 10^3$	$1.4 \times 10^3$	$5.2 \times 10^2$
	7	$5.5 \times 10^3$	$1.9 \times 10^3$	$3.6 \times 10^3$	$7 \times 10^2$	$3.4 \times 10^1$
	14	$3.5 \times 10^5$	$2.2 \times 10^5$	$1.3 \times 10^5$	$1.7 \times 10^3$	$5.7 \times 10^1$
	21	$6.5 \times 10^6$	$3.5 \times 10^6$	$2 \times 10^6$	$1.8 \times 10^4$	$1.7 \times 10^2$

Overall results of the present study suggested that gamma irradiation (3 kGy) could be a potential tool to extend shelf life of hilsa shad at 4 °C temperature along with improving microbial safety by inactivating food-borne pathogens.

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