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Microbial Profiles of Non-Irradiated and Irradiated Semi-dried Fish

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Semi-dried non-irradiated anchovies (*Anchoviella commersoni*), initially exhibited the presence of *Micrococcus* (15%), *Bacillus* (60%) and *Staphylococcus* (20%) microflora. On the other hand, Bombay duck (*Harpodon nehereus*) and Vietnam scads (*Alepes mate*) were found to be contaminated predominantly by *Bacillus* and *Staphylococcus* sp. respectively. Irradiation (3 kGy) of semi-dried anchovies resulted in modification of microbial composition which comprised of *Bacillus* (50%) and *Staphylococcus* (50%), while microflora of semi-dried irradiated Bombay duck consisted of *Bacillus* sp. only. These results suggested that irradiation of semi-dried fish resulted in the elimination of *Micrococcus* leaving the survivors belonging to *Bacillus* sp. and *Staphylococcus* sp.

Key words : Semi-dried fish, microbiological evaluation, gamma radiation preservation.

Gamma-irradiation of semi-dried fish has been advocated to prevent infestation as well as to reduce bacterial contamination to a great extent, so that extension in shelf-life of the product could be achieved (Guevera *et al.*, 1989). In our laboratory at Bhabha Atomic Research Centre, Bombay, dehydro-irradiation processes involving combination of heat and radiation have been developed for sea-foods, viz. Bombay duck, white pomfret and shrimps (Doke *et al.*, 1978; Agarwal *et al.*, 1972; Gore *et al.*, 1970). These products are quite stable at ambient temperature over a long period of time. A packaging system was also developed which could reliably be used to prevent recontamination of semi-dried anchovies and Bombay duck (Doke, 1990) with extended shelf-life without infestation. We have also investigated physical properties of irradiated packaging materials for semi-dried fish (Vinh *et al.*, 1992) and storage properties of non-irradiated and irradiated semi-dried fish in terms of microbial load, bio-chemical changes as well as sensory attributes during storage (Vinh *et al.*, 1993a; 1993b). The present communication deals with microflora of semi-dried non-irradiated and irradiated (3 kGy) anchovies (*Anchoviella commersoni*), Bombay duck (*Harpodon nehereus*) and Vietnam scad (*Alepes mate*).

Materials and Methods

Semi-dried Bombay duck (*Harpodon nehereus*) and anchovies (*Anchoviella commersoni*) were procured from local fish market at Bombay while semi-dried Vietnam scads (*Alepes mate*) were brought by one of authors from Vietnam.

Semi-dried Indian sea-foods have been reported to contain 5-7% salt and 20% moisture (Solanki & Sankar, 1987) while dried salted Vietnam scads contained 18% salt and 20-22% moisture.

Semi-dried fishes were packed in polyethylene pouches (400 gauge) in 25 g quantity and sealed. Sealed pockets were exposed to gamma radiation dose of 3 kGy in a ^{60}Co package irradiator (dose rate, 0.05 kGy min^{-1}). Another set of packets were maintained as unirradiated controls.

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10 g of non-irradiated and irradiated (3 kGy) semi-dried fishes were blended with 90 ml sterile saline in a Sorvall omni-mixer for 2 min (5000 rpm). The homogenate thus obtained was serially diluted in sterile physiological saline. Appropriate dilutions were plated on nutrient agar (Difco) plates by streak plate method. After incubation of plates at 30°C for 48 h at least 40-50 distinct colonies were picked up from petridishes and transferred to nutrient agar slants. After 24 h of incubation, the cultures, were tested for purity by simple staining. Pure cultures, thus obtained were subjected to morphology, Gam-staining, motility and biochemical tests, as described by Alur & Lewis (1980). Fermentation - Oxidation of carbohydrates for cultures were performed using Hugh - Leifson medium. Kovacs oxidase test, Urease, MRVP etc., tests were also performed. Cultures were identified to the generic level, based on the observations made by morphological and biochemical tests. The scheme employed in identification of bacteria was that of Harrison *et al.* (1981).

Results and Discussion

As shown in Table 1, initial bacterial load of non-irradiated semi-dried fish ranged from $7x10^2$ - $5.4x10^2$ bacteria g^{-1}.

Table 1. Bacteriological status of non-irradiated and irradiated semi-dried fish during storage at ambient temperature (26°C)

Radiation dose, kGy	Ambient storage period, months	Total bacterial count @, cfu g^{-1} Anchovies	Bombay duck	Vietnam scad
0	0	$5.4x10^2$	$7x10^2$	$2.60x10^3$
	2	$1.9x10^4$	$2x10^3$	$2.81x10^3$
3	0	Nil	20	40
	2	$3x10^3$	$1x10^3$	20

@ Values are the average of three independent experiments

However, irradiation at 3 kGy inactivated bacteria by approximately 2 log cycles. During storage for 2 months at ambient temperature, total bacterial count (TBC) increased by 2-3 log cycles in both non-irradiated and irradiated semi-dried fish. Distinct colonies from these plates were picked up and maintained on nutrient agar slants for further tests.

Table 2. Composition of microflora in non-irradiated and irradiated semi-dried fish

Fish variety	Microbial groups	Percent of total number of isolates Non-Irradiated control	Irradiated (3 kGy)
Anchovies	*Micrococcus*	15	nd
	Staphylococcus	20	50
	Bacillus	60	50
	Yeasts	05	nd
Bombay duck	*Micrococcus*	nd	nd
	Staphylococcus	nd	nd
	Bacillus	100	100
Vietnam scad	*Micrococcus*	20	nd
	Staphylococcus	60	nd
	Bacillus	20	100

nd - not detected

Table 2 incorporate the data on microbial profiles of non-irradiated and irradiated (3 kGy) anchovies, Bombay duck and Vietnam scads during storage at ambient temperature (26°C). It may be noted that non-irradiated anchovies exhibited diverse microflora consisting of *Micrococcus* (15%), *Staphylococcus* (20%) and *Bacillus* (60%). On the other hand, non-irradiated semi-dried Bombay duck was found to be contaminated by *Bacillus* only. Microflora of Vietnam scad was predominated by *Staphylococcus* sp. (60%).

Irradiation of semi-dried fish modified the composition of microflora. Thus, in irradiated anchovies, *Micrococcus* and

yeasts were elimimated. In irradiated Vietnam scad also both *Micrococcus* and *Staphylococcus* were completely eliminated leaving only *Bacillus* sp. as survivors.

Microorganisms show enormous differences in their response to gamma radiation. Gram-negative organisms such as *Pseudomonas, Aeromonas* sp. show D_{10} values 0.4 - 0.19 kGy, while Gram-positive *Micrococcus radiophilus* isolated from radurized Bombay duck exhibits extreme radiation-resistance which could be expressed in terms of D_{10} value of the order of 15 kGy (Alur *et al.*, 1971; Lewis, 1971). Based on differential radiation sensitivity among bacteria, the composition of microflora has also been reported to be modified by gamma radiation in Indian mackerel (*Rastrelliger kanagurta*) Bombay duck (*Harpodon nehereus*) and shrimps (*Penaeus indicus* (Ghadi *et al.*, 1978; Mavinkurve *et al.*, 1967; Kamat and Kumta, 1974). In most of these studies, fresh fish has been shown to harbour diverse microflora comprising of *Pseudomonas, Aeromonas, Micrococcus, Bacillus,* etc. However, gamma irradiation knocks off Gram-negative organisms which are sensitive to radiation, while radiation survivors such as *Micrococcus* sp. and *Bacillus* sp. have been reported to predominate in irradiated fish (Lewis *et al.*, 1973).

In the present investigation, Gram-negative organisms were not detected as the water activity a_w required for their growth was inadequate in semi-dried fish (Troller, 1980). Handling practices might have introduced *Staphylococcus, Micrococcus* and *Bacillus* in these products.

It was of interest to note that micrococci were completely eliminated by gamma radiation, survivors being either *Bacillus* or *Staphylococcus* sp. in irradiated semi-dried fish. Microbial profiles did not alter during storage.

Korean researchers have reported the predominance of *Staphylococcus* and *Micrococcus* in addition to Enterobacteriaceae in dried file fish fillet (Yoo *et al.*, 1985). The contamination in Korean dried file fish was reported to be in the order of 10^3 - 10^8 cfu g^{-1}, whereas commmercially dried Indian fish harbor comparatively lower bacterial contamination (10^4 bacteria g^{-1}).

Studies on microbial flora of dehydro-irradiated shrimps in our laboratory suggested that *Micrococcus* and *Staphylococcus* predominate in non-irradiated dried shrimps while irradiation (2.5 kGy) resulted in predominance of *Micrococcus* and *Bacillus* sp. (Venugopal & Lewis, 1978).

In conclusion, semi-dried anchovies, Bombay duck and Vietnam scad consisted of diverse microflora before irradiation. Irradiation at a dose of 3 kGy resulted in alteration of microflora in semi-dried fish which could be assigned to the genera *Bacillus* and *Staphylococcus.*

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