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Effect of gamma radiation on microbiological changes in dry salted ribbon fish (*Lepturacanthus savala*, Cuvier, 1829)

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SUMMARY :

A study was conducted to determine the effects of gamma radiation on microbiological qualities of dry salted ribbon fish (*Lepturacanthus savala*) collected from fish drying yard. Results of microbiological analysis showed that total plate count in non-irradiated ribbon fish was 6.9±0.15 log cfug^{-1} which was significantly reduced by 1 logs at 1.0 kGy, 2 to 3 logs at 3.0 kGy and 3 to 4 logs at 5.0 kGy gamma radiation. Maximum staphylococci count was noted in non-irradiated ribbon fish (3.17±0.17 log cfug^{-1}) compared to irradiated with 1.0 kGy (2.63±0.50 log cfug^{-1}), 3.0 kGy (2.35±0.35 log cfug^{-1}) and 5.0 kGy (1.95±0.50 log cfug^{-1}) on 0th day. At the end of experiment, the halophilic count was 4.98±0.40 log cfug^{-1} in control while in 5.0 kGy irradiated fish sample it was 1.49±0.49 log cfug^{-1}. *Vibrio cholera*, *Salmonella* and *Shigella* and *E.coli* were not detected in experimental fish samples during investigation. Total fungal count recorded was 2.48±0.03 log cfug^{-1} in non-irradiated ribbon fish but it was totally eliminated in 5.0 kGy irradiated fish samples. Gamma irradiation doses (1.0, 3.0 and 5.0 kGy) had no significant effect (P>0.05) on sensory properties (appearance, colour, odour and texture) of dry salted ribbon fish sample neither at zero time nor during nine month storage periods. It can be concluded that, gamma irradiation dose of 3.0 and 5.0 kGy had a significant (P<0.05) effects on decreasing microbial load and improve the microbiological safety of dry salted ribbon fish without any adverse effects on their sensory quality.

KEY WORDS : Dry fish, Gamma irradiation, Microbiological, Sensory attributes, Ribbon fish

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Fishes have usually played an essential part of human diet. Most people of developing countries are still depending almost entirely on fish as a source of animal protein. Bacteria are one of the major organisms contributing to the rapid deterioration of fish quality (Laycock. and Regier, 1971). When fish dies, bacteria present on the surface and in the guts multiply rapidly and invade the flesh, which provides an ideal medium for

growth and multiplication (Govindan, 1985). The micro-organisms present in fish are closely connected to the micro flora of the environment (Prakash *et al.*, 2011). Fish is one of the most perishable and difficult to handle of all foods. Bacterial activity is by far the most important factor influencing fish quality (Gram and Huss, 1996). Therefore, it is logical to use bacteria numbers as an index of quality (Sikorski, 1990). Spoilage due to microbial activity is the main limitation of the shelf-life of fish. The deterioration of fish is due to a combination of chemical, autolytic and microbiological changes, but the deterioration rate can be reduced by taking different preservation measures.

Food irradiation is the process of exposing food to ionizing radiation at different doses in order to disinfest, sterilize, or preserve food. Further, it is the only novel method of food preservation suggested for many countries (Clucas and Ward, 1996). It has been considered that ionization radiation or energy exerts its lethal effects on bacteria mainly through direct action.

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). Hence, estimation of bacterial numbers in fish is being used to assess microbiological quality as well as to assess the presumptive safety of the fish product for human consumption (Yousuf *et al*., 2008). Gamma irradiation can be effectively applied for ensuring the hygienic quality of dried fishes (Roksana *et al*., 2013). The safety of irradiated foods for human consumption has been questioned because ionizing radiation can lead to chemical changes. The wholesomeness of irradiated foods has, therefore, been the subject of considerable national and international researches, which has been reviewed and evaluated by joint expert committees of the International Atomic Energy Agency (IAEA), the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations. These expert groups have uniformly concluded that the food irradiation process does not present any enhanced toxicological, microbiological, or nutritional hazard beyond those brought about by conventional food processing techniques (Diehl, 1995). Hence, the objective of this study was to observe the effect of controlled gamma radiation of 1.0, 3.0 and 5.0 KGy on microbiological and sensory changes of dry salted ribbon fish. In addition, the study aimed to find out the optimum dose of Gamma irradiation that does not adversely affect the quality of dry salted fish during long term storage at ambient temperature.

EXPERIMENTAL METHODS

Sample collection, gamma irradiation and storage conditions:

In this study, ribbon fish (*Lepturacanthus savala*) was procured from the local fish drying yard of Dalda Bandar, Okha (Lat.22° 28' 9" N; Longi. 69° 3' 38" E), Gujarat. Collected dry salted fish was transferred to the laboratory of Fish Processing Unit, Fisheries Research Station, Junagadh Agricultural University, Okha. It was packed in polythene bags (50µ). Entire polythene bags with dried fish except control were transported to the irradiation facility at Universal Medicap limited (UML), At Dasharath, Vadodara. The irradiated samples were then transported back to the laboratory and stored at ambient temperature.

Dried fish samples were irradiated using gamma radiation from Cobalt-60 source. Except the control sample, the other samples were subjected to irradiation in a 800000 curie Co-60 source. The applied doses were 1.0, 3.0 and 5.0 kilo Gray (kGy). Fish samples were maintained at 20±2°C during irradiation. After irradiation, the non-irradiated and irradiated samples were stored at room temperature in polypropylene bags. The storage duration for all samples lasted 9 months and samples from each lot were taken every 30 days interval of storage periods for microbiological and sensory study.

Microbiological analysis and sensory evaluation :

The samples were analyzed for total plate count (TPC), *Salmonella* and *Shigella*, *E. coli*, *Vibrio cholera,* Staphylococci, total fungal count (TFC) and halophilic bacterial count(HC) using Plate Count Agar, Salmonella Shigella Agar, EMB Agar, TCBS Agar, Baird-Parker Agar, Potato Dextrose Agar and Halophilic Agar, respectively following the standard methods recommended by APHA (1975). All microbiological media were procured from HiMedia Laboratories, Mumbai, India. The results were expressed as colony forming unit per gram of sample (cfu/g). The sensitivity to the radiation is expressed as D_{10} value, (the dose required for killing 90 % of microbial population). D_{10}

values were calculated by following equation; D_{10} = dose/(log *N0*-log*N*), where *N0* was the initial number of microbes and *N* was the number of microbes surviving the dose of 5.0 kGy.

The sensory characteristics appearance, colour, odour and texture of dry salted fish were assessed by a panel of 9 trained members belonging to the Fisheries Research Station, Okha on the basis of 9 point Hedonic scale (Peryam and Pilgrims, 1957).

Statistical analysis :

Data of dry salted fish were subjected to statistically analysis as per the procedure of Snedecor and Cochran (1967). The analysis was carried out by computer system at the computer center, Department of Agricultural Statistics, Collage of Agriculture, Junagadh Agricultural University, Junagadh Campus, Junagadh. Critical difference (C.D.) was worked out. Level of significance was set at $P< 0.05$.

EXPERIMENTAL FINDINGS AND ANALYSIS

The TPC of the non-irradiated dried ribbon fish was about 6.90±0.15 log cfug^{-1} on initial day (Fig. 1). These associated micro-organisms indicated the presence of indigenous micro-organisms and further contamination during harvesting, processing and drying of these fishes. Previous reports presented the abundance of total aerobic bacteria at 4-6 logs of different dried fishes (Kumar, 2008; Saritha *et al*., 2012; Sulieman and Mustafa, 2012). The results indicate that microbiological safety of dry salted ribbon fish cannot be maintained by an ordinary processing method such as the salting and drying.

To reduce/eliminate micro-organisms, dried ribbon fish was irradiated at different doses of gamma radiation. The level of viable micro-organisms decreased immediately after irradiation, depending upon absorbed dose. At control, 1.0, 3.0 and 5.0 kGy irradiation dose TPC of dried ribbon fish was 6.95± 0.05, 5.88±0.07, 3.96±0.04 and 2.29±0.05 log cfug^{-1}, respectively on first day of irradiation (Fig. 1). Tabiri (2004) also reported similar rate at of reduction of TPC in response to irradiation. It gradually reduced to the level of 5.51± 0.51, 4.49±0.49, 2.69±0.60 and 1.87±0.30 log cfug^{-1}, respectively at the end of nine months storage. The results are in agreement with the observations of Prasad *et al*. (2007) and Bhalala *et al*. (2011). As per Indian standards (ISI 4950: 2001), a TPC of 5 log cfug^{-1} is acceptable for dried/cured fish in domestic trade. In the present study, TPC in all the gamma irradiated samples were far below this level.

D_{10} value of total plate count on irradiation of dry

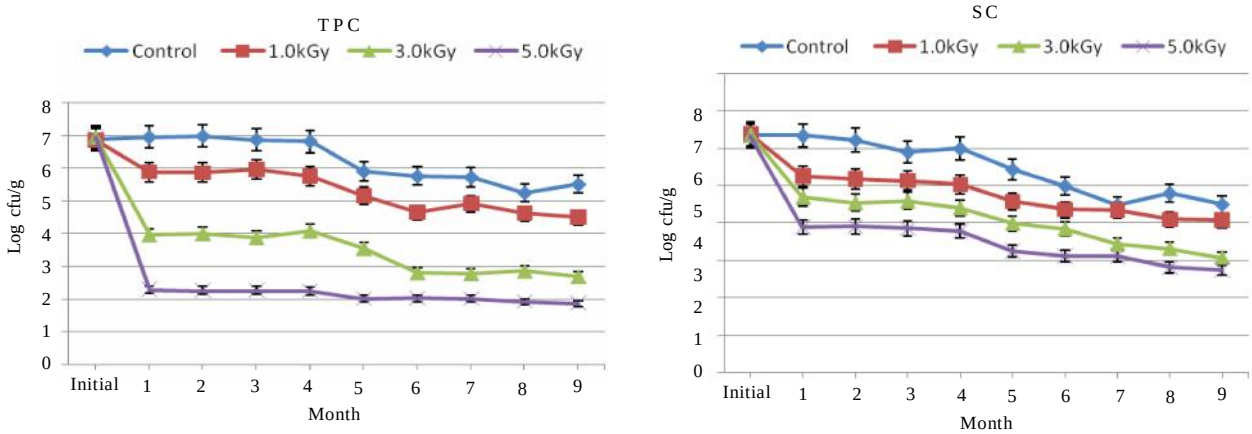

Fig. 1 : The effect of gamma irradiation on total plate count (TPC) and staphylococci count (SC) (log cfug^{-1}) in dry salted ribbon fish during storage (Mean±SD, n=3)

Table 1 : The analysis of variance (ANOVA) table – Total plate count

Source of variation	DF	SS	MS	F CAL	F TAB	S EM	CD
Dose (D)	3	148.49	49.497	369.54	2.70	0.067	0.188
Storage (T)	9	95.35	10.59	79.095	1.99	0.106	0.298
D × T	27	20.380	0.755	5.635	1.62	0.211	0.596
Error	80	10.71					

DF- Degree of freedom, SS- Some of square, MS- Mean square, CAL-F Calculated, F TAB- F Tabulated, S EM - Standard error of mean

salted ribbon fish was about 1.07 kGy. Lee *et al*. (2002) noted that D_{10} value of total aerobic bacteria on irradiation was about 2.37 kGy during their investigation. Radiation sensitivity of micro-organisms differ with genera and species with all the extrinsic condition (media, temp, oxygen, pH, water activity etc.) being equal (Lagunas-Solar, 1995; Monk *et al*.,1995).

Observation showed that gamma irradiation treatment had a significant (P<0.05) effects on microbial load of dry salted ribbon fish at zero time and during storage period (Table 1). The same trend of results was confirmed by Arvanitoyannis and Stratakos (2010) and Michele *et al.* (2013). Irradiation treatments induced ionization for the cell of bacteria and direct effects on DNA of nucleus cells (Shea *et al*., 2000; Temur and Tiryaki, 2013) resulting in death of cell.

Gamma irradiation treatment of dried ribbon fish with 1.0, 3.0 and 5.0 kGy resulted in about 1 to 2 log reduction in staphylococci count (SC). Reduction of SC at similar rate at the response to irradiation was also reported by Tupe *et al.* (2012). SC of control, 1.0, 3.0 and 5.0 kGy ribbon fish was 3.18±0.03, 2.63±0.50, 2.35±0.35 and 1.95±0.50 log cfug^{-1}, respectively on first day of irradiation (Fig. 1). Result showed that gamma irradiation treatment had a significant (P<0.05) effects on SC of dry salted ribbon fish during storage period (Table 2). Staphylococci were eliminated by gamma irradiation with D_{10} value of 4.13. Gram positive organisms had higher D_{10} value as compared with gram negative organisms (Vazhiyil, 2006).

The bacterial floras that are concerned with salt cured fish are of halophilic in nature. In the present study, their quantitative analysis was carried out and the results are presented in Fig. 2. At control, 1.0, 3.0 and 5.0 kGy irradiation dose halophilic count of dried ribbonfish fish was 6.43±0.03, 5.36±0.04, 3.44±0.04 and 1.76±0.02 log cfug^{-1}, respectively on first day of irradiation. It was reduced to the level of 4.98±0.40, 3.97±0.47, 2.17±0.17 and 1.49±0.49 log cfug^{-1}, respectively at the end of nine

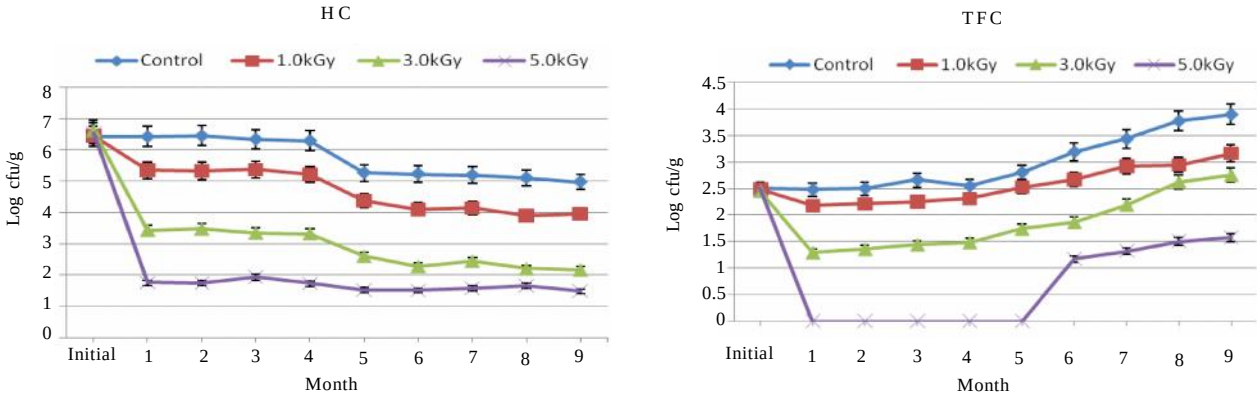

Fig. 2 : The effect of gamma irradiation on halophilic count (HC) and total fungal count (TFC) (log cfug^{-1}) in dry salted ribbon fish during storage (Mean±SD, n=3)

Table 2 : The analysis of variance (ANOVA) table – Staphylococcus count

Source of variation	DF	SS	MS	F CAL	F TAB	S E. ±	CD
Dose (D)	3	13.79	4.59	35.29	2.70	0.066	0.186
Storage (T)	9	18.56	2.06	15.83	1.99	0.104	0.294
D × T	27	2.02	0.075	0.574	1.63	0.208	NS
Error	80	10.42					

NS=Non-significant

Table 3 : The analysis of variance (ANOVA) table – Halophilic count

Source of variation	DF	SS	MS	F CAL	F TAB	S.E. ±	C.D. (P=0.05)
Dose (D)	3	237.59	79.19	765.74	2.70	0.059	0.166
Storage(T)	9	109.12	12.13	117.23	1.99	0.093	0.262
D × T	27	33.57	1.24	12.02	1.63	0.186	0.523
Error	80	8.27					

month storage. Halophilic bacteria were eliminated by gamma irradiation with D_{10} value of 1.05.

It was noticed that there was higher halophilic count in control fish than treated with gamma irradiation. There was significant effect ($P< 0.05$) of irradiation on the halophilic count of ribbon fish (Table 3). So, it is revealed that the irradiation treatment has played a role in retarding the growth of halophilic bacteria in the dry salted fish.

Total Fungal Count (TFC) of the untreated ribbon fish was 2.48±0.48 log cfug^{-1}. Previous report showed the presence of fungi in dried fish at 2 log cfug^{-1} concentration (Tabiri, 2004). At 1.0, 3.0 and 5.0 kGy irradiation dose TFC of dried ribbonfish was 2.18±0.03, 1.30±0.01 and nil log cfug^{-1}, respectively (Fig. 2). Mold and yeast have lower resistance as compared with bacterial spores. A dose level of 3.0 to 5.0 kGy may be required to control mold growth in dried fish (Vazhiyil, 2006).

There was significant effect ($P< 0.05$) of irradiation on the TFC of dry fish samples (Table 4). Reduction of TFC at similar in the response to irradiation was also reported by Huque *et al*. (2013). In case of fish treated with 5.0 kGy irradiation TFC was nil up to four months, however, during subsequent storage of dry salted fish it gradually increased up to the end of the storage period (Fig. 2). It has been stated that yeasts and molds are sensitive to the irradiation process because of their large genomic structure (Fallah *et al*., 2010). Ahmed *et al.* (2009) reported that in evaluating the efficiency of gamma radiation (3.0, 5.0 and 8.0 kGy) in combination with low

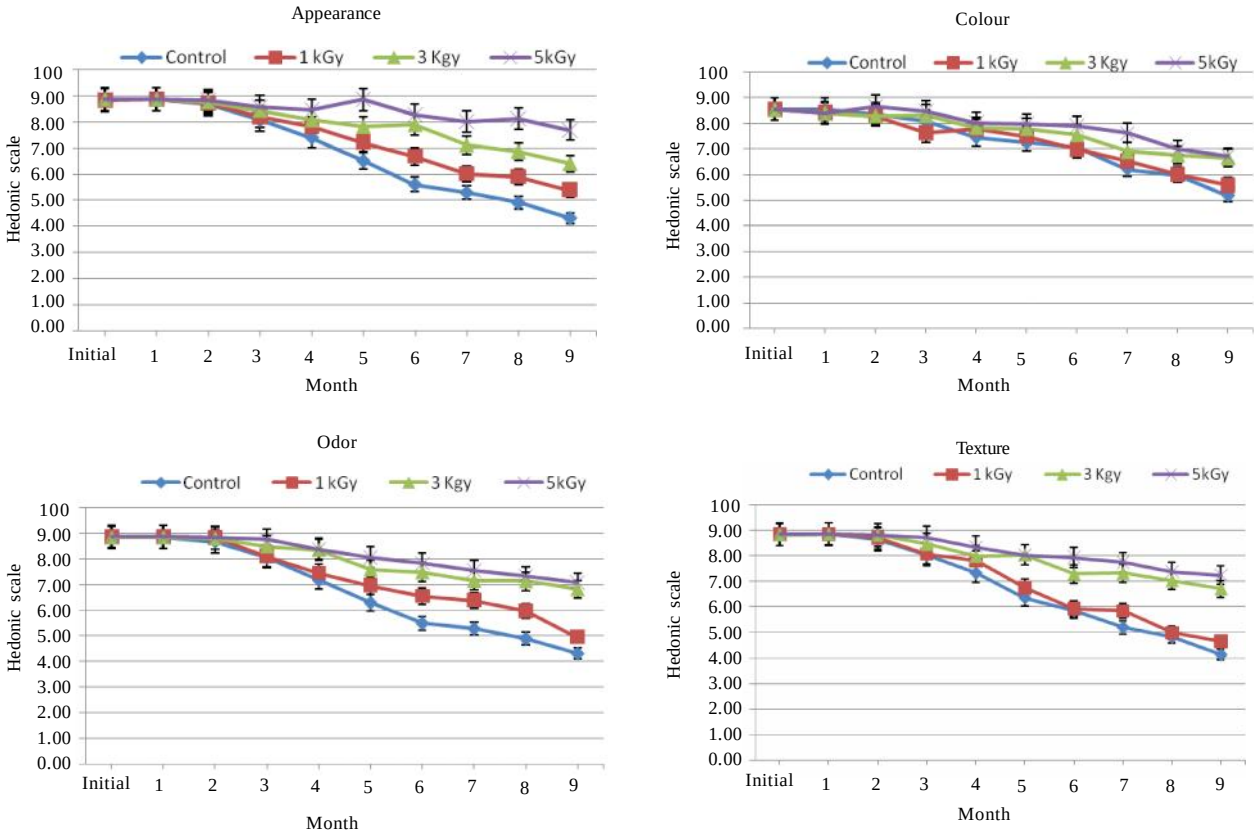

Fig. 3 : Sensory attributes of dry salted ribbon fish as affected by gamma irradiation during storage (Mean±SD, n=3)

Table 4 : The analysis of variance (ANOVA) table – total fungal count

Source of variation	DF	SS	MS	F CAL	F TAB	S EM	CD
Dose (D)	3	80.26	26.75	308.19	2.70	0.054	0.152
Storage(T)	9	30.79	3.42	39.42	1.99	0.085	0.240
D × T	27	11.56	0.42	4.93	1.63	0.170	0.480
Error	80	6.95					

temperature storage of degutted fresh *Pampus chinensis*, the total mold count (TMC) increased with the increase of storage period. One of the fungi, *Aspergillus* spp. is responsible for the production of aflatoxin which causes food borne intoxication and may lead to serious health hazards. *Aspergillus* is the main genus which is commonly involved in the production of mycotoxin (Hashem, 2011). *Vibrio cholera*, *Salmonella* and *Shigella* and *E. coli* were not detected in experimental dry salted fish samples, it may be due to good hygienic quality of production, fishing, handling, salting, washing and drying.

Sensory evaluation of dry salted ribbon fish :

Sensory attributes showed the changes in sensory evaluation of dry salted ribbon fish as positively affected by gamma irradiation during storage period (Fig. 3). It was noticed that gamma irradiation doses (1.0, 3.0 and 5.0 kGy) had not significant effect (P>0.05) on sensory properties like appearance, colour, odour and texture of fish samples under investigation at zero time and during two month storage periods.

Appearance of dried ribbon fish was about 8.85±0.50 on first day of irradiation. At control, 1.0, 3.0 and 5.0 kGy irradiation doses, it was 4.31±0.31, 5.38±0.38, 6.39 ±0.39 and 7.68±0.50, respectively on end of the nine months of storage. In case of colour and odour highest score was observed for fish treated with 3.0 and 5.0 kGy irradiation dose. In case of texture initial score was about 8.84±0.52 and it was 4.13±0.53, 4.65±0.26, 6.71±0.71 and 7.25±0.45 of control, 1.0, 3.0 and 5.0 kGy irradiation dose treated ribbon fish, respectively at the end of the nine months. Thus, subsequent storage of fish sensory properties was significantlty (P<0.05) affected by the gamma irradiation doses (3.0 and 5.0 kGy). Similar result on irradiation (2.5 and 5.0 kGy) of dry salted mullet fish during storage was noted by Aly *et al.* (2014). Sensory changes involved in fish are results of the value of microbial changes taking place during storage period. Microbial count was inversely proportional to the gamma irradiation dose. Sensory attributes of dry salted ribbon fish got highest score in 3.0 and 5.0 kGy irradiation treated fish. There was no adverse effect of gamma irradiation on sensory quality parameters of dry salted ribbon fish during nine months storage period.

Conclusion :

The present investigation ascertained that gamma radiation effectively extended the self-life of dry salted ribbon fish by reducing or eliminating fish spoilage micro-organisms. Present investigation revealed that 3.0 and 5.0 kGy irradiated dry salted ribbon fish samples gave the best result. Finally, the effect of gamma radiation during storage was significantly effective in reducing TPC, SC, hallophilic and total fungal count, which proves the necessity of gamma radiation for quality products in addition to drying preservation. So, this treatment can be applied on large scale preservation of dry fish for long time preservation without any significant loss of quality and economics during long term storage of cured fish.

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