Fishery Technology
2001, Vol. 38(1) pp : 8 - 13

Radiation Technology for control of *Listeria monocytogenes* and *Yersinia enterocolitica* in Fish

Anu Kamat* and Dilip Bongirwar
Food Technology Division
Bhabha Atomic Research Centre
Mumbai-400 085, India

The efficacy of the radicidation as a method of preservation of fish was evaluated by artificially inoculating *Listeria monocytogenes* or *Yersinia enterocolitica* cells prior to irradiation and detecting the respective pathogen during storage at 2-4°C. These studies when repeated with two serotypes of *L. monocytogenes* clearly suggested the need of a dose of 3 kGy for elimination of 103 cfu.g^{-1} from air packed frozen shrimp. In case of *Y. enterocolitica*, a dose of 1 kGy was sufficient to eliminate naturally occurring low numbers from shrimp without any revival during storage at chilled temperature for two weeks. The initial level of contamination was an important factor in deciding the dose of radiation for effective control of growth of these organisms.

Key words : Gamma irradiation, *Listeria monocytogenes*, *Yersinia enterocolitica*,

Yersinia enterocolitica and Listeria monocytogenes are potential pathogens having occurrence in a variety of foods and meat (Farber & Peterkin, 1991, Palumbo, 1986). The organisms, being psychrotrophs, can grow in foods at refrigeration temperature and cause food borne diseases. The major outbreaks of listeriosis and yersiniosis in different parts of the world were associated with consumption of foods of animal origin. This has led to increased concern world wide for detecting these organisms in the foods and investigating means to eliminate them. Many studies conducted over 40 years have revealed irradiation as one of the cost-effective technologies in processing wholesome and safe foods (Farkas, 1990; Radomysky *et al.*, 1994; Anon, 1993). One advantage of this technology is that irradiation, being a nonthermal process, can bring about extension of shelf life of raw fish/meat at refrigeration temperature and help in marketing the product as chilled (0 - 10°C) instead of frozen, thus meeting the demand for fresh foods. Reports from our laboratory (Kamat *et al.*, 1991; Kamat & Nair, 1995; Kamat *et al.*, 1997) as well as from elsewhere (Diehl, 1994 and Radomysky *et al.*, 1994) have demonstrated that gamma irradiation in the range of 2-7 kGy, depending upon the level of natural contamination and irradiation temperature, eliminated many hazardous food borne pathogens from sea food and made the product microbiologically safe. The results of the work carried out to establish the minimum radiation dose to decontaminate fish product from *Listeria monocytogenes* and *Yersinia enterocolitica* are presented in this paper

Materials and Methods

L. monocytogenes and other strains of *Listeria*; as well as *Y. enterocolitica* and other strains of *Yersinia* used in the studies were obtained from Public Health Laboratory Services, U.K.

Fresh, frozen and dry varieties of fish from local market were collected in sterile

*Corresponding author

plastic bags. The fresh and frozen fish samples were transported to the laboratory in boxes containing ice or dry ice. Ten grams of sample were aseptically added to 90 ml sterile BHI (brain heart infusion medium) and homogenized. Two stage enrichment procedure, including primary cold enrichment in BHI followed by secondary enrichment in selective medium was adopted for isolation of *Listeria* (Kamat & Nair, 1994) and *Yersinia* (Khare *et al.*, 1996). The identification of the pathogens to species level was done on the basis of biochemical tests by comparing with reactions of standard type cultures.

Overnight grown cells of the required cultures *(L. monocytogenes* or *Y. enterocolitica)* were washed with phosphate buffer (pH 7.00). The shrimp samples were completely submerged in cell suspensions at a concentration of 10^3 - 10^4 organisms/ml. After 1 h at 0°C the samples were drained, packed aseptically in sterile polyethylene bags and frozen at -40°C prior to irradiation.

Shrimp samples packed in polyethylene bags were exposed to 1,3,4 and 6 kGy doses in a Co^{60} food package irradiator (Atomic Energy Canada) at a rate of 0.05 $kGy.min^{-1}$ (IAEA, 1977). Unirradiated products served as controls. All the samples were stored at 2 - 4°C in separate containers and periodically checked for the concentration of the inoculated organism.

Table 1. Incidence of *Listeria* spp. in fish and shellfish

Product	No. of samples	No. of isolates	Number of isolates of						
			L. monocy-togenes	*L. ivanovii*	*L. innocua*	*L. seeligeri*	*L. murrayi*	*L. grayi*	Un identified
Frozen									
Shrimp	4	65	-	-	21	10	-	34	-
Fresh fish									
Dhoma	3	5	-	2	-	-	-	3	-
Anchovy	2	5	-	-	-	-	3	2	-
Golden anchovy	2	10	-	-	2	-	3	4	1
Pomfret	2	10	-	-	2	-	3	5	-
Indian Salmon	1	5	-	-	-	2	-	1	2
Threadfin (Raja Rani)	3	15	-	-	5	-	5	2	3
Mackerel	3	10	-	-	4	-	-	2	4
Bombay duck	2	10	-	-	-	-	4	4	2
Crab	2	15	-	2	3	1	2	2	5
Dry fish									
Shrimp	3	10	-	2	4	-	2	2	-
Mackerel	2	8	-	-	-	-	-	6	2
Bombay duck	3	14	-	-	8	-	-	4	2
Golden Anchovy	2	10	-	-	2	-	-	6	2
Anchovy	3	9	-	-	-	-	-	9	-
Total	38	201	-	6	51	13	22	86	23

1. Many of the isolates remained unidentified but they remain nonhaemolytic
2. Many isolates belonging to nonhaemolytic *L. innocua* ferment rhamnose

Table 2. Profile of *Yersinia* species isolated from fish

Product	No. of samples	CIN (count g^{-1})	Total number of isolates	Number of *Y. intermedia* isolates	Number of *Y. pestis* isolates	Number of *Y. pseudotuber-culosis* isolates	Number of *Y. enterocolitica* isolates	Number of *Y. fredriksenii* Isolates	Number of unidentified (Mannitol negative) isolates
Fresh finfish									
Anchovy	2	$7x10^4$	6	4(66.6%)	-	-	-	-	2
Bombay duck	6	$3x10^6$	14	8(57.14%)	-	-	-	-	6
Mackerel	5	$2.5x10^5$	8	8(100%)	-	-	-	-	-
Pomfret	1	$6x10^3$	7	4(57.14%)	-	-	1(14,28%)	-	2
Total	14		35	24	-	-	1	-	10
Fresh shellfish									
Shrimp - 1	1	$6x10^4$	18	5(27.77%)	1(5.5%)	1(5.5%)	-	1(5.5%)	10
Shrimp - 2	2	$1.2x10^5$	18	-	-	-	-	-	-
Crab	2	$1.5x10^5$	10	2(20%)	-	-	2(20%)	-	6
Oyster	5	$3.5x10^4$	5	-	-	-	1(20%)	-	4
Prawn	3	$2.5x10^4$	14	3(21.42%)	-	-	-	-	11
Total	12		47	10	1	1	3	1	31
Dried fish and shellfish									
Golden anchovy	5	$2x10^5$	5	3(60%)	-	-	-	-	2
Anchovy	5	$5x10^4$	10	4(40%)	4(40%)	-	1(10%)	-	1
Mackerel	3	$4x10^5$	6	5(83.33%)	-	-	-	-	1
Shrimp	3	$2.2x10^6$	11	10(90.90%)	-	-	-	-	1
Prawn	3	$5.1x10^5$	11	9(81.1%)	-	-	1(9.01%)	-	1
Bombay duck	5	$2.5x10^4$	13	9(69.23%)	-	-	-	-	4
Total	24		56	40	4	-	2	-	10

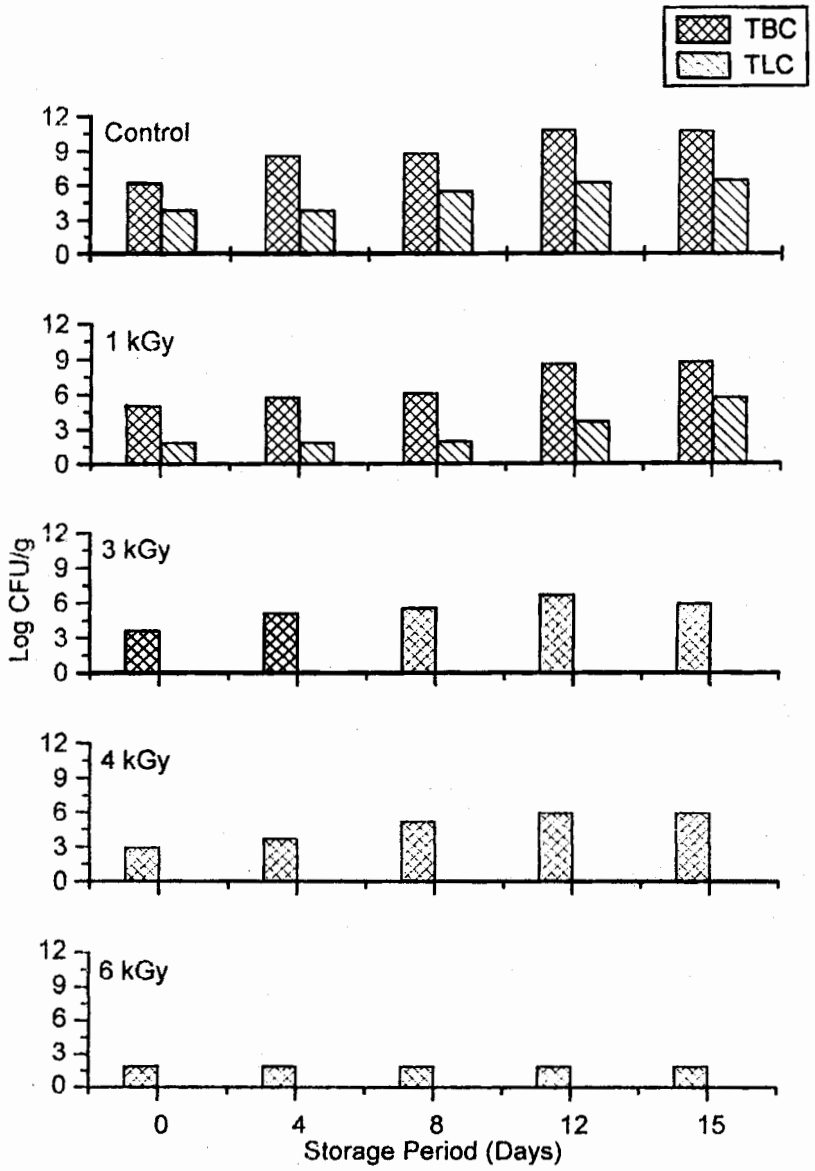

Fig. 1. *L. monocytogenes* in irradiated and unirradiated inoculated fish during storage at 2 - 4°C

The packages were opened periodically, and 10 g of the product were aseptically transferred to 90 ml sterile saline and homogenized for 3 min. Serially diluted shrimp samples were plated in triplicate on the plate count agar (PCA) and respective selective enrichment medium for detection of *L. monocytogenes* or *Y. enterocolitica.*

Results and Discussion

Data on incidence of Listeria in 38 samples of fish are given in Table 1. Out of 38 fish samples, 10 were contaminated with *L. innocua* and 7 samples with *L. murrayi* while *L. grayi* was detected in all samples. However *L. monocytogenes* was not found in any of the samples tested. The findings are in agreement with the only report regarding incidence of this organism in Indian fishery products by Fuchs & Surendran (1989) who examined the incidence of Listeria in local markets of Cochin. However, later it was reported that the recovery of *L. monocytogenes* from Indian fish is possible by modifying the isolation procedures (Jayasekaran *et al.*, 1996).

Table 2 gives the details of *Yersinia* species isolated from fish samples on Cefsiodin-Irgasan - Novobiocin (CIN) agar plates and identified to species level. Out of 138 *Yersinia* isolates recovered from 15 samples, 74 isolates belonged to *Y. intermedia*. Thus the predominant species was non-pathogenic in nature. Nevertheless, it can be seen that seafood like pomfret (14.28%), oyster and crab (20% each) and some dry fish (10%) harboured *Y. enterocolitica*. These findings of incidence of *Y. enterocolitica* in fish are similar to the reports from Canada (Toma, 1973), USA (Lee, 1977) and other parts of the world (Gerard & Leland, 1992).

The D_{10} values of *L. monocytogenes* and *Y. enterocolitica* in fish homogenate at 40°C was observed to be 0.25 - 0.3 kGy (Kamat & Nair, 1992). It is evident from Fig.1 that the initial total bacterial count (TBC) increased to 10^9 cfu. g^{-1} in the case of the control and 1 kGy samples after 4 and 12 days of storage respectively. In contrast, the growth of *L. monocytogenes* was comparatively slow in control and 1 kGy samples which could be due to presence of fast growing organisms surviving in 1 kGy samples (82 cfu. g^{-1}). Shelef (1989) has reported poor growth of *L. monocytogenes*, ATCC 35152, in ground beef in presence of fast growing bacteria. Listeria showed growth to $5.3x10^3$ and 7.0×10^3 in 12 and 15 days, respectively in control and 1 kGy samples and irradiation resulted in lag of 8 days as against 4 days in unirradiated samples. However, at doses 3 kGy and above Listeria were completely eliminated and its revival was not noticed during storage for 15 days. The storage studies of samples artificially inoculated with *Y. enterocolitica* showed the ability of this organism to revive in inoculated fish during post irradiation storage (Table 3). However, this phenomenon was not noticed when naturally contaminated fish was irradiated and stored at chilled temperature, suggesting the significance of initial level of contamination in determining the optimum dose for radiation.

From the results of this investigation it can be safely concluded that irradiation at a dose of 3 kGy, efficiently eliminates *L. monocytogenes* and *Y. enterocolitica* from frozen fish and makes it safe for consumption.

References

Anon (1993) *Food Irradiation News Lett.* **17**, 39

Diehl, J.F. (1994) *Safety of irradiated foods.* Marcel Dekker Inc, New York

Farber, J.M. & Peterkin, P.I. (1991) *Microbiological Rev.*, **55**, 476

Table 3. Fate of *Yersinia enterocolitica* in naturally contaminated and inoculated fish during irradiation and storage at 2 - 4°C.

Irradiation dose (kGy.min^{-1})	Storage time (Weeks) Natural				Storage time (Weeks) Inculated (10^6 cell.g^{-1})			
	0	2	3	4	0	1	2	3
0 (Control)	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+
3	+	+	-	-	-	+	+	+
4	-	-	-	-	-	+	+	+
6	-	-	-	-	-	+	+	+

+ = detected; - = not detected

Farkas, J. (1990) *Radiation Effects on Organisms of Public Health Significance.* Training on Food Irradiation by IFFIT at Wageningen, The Netherlands

Fuchs, R.S. & Surendran, P.K. (1989) *Lett. Appl Microbiol.* **9**, 49

Gerard, N.S. & Leland, J.M. (1992) *J. Food Protect.* **55**, 649

IAEA (1977) *Manual of Food Irradiation Dosimetry,* Technical Report Series No.178, 1977, IAEA, Vienna

Jeysekaran, G. & Karunasagar, I. (1996) *Int. J. Food Micro.* **31**, 333

Kamat, A.S. & Nair, P.M. (1994) J. *Food Safety,* **14**, 117

Kamat, A.S., Alur, M.D., Nerkar, D.P. & Nair, P.M. (1991) *J. Food Safety,* **12**, 59

Kamat, A.S. & Nair, P.M. (1992). Paper presented at the Eleventh International Symposium on Problems of Listeriosis. Copenhagen 11-14 May 1992.

Kamat, A.S. & Nair, P.M. (1995) *J. Sci. Food Agric.* **69**, 415

Kamat, A.S., Khare, S., Doctor, T. & Nair, P.M. (1997) *Int. J. Food Micro.,* **36**, 64

Khare, S.S., Kamat, A.S., Doctor, T.R. & Nair, P.M. (1996) *J. Sci. Food Agric.* **72**, 187

Lee, W.H. (1977) *J. Food Prot.,* **40**, 456

Palumbo, S.A. (1986) *J. Food Protect.* **49**, 1002

Radomysky, T., Murano, E.A., Olson, G.D. & Murano, P.S. (1994) *J. Food Protect.* **55**,73

Shelef, L.A. (1989) *J. Food Protect.* **55**, 379

Toma, S. (1973) *Can. J. Public Health,* **64**, 477