

EFFECTS OF FROZEN STORAGE, RADIATION AND THEIR COMBINED TREATMENTS ON MICROORGANISMS OF FRESHWATER MOLA FISH *AMBLYPHARYNGODON MOLA*

MD. MANSURUL HAQUE*, M. G. SORROWAR AND HARUN-UR- RASHID¹

Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

ABSTRACT

The study revealed that the total viable bacterial count, staphylococcal count, coliform count, faecal coliform count, *Aeromonas* count and total fungal counts varied from 8.8×10^7 to 1.27×10^8 , 2.7×10^6 to 4.2×10^6 , 9.0×10^4 to 1.03×10^5 , 4.8×10^5 to 7.3×10^5 , 2.7×10^5 to 5.0×10^5 and 1.6×10^3 to 3.5×10^3 cfu/gm, respectively. Seventy six bacterial strains were isolated and identified, where *Staphylococcus aureus*, *Micrococcus varians*, *Aeromonas hydrophila*, *Klebsiella ozaenae*, *Bacillus subtilis*, *Escherichia coli*, *Bacillus megaterium*, *Klebsiella edwardsii*, *Pseudomonas aeruginosa* and *Micrococcus radiodurans* were 16 (21.06%), 11 (14.47%), 9 (11.82%), 6 (7.89%), 6 (7.89%), 8 (10.53%), 6 (7.89%), 4 (5.26%), 7 (9.21%) and 3 (3.95%), respectively. Total coliform, faecal coliform, *Aeromonas* bacteria and total fungi were found to be totally inactivated by the irradiation dose of 2.5 kGy onwards and total viable bacteria and staphylococcal bacteria were eliminated completely at the irradiation dose of 7.5 kGy. The bacterial counts were shown to be gradually decreased in all the samples from initial to final storage. The combined treatments were found more effective than that of the single treatment for reducing or eliminating the associated bacteria. The moisture contents of the studied fish were found nearly similar during initial and final storage periods and also at different radiation doses but the total protein contents decreased during final storage period and also at different radiation doses.

Key words : Mola fish, Coliform, Microorganisms, Radiation treatment, Frozen storage

INTRODUCTION

Fish is highly nutritious but very perishable that cannot be kept for a long time without any treatment for further consumption. Spoilage is the result of a series of complicated changes brought about in the dead fish mainly by enzymes and bacteria (Dyer 1942). Immediately after the fish dies, certain irreversible changes begin to take place. Within a few hours or more, the muscles gradually harden along the fish until it is quite stiff. The fish can remain rigid for a number of hours or a few days depending in various factors. The muscles then soften or become pliable again. This stiffening is known rigor mortis and is brought about by enzymes in the muscles (Hobbs 1982). Fish is more susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage

* Corresponding author: <mansurulju@yahoo.com>.

¹ Microbiology and Industrial Irradiation Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka-1000, Bangladesh.

(Frazier and Westhoff 1988). The deterioration is believed to be caused mainly by the bacterial activity, which brings about very noticeable changes in the texture, flavor, odor and general appearance of the product. The microorganisms present in fish include those which are associated with the raw material and acquired during harvesting, handling and processing (Banwart 1979). Mola fishes (*Amblypharyngodon mola*) are small freshwater indigenous fishes containing more nutrients which are cheaper and easily available in the fish markets. The protein content in mola fish is 15% (Rubbi *et al.* 1987). They inhabit in the rivers, canals, beels, ponds haor, baor and inundated floodplains throughout Bangladesh (Rahman 2005). They generally eat algae, protozoa, aquatic insects, rotifers and crustaceans (Rahman 2005). Microorganisms differ in their responses to freezing, some survive virtually unharmed, some resist freezing but are susceptible to damage during frozen storage. Gram-negative organisms such as *Escherichia*, *Pseudomonas*, *Alcaligenes*, *Vibrio* and *Salmonella* are more sensitive to freezing than Gram-positive organisms (Thomson and Thacker 1973). Hitherto there is no enough data regarding preservation of mola fish by radiation, low temperature and their combined treatments.

The present study was carried out to oversee the effectiveness of frozen storage, radiation and their combined treatment in the stated fish which would be an effective means of increasing the shelf life of that fish and other fishes as well.

MATERIALS AND METHODS

The fishes were collected from various fish markets of Savar and Nabinagar. All the samples were aseptically packed in pre-sterilized polythene bags supplied from the Laboratory of Microbiology and Industrial Irradiation Division (MIID), IFRB, AERE. The polythene bags were sealed air tight after collecting the samples. The samples were immediately transported to the laboratory in chilled condition (in insulating foam box with ice) for microbiological analysis. Around 80 polythene bags were prepared and they were sealed properly with the help of sealer machine. Then they were irradiated in the Gamma Irradiation Laboratory to make them aseptic. For six-month-sampling, six sets of polythene bags were prepared. In each polythene bag 200 gram sample was taken. The plate count was performed following the method of Sharp and Lyles (1969). The media used were nutrient agar (pH 7.0 - 7.4), MacConkey agar, mFC agar, staphylococcal agar, potato dextrose and starch ampicillin agar media for total viable bacterial count (TVBC), total viable coliform count (TCC), total faecal coliform count (TFCC), total staphylococcal count (TSC), total fungal count (TFC) and total *Aeromonas* count (TAC), respectively. Plates were incubated at 37°C for 24 hrs and the count was expressed as colony forming unit per gram. Various morphological characteristics were observed and biochemical tests were performed for the isolation and identification of microorganisms. All the bacterial isolates were identified according to the Bergey's Manual of Determinative Bacteriology (1974). For fungal count, the plates were incubated at 28°C

and counts were recorded after 5 days of incubation. For determination of the moisture content of the sample porcelain made crucible was heated at 105°C for 1 hr and then kept at desiccators until the crucible reached at room temperature, 4 gm of sample was taken in the crucible and dried at 105°C for 3 hrs. Then the sample was taken at desiccators until it reached at room temperature and weight of the sample was taken. The process was continued until constant weight was obtained. The loss of moisture from the sample was then determined and expressed in percentage using the following formula.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight (i.e. weight loss)}}{\text{Original weight of the sample}} \times 100$$

RESULTS

The total viable bacterial count (TVBC) of the fish samples varied from 8.8×10^8 to 1.27×10^8 cfu/gm and the average count of the five samples was 9.7×10^7 cfu/gm. The highest TVBC was observed in the sample 1 and lowest in sample 5 (Fig. 1). The total staphylococcal count of the fish samples varied from 2.7×10^6 to 4.2×10^6 cfu/gm and the average count of the five samples was 3.6×10^6 cfu/gm. The highest total staphylococcal count (TSC) was observed in the sample 1 and lowest in sample 5 (Fig. 1).

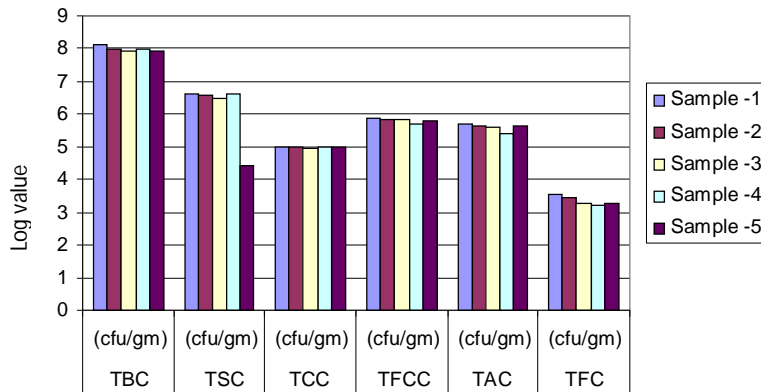


Fig. 1. Total count of associated microorganisms in freshwater mola fish.

The total coliform count (TCC) of the samples varied from 9.0×10^2 to 1.03×10^5 cfu/gm and the average count of the five samples was 9.7×10^4 cfu/gm. The highest total coliform count (TCC) was observed in the sample 2 and lowest in sample 3 (Fig. 3). The total faecal coliform counts (TCC) of the samples were varied from 4.8×10^5 cfu/gm to 7.3×10^5 and the average count of the five samples was 6.4×10^5 cfu/gm. In case of total faecal coliform, the highest count was detected in the sample 1 and lowest was in sample 4. (Fig. 4). The total *Aeromonas* counts (TAC) were varied from 2.7×10^5 to 5.0×10^5 cfu/gm and the average count of the five samples was 4.0×10^5 cfu/gm. The highest TAC was observed in the sample 1 and lowest in sample 4 (Fig. 5). The total fungi count

(TFC) of the fish samples varied from 1.6×10^3 to 3.5×10^3 cfu/gm and the average count of the five samples was 2.3×10^3 cfu/gm. The highest TFC was observed in the sample 1 and lowest in sample 4 (Fig. 1).

Changes of the microbial count during frozen storage of fish samples were observed monthly. The initial total viable bacterial count (TVBC) was found to range from 8.8×10^7 to 1.27×10^8 cfu/gm and the average was 9.7×10^7 cfu/gm and after six months storage the average was 5.6×10^6 cfu/gm (Fig. 2). The initial total staphylococcal count (TSC) was ranged from 2.7×10^6 to 4.2×10^6 cfu/gm, the average was 3.6×10^6 cfu/gm and after six months of storage the average count was 4.2×10^4 cfu/gm (Fig. 2). The initial total coliform count (TCC) ranged from 9.0×10^4 to 1.03×10^5 cfu/gm, the average was 9.7×10^4 cfu/gm and after six months of storage its average was 6.8×10^3 cfu/gm (Fig. 2). The initial total faecal coliform count (TFCC) ranged from 4.8×10^5 to 7.3×10^5 cfu/gm, the average was 6.4×10^5 cfu/gm and after six months of storage the average was 5.2×10^4 cfu/gm (Fig. 2). The initial total *Aeromonas* count (TAC) ranged from 2.7×10^5 to 5.0×10^5 cfu/gm, the average was 4.0×10^5 cfu/gm and after six months of storage the average was 3.8×10^4 cfu/gm (Fig. 2). The initial total fungi count (TFC) ranged from 1.6×10^3 to 3.5×10^3 cfu/gm, the average was 2.3×10^3 cfu/gm and after six month of storage the total fungi count was nil. The fungus count of all the fish samples decreased gradually and after two months the count was nil in all the samples. So, it has been found that during frozen storage, the bacterial count was gradually decreased in all the samples (Fig. 3).

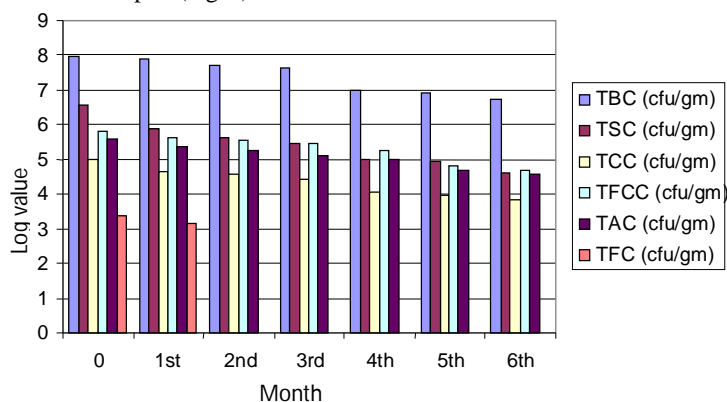


Fig. 2. Storage effect (-20°C) on associated microorganisms in fresh water mola fish.

In the non-irradiated samples the average of total viable bacterial count (TVBC) was 9.7×10^7 cfu/gm and at the irradiation doses of 2.5 and 5.0 kGy, the count decreased to 4.5×10^3 cfu/gm and 3.4×10^3 , respectively. The count was nil at the irradiation doses of 7.5 kGy and 10.0 kGy. In the non-irradiated samples, total staphylococcal count (TSC) was 3.6×10^6 cfu/gm. The count decreased to 5.2×10^4 and 4.1×10^3 cfu/gm at the irradiation doses of 2.5 and 5.0 kGy, respectively. At the irradiation doses of 7.5 and 10

kGy, no count was observed (Fig. 3). Total coliform count (TCC) was 9.7×10^4 cfu/gm at the non-irradiated samples. At the irradiation doses of 2.5, 5.0, 7.5 and 10.0 kGy, the count was nil in all the samples (Fig. 3). In case of faecal coliform, it has been found that in the non-irradiated samples total faecal coliform count (TFCC) was 6.4×10^5 cfu/gm. At the irradiation doses of 2.5, 5.0, 7.5 and 10.0 kGy, the count decreased to nil (Fig. 3). In non-irradiated samples the total *Aeromonas* count (TAC) was 4.0×10^5 and at the irradiation doses of 2.5, 5.0, 7.5 and 10.0 kGy, the count decreased to nil. In non-irradiated samples the total fungus count (TFC) was 2.3×10^3 . At the irradiation doses of 2.5, 5.0, 7.5 and 10.0 kGy, the count decreased to nil (Fig. 3).

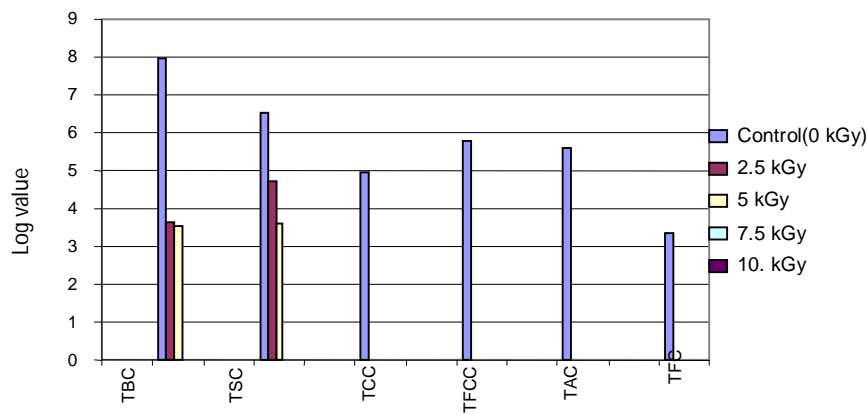


Fig. 3. Irradiation effect on microorganisms associated with fresh water mola fish.

In case of combined treatment (irradiation and low temperature storage), the fish samples were irradiated at 0 kGy (control), 2, .5, 5.0, 7.5 and 10.0 kGy of irradiation doses and then kept at -20°C for six months and the microbial counts were observed in each month.

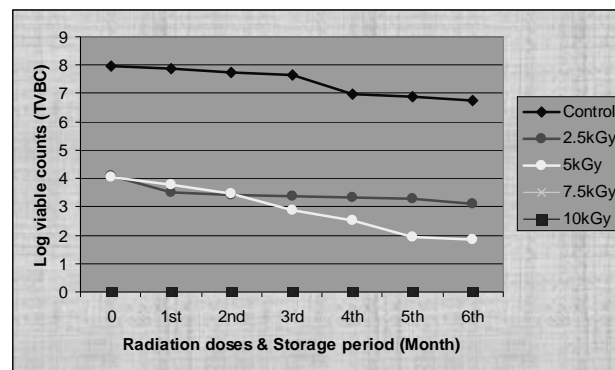


Fig. 4. Effects of combined treatment on total viable bacteria in fish samples.

In case of total viable bacterial count (TVBC), the initial bacterial count at 0 month was 9.7×10^7 cfu/gm. At the radiation doses of 2.5 and 5.0 kGy in 0 month, the TVBC reduced to 1.3×10^4 and 1.1×10^4 cfu/gm, respectively. Thus, no count was observed after the irradiation doses of 7.5 and 10kGy at the 0 month. After 1 month of frozen storage, the count of TVBC for control sample was 7.8×10^7 . At the irradiation doses of 2.5 and 5.0 kGy, the reading decreased to 3.2×10^3 and 5.9×10^3 cfu/gm, respectively. Similarly, after 2, 3, 4, 5 and 6 months of storage, control sample and at the irradiation doses of 2.5 and 5.0 kGy, the TVBC decreased to 5.6×10^7 , 4.3×10^7 , 9.9×10^6 , 7.9×10^6 , 5.6×10^6 and 2.7×10^3 , 2.4×10^3 , 2.1×10^3 , 1.9×10^3 , 1.3×10^3 and 3.0×10^3 , 7.6×10^2 , 3.4×10^2 , 8.8×10^1 cfu/gm, 6.7×10^1 cfu/gm, respectively (Fig. 4). In case of total staphylococcal count (TSC), the total staphylococcal count at first month was 3.6×10^6 cfu/gm.

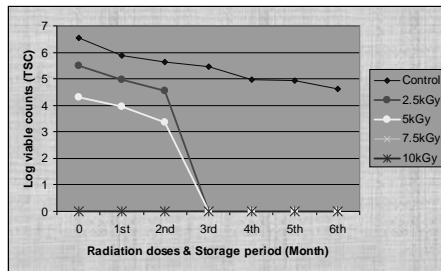


Fig. 5. Effect of combined treatment on total staphylococci bacteria in fish samples.

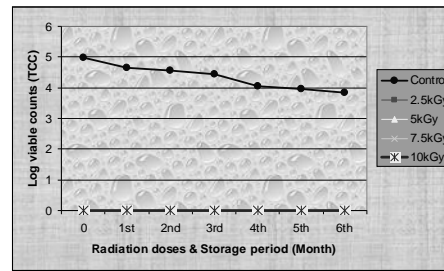


Fig. 6. Effect of combined treatment on total coliform bacteria in the fish samples.

At the radiation doses of 2.5 and 5.0 kGy, at the 0 month, the count reduced to 3.1×10^5 and 2.1×10^4 cfu/gm, respectively. After first month of frozen storage, count of TSC for control sample was 7.8×10^5 and at the irradiation doses of 2.5 and 5.0 kGy, the count decreased to 9.7×10^4 and 7.3×10^3 cfu/gm, respectively. After two months of frozen storage, count of TSC for control sample was 4.2×10^5 cfu/gm and at the irradiation doses of 2.5 and 5.0 kGy, the count decreased to 3.6×10^4 cfu/gm and 3.1×10^3 cfu/gm, respectively. Similarly, after 3, 4, 5 and 6 months of storage, the count of total staphylococcal in control sample decreased to 2.9×10^5 , 9.4×10^4 , 8.8×10^4 and 4.2×10^4 cfu/gm, respectively. No count was observed at the irradiation doses of 2.5 and 5.0 kGy in case of 4, 5 and 6th month. Similarly, at the irradiation doses of 7.5 and 10.0 kGy in all months, the count was nil (Fig. 5). In case of combined treatment, initial total coliform count (TCC) was 9.7×10^4 cfu/gm in the non-irradiated samples whereas the samples, irradiated with 2.5, 5.0, 7.5 and 1.0 kGy, all counts were nil for every month (Fig. 6). In case of faecal coliform, in the non-irradiated samples the initial total faecal coliform count (TFCC) was 6.4×10^5 cfu/gm in the 1st month, whereas the samples irradiated with 2.5, 5.0, 7.5 and 1.0 kGy all counts were nil for every month (Fig. 7). In case total

Aeromonas count (TAC), at first month of storage in the non-irradiated samples initial total *Aeromonas* count (TAC) was 4.0×10^5 cfu/gm whereas the samples irradiated with 2.5, 5.0, 7.5, 1.0 kGy there was none (Fig. 8).

In case of total fungi count (TFC), at the first month of storage in the non-irradiated samples initial total fungi count (TFC) was 2.3×10^3 cfu/gm, whereas the samples irradiated with 2.5, 5.0, 7.5, 1.0 kGy all counts were nil for every month (Fig. 9).

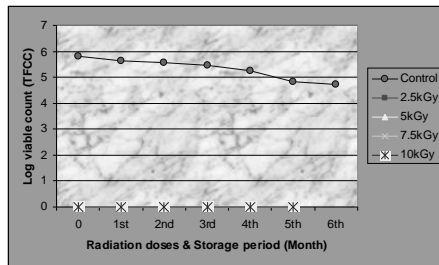


Fig. 7. Effects of combined treatment on total faecal coliform bacteria in fish samples.

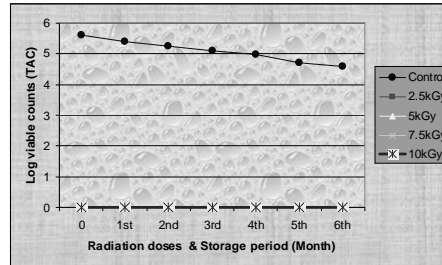


Fig. 8. Effects of combined treatment on total *Aeromonas* bacteria in fish samples.

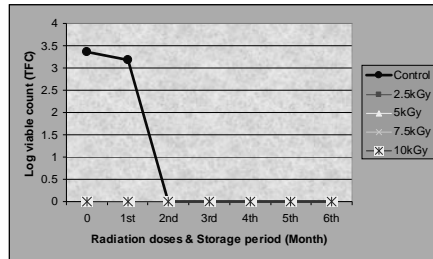


Fig. 9. Effects of combined treatment on total fungi in fish samples.

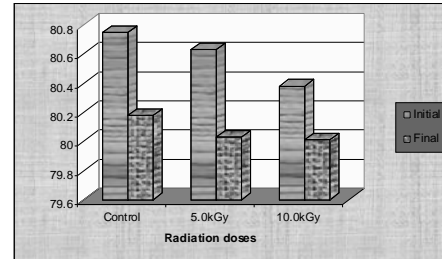


Fig. 10. Changes in moisture content of non-irradiated and irradiated samples.

Initially the bacteria which were associated with the fish samples were *Staphylococcus aureus*, *Micrococcus varians*, *Klebsiella ozaenae*, *Bacillus subtilis*, *Escherichia coli*, *Bacillus megaterium*, *Klebsiella edwardsii*, *Pseudomonas aeruginosa*, *Aeromonas hydrophilla* and *Micrococcus radiodurans*, but after six months of storage *Klebsiella edwardsii* and *Micrococcus radiodurans* became eliminated. According to Bergeys Manual of Determinative Bacteriology (Bauman and Schuber 1984), there were 76 identified strains of which strain No. 101, 104, 113, 202, 206, 208, 302, 303, 401, 403, 410, 412, 501, 503, 602 and 603 were identified as *Staphylococcus aureus*, 102, 201 and 304 were *Micrococcus radioduran*, 103, 203, 207, 305, 402, 411, 413, 502, 504, 505 and 601 were *Micrococcus varians*, 106, 209, 210, 309, 405, 406, 508, 509 and 609 were *Aeromonas hydrophila*, 109, 213, 311, 407, 511 and 608 were *Klebsiella*, 108, 211, 306, 408, 506 and 607 were *Bacillus subtilis*, 110, 111, 307, 409, 512 and 605 were *Bacillus*,

107, 112, 205, 301, 312, 414, 507 and 606 were *Escherichia coli*, 115, 214, 310 and 404 were *Klebsiella* and strain No. 105, 114, 204, 212, 308, 510 and 604 were identified as *Pseudomonas aeruginosa*.

The changes in moisture content (gm %) of non-irradiated and irradiated samples have been shown in figures. The initial moisture content of the fish sample (control) was 80.75 gm%. After six months of storage the final moisture contents was 80.18 gm%, whereas in 5.0 kGy irradiated samples the initial value was 80.63 gm%, which decreases slightly during storage and finally the value was 80.03 gm%. In case of 10.0 kGy irradiated samples, the initial value was 80.38 gm% which decreased slightly during storage period and finally the value was 80.01 gm samples in low temperature.

The changes in protein content (gm %) of non-irradiated and irradiated fresh water mola fish samples have been shown in Fig. 10. The initial protein content of the fish sample (control) was 21.89 gm% which decreased during storage and after six months of storage the final moisture contents was 17.87 gm%, whereas in 5.0 kGy irradiated samples the initial value was 21.65 gm% which also decreased during storage and finally the value was 17.53 gm%. In case of 10.0 kGy irradiated samples, the initial value was 21.47 gm% which decreased to 17.76 gm% in final storage time. For all of the samples the initial and final counts were slightly higher in non-irradiated samples compared to irradiated samples (Fig. 11).

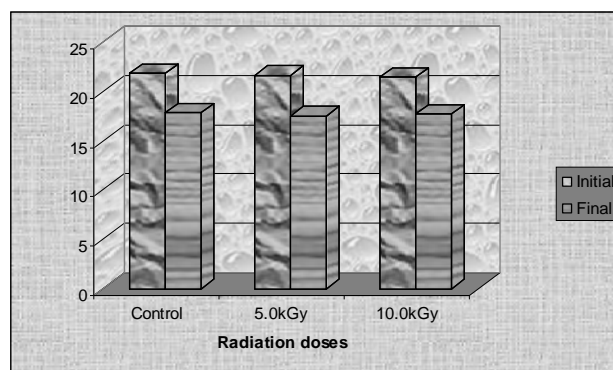


Fig. 11. Changes in protein content of non-irradiated and irradiated samples in low temperature.

DISCUSSIONS

In the present investigation, the total viable bacterial count (TVBC) of the sample varied from 8.8×10^7 to 1.27×10^8 cfu/gm. The highest TVBC in sample 1 was 1.27×10^7 . Similar results have been reported by Cho *et al.* (1992) and Ito *et al.* (1984). The

total staphylococcal count (TSC) varied from 2.7×10^6 to 4.2×10^6 cfu/gm in the present study. 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). Total coliform counts (TCC) in the sample varied from 9.0×10^5 to 1.03×10^5 cfu/gm of the sample. Presence of coliform in food has been linked with the practice of inadequate hygienic measure, mishandling, improper storage and use of dirty water during marketing and all unhygienic condition of the shops (Munce 1980). This finding is supported by Rashid *et al.* (1996). The total *Aeromonas* count (TAC) varied from 2.7×10^5 to 5.0×10^5 cfu/gm in the present study which was similar to the finding of Cho *et al.* (1992) and Ito *et al.* (1984). Total faecal coliform counts (TFCC) varied from 4.8×10^5 to 7.3×10^5 cfu/gm in the present study which is similar to the findings of Munce (1980).

The result regarding isolated strains of bacteria in the present study is supported by several workers such as Islam *et al.* (2001), Anwar *et al.* (1988), Jay (1977), Banwart (1979), Dyer (1942), Liston (1960) and Shewan and Hobbs (1967), Lee and Pfeifer (1977), Burke *et al.* (1983), Clark and Pegel (1977). Anwar *et al.* (1988) reported that freezing reduced bacterial load of shrimps which is more or less similar to the findings of present study. Total *Aeromonas* count (TAC) decreased gradually from initial to final storage period in all the samples. Similar results have been reported by Anwar *et al.* (1988). Total viable bacterial count (TVBC) was reduced one to two-folds at 2.5, 5.0 kGy of irradiation doses and become nil after the radiation doses of 7.5 kGy. The result is supported by Ito *et al.* (1993) and Khatun *et al.* (1996). Total Staphylococcal count (TSC) became reduced at 2.5 and 5.0 kGy of irradiation doses and became nil at the radiation doses of 7.5 kGy which is similar to the findings of Ito *et al.* (1993) and Hossain *et al.* (1979). Total coliform count (TCC), total faecal coliform count (TFCC), total *Aeromonas* count (TAC) and total fungi count (TFC) decreased to nil at the radiation doses of 2.5 kGy which is similar to the data of Ito *et al.* (1993).

Bacteria survived after irradiation also gradually decreased during frozen storage, and after six months of storage it decreased about three to four log more in all the samples. Similar results have been reported by Thampuran and Gopakumar (1991) and Anwar *et al.* (1988). Brogstorm (1961) reported that the moisture contents in fresh fishes were 66 to 84%. The present findings of biochemical compositions of mola fish were within of the range of the findings of that author. INFS (1980) reported that freshwater fishes contained 70 to 80% moisture which supports the present findings. Initially total protein value of control (0 kGy) mola fish samples was 21.89% which is within the findings of Brogstorm (1961) and Rubbi *et al.* (1987).

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