The Asia Journal of Applied Microbiology
2014 Vol.1, No.3, pp.26-48
ISSN(e): 2313-8157
ISSN(p): 2409-2177

EFFECT OF GAMMA IRRADIATION ON THE MICROBIAL QUALITY OF DRIED FISHES

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ABSTRACT

Sun dried fishes such as C. malabaricus, S. lysan, S. acutipinnis, L. dussumieri, L. platypterus, L. rubrioperculatus and A. thazard were irradiated using gamma radiation (5 kGy) and the microbial quality and shelf life were assessed in the irradiated and non-irradiated dry fish samples stored under ambient temperature for six months. Irradiation had significant effects on the reduction of microbial population. The total bacterial count in was in acceptable condition until the end of the sixth months of storage. Total fungal counts were below detectable level throughout the storage period. Growth of Salmonella and Vibrio were observed in the non- irradiated samples where as it was not observed in the irradiated dry fishes.

Keywords: Gamma irradiation, Ambient storage, Sun dried fishes, Microbiological analysis.

Contribution/ Originality

This study is one of the very few studies which have investigated the microbial load in irradiated dry fishes. This study documents irradiation of dry fish with high moisture content provides safety, taste like fresh fish and extended shelf life due to the irradiations effectiveness in inactivating pathogenic and spoilage microorganisms.

1. INTRODUCTION

Fish is one of the most important sources of animal protein in the tropics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body. Fish is a perishable food which needs processing or storage [1]. Drying could be used for enhancing the resistance of high humid products to the degradation by decreasing their water activity. Sun drying of fish is an old traditional practice in many parts of the world, which is considered an efficient technique for improving stabilization and storage. Dried fishes are not only economically important but also an important source of animal protein. Graikoski [2] reported that, dried fish products are the pre-dominant food bringing vital protein to people in the rural areas. Besides

protein source dried fishes are also rich in vitamins and minerals, which are often overlooked in developing countries [3].

Drying of fishes is susceptible to many types of spoilage which can affect the quality and shelf life. Physical and organoleptic qualities of many traditional sun-dried products are unsatisfactory for human consumption [4]. Damages occurring due to flies and insects are of great significance in open sun drying and this is a serious problem in traditional drying. Major problem with respect to distribution of seafood or fishery products is their susceptibility to spoilage, mainly due to the contamination of spoilage and pathogenic microorganisms [5]. Fish spoilage occurs following the growth and activity of special microorganisms and lipid oxidation which cause off odor and taste by the production of some metabolites changing sensory characteristics and customer acceptability [6, 7]. During rainy season, humidity levels are high, sufficient drying cannot be achieved using traditional methods, processed and stored dried fishes reabsorb moisture and become susceptible to insect attack. Losses during storage result from the attack of pests. The amount of quantitative loss by insect infestation was nearly 10%. This amount increases during rainy season (15 - 20%) in the drying yards. Traditional methods such as ice storage, rapid chilling, freezing [8], salting and sun drying [9] and smoking and drying [10] have been used to extend the shelf life of fish and fishery products.

New technologies like ionizing radiation have been proposed to extend the shelf life of fish and fishery products [11]. The irradiation of food products is a physical treatment involving direct exposure to electron or electromagnetic rays, for their long time preservation and improvement of quality and safety [12]. Food irradiation is a process that has proven to be successful, not only in ensuring the safety, but also in extending the shelf life of fresh meats because of its high effectiveness in inactivating pathogens without deteriorating product quality [13]. Food irradiation provides safety and extends the shelf life of fishery products because of its high effectiveness in inactivating pathogenic and spoilage microorganisms without deteriorating product quality [14]. The demand for dried fishes in Tamil Nadu especially in Tuticorin markets has increased significantly over the past decade and this could be due to its desirable characteristics of taste and aroma resulting in a high - quality product and nutritional value. Also, from Tuticorin nine companies are exporting sun dried fishes. The export inspection company of India is checking the moisture content and sand content in the dried fishes before permitting to export the products. So the fishes are properly dried by keeping on coir mats. Over drying reduces the weight of the products and exporters are suffering since the high value of the fresh fishes. If the fishes are semi-dried their weight won't be decreased but they are susceptible to microbial spoilage.

The aim of the present study was to analyze the effect of irradiation of sun dried fishes with 25% moisture on their microbial quality and shelf life of the products. The changes were compared with non-irradiated samples and shelf life of the products was also analyzed for both the products.

2. MATERIALS AND METHODS

2.1. Sample Preparation and Irradiation

Fish samples such as Istiophorus *platypterus*, *Carangoids malabaricus*, *Scomberoides lysan*, *Lethrinus rubrioperculatus*, *Auxis thazard*, *Leiognathus dussumieri* and *Sphyraena acutipinnis*were bought from fish landing center of Tuticorin and were ice packed and taken to Refon Impex Dry Fish Export Company's processing center. Their fishes were cut, washed and salted for two days and sundried. The sun dried fishes with a moisture content of 25 to 30% were brought to laboratory and were packed in polythene bags and all the four sides were electrically sealed and then repacked into another cover and that too was sealed. The samples were kept in the refrigerator until irradiating. Samples were ice packed and air lifted to Bhabha Atomic Research Center, Mumbai and their the sample packets were removed from the ice and the surface of the covers were wiped using tissue paper and irradiated using a Gamma radiation in a 60 Co source of radiationat a dose of 5 kGy. Once samples were irradiated the control samples were taken out from the refrigerator and kept in room temperature. The irradiated samples were packed in cardboard box and air lifted to the laboratory and kept in room temperature (37°C). The microbial quality of the dried fishes were analyzed for the first day of storage and then at monthly intervals for 6 months for both the samples in duplicate.

2.2. Microbial Analysis

2.2.1. Moisture

Moisture of the fish samples (5 g) were determined according to the AOAC (Association of Official Analytical Chemist) [15] method by drying in an oven at 105°C. Results were expressed as percentage of wet weight.

2.2.2. Determination of pH

pH of the samples were determined by the method of Goulas and Kontominas [16]. 10 g of the sample was homogenized with 50 ml of distilled water and the pH value of the homogenate was measured by means of a glass electrode pH meter (HANNA pH213) that was previously standardized.

2.2.3. Total Plate Count (TPC) in the Dry Fishes

Bacterial counts were analyzed by spread plate method using Plate Count Agar [17]. 10 g of dry fish sample was macerate with 90 ml of saline water using mortar and pestle. It is serially diluted and 1 ml of the supernatant was mixed with 9 ml of saline water (10^{-2}), and it was serially diluted as 10^{-3} and 10^{-4}. After serial dilutions inoculate 0.5 ml of each of the dilutions was poured on agar plates in duplicates. Using a sterile bent glass rod spread the inoculums uniformly on the surface of the plates. Incubate the plates at 37°C for 48 hours. After 48 hours of incubation, the

colonies developed on each plate are counted using a colony counter. The colony counts of duplicate plates (TPC/g) were calculated by Average count × dilution factor.

2.2.4. Total Fungal Count (TFC)

Total Fungal Count was assessed using Potato Dextrose agar [18]. 25 g of the sample was blended with 225 ml of 0.1% peptone water in a blender for 30 seconds. 0.5 ml of the appropriate dilutions of the sample was spread on the surface of the medium. Incubate the plates at room temperature (28 ± 1ºC) for 3 - 5 days and examine the plates for fungal colonies and record the number of colonies per gram of the samples. The colony counts of duplicate plates were calculated by the Average number of colony × dilution factor.

2.3. Detection and Identification of *Salmonella*

For *Salmonella* detection macerate 25 g of the dry fish samples with 225 ml of Lactose broth for pre enrichment [19]. Incubate at 36 ± 1ºC for 18 - 24 hours. Pipette 1 ml of culture from the pre enriched medium and transferred in to 10 ml of selective enrichment medium tetrathionate broth. It was incubated at 36 ± 1ºC for 18 - 24 hours. Also it was inoculated in 0.1 ml culture tube of Rappaport-Vassiliadis medium (RV) and was incubated at 42º C in a water bath for 24 hours. After that one loopful of the selective enrichment medium was streaked on the pre-dried selective plating medium, viz.(1) Bismuth sulphite agar (BSA), (2) Hekton's Enteric agar (HEA), (3) Xylose Lysine Deoxycholate agar (XLD). All were incubated at 36 ± 1º C for 24 hours. Typical and atypical colonies were isolated and identified by biochemical test.

2.4. Detection and Identification of *Vibrio*

For *Vibrio* detection and identification 25 g of the dry fish sample is blended with 225 ml alkaline peptone water (APW) and incubated at 36 ± 1ºC for the enrichment. After 6 - 8 hours of incubation loopful of culture from the enrichment medium was streaked on the surface of Thiosulphate citrate bile salt sucrose agar (TCBS) and Cellobiose-Polymixin - B Colistin agar (CPC) plates. Then both were incubated at 36 ± 1ºC for 18 - 24 hours. The colonies were inoculated in to Triple sugar iron agar by stabbing butt and streaking slant and incubated for 36 ± 1º C for 18 - 24 hours. Typical and atypical colonies were isolated and identified by biochemical test [20].

2.5. Organoleptic Analysis

The organoleptic quality of the non irradiated and irradiated dry fish cooked products were served to a taste panel of 6 to 8 members and appearance, colour, odour, taste texture and overall acceptability was determined by using hedonic scale of 1 to 9 [21] and the dishes were rated as 9 for excellent, 6 for good and below 4 as poor or unacceptable.

3. RESULTS

The moisture content of the fresh fish, dry fishes and irradiated dry fishes were shown in Table 1. The results showed that the moisture content was above 70% in all the fresh fishes, while in sundried fishes it was reduced to nearly to a range of 25 to 29%. In the irradiated fishes moisture content slightly decreased and it was ranged from 24 to 27%.

The pH of the non-irradiated and irradiated dry fish samples was assessed and the results are presented in Table 2. Decrease of pH was noted in the irradiated dry fishes and all are in acidic nature. The Total Bacterial Count was enumerated in the non- irradiated and irradiated samples and the results were presented in Table 3 and Fig.1. Initial bacterial load of all the samples were and two samples such as *L. rupioperculatus* (4.93×10^8) and *L. dussumieri* (5.49×10^8)had too numerous countofbacteria. The irradiated samples had very low bacterial counts and it was noted as too low to count (TLTC) in *L. dussumieri* and *C. malabaricus*. Fig. 1 shows the high bacterial content of non- irradiated and meager counts in the irradiated *I. platypterus*. Microbial load of the irradiated samples atthe end of six months storage were 10^2 to 10^3, but in all the control sample bacterial count was in increasing trend and it was above 10^8 (too numerous to count).

Total fungal count (yeast and molds) increased in the non-irradiated samples and were not detected in the irradiated samples and the results were shown in Table 4 and Fig.2. In the irradiated dry fishes fungal colony was not detected until the end of the six months storage. The population of fungi were increased in the non-irradiated sample and it was too numerous to count during the six months of storage.

The pathogenic bacteria such as *Salmonella* and *Vibrio in* 25g of the non- irradiated and irradiated samples were assessed and the results were presented in Table 5. Both the pathogens are present in *I. platipterus*, *S. acutipinnis* but in *C. malabaricus* ent but in the rest of the samples any one of the pathogen is present but in the irradiated dry fish samples both the pathogens were absent. *Salmonella* and *Vibrio* in selective plates were identified as respective pathogen by the biochemical test.

Biochemical test for suspected *Salmonella* culture were presented in Table 6. Typical and atypical colonies were isolated from 3 selective plates of irradiated and non-irradiated fish cultures. Eight typical colonies were taken as suggested in FDA BAM as *Salmonella* suspects. Four isolates were gram positive and the remaining four cultures, one was deleted as it tested negative for both TSI and LIA. The remaining three suspected *salmonella* cultures showed positive results for *Salmonella*

Biochemical test for suspected *Vibrio* culture were presented in Table 7. From irradiated and non irradiated fish sample eight typical colonies were isolated from TCBS and CPC selective isolation plates. Only three cultures were gram negative showed positive results to *Vibrio* based on the Marine Vibrios given in Bergey's Manual of Determinative Bacteriology based on their Carbohydrate utilization and gram staining. .

Organoleptic score of cooked irradiated and non-irradiated dried fish were presented in Table 8 and 9. The sensory assessment of irradiated and non-irradiated samples was investigated respect of sensory variables such as external appearance, taste, odour, color, texture of the fishes. Average score for non- irradiated sample was 7 - 8. For irradiated sample it was 9. This was the highest score for organoleptic property. The acceptable limit of sensory score being fixed at 5.0.

Changes of pH were noticed in the irradiated and non irradiated dry fish samples during the storage period were presented in Table 10. The pH of the samples is ranged between 6.13 to 6.79 and it reveals samples are in good condition. No significant difference was observed in both non-irradiated and irradiated samples. The pH of the samples are slightly increased or decreased in the irradiated fishes and it is inacceptable condition.

Changes of microbial load were noticed both from the irradiated and non irradiated dried fish during the storage period were presented in Table 11 and 12. TFC and TPC were increased in non- irradiated samples, in the case of TFC some samples showed visible fungal colonies during the second month of storage. In the irradiated samples very low bacterial count was observed during the two months of storage. But in the case of TFC in irradiated fishes were absent up to two months of storage. These bacterial and fungal growths may be due the moisture content of the samples**.** The non- irradiated control samples moisture content may be the reason for the bacterial and fungal growth. In the irradiated samples also had the same moisture content but we dint absorb absence of fungal growth and very little bacterial growth due the effect irradiation.

Prevalence of pathogens such as *Salmonella* and *Vibrio* during the storage period of irradiated and non- irradiated dry fishes were shown in Table 13 and 14. Pathogens were observed in the irradiated samples throughout the storage period, but pathogens were not detected in all the irradiated samples throughout the storage.

Table-1. Moisture content of the final batch sun dried fishes

Species Name	Fresh fish	Sun dried fish (%)	Irradiated Sun dried fish
I.platypterus	76.87	28.26	26.22
C.malabaricus	71.19	28.63	26.59
S. lysan	70.32	25.12	24.04
L.rubrioperculatus	71.58	25.17	25.12
A.thazard	75.0	28.74	25.70
L.dussumieri	70.23	27.12	27.08
S.acutipinnis	73.47	26.50	25.00

Table-2.pH values of the final batch sun dried fishes

Name of the species	pH of control fishes	pH of the irradiated fishes
I.platypterus	6.39	6.29
L.rubrioperculatus	6.39	6.30
L. dussumieri	6.52	6.42
S. lyzan	6.58	6.53
A. thazard	6.73	6.68
S.acutipinnis	6.88	6.72
C.malabaricus	7.0	6.99

Table-3. Total plate count of irradiated and non-irradiated dried fish samples

Dried fishes	Control	Irradiated
C.malabaricus	2.20×10^5	00
S. lysan	6.0×10^5	1.7×10^2
S.acutipinnis	2.40×10^6	1.0×10^2
L.dussumieri	5.49×10^8	00
L.platypterus	6.38×10^7	2.1×10^2
L.rubrioperculatus	4.93×10^8	3.8×10^2
A.thazard	4.1×10^6	2.3×10^2

Table-4. Total fungal count of irradiated and non-irradiated dried fish samples

Dried fishes	Control	Irradiated
C.malabaricus	1.0×10^2	ND
S. lysan	6.0×10^2	ND
S.acutipinnis	1.5×10^3	ND
L.dussumieri	8.0×10^2	ND
L.platypterus	1.1×10^3	ND
L.rubrioperculatus	5.0×10^2	ND
A.thazard	3.0×10^2	ND

Table-5. Pathogenic bacteria count in the final batch control and irradiated dry fish samples

Samples	Pathogens in control samples		Pathogens in irradiated samples	
	***Salmonella* (25 g)**	***Vibrio* (25g)**	***Salmonella* (25 g)**	***Vibrio* (25g)**
C.malabaricus	Absent	Absent	Absent	Absent
S.lysan	Present	Absent	Absent	Absent
A.thaard	Absent	present	Absent	Absent
L.dussumieri	Absent	Present	Absent	Absent
I.platipterus	Present	Present	Absent	Absent
L.rupioperculatus	Absent	Present	Absent	Absent
S.acutipinnis	Present	Present	Absent	Absent

Table-6. Biochemical test for identification of *Salmonella*

Bio-chemical test		**Strain 1**	**Strain 2**	**Strain 3**
Gram		Negative	Negative	Negative
TSI	Butt	A	A	A
	Slant	AK	AK	AK
	H2S	-	-	-
LIA	Butt	A	A	A
	Slant	AK	AK	AK
	H2S	-	-	-
Urease		+	-	-
Lactose		-	-	-
Sucrose		-	-	-
Indole		-	+	-
MR		-	+	+
Dulcitol		-	-	-
Malonate		-	-	-
Xylose		+	+	+
Oxidase		-	-	-
Catalase		+	+	+
Motility		+	+	+
Gelatinase		-	-	-

Table-7. Biochemical test for identification of *Vibrio*

	Strains				
Bio chemical test		**Strain 1**	**Strain 2**	**Strain 3**	**Strain 4**
Carbohydrate utilization test	Sucrose	+	+	-	-
	Mannose	-	+	+	+
	Lactose	-	+	+	-
	Arabinose	-	+	+	+
Amino acid utilization	Arginine dihydrolase	-	-	-	-
	Ornithine dihydrolase	+	+	-	+
	Lysine decarboxylase	+	-	-	+
Salt tolerance	0%	-	-	-	-
	3%	-	+	-	-
	6%	+	+	+	-
	8%	+	+	+	-
	10%	+	+	+	+
	VP	-	-	+	+
	Urease	-	+	+	-
	Oxidase	+	+	+	+
	ONPG	+	-	-	-

Table-8. Organoleptic properties of non –irradiated dried fish

Appearance	8	7	6	8	7	8	8	9	8	6	6	6
Colour	7	7	5	7	7	7	8	8	9	7	9	6
Taste	6	6	5	8	6	8	8	9	8	7	7	5
Texture	5	6	4	8	7	7	8	8	7	7	6	6
Flavour	5	6	4	7	7	7	8	9	8	6	6	7
Overall Acceptability	6	6	5	8	7	8	8	8	8	7	8	6

Table-9. Organoleptic properties of irradiated dried fish

Appearance	9	8	8	9	9	9	9	9	9	9	9	9
Colour	9	9	9	9	9	9	8	9	9	9	9	9
Taste	9	9	9	9	9	9	9	9	9	8	9	8
Texture	8	8	8	9	8	8	8	9	8	6	9	8
Flavour	8	8	8	9	8	8	8	8	9	8	9	8
Overall Acceptability	8	8	8	9	8	8	8	9	8	9	9	8

Table-10.pH in non- irradiated and irradiated dried fishes during storage at room temperature

Samples	**Storage months**						
	Apr-2012	**May-2012**	**Jun-2012**	**Jul-2012**	**Aug-2012**	**Sep-2012**	**Oct-2012**
	0	**Ist month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
C.malabaricus							
Control	7.0	7.17	7.31	7.50	7.86	7.90	8.21
Irradiated	6.99	6.96	6.96	6.95	6.95	6.96	6.97
S.lysan							
Control	6.58	7.32	7.25	7.35	8.0	8.07	8.10
Irradiated	6.53	6.55	6.10	6.31	6.35	6.35	6.45
S.acutipinnis							
Control	6.88	7.10	8.12	8.15	8.20	8.23	8.30
Irradiated	6.72	6.71	6.70	6.72	6.80	6.81	6.85
L.dussumieri							
Control	6.52	7.32	7.40	7.55	7.56	8.18	8.22
Irradiated	6.42	6.43	6.44	6.44	6.45	6.45	6.45
L.rubrioperculatus							
Control	6.39	7.66	8.18	8.21	8.25	8.28	8.33
Irradiated	6.30	6.34	6.43	6.45	6.45	6.50	6.50
I.platypterus							
Control	6.39	6.48	6.49	7.0	7.6	7.68	8.09
Irradiated	6.29	6.40	6.53	6.54	6.54	6.55	6.55
A.thazard							
Contorl	6.73	7.25	7.85	8.19	8.20	8.20	8.24
Irradiated	6.68	6.74	6.75	6.81	6.81	6.90	6.90

Table-11. Total plate count (cfu/g) in non- irradiated and irradiated dried fishes during storage at room temperature

Samples	**Storage months**						
	Apr-2012	**May-2012**	**Jun-2012**	**Jul-2012**	**Aug-2012**	**Sep-2012**	**Oct-2012**
	0	**Ist month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
C.malabaricus							
Control	2.20×10^5	6.5×10^5	5.0×10^7	1.39×10^8	1.5×10^9	TNTC	TNTC
Irradiated	-	1.1×10^2	1.5×10^2	1.9×10^2	8.0×10^2	1.1×10^3	1.7×10^3
S.lysan							
Control	6.0×10^5	8.9×10^6	4.0×10^8	6.38×10^8	TNTC	TNTC	TNTC
Irradiated	1.7×10^2	1.9×10^2	2.2×10^2	2.5×10^2	2.5×10^2	2.7×10^2	3.3×10^2

S.acutipinnis							
Control	2.40×10^{6}	3.5×10^{7}	2.2×10^{8}	5.07×10^{8}	8.9×10^{8}	TNTC	TNTC
Irradiated	1.0×10^{2}	2.3×10^{2}	2.5×10^{2}	2.8×10^{2}	3.6×10^{2}	1.1×10^{3}	2.3×10^{3}
L.dussumieri							
Control	5.49×10^{8}	6.0×10^{8}	8.8×10^{8}	1.7×10^{9}	6.4×10^{9}	TNTC	TNTC
Irradiated	-	1.0×10^{2}	1.3×10^{2}	1.9×10^{3}	2.5×10^{3}	2.5×10^{3}	3.1×10^{3}
L.rubrioperculatus							
Control	6.38×10^{7}	8.11×10^{7}	3.2×10^{8}	7.0×10^{8}	TNTC	TNTC	TNTC
Irradiated	2.0×10^{2}	2.5×10^{2}	3.4×10^{3}	4.1×10^{3}	4.3×10^{3}	4.5×10^{3}	5.0×10^{3}
I.platypterus							
Control	6.38×10^{7}	2.5×10^{8}	6.1×10^{8}	2.0×10^{9}	5.9×10^{9}	TNTC	TNTC
Irradiated	2.1×10^{2}	2.8×10^{2}	2.1×10^{3}	2.3×10^{3}	2.3×10^{3}	6.0×10^{3}	6.2×10^{3}
A.thazard							
Contorl	4.1×10^{6}	1.7×10^{7}	3.3×10^{8}	6.7×10^{8}	TNTC	TNTC	TNTC
Irradiated	2.3×10^{2}	2.5×10^{2}	2.8×10^{2}	1.0×10^{3}	1.4×10^{3}	1.5×10^{3}	2.7×10^{3}

Table-12. Total fungal count (cfu/g) in non- irradiated and irradiated dried fishes during storage at room temperature

Samples	**Storage months**						
	Apr-2012	**May-2012**	**Jun-2012**	**Jul-2012**	**Aug-2012**	**Sep-2012**	**Oct-2012**
	0	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
C.malabaricus							
Control	1.0×10^{2}	7.0×10^{2}	4.4×10^{3}	5.7×10^{3}	6.0×10^{4}	2.0×10^{6}	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
S.lysan							
Control	6.0×10^{2}	6.5×10^{2}	3.8×10^{3}	2.5×10^{4}	2.8×10^{4}	TNTC	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
S.acutipinnis							
Control	1.5×10^{3}	5.0×10^{4}	2.0×10^{5}	3.3×10^{5}	4.0×10^{5}	TNTC	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
L.dussumieri							
Control	8.0×10^{2}	1.6×10^{3}	1.9×10^{3}	3.5×10^{4}	6.2×10^{4}	2.0×10^{7}	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
L.rubrioperculatus							
Control	1.1×10^{3}	2.0×10^{3}	5.1×10^{3}	2.4×10^{4}	3.8×10^{4}	1.7×10^{9}	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
I.platypterus							
Control	5.0×10^{2}	2.3×10^{3}	5.0×10^{4}	5.8×10^{4}	1.4×10^{6}	TNTC	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND
A.thazard							
Contorl	3.0×10^{2}	6.0×10^{2}	9.0×10^{2}	2.7×10^{4}	8.0×10^{5}	TNTC	TNTC
Irradiated	ND	ND	ND	ND	ND	ND	ND

Table-13. Pathogenic *Salmonella* /25g in non- irradiated and irradiated dried fishes during storage at room temperature

Samples	Storage months						
	Apr-2012	May-2012	Jun-2012	Jul-2012	Aug-2012	Sep-2012	Oct-2012
	0	1st month	2nd month	3rd month	4th month	5th month	6th month
C.malabaricus							
Control	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.lysan							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.acutipinnis							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
L.dussumieri							
Control	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
L.rubrioperculatus							
Control	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
I.platypterus							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
A.thazard							
Contorl	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table-14. Pathogenic *ibrio* /25g in non- irradiated and irradiated dried fishes during storage at room temperature

Samples	Storage months						
	Apr-2012	May-2012	Jun-2012	Jul-2012	Aug-2012	Sep-2012	Oct-2012
	0	1st month	2nd month	3rd month	4th month	5th month	6th month
C.malabaricus							
Control	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.lysan							
Control	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
S.acutipinnis							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
L.dussumieri							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
L.rubrioperculatus							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
I.platypterus							
Control	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent
A.thazard							
Contorl	Present	Present	Present	Present	Present	Present	Present
Irradiated	Absent	Absent	Absent	Absent	Absent	Absent	Absent

4. DISCUSSION

The moisture content is directly linked with microbial activity but the growth of microorganisms is inhibited as water activity falls below 10% [22]. However some fungi and halophilic bacteria can grow at water activity level below 8% [23]. Although the low water activity suppresses the growth of a wide range of spoilage microorganisms, some persist over time in dried product and reduce the economic value due to less weight, taste and very hard nature. Hence the need for additional treatment for effective against microbes with high moisture content is necessary. Radiation processes control the microbial population under high moisture condition [24]. In the present study fresh fishes had higher moisture content (70.23 to 76.87%), and it was reduced during salting and sun drying (25.12 to 28.74%) and after irradiation it was slightly decreased due to irradiation parallel to changes of protein, lipid, amino and fatty acid composition and vitamins.

pH in fresh fish is almost neutral. The post mortem change in the pH of fish muscle has an effect on the physical properties of the muscle texture [25] and quality of the fish. The pH of the fish meat gives valuable information about the condition of fish [26]. Too much increase or decrease of pH in fish muscle definitely spoils the seafood quality. pH 8 to 9 is favorable for the growth of actinomycetes and lower pH is favorable for the growth of fungi and some fungi grow even in pH 2 but bacteria grow well in neutral pH. The pH of the non- irradiated and irradiated dry fish samples ranges between 6.39 to 7.0. Irradiated samples had low pH than the non-irradiated samples due to the reduced moisture and bacterial population. Brewer [27] reported meat contain higher bacterial population could increase the pH. Samira, et al. [28] reported slight decrease in pH in the irradiated and vaccum wrapped chicken breast meat. In the present study regarding pH, significant differences were determined between non-irradiated and irradiated sample at the end of the storage period. The pH increases are in agreement with the findings of some research [29-35] for sea bass species stored in ice. Increases of pH indicate the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, mainly derived from microbial action [36]. The pH of live fish muscle is close to the value of 7.0. However, post-mortem pH can vary from 6.0 to 7.1 depending on season, species and other factors.

Sinduja Prakash, et al. [37] stated that salted and sun dried fishes sold in Tuticorin fish markets were contaminated with pathogenic bacteria and fungal agents in the different seasons due to unhygienic handling of the fisher folks, improper processing and unhygienic vendors and venting area. Extensive contamination of coastal water along the major landing center of Thirespuram of Tuticorin coast was reported by Christolite [38], [39], [40] isolated antibiotic resistive pseudomonas from dried sea food sold in Tuticorin. Immaculate, et al. [41] reported quality and shelf life status and sundried fishes of Tuticorin fishing villages in different season was in very poor in condition. So elimination of pathogens from the sea food is must. In this study also non- irradiated fishes had high microbial population and gamma irradiation caused a

reduction of total bacterial count in dry fish samples. The results of total bacterial count showed that the microbial loads of the irradiated samples were lower than control and this finding confirms the reduction of microbial count after irradiation of the dry fish samples. Food spoilage microorganisms are generally susceptible to irradiation; 90% reduction of most vegetative cells can be accomplished with 1–1.5 kGy [27]. Ozkan, et al. [42] reported reduced microbial count after irradiation of refrigerated sea bream (*Sparus aurata*).Moini, et al. [6] reported that irradiation at 1, 3 and 5 kGy doses had a significant reduction effect on the total viable count in rainbow trout fillets.Noomhorm, et al. [43] reported reductions of microbes in the irradiated meat and fishery products. Javanmard, et al. [44] reported that irradiation has a significant reduction effect on the microbial load in chicken meat. Reduction of total bacterial and mould counts of fresh Chinese pomfret, *Pampus chinensis* was observed after gamma radiation [45]. Mendes, et al. [46] reported that mesophilic bacteria count of irradiated shrimp, crab and fish were lower than those of non-irradiated samples during the storage at 4°C. In this study storage time caused a significant reduction in the microbial population in the irradiated samples compared to the non-irradiated dry fish samples. Irradiation is known to reduce microbial population with extended storage causing additional time dependent reductions. TBC values in their investigation suggest that the irradiated sample remain acceptable even after 6 months of storage at 37°C. Sedeh, et al. [47] reported that irradiation with a dose of 0.5, 1, 2 and 3 kGy and storage at low temperature had a significant reduction effect on microbial loads of bovine meat. They reported that the combined effect of irradiation and frozen storage was more effective than each treatment alone on decreasing total bacteria counts. In the present study combined effect of salted sun drying and irradiation had effective to the reduction of bacterial counts. Combination of irradiation with any one of the preservation method like icing, salting, drying, smoking and irradiation resulted in greater reductions of microbial loads, extending shelf life of meat for commercial application and critical conditions. Jørgensen and Hansen [48] reported that the irradiation of vacuum-packed gutted trout the total viable aerobic count of 10^6 CFU/g was not reached within 4 weeks in ice storage. At doses of 1 and 0.5 kGy this count was reached after 26 and 23 days, respectively. Non-irradiated fishes were spoiled in the third week of ice storage reached a count of 10^6 CFU/g after 15 days.

In the present study total fungal count (yeast and molds) were not detected in irradiated samples until the end of the six months of storage at ambient temperature. It has been stated that yeast and molds are sensitive to irradiation process because of their large genomic structure [49]. Fungal growth and production of fungal toxin in the fish favored by the hot and humid climate, moisture content of >16% and insect damage[50]. Fungal growth in the dried fishes produce fungal toxin with melting point at 250°C. It has been proved that food items do carry residue of the toxin namely Murgani [51]. Human beings are exposed to aflotoxin through contaminated food item among which fish is an important component [52]. The growth of filamentous fungi in food and food products results in waste and is costly as well as sometimes hazardous. Improper

salting and drying of fishes may lead to insect and fungal attack, fragmentation and degradation of products [53]]. In the present study the moisture content of the dried fishes were 25 to 30% and are semi dried samples, could be preferred for the benefit of consumers and processors. For the processors, the dried fish with high moisture content increases the weight of dried fish during sales and for the consumer's fish's shows the taste of fresh fishes. In the present study none of the dried fish samples cannot exceed the count 10^4 in the irradiated fishes and which indicates that the irradiated fishes were in good condition. Eyo [53] reported moisture content ranging from 22.7 - 27.6% in fish was easily susceptible to fungal contamination. In the present study all the dried fishes had 25 to 30% moisture. The control group samples with this moisture content had fungal colonies but in the irradiated samples with same moisture content fungus was absent. Aziz, et al. [54] concluded that doses of up to 10 kGy are highly effective in fungal decontamination and have no adverse effects on the nutritional quality of cereal grains. Regarding aromatic herbs or powdered spices, it has been reported that exposure to gamma irradiation in the dose range from 6.0 to 10.0 kGy is adequate to sterilize pepper, cardamom, nutmeg, cinnamon, fennel and turmeric without causing significant chemical or sensory alterations [55, 56]. Badr [57] reported that irradiation of rabbit meat significantly reduced the counts of yeasts and molds by 84 and 94%, respectively. Ahmed, et al. [58] also reported that 4 kGy was needed to control fungal growth of sun dried fish. Ahmed, et al. [45] reported the efficiency of gamma radiation (3, 5 and 8 kGy) in combination with low temperature (-20° C) storage of degutted fresh *Pampus chinensis*, the total mold count (TMC) increased with the increase of the storage period. In contrast dry fishes with 20 - 25% moisture, irradiated with a dose of 5kGy and stored at ambient temperature reduce the fungal population throughout the storage period. Fallah, et al. [49] reported that the irradiation at a dose of 1.5 kGy reduced the initial counts of yeasts and molds by 2 Log units, while at irradiation at a dose of 5 kGy yeasts and molds were below the detection levels during the storage period of 6 days. Badr [54] reported that the irradiation of rabbit meat at dose of 5 kGy significantly reduced the counts of yeasts and molds by 84 and 94% respectively.

H_2S producing bacteria such as *Salmonella* are generally predominant in spoiled fish flora [6]. Aquatic environments are the major reservoir of *Salmonella*. Therefore, fishery products have been recognized as a major carrier of food borne pathogens [59]. Effect of irradiation of pork and chicken [60], poultry [61], mechanically deboned chicken [62], beef jerky [63], and semi-dried seafood [64] were reported earlier. In the present study it was observed processing was carried out especially degutting was done on the soil, using of dirty salt and coir mat for drying were the factors may be responsible for pathogenic contamination. Up to 10 -15% of fish samples from India and Mexico were positive of *Salmonella* which has also been detected in several crustacean and molluscan products from India and Malaysia [65]. Pathogenic bacteria associated with fish and fishery product can be categorized into three general groups: (1) bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* sp., *Aeromonas hydrophila*); (2) enteric bacteria (non-indigenous bacteria) that are present due to fecal

contamination (*Salmonella* sp., *Shigella* sp., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and (3) bacterial contamination during processing, storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* sp.) [66]. The U.S. Food and Drug Administration's (FDA) data showed that *Salmonella* was the most common contaminant of fish and fishery products [67]. *Salmonella* contamination in fish and fishery products has also been reported from other countries like Thailand, Hong Kong, Spain and Turkey [68, 69]. The highest *Salmonella* incidence in fishery products was determined in Central Pacific and African countries while it was lower in Europe and including Russia, and North America [70].

Vibrio species are Gram negative rod (or curved rod) shaped bacteria are known to occur naturally in marine and freshwater environments and thus are commonly associated with seafood and/or food of freshwater origin [71, 72]. Most cases of reported diseases are attributed to *Vibrio* are associated with recent consumption of seafood particularly dried fish [73]. The level of *Vibrio* sp. in seafood can also affect survival quality of the product. The Fresh and salted and dried fishes are intended for consumption and the presence of *Vibrio* spp. has a direct impact on food safety. Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp. [74]. This bacterium is recognized as the leading cause of human gastroenteritis associated with seafood consumption in United States and important seafood borne pathogen throughout the world [75]. In the present study dry fishes processed and sun dried by the exporters are having *Vibrio* contamination. Since *Vibrio* can occur naturally in an aquatic environment and the presence of these organisms in raw and processed seafood may be expected [76, 77] FDA'S seafood HACCP program concluded that irradiation process with a maximum of 5.5 kGy dose is sufficient for the elimination of *Vibrio* from sea foods [78]. Because of unhygienic quality of production, fishing, handling, filleting, washing and packaging *Salmonella* and *Vibrio* were detected in control samples. Radiation sensitivity of non spore forming pathogenic bacteria, salt loving bacteria *Vibrio* in meat and fishery products is well documented [6, 49, 57]. In the present study confirms that like other gram negative bacteria, *Salmonella* and *Vibrio* have a very low resistance to radiation. Therefore elimination of these bacteria by radiation could be beneficial to the preservation of fish products in view of the major role that these species play in the spoilage of fish. Moini, et al. [6] have reported that the H_2S producing bacteria in the rainbow trout samples reached a maximum count of 4.89 Log CFU/g on day 35 and were not observed at the dose levels of 1, 3 and 5 kGy for 7, 21 and 42 days respectively. Noorlis, et al. [79] reported *Vibrio's* are halophilic bacteria are able to grow at high NaCl concentration, so some *Vibrio* cannot be eliminated by salting and this type of the species are killed by irradiation. Fallah, et al. [49] have reported that absence of Coliforms, *Vibrio* in the irradiated camel meat during refrigerated storage at 3 ± 1°C. In the present study *Salmonella*,*Vibrio* was not detected in the dried fishes irradiated and stored at ambient temperature for 6 months. Sedeh, et al. [47] reported that the optimum dose of gamma radiation decrease

pathogen especially for the elimination of *Salmonella* inred meat. Irradiation doses in the range of 1.5 – 2.0 kGy effectively control all pathogenic bacteria in shellfish except *Salmonella* species which requires 5.0 kGy [80]. Vibrionaceaewere more radiation sensitive than the *Enterobacteriaceae* [81]. Badr [57] reported that the irradiation at a dose of 3 kGy was not enough for complete elimination of *Salmonella and Vibrio* in rabbit meat, while at irradiation with a dose of 5 kGy pathogens *was* not detected. Clavero, et al. [82] reported *Salmonella* present in the ground beef eliminated by gamma irradiation at a dose of 5 kGy. Application of gamma radiation up to a dose level of 10 kGy can be used to eliminate or greatly reduce the numbers of food spoilage micro-organisms as well as food borne pathogens in food products without compromising the nutritional or sensory quality [83-85]. In the present study both thepathogenic bacteriawere absent in sun dried fishes irradiated with a dose of 5 kGy.

The sensory assessment of irradiated and non-irradiated samples was investigated respect of sensory variables such as external appearance, taste, odour, color, texture of the fishes. Average score for non- irradiated sample was 7 - 8. For irradiated sample it was 9. This was the highest score for organoleptic property. The acceptable limit of sensory score being fixed at 5.0. Both irradiated and non irradiated samples were in acceptable condition. Irradiation process improves the taste and texture of dried fish through the amino acid composition increases. Many amino acids, such as glutamic acid, aspartic acid, alanine and glycine are responsible for flavor and taste [86]. All these amino acid except glycine were rich in irradiated dried fish of *S. acutipinnis* compare to non irradiated fish. Our results agreed with the results of Ahmed, et al. [45]. Golge and Ova [87] reported that irradiation processing did not affect the sensory attributes of pine nuts. Ahmed, et al. [45] showed that irradiation doses up to 5kGy had significant effect on the visual quality, decay, colour and texture of minimally processed foods. Medium dose of irradiation (4-5 kGy) preserved the sensory attributes of dried fishes and extent their acceptability compares to non- irradiated dried fish [88]. Irradiation of fish and fish products at very high doses 10 kGy has led to changes in the food structure. If administered dose is too high from requirement the colour and the taste of the fish start to change [89]. Organoleptic quality changes were observed in irradiated dose 8kGy higher that of irradiated dose at 5kGy [90].

5. CONCLUSION

During irradiation treatment, DNA molecules undergo swelling and break alongside the chain, preventing them from functioning normally. As a result, the parasites and microorganisms that have been affected are no longer capable of reproducing themselves and so they die [91] . Therefore food irradiation provides safety and extends the shelf life of fisheries products because of its high effectiveness in inactivating pathogenic and spoilage microorganisms without deteriorating quality of the product. According to all obtained data from microbial analysis, gamma irradiation especially 5 kGy can be applied for microbial control and the safety of dried fish and shelf life extension in ambient temperature. In addition, the current study showed the

synergistic effect of irradiation of salted and sundried fishes with 25% moisture stored at ambient temperature s extent the shelf life by reducing the microbial load.

6. ACKNOWDEMENT

The authors are grateful to the Director, Dr. J.K. Edward Patterson, Suganthi Devadason Marine Research Institute, Tuticorin, for his excellent encouragement and support to carry out this work.

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Illustrations

Fig-1. TPC in non- irradiated and in irradiated *I.platypterus*

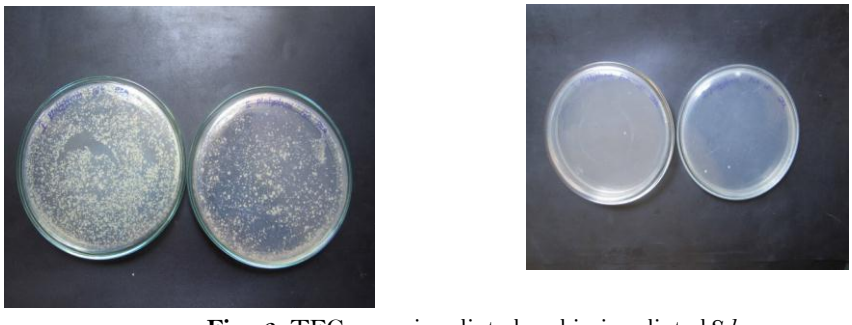

Fig- 2. TFC non - irradiated and in irradiated*S.lysan*