Radiation Preservation of Seafoods: A Case Study on Indian Mackerel

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Several aspects on the use of low dose gamma radiation for shelf life extension of Indian mackerel in ice have been examined to understand its amenability for the radiation process. These included, apart from determining the optimum dose (1.5 kGy) for maximum shelf life, storage studies in terms of sensory and chemical indices of freshness of the treated fish, influence of radiation on fish lipids, physico-chemical and functional properties of the fish proteins, textural attributes of the muscle and microbiological as well as wholesomeness aspects of the irradiated fish. Stability of the fish irradiated under non-packaged condition has also been studied with a view to adopt the method for large scale processing. This article consolidates these data and points out that Indian mackerel in ice could be irradiated at landing centres to achieve quality retention and hence enhanced marketability of the catch.

Although world fish production is steadily increasing, preservation of the commodity still remains to be a challenging problem. Susceptibility of fish to rapid spoilage has been attributed to its intrinsic characteristics and to possibilities of microbial contamination from a variety of sources (Venugopal, 1990; Ward & Baj, 1988). Conventional chilling and refrigeration have limited effects on psychrotrophs and several pathogenic microorganisms harbouring the fish (Lecowich, 1988; Palumbo, 1986).

Treatment of foods by ionizing radiation (gamma rays, electrons or x-rays) has recently emerged as a promising method for control of contaminant microorganisms in foods particularly after the declaration of JECFI (1981) that irradiation of any food commodity up to an overall average dose of 10 kGy presented no toxicological hazard. The process reduces both post-harvest food losses and public health hazards arising from pathogens (Chinsman, 1987). Low dose of radiation is also useful for disinfestation of dried fishery products (Giddings, 1984; Nickerson *et al.*, 1983). An average dose of 3 kGy can extend the chilled storage life of several wet fish by 2 to 3 fold (Giddings, 1984; Nickerson *et al.*, 1983; Kumta & Sreenivasan, 1970). Lean fish species show the least irradiation - induced rancidity, while fatty fish can be treated only if adverse sensory changes as a result of irradiation are insignificant. In developing irradiation processing for fishery products, several other aspects including physico-chemical, microbiological, safety and techno-economic feasibility of the process have to be examined before commercialisation of the process. Many of these aspects have been studied with respect to Indian mackerel. This article is intended to consolidate these data and to assess the feasibility of irradiation treatment for the fish.

Indian mackerel (*Rastrelliger kanagurta*) widely distributed in the Indo-Pacific ocean (Noble, 1972), is one of the most popular fish varieties in India. The annual catch of the fish in the country fluctuates widely, but is estimated to be about 69,000 t constituting about 9% of the total marine landings. The fish is normally caught between August and April on the western coast. More than 50% of the catch is sold fresh, 25% is salted and dried and a negligible amount is canned. About half of the fresh fish is makreted in ice while the remaining is sold uniced. The fat content of the fish varies with breeding area and season (Venkatraman & Chari, 1950). The fish used in the present studies had a maximum of about 13% lipids (Rao & Bandyopadhyay, 1982). The muscle fat contained 14.9% $C_{22:6}$ and 3.3% $C_{20:5}$ poly- unsaturated fatty acids (Rao *et al.*, 1988) while palmitic and stearic were the main saturated fatty acids (Rao & Bandyopadyay, 1982, Rao, *et al.*, 1988).

The radiation process

The process (Venugopal *et al.*, 1973) consists of evisceration of the fish, washing in potable water, sealing in polythene (500 gauge) pouches icing the bags in aluminium boxes and exposure to an average dose of 1.5 kGy (^{60}Co pencil source, 75,000 Curie, Food Package Irradiator of Atomic Energy of Canada Ltd.). The irradiator has 1.5 t capacity per day. A dose of 1.5 kGy was found to be ideal for maximum shelf life extension of the fish causing minimum changes as judged by sensory and chemical criteria (Venugopal *et al.*, 1973).

Storage studies

The irradiated fish held in ice remained in acceptable condition up to 28 days as against 12 days for unirradiated mackerel under the same conditions. The shelf life was determined by sensory analysis by an experienced panel of 8 members. A 9 point hedonic scale was employed to rate liking for the fish. Total volatile basic nitrogen (TVBN) and total volatile fatty acid (TVA) - mainly formic and acetic acids - contents were also measured as chemical indices of freshness. Acceptability of the fish decreased with the rate of increase in TVBN and TVA values (Venugopal *et al.*, 1981). The spoilage rates taken as rates of increase in TVBN or TVA were less than half in irradiated fish in comparison with those of unirradiated fish.

It is known that storage temperature has a profound influence on the shelf life of fresh fishery products. A reduction of storage temperature from 5° to 0.6°C doubles the shelf life of several fish varieties (Nickerson *et al.*, 1983). In the case of Indian mackerel, changes with time of chemical, microbiological (total colony forming units) and sensory criteria were essentially linear for both unirradiated and irradiated fish stored between 0 to 15°C. Irradiation at 1.5 kGy suppressed the spoilage rates throughout the temperature range; however the relative spoilage rates, namely, the ratio of spoilage rates with respect to each specific parameters at a particular temperature to that at 0°C for the same parameter was similar for both unirradiated and irradiated mackerel (Venugopal *et al.*, 1982). The data could also be used to predict the shelf life of unirradiated or irradiated fish at any temperature between 0° and 15°C using the Spencer and Baines (1964) equation. These studies also led to identification of volatile acids content as a quality index for unirradiated and irradiated mackerel (Venugopal *et al.*, 1981).

As the initial quality of the fish is of utmost importance for getting maximum extension of shelf life only Grade A fish having characteristic flavour and odour of the variety (Ronsivalli, 1982) should be used. In many instances like continuous fishing operations, it is likely that some fish may lose initial quality before irradiation on shore. Therefore, the influence of pre-irradiation ice storage on post-irradiation extension in shelf life was examined. Freshly caught mackerel gave a maximum post-irradiation shelf life in ice of 28 days, but the fish held in ice for 3, 5, 7 and 9 days before irradiation, shelf life was reduced to 25, 23, 15 and 13 days respectively (Venugopal, *et al.*, 1973).

Packaging

A number of laminates were screened as packaging material for the fish. It was found that polythene (500 to 700 gauge) or polythene-cellophane (polycel) bags were acceptable for the purpose, as also observed by Agarwal & Sreenivasan (1973). The influence of irradiation on lipid peroxidation is important in determining sensory properties since the fish contains significant amount of lipids (Rao & Bandyopadhyay, 1982). Although vacuum packaging reduced lipid peroxidation, it is not advisable for radurized fishery products since it may favour growth of anaerobic organism like *Clostridium botulinum* (Giddings, 1984). Sensory evaluation of both vacuum packaged and air packaged irradiated mackerel after immersing the pouches in boiling water for 20 min did not show significant differences (Venugopal *et al.*, 1973) suggesting that air packaging would be sufficient for radurization of the fish.

Microbiology

The microbial profiles of Indian mackerel have been studied by Surendran & Gopakumar (1982, 1983) and Alur & Lewis (1980). The skin of freshly caught mackerel harboured a high proportion of *Vibrio* followed by *Moraxella, Acinetobacter, Pseudomonas* and *Micrococcus* (Table 1). Gram-positive organism consisting of *Corynebacterium, Bacillus* and *Micrococcus* usually ranged between 10-15% (Surendran & Iyer, 1976). After storage in ice for a period of 24 days, when the fish has spoiled, *Pseudomonas* became the predominant flora while *Vibrio* almost disappeared. Other surviving flora consisted of *Moraxella, Acinetobacter, Flavobacterium* and *Micrococcus*. The total flora of the spoiled fish comprised of gelatin liquefiers and putrefiers and had the capacity to grow at 0°C (Surendran & Gopakumar, 1982). Alur & Lewis (1980) showed that storage of the fish at 10 and 15°C resulted in predominance of *Micrococcus* and *Bacillus* instead of *Pseudomonas*.

Table 1. *Bacterial flora of fresh Indian mackerel and approximate radiation doses needed to reduce microorganisms by 10^6 fold*

Spices	Percent of total isolates* Fresh	Radiation dose** (kGy)
Pseudomonas	10	0.5-2.0
Moraxella	18	5.0-7.5
Acinetobacter	26	0.5-1.0
Vibrio	32	0.5-1.0
Bacillus	-	2.0-3.0
Flavobacter/cytophaga	5	-
Micrococcus	5	3.0-5.0
Gelatine Liquefiers	82	
Putrefiers (fish media)	14	
Capable of growth of 0°	7	

* Cited from Surendran & Gopakumar (1982)

** Cited from ICMSF (1980)

Irradiation of mackerel at 1.5 kGy resulted in preferential elimination of gram-negative spoilage organisms. These organisms are comparatively more sensitive to radiation (ICMSF, 1980). Radiation doses required for 90% reduction in viable counts (D_{10} values) of predominant flora of Indian mackerel are given in Table 1. It can be seen that species like *Pseudomonas, Vibrio* and *Acinetobacter* are comparatively sensitive to radiation. Thus *Micrococcus* was predominant with smaller proportion of *Pseudomonas* and *Enterobacteriace* in fish after irradiation at 1.5 kGy (Alur & Lewis, 1980). The treatment resulted in reduction in total count by about one log cycle (Venugopal *et al.*, 1973). Spoilage potentials of some typical bacterial isolates from unirradiated and irradiated mackerel were examined by Alur *et al.* (1989) and Venugopal *et al.* (1983). It was found that the spoilage potentials of organisms varied with *Aeromonas* and *Pseudomonas* causing more spoilage of fish as compared with *Bacillus* and *Micrococcus*.

The radiation treatment of the fish also ensures higher safety since it simultaneously reduces the levels of pathogenic organisms like *Vibrio, Aeromonas, Salmonella* and *Listeria* species, if present in the fish, which are comparatively sensitive to radiation (ICMSF, 1980). The problem of survival of *Clostridium botulinum* in irradiated seafoods has received much attention. The possibility of growth and lethal toxin formation by spores of the organism in treated fish was examined by carefully designed inoculated pack studies. These studies showed that botulism hazard would result only if the fish carried a high initial level of *C.botulinum* spores (>10^3 spores per fish) and the storage temperature exceeded 10°C (Hobbs, 1977). Surveys todate indicate that the contamination level is about one spore per gram of edible flesh (Giddings, 1984). Survey for the presence of *C.botulinum* in India has shown negative results (Bhattacharjee, 1981). Further, the general Indian habit of consumption of well cooked foods including fishery products ensures additional protection against the toxin which is heat sensitive. The microbiological safety of irradiated foods had been evaluated and concluded that there was no cause for concern (ICFMH, 1983; Farkas, 1989).

Radiation induced changes

Ionizing radiations are known to cause changes in food constituents. The direct effect of radiation lead to generation of several radiolytic products while indirect effects are due to the interaction of these moieties with the fish constituents (Taub *et al.*, 1979). It has been estimated that a dose of 10 kGy may yield one millimole of radiolytic products per kg of irradiated food (Takeguchi, 1983). King *et al.*, (1972) reviewed the chemical changes in irradiated seafoods, and observed that, depending upon composition, the dose employed and the condition of irradiation, some changes occurred in the fish constituents. At lower doses used for radurization purposes such changes were not significant. Dubravic & Magaar (1969) observed that irradiation of Atlantic mackerel resulted in the formation of long chain compounds from cleavage of fatty acids. The influence of irradiation of 1.5 kGy on properties of lipids and proteins from mackerel were studied in order to assess the stability of the fish to radiation treatment.

Lipids

The influence of irradiation on the fish lipids was investigated with a view of ascertaining the release of oxidation products as a result of the treatment and subsequent ice storage. The treatment caused lipid oxidation as determined by thiobarbituric acid and peroxide values, which was more under aerobic condition than in vacuum packaged fish (Venugopal *et al.*, 1973). Rao & Bandyopadhyay (1982) studied the lipid profiles of the fish and showed that there was a progressive decrease in the initial content of triglycerides (85 g per 100 g lipids), with increase in free fatty acids during storage of both unirradiated and irradiated mackerel. However, the overall changes in fatty acid composition of irradiated mackerel during storage at either 0 or 10°C were similar to those of unirradiated fish. Ghadi & Venugopal (1991) showed that using thiobarbutiric acid value as index, irradiation at doses up to 5 kGy did not produce any appreciable level of lipid oxidation in either skin or muscle lipids of Indian mackerel.

Proteins

Interactions of proteins and amino acids with peroxidized lipids may lead to a variety of adverse chemical changes (Gardner, 1979). These may reflect changes in the properties of proteins in the food system. The influence of irradiation on several properties of mackerel proteins were, therefore, examined. These studies showed that the fish proteins were generally stable to irradiation at 1.5 kGy. Solubilities and denaturation characteristics of the proteins as a function of pH, temperature and ionic strength were not affected (Venugopal, 1981). This is in contrast to the proteins of Bombay duck which were highly sensitive to radiation-induced denaturation (Warrier *et al.*, 1973). However, the sarcoplasmic proteins from irradiated mackerel were slightly more sensitive to digestion by trypsin. Further, myosin from the treated fish also showed some enhanced ATPase activity (Venugopal, 1981).

Functional properties of food proteins include organoleptic, textural, rheological and hydration characteristics which decisively influence the acceptability of the food. These properties are governed by composition, structure and conformation of the proteins and also the environment in the food system (Kinsella, 1976). Any processing techniques that cause changes in the proteins may therefore lead to alterations in the functional properties. The functional properties of irradiated mackerel were determined in order to assess radiation induced changes in the fish proteins. Some of the properties studied included extractability of protein fractions during the course of storage at 0°C, oil emulsification properties and gel forming abilities of the proteins. It was found that the treatment did not significantly affect extractability of proteins, their emulsification capacity, emulsion stability and gel forming ability (Venugopal & Lewis, 1983).

The radurized fish had better textural properties like sheer force (SF), plasticity index (PI) and water holding capacity (WHC) compared with untreated mackerel (Ghadi & Lewis, 1979). The sheer force value both in control and radurized samples indicated a reduction during storage at 0-2°C. However, the initial SF of 2.6 kg in the control was reduced to 1.3 kg after 10 days ice storage, while a similar effect was observed in the radurized sample only after 20 days. WHC values also reduced both in control and irradiated fish during storage of 5 and 10 days, respectively. PI increased concomitantly with the decrease in water holding capacity. The drip loss was similar in both control and radurized samples, in the range of 3-5%, while pH of the muscle in both the fish varieties increased from 5.9 to 6.3 within the first 5 days. These studies suggested better texture retention of irradiated fish muscle during ice storage.

Safety

Food irradiation being comparatively a new process, has to satisfy many criteria from the regulatory point of view before its large scale application. Evaluation of wholesomeness is essential to convince health officials and the consumers of nutritional, microbiological and toxicological safety of the processed food. Rigorous feeding trials in which several irradiated foods were fed to mammals have failed to show any adverse effects of consumption of these foods (Brynjolfsson, 1986). These studies led the FAO/IAEA/ WHO Joint Expert Committee to conclude that foods treated to an overall average dose of 10 kGy presented no toxicological hazard (JECFI, 1981).

Detailed wholesomeness studies of radurized Indian mackerel were carried out (Aravindakshan *et al.*, 1978; Chaubey *et al.*, 1978). A 90 day feeding study including single reproduction study in which Wister rats were fed with diet containing irradiated

Table 2. *Influence of irradiation (1.5 kGy) of Indian mackerel on the fish quality*

Parameter studied	Observation	References
Shelf life on ice	Extended up to 28 days, spoilage rate reduced by more than half that of control	Venugopal *et al.*, 1973; 1982
Microflora	Shift in microflora. Reduction in spoilage causing organisms	Alur & Lewis, 1980
Lipids	Rancidity not significant in air packaged fish as judged by sensory evaluation. Triglycerides hydrolysis during storage not affected	Venugopal *et al.*, 1973 Rao & Bandyopadhyay 1982
Physico-chemical properties of proteins	Protein generally stable. No changes in extractability and solubility characteristics, amino acid composition and spectra of myoglobin. However, enhancement in ATPase activity of myosin and tryptic digestibility of sarcoplasmic proteins	Venugopal, 1981
Functional properties of proteins	No change in oil emulsification properties of sarcoplasmic and salt soluble protein fractions. Gel forming capacities of salt soluble proteins not affected	Venugopal & Lewis, 1982
Textural properties of muscle	Retention in sheer forece, water holding capacity and plasticity of muscle of irradiated fish. Drip formation not affected by irradiation	Ghadi & Lewis, 1979
Wholesomeness	Multigeneration feeding studies did not show any abnormal effects in rats	Aravindakshan *et al.*, 1978); Chaubey, *et al.*, 1978

mackerel at 35% level (protein content, 26%) did not show any adverse effects. There was no significant effect on the body weight gain, protein efficiency ratio or fertility. Similarly no changes were noticed in hematological profiles, liver enzyme activities or in pathological data. Mutagenicity studies by Chaubey *et al.* (1978) showed that consumption of irradiated mackerel had no effects on induced dominant lethality in the male germ cells or in the micronuclei of the bone marrow. These results established the wholesomeness of Indian mackerel irradiated at 1.5 kGy.

Table 2 summarises the influence of radiation of the fish on various parameters.

It can be concluded that Indian mackerel exposed to an overall average dose of 1.5 kGy is generally stable against radiation -induced changes while giving the benefit of enhanced refrigerated shelf life.

Irradiation detection

Detection of irradiated foods is essential for proper process control as well as identification of the processed items during marketing. As discussed above, since low dose irradiation does not cause significant changes in fresh fishery products, development of simple and rapid methods for identification of irradiated fish has not been successful. Some of the methods developed recently for this purpose are based on electron spin resonance, radiolytic products etc. (Bradford, 1989). Gore *et al.*, (1982) showed that irradiation resulted in enhanced release of lysosomal enzymes in the press juice of irradiated Bombay duck, pomfret and tilapia, which could be measured to identify the treated fishery items. Alur *et al.*, (1991) suggested that microbial spoilage profiles could be used to identify irradiated fishery products. It was observed that certain spoilage causing microorganisms produced significantly less volatile bases and acids when allowed to grow in fish items including mackerel irradiated at doses of 1 to 5 kGy.

Transportation trials

Investigations on transportation of wet fish varieties including Indian mackerel have been carried out by Chatopadhyay & Bose (1978) with a view to identify the efficacy of different containers, the requirement for ice and the effects of storage on the fish quality. The fish was eviscerated and aerobically packaged in polythene bags (500 gauge). The packages containing 20-25 kg of irradiated (1.5 kGy) mackerel were shipped by rail to Calcutta in polyurethane insulated (20 mm thickness) plywood boxes (60x50x40 cms). The fish to ice ratio was in the range of 1:3 to 1:4. Analysis of the sample at destination employing sensory, chemical and microbiological criteria indicated an acceptable shelf life of four weeks for the transported fish. These studies further showed that if mackerel is iced properly at the processing plant, subsequent re-icing is not necessary during shipment in spite of the ambient conditions (26 to 35°C) of transport, pre-irradiation storage of one to two days does not significantly reduce the marketable quality of irradiated Indian mackerel, and transported irradiated mackerel and the laboratory held counterparts have similar shelf life. It should be emphasised that in the absence of a refrigerated transport system, the irradiated fish should be always maintained in ice to prevent any botulism hazard that might result due to high ambient storage temperatures.

Bulk irradiation

Conventionally, extension of shelf life of fresh fish by radiation treatment stipulates pre-packaging of the fish in pouches in order to prevent bacterial contamination during post- irradiation storage. However, from a commercial point of view, packaging of large amounts of fish at the landing centres has several limitations. These include cost of packaging material and labour, unattractive appearance of certain types of eviscerated fish species in the pounches due to drip and oozing of blood, and possibility of the pouches favouring growth of anaerobic organisms like *C.Botulinum* under reduced oxygen levels. Therefore, irradiation of Indian mackerel under non-packaged condition was investigated with a view to scale up the process for large scale operation (Venugopal *et al.*, 1987). The eviscerated and washed Indian mackerel was held under ice in perforated boxes (30x13x20 cm). The perforated boxes were carried to the irradiator in aluminium containers which collected the melt water from ice during irradiation. After the treatment, the fish boxes were held in a cold room maintained at 0°C. Ice was replenished and the melt water was removed periodically. It was found that the non-packaged irradiated fish had a shelf life of 20 days while the non-packaged unirradiated fish had a shelf life of about 14 days, as judged by sensory evaluation (Venugopal *et al.*, 1987). The extension in shelf life was achieved as a result of radiation dependent reduction of bacterial level on the fish coupled with the washing action of melt water from ice on the surviving flora. The results suggested a beneficial effect of melting ice in conjunction with radiation in enhancing the acceptability of the fish under non-packaged condition. The quality of ice is of utmost importance in this situation in order to minimise bacterial contamination of the fish. It is recommended that flake ice with less than 100 organisms per g (Hobbs, 1983) should be ideal for the purpose. Further, the rate of melting of the ice could be controlled by storing the non- packaged irradiated fish in insulated boxes. These boxes could be carried to destinations in refrigerated truck for commercial distribution of the processed fish.

Economics

Indian mackerel, one of the seasonal fish varieties, fetches good price due to its high consumer appeal. It is, therefore, possible that the commodity can be economically irradiated at processing costs comparatively much less than the cost of the commodity. Krishnamurthy & Bongirwar (1987) have worked out the cost of irradiation with respect to Indian conditions for different food items. According to them, the cost of fresh fish radurization at 2.5 kGy, would amount to 35.4 Indian paise per kg of fish, considering a throughput of 12,000 t per year in a plant having an installed capacity of 500 kCi of Cobalt-60. This figure is comparable to that of Giddings (1984) who extended the 1966 cost-benefit study of Yankelowich to the current price situation. It was observed that irradiation processing of fresh fish was commercially feasible at a cost of 10 cents per kg. According to him, at least 4000 t of fish should be available annually to justify the capital investment.

In India, radiation processing has been cleared for the treatment of frozen seafoods for elimination of pathogens. It is anticipated that multipurpose irradiation plants with attached refrigeration facilities intended to treat frozen fishery products could also be used for the purpose of shelf life extension of Indian mackerel. Installation of such irradiators at the landing centres would help treatment of fresh Indian mackerel so as to provide economically viable operation of the plant. It should be emphasized that adherence to high initial quality of the raw material and good manufacturing practices including stringent temperature control are necessary to make preservation of Indian mackerel by radiation feasible.

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