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EFFECT OF RADIATION AND LOW TEMPERATURE ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF SALTED HILSA (*TENUALOSA ILISHA*)

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Abstract: Investigations were made on the changes of quality of salted hilsa (*Tenualosa ilisha*) and also to evaluate the effect of irradiation for their preservation at low temperature (0-4°C). Prior to storage, total viable bacterial count (TVBC), total psychrophilic bacterial count (TSBC) and total fungal count (TFC) were 2.8×10^6, 9.52×10^4 and 8.12×10^4 cfu/g, respectively. TVBC, TSBC and TFC were found to decrease gradually after six months of storage. TVBC and TSBC decreased after treatment with gamma radiation doses at 2.5 and 5.0 kGy, respectively and no count was found when treated with gamma radiation dose at 7.5 kGy. Total fungi were completely eliminated at 5.0 and 7.5 kGy. Counts of total viable bacteria and psychrophilic bacteria of all the fish samples decreased to below 10^4 /g at 5.0 kGy of irradiation. During storage period at low temperature (0-4°C) the residual bacteria decreased more rapidly in irradiated samples than in non-irradiated samples. Different physico-chemical parameters regarded as the indices of spoilage were studied from the non-irradiated and irradiated fish samples during storage period. Moisture content, protein content were found to decrease, while tyrosine values (TV) were found to increase, during storage compared to non-irradiated samples. The initial values of all the indices were less in irradiated samples except protein content and the increasing or decreasing rate was also affected by the radiation during storage period.

mvi-ms‡¶ct jeY Bwj‡ki msi¶YKvjxb mg‡q RxevbMZ I MvVwbK-ivmvqwbK cwieZb ch‡e¶Y Kiv nq| (0-4)^{o}C G jeY Bwj‡ki msi¶YKvjxb mgq ¸YMZ gv‡bi cwieZb Ges we‡KiY cwµqvi gv‡bi DbœZK‡í M‡elYv Kiv nq| msi¶Y c‡e gv‡Qi †gvU Rxevb msL¨v, †gvU wbg Zvcgv‡Îvq mnbkxj Rxevb Ges †gvU Q‡Îv‡Ki msL¨v wbYq K‡i †`Lv †M‡Q $2.8\times10^6$, $9.52\times10^4$ Ges $8.12\times10^4$ cfu/g QqgvmKvjxb msi¶‡Yi c‡i †gvU Rxevb, wbgZvcgv‡Îv mnbkxj Rxevb Ges †gvU Q‡Îv‡Ki msL¨v µgvMZfv‡e K‡g‡Q| †gvU Rxevb Ges wbgZvcgv‡Îv mnbkxj Rxevbi †¶‡Î †`Lv †M‡Q gvQ‡K 2.5 Ges 5.0 kGy we‡KiY Zvcgv‡Îv c‡qvM Kivq Rxevbgv‡Îv K‡g Ges 7.5 kGy ‡Z †Kvb Rxevb cvIqv hvqwb| 5.0 Ges 7.5 kGy †Wv‡Ri †¶‡Î Q‡ÎvK m¤úYfv‡e we`¨wiZ n‡q‡Q| me jeY gv‡Qi bgbv‡ZB 5.0 kGy we‡KiY gv‡Îv c‡qv‡M †gvU Rxevb Ges wbg Zvcgv‡Îv mnbkxj Rxevbi msL¨v $10^4$/g Gi wb‡P †b‡g‡Q| msi¶YKvjxb mgq wbgZvcgv‡Îvq (0-4)$^{o}$C we‡KwiZ bgbvq wU‡K hvIqv e¨K‡Uwiqv AwewKwiZ bgbvi Zjbvq `“Z nvm †c‡q‡Q| we‡KwiZ I AwewKwiZ gv‡Qi bgbv‡Z we‡fb MvVwbK-ivmvqwbK c¨viwgUvi †h¸‡jv cPbkxjZv wbY‡qi gv‡Îv wn‡m‡e cwiMwYZ †m¸‡jv‡K msi¶YKvjxb mg‡q ch‡e¶Y Kiv nq| msi¶YKvjxb mgq AwewKwiZ bgbvi Zjbvq we‡KwiZ bgbvq Av`Zvi cwigvY Ges Awg‡li cwigvY K‡g hvq, Ab¨w`‡K UvB‡ivwmb f¨vji cwigvY ev‡o| we‡KwiZ bgbvq cPbkx‡ji ïi“‡ZB cPbkxjZvi gv‡Îv †cwUb Gi cwigvY Qvov me‡¶‡Î Kg wQj| msi¶YKvjxb mg‡q, we‡KiY gv‡Îvi Dci Gi ew× ev nvm wbfi K‡i|

Key words: Salted Hilsa, microbial contamination, physico-chemical properties, radiation treatment.

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INTRODUCTION

Among fishes hilsa (*Tenualosa ilisha*) has an important role in earning foreign currency and are usually exported in salted conditions. Salted hilsa is usually spoiled by various microorganisms and by their metabolic activities that lead to the formation of gases and foul smelling compounds and eventually deteriorate fish quality (Shewan 1976).

The preservation of hilsa (*T. ilisha*) fish to retain its edibility and controlling its quality to the general level of acceptance of consumers is mainly concerned with arresting the bacterial contamination and growth, simultaneously with the retardation of autolysis which singly or in combination eventually make the fish unmarketable, inedible and unacceptable for human consumption (Kreuzer 1972). Though the spoilage of salted fish is less severe but it is usually contaminated with the harmful bacteria like *Bacillus* spp., *Staphylococcus* spp., *Micrococcus varians*, *Micrococcus luteus*, *Pseudomonas mallei*, and the fungi like *Aspergillus niger*, *Aspergillus* flavus and *Rhizopus* spp. (Pablo *et al.*1977 and Pitt 1975). The important constituents of the fish muscle in their order of magnitude are moisture, proteins, fats and minerals (Govindan 1985). Information on the chemical composition of fish in respect to the nutritive value is important to compare with other source of animal protein, food such as meat and poultry products (Stansby 1954). There are some information on the biochemical and nutritional studies of some fish species, namely Khuda *et al.* (1962), Kamaluddin *et al.* (1977), Gheyasuddin *et al.* (1979) and Rubbi *et al.* (1987). Biochemical composition of fish shows wide variations of nutrients from species to species, within the species and in different parts of some species as well as from different geographic regions, season, age, size and growth (Govindan 1985). The principle aim of preservation is to avoid the spoilage of fish and loss of protein component of all the flesh foods. The fish is most susceptible to autolysis oxidation and hydrolysis of fats and microbial spoilage (Frazier and Westhoff 1988).

OBJECTIVES

For this specific purpose, this investigations were carried out to know : (1) the effect of storage period at low temperature (0-4°C) and gamma irradiation on micro-organisms in salted hilsa fish samples, (2) Isolation and identification of micro-organisms from the salted hilsa fish samples and (3) physico- chemical analysis of salted hilsa fish and changes in physico-chemical properties during storage period and gamma irradiation.

MATERIAL AND METHODS

Sample collection: Salted hilsa (*T. ilisa*) fishes were collected from different markets of Dhaka city. One kilogram of salted hilsa was collected in a sterile polyethene bag from each market. The samples were placed in insulated boxes and transported immediately to the laboratory of Microbiology and Industrial Irradiation Division of Atomic Energy Research Establishment (AERE), and sample preparation was started within three hours of collection. The samples were prepared for microbiological analysis according to the procedure described by ICMSF (1978).

Microbiological analysis: Total viable bacterial count (TVBC) was done by the standard plate count method following the method described by Sharp and Lyles (1969). All the bacterial isolates were identified following Buchanan and Gibbon (1974).

Potato Dextrose Agar (PDA) was used for total fungal count respectively. The plates were incubated at 28°C and counts were recorded after five days of incubation. The fungal isolates were identified following the procedures described by Gilman (1991), Raper and Fennell (1977) and Koneman *et al.* (1978).

Gamma irradiation: Samples were subjected to 0, 2.5, 5.0 and 7.5 kGy of ionizing radiation. The samples were irradiated at a dose rate of 1.25 Mrad/hr from a 50,000 curie Co^{60} source (Gamma beam, 650, AECL, Canada) situated at the Institute of Food and Radiation Biology of AERE.

Storage condition: After irradiation the non-irradiated and irradiated samples were kept in low temperature (4-10^{0}C)) for six months for observing the change in microbiological and physico-chemical quality during storage.

Physico-chemical analysis: Fishes were first beheaded, degutted, washed and drained. Then only the muscles were collected for physico-chemical analysis.The following methods were adopted for the estimation of different constitutents:-

The change of weight is estimated under certain temperature. Moisture of fish is commonly determined by drying a sample at some elevated temperature and reporting the loss in weight as moisture (AOAC 1975). The universally accepted method for determining total nitrogen of crude protein in fish is the “Macro-Kjeldahl” method. Tyrosine value was determined following the method as described by Wood *et al.* (1942).

RESULTS AND DISCUSSION

Microbiological and physico-chemical properties of the salted hilsa fish collected from different markets were analyzed and the properties were studied in storage condition at low temperature (0-4°C).

Fig. 1a represents total viable bacterial count (TVBC) prior storage was 2.8 × 10^6 cfu/g but the number decreased to 2.2 × 10^4 cfu/g after six months of storage period. On the other hand, the number was reduced to 8.25 x 10^4 and 7.32 × 10^3 cfu/g and non detectable for the samples irradiated with 2.5, 5.0 and 7.5 kGy, respectively. The number was gradually decreasing in 2.5 kGy and it was viable 2.15 × 10^2 cfu/g upto six months respectively and the number was decreasing during storage and it was 1.01 × 10^1cfu/g at the four months count. No viable bacterium was detected in 7.5 kGy treated samples during the storage period.

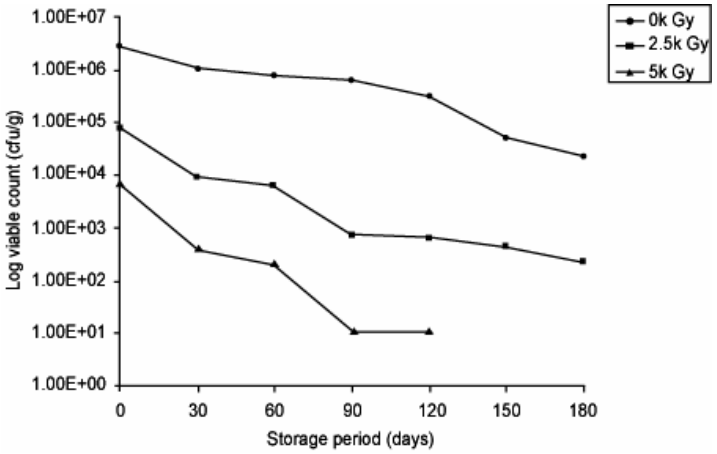

Fig.1(a): Changes of TVBC of salted hilsa fish samples during storage period.

Initial total psychrophilic bacterial count (TSBC) was 9.52 × 10^4 cfu/g was reduced to 3.1 × 10^2 cfu/g after six months storage at low temperature. The psychrophilic bacteria was found decrease 4.3 × 10^4 cfu/g after 2.5kGy and it was reduced to 1.21 × 10^1 cfu/g respectively after the end of six months storage. The psychrophilic bacterial count of 5.0 kGy treated samples was 8.27 × 10^2 cfu/g but the number was decreasing during storage and it was 1.19 × 10^1 cfu/g at three months count. No viable psychriphilic bacteria was detected in the samples with the radiation dose of 7.5 kGy. Similar results have been reported by Hains 1938, Anwar *et al.* 1988a, Anwar *et al.* 1988b, Liston 1980, Novak 1973, Hobbs1976, Thampuran and Gopakumar 1991. The declation in the rate of reduction of bacteria with time indicated their gradual adaptation to the storage temperature. Slow freezing is more ditrimental than quick freezing,

because of the formation of large ice crystals, which disrupt cell membranes as well as bring outsolute of the cells. Thus freezing causes the death of bacterial cells (Fung 1987).

Micro-organisms differ in their responses to freezing. Some survive virtuall unharmed, some resist freezing but are susceptible to damage during frozen storage or thawing; others are sensitive to freezing, storage and thawing under only some conditions, others are inactivated by freezing under nearly all conditions. Most spores and vegetative cells survive virtually unchanged. Most other non-spore forming organisms are sensitive to one or more steps of the freezing process (Fennema *et al.* 1973). The different types of bacteria associated with the salted hilsa fish are *Staphylococcus aureus, Bacillus subtilis, B. megaterium*, *Micrococcus varians* which can survive at low temperature. Similar results have been reported by Hossain *et al.* (1990). Micro-organisms maintained at freezing and sub-freezing temperature may be considered dormant, they perform no detectable metabolic activity. Micro-organisms can tolerate -20°C or lower temperature. Here the initial freezing kills afraction of the population, where the survivors may remain viable for long period (Pelczar *et al.* 1977).

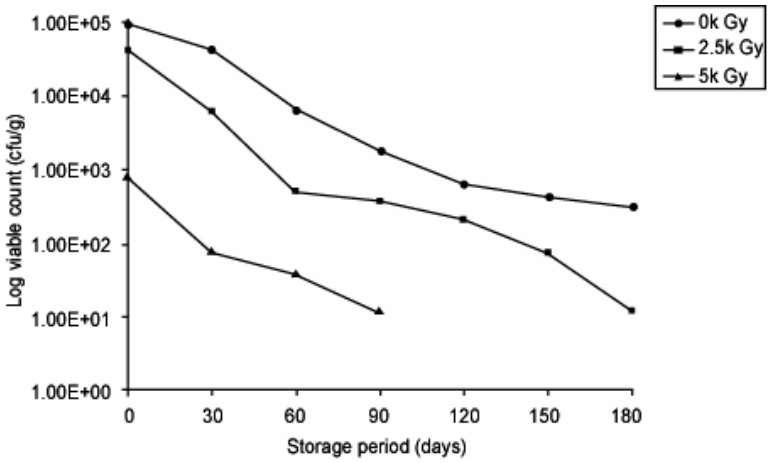

Fig.1(b): Changes of TSBC of salted hilsa fish samples during storage period.

The initial total fungal count was 8.12 x 104 cfu/g in the non-irradiated samples and the number was gradually reduced to 1.12 × 102 cfu/g after six months storage (Fig. 1C). The fungal count in 2.5 kGy treated samples was 6.15 × 104 cfu/g but the number was decreasing during the storage and it was 1.01 × 104 cfu/g at five months count. No viablew fungal was detected in 5.0 kGy and 7.5 kGy treated samples during the storage.

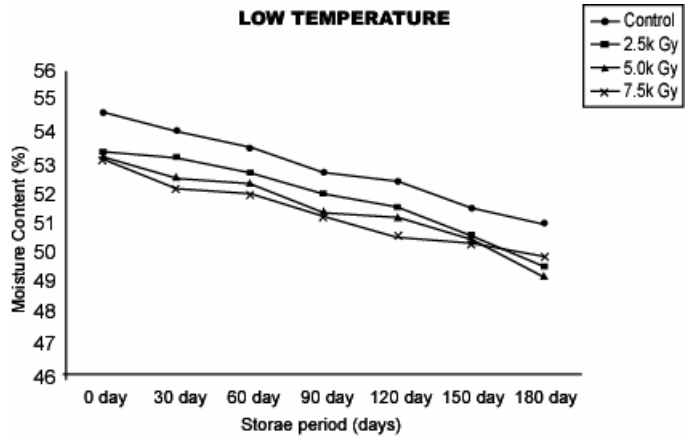

Fig.1(c): Changes of total fungal count of salted hilsa fish samples during storage period.

Results present in the Fig. 2A indicate that the physico-chemical properties of the salted hilsa were remarkably fluctuated during the storage of six months. Initial moisture of salted hilsa was 54.63 g% in the non-irradiated samples and the number was gradually reduced to 50.96 g%, whereas in 2.5 kGy irradiated smaples the initial value was 53.34 g% which decreased during storage and finally the value was 49.97 g%. In case of 5.0 kGy irradiated samples, the initial value was 53.14 g% which decreased during storage period and finally the value was 49.18 g%. In case of 7.5 kGy irradiated samples, the initial value was 53.07 g% which during time of storage decrease to 49.83 g%. This findings shows some similarity with the findings of Probhakara and Satyanarayana (1990). During freezing ice crystals are formed from the free water and fish muscle and thereby decreasing of the moisture contents of the fish and microbes.

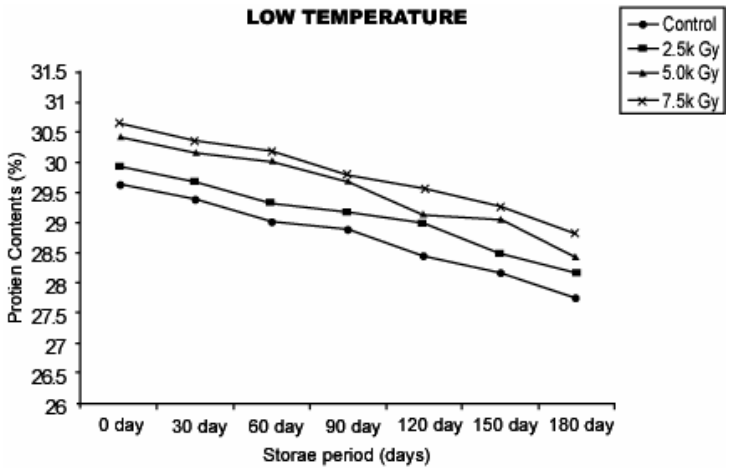

Fig. 2(a): Changes of moisture content of salted hilsa fish samples during storage period

The crude protein content of the salted hilsa fish was 29.63 gm% and the number was found to decrease gradually and it was 27.78 gm% at the count of six months storage (Fig. 2b). Vlieg (1984) determined the protein content of the New Zealand marine fish species which was 17.1-21.6 g% of the fillet. Govindon (1985) analyzed the amount of protein content that was present in different fresh water and marine fishes, and obtained the result of the fish contained nine to 25% of protein. The initial values of different irradiated (2.5, 5.0 and 7.5 kGy) salted hilsa fish were 29.94, 30.42, 30.65 g%, respectively which decrease during storage period and finally the values were 28.16, 28.42, 28.81 g%, respectively. For all the samples the initial and final counts were higher in the irradiated samples compared to the non-irradiated samples. During the storage period protein content gradually decreased in both untreated and irradiated samples. But the rate of degradation was higher in non-irradiated than irradiated samples.

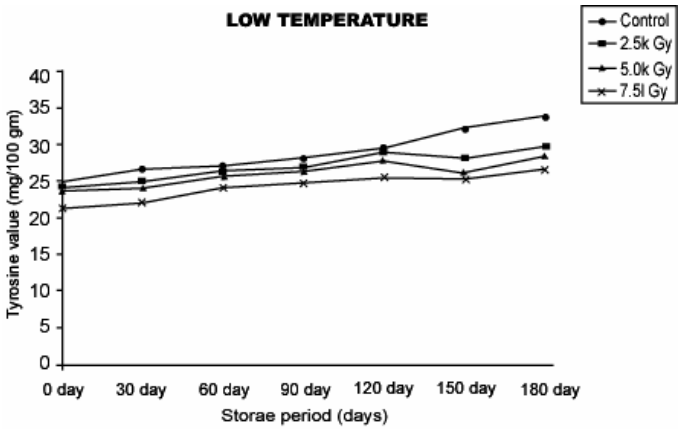

Fig. 2(b): Changes of protein content of salted hilsa fish samples during storage period

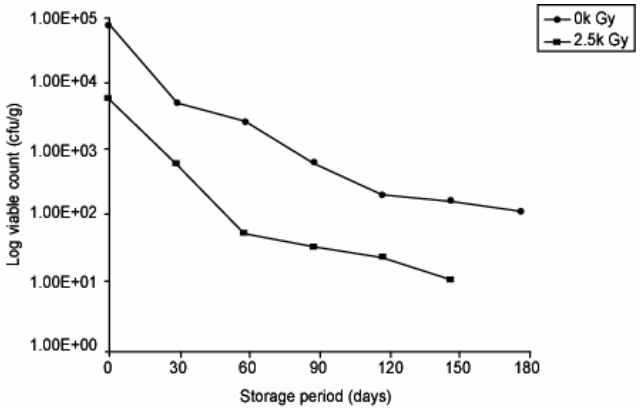

Fig. 2(c): Changes of tyrosine value of salted hilsa fish samples during storage period

The initial tyrosine value of the non-irradiated salted hilsa fish samples was 24.8 mg/100 g (Fig. 2c). After six month of storage the final tyrosine increased to 34.8 mg/100 g. Whereas, the initial values of different types of irradiated (2.5kGy, 5.0kGy, 7.5kGy) salted hilsa fish were 24.2mg/100g, 23.64 mg/100g, 21.2 mg/100g, respectively which gradually increased during the storage period and after six months the values were 30.48mg/100g, 29.64mg/100g and 27.7mg/100g, respectively. In the present study, it has been found that tyrosine value has been increased with the non-irradiated samples, the increases were low in irradiated samples and the decrease was directly proportional to radiation doses. Similar results have been reported by Pearson (1968a) and Bose (1969.)

CONCLUSIONS

Salting with radiation is effective for long term preservation. Again for storage, low temperature is effective and since all the microbes were controlled at 5.0 kGy of irradiation. But in terms of microbiological and nutritional conditions the best results were observed from the samples irradiated at 7.5 kGy. In this regard from our observation it can be concluded that irradiation at low temperature is useful to improve the nutritional qualities and lower the food borne illness caused by micro-organisms.

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