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Effect of Gamma-irradiation on the Fatty Acid Composition of Salted, Semi-dried Vietnamese Scad and Bombay duck

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Effect of gamma irradiation on the lipid and fatty acid composition of semi-dried Bombay duck (*Harpodon nehereus*) and Vietnamese scad (*Alepes mate*) was studied. In both these dried fishes, $C_{16:0}$ aand $C_{18:1}$ were the predominant fatty acids and the fishes contained significant quantities of the n-3 polyunsaturated fatty acids (PUFAs), $C_{20:5}$ and $C_{22:6}$. Irradiation at a dose of 3 kGy, did not cause any alterations in the total lipid content and the overall lipid class profile in these fishes. However, in the Vietnamese scad, $C_{18:3}$, $C_{20:4}$ and $C_{22:6}$ were significantly decreased in the irradiated samples. In spite of this decrease the irradiated semi-dried scad retained 82% of the initial amount of n-3 PUFAs.

Fish has traditionally been considered as a dietary source of proteins and minerals, especially phosphorous. In recent years, consumption of fish has gained importance also because of its role in preventing cardiovascular disease (Kinsella *et al.*, 1990) due to the presence of significant amounts of the n-3 polyunsaturated fatty acids (n-3 PUFAs) in fish lipids. Preservation and processing of fish to extend its shelf life is therefore important in order to increase its availability.

In many of the developing countries, chilling and freezing facilities are limited and hence traditional methods such as curing are still used to preserve fish. However, fish preserved by such methods and stored at room temperature is susceptible to spoilage by microbes and insects. For example, in Vietnam, 20% of the total fish catch is dried and stored at ambient conditions of which nearly 60% is lost due to microbial contamination and insect infestation (Anon, 1990). In Bangladesh, Indonesia and other countries, the loss of dried fish is between 20-40% (Anon, 1989).

Gamma irradiation of foods at low and moderate doses has been established as an effective method to prevent spoilage in a variety of food products including cereals, vegetables and fish, both fresh and dried (Urbain, 1986). Studies carried out in India (Doke, 1990) and Bangladesh (Ahmed, 1977) have shown that irradiation of dried seafood at doses between 0.1-1.0 kGy completely prevented insect infestation. Thus, Vietnamese salted semi-dried herring when irradiated at 3 kGy could be preserved up to 3 months; whereas irradiation (up to 4 kGy) and use of oxygen-free packaging was found to extend the shelf life of dried fish up to 6 months (Gneran, 1989).

The wholesomeness and nutritional adequacy of irradiated foods including fish has been demonstrated by a number of studies carried out the world over (Farkas, 1987). Earlier studies have shown that

irradiation at 3 kGy did not alter the acceptability of semi-dried fish during prolonged storage (Vinh *et al.*, 1993 a, b). Since fish is an important source of n-3 PUFAs, it is necessary to ascertain the effect of irradiation on the lipid profile of fish. The present study was undertaken to assess the changes in the lipid and polyunsaturated fatty acid composition of dried Bombay duck and Vietnamese scad due to irradiation. Both these fishes are available in plenty during the season and are preserved by drying.

Materials and Methods

Two varieties of fish, namely, Vietnamese scad (*Alepes mate*) and Bombay duck (*Harpodon nehereus*) were used in the study.

For preparing dried scads, fresh scad was purchased one day after landing of the catch in chilled condition. The fish was cleaned, eviscerated, washed thoroughly with tap water and mixed with dry solar salt (Fish : salt ratio, 1:5). After 24 h, the salted fish was transferred to 15% brine and kept under pressure for another 48 h. The fish was then rinsed with 5% brine, drained to remove excess brine and dried in a traditional dryer chamber (Anon, 1989) at 60°C for 6 h daily for 4 days. The final dried product containing 20-24% moisture and 18% salt was packed in polyethylene pouches (0.1 mm thickness) and irradiated at a dose of 3 kGy. Irradiated and unirradiated (control) samples were transported by air to Bhabha Atomic Research Centre, Bombay, India for further analysis.

Semi-dried Bombay duck was purchased from local market in Bombay. The fish was packed in polyethylene pouches (0.2 mm thickness) and irradiated at a dose of 3 kGy. Irradiation was carried out in a Co^{60} package irradiator at Bhaba Atomic Research Centre, Bombay with a dose rate of 0.3 kGy h^{-1}.

Moisture was determined by the air-oven (110°C) method (AACC, 1983).

Extraction of lipids from the dry fish samples was carried out by the procedure of Bligh & Dyer (1959). The fish samples were powdered and 10 g of the powder was blended in a omnimixer with 100 ml chloroform : methanol (2:1, vol./vol.) mixture. The mixture was filtered, the residue re-extracted with 50 ml chloroform : methanol and filtered. The filtrates were mixed, washed twice with Folch's reagent (Folch *et al.*, 1957) and the chloroform phase collected and dried over anhydrous Na_2SO_4. The solvent was then evaporated and the total lipids weighed. Lipids were redissolved in $CHCl_3$ and stored under nitrogen at 0-4°C until further analysis.

For gas chromatographic analysis of the fatty acids, methyl esters of the fatty acids were prepared by transmethylation using dry methanolic HCl (Lepage & Roy, 1986). A aliquot of the lipid extract (equivalent to 200 µg lipids) was introduced into a vial, evaporated to dryness under N_2 and 0.4 ml methanolic HCl was added to it. The vial was sealed and kept at 80°C for 2 h. The methyl esters formed were extracted by adding 0.4 ml hexane and aspirating the upper layer after throughly mixing the contents with hexane. The hexane extraction was repeated twice. The pooled extract was evaporated to dryness, the residue redissolved in 50 µl distilled hexane and was used for the gas chromatographic analysis.

Separation of the methyl esters was achieved by gas chromatography on a Shimadzu GC-7A model using 10% silar 10-C on gas chrome Q (100-200 mesh) glass column (3 mm x 2.5 m) with temperature

programming (Column initial temp. 140°C; final temp. 210°C; rise in temperature 4°C min^{-1}; injection port and detector temperature 230°C). Nitrogen was used as the carrier gas with a Flame Ionization Detector. Identification of fatty acid peaks was done by comparing the retention times of methyl esters in a standard mixture (Sigma Chemical Co., USA, 1993 Catalogue, No. ME-14 and ME-19). Percentage distribution of fatty acid was calculated by peak area distribution obtained using a varian integrator. The fatty acid composition was also expressed as mg fatty acid per 100 g dry fish. Polyene index was calculated as the ratio $(C_{20:5} + C_{22:6}) / C_{16:0}$.

Statistical differences between the control and irradiated samples were calculated using the student's *t* test (Snedecor & Cochran, 1976).

Results and Discussion

Table 1 shows the results on moisture and total lipid content of the unirradiated and irradiated fish. The dried fish samples contained about 19% moisture. The high moisture content was probably one of the reasons for the observed mould growth in the unirradiated samples. No mould growth was observed in the irradiated samples. Lipid content of the Vietnamese scad was about 2.7% while Bombay duck showed slightly higher content of lipids (3.2%). Irradiation did not cause any changes in either the moisture or total lipid content of both fishes. Thin layer chromatographic analysis of the lipids further showed that irradiation did not alter the polar and non-polar lipid composition of both the fishes (data not given).

Table 2 gives the data on the percentage composition of fatty acids of Vietnamese scad and Bombay duck. The major fatty acids present in semi-dried scad were $C_{16:0}$, $C_{18:1}$ and $C_{22:6}$ whereas those in the Bombay duck were $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{22:6}$. It must be pointed out that both the fishes contained significant amounts of the n-3 PUFAs, $C_{20:5}$ and $C_{22:6}$, indicating that the drying process left substantial amounts of these fatty acids in both the fish varieties. These results are in agreement with those reported earlier for these and other fishes (Thomas *et al.*, 1987; Ackman & McLead, 1988; Manif *et al.*, 1990).

Table 1. *Moisture and total lipid content of dried fish in g $100g^{-1}$*

	Dried scad		Dried Bombay duck	
	Control	Irradiated	Control	Irradiated
Moisture	19.79±0.36	19.08±0.79	19.17±0.76	19.42±0.52
Total lipid	2.72±1.41	2.69±0.78	3.21±1.36	3.78±0.89

Values are averages of 4 independent determinations ±S.E.

Total 2. *Fatty acid composition for dried fish (All values are expressed as percent of total peak area)*

Fatty acid	Vietnamese scad		Bombay duck	
	Control	Irradiated	Control	Irradiated
$C_{14:0}$	4.84	3.97	4.42	4.16
$C_{16:0}$	27.49	28.18	29.85	30.82
$C_{16:1}$	9.93	10.74	11.52	11.77
$C_{18:0}$	10.84	12.20	8.69	9.41
$C_{18:1}$	18.75	22.73	16.24	18.99
$C_{18:2}$	1.62	1.15	1.17	1.12
$C_{18:3}$	2.37	1.28	1.39	1.07
$C_{20:4}$	3.48	1.90	4.24	4.54
$C_{20:5}$	3.22	3.46	5.47	4.89
$C_{22:6}$	10.65	8.68	11.39	10.20
Unidentified	8.96	7.30	7.24	6.36

The significance of a fish as a source of n-3 PUFAs would depend upon how much quantity of the fatty acids will be available to the consumer upon consumption of reasonable amount (e.g. 100 g) of the fish.

Table 3. *Effect of irradiation on the content of fatty acids in semi-dried fish (in mg 100 g^{-1})*

Fatty acid	Vietnamese scad		Bombay duck	
	Control	Irradiated	Control	Irradiated
$C_{14:0}$	131.6±2.72	108.8±2.44	141.9±3.58	157.2±4.47
$C_{16:0}$	747.7±17.09	766.4±7.86	958.2±40.90	1165.0±48.61
$C_{16:1}$	270.1±15.80	292.1±9.47	369.8±5.59	444.9±7.50
$C_{18:0}$	294.8±4.56	331.8±4.78	279.0±19.09	355.7±40.12
$C_{18:1}$	510.0±9.73	618.3±7.32	521.3±38.47	717.8±63.73
$C_{18:2}$	44.1±6.09	31.3±4.80	37.6±10.91	12.3±6.02
$C_{18:3}$	64.4±3.06	34.8±2.63*	44.6±9.18	40.4±5.09
$C_{20:4}$	94.6±6.68	51.7±1.86*	136.1±3.17	171.6±20.41
$C_{20:5}$	87.5±2.61	94.1±3.08	175.6 7.94	184.8±10.69
$C_{22:6}$	289.7±6.62	236.1±5.47*	365.6±15.22	385.6±28.08
Polyene index	0.50	0.43	0.56	0.49

Values are average of 6 independent determinations; *Significant at $p < 0.001$

Table 3 shows the content of various fatty acids in Vietnamese scad and Bombay duck, expressed as mg 100g^{-1} dried fish. Irradiation did not cause any significant alteration in the overall fatty acid pattern of these fishes except that in Vietnamese scad, the contents of $C_{18:3}$, $C_{20:4}$ and $C_{22:6}$ were significantly ($p < 0.001$) reduced in the irradiated samples. Such decreases were not observed in irradiated Bombay duck samples.

Both Vietnamese scad and Bombay duck after drying, were good sources of the n-3 PUFAs, $C_{20:5}$ and $C_{22:6}$. Irradiation did not cause any alterations in the content of $C_{20:5}$ in both the fishes (Table 3). This differential response of the two products to irradiation treatment cannot be explained; presumably the difference in the processing parameters used to prepare the dried fish may be responsible for it. However, in spite of the reduction in $C_{22:6}$ content in irradiated Vietnamese scad, it still retained a substantial amount of this fatty acid.

The polyene index, which is indicative of the content of $C_{20:5}$ and $C_{22:6}$ in comparison with the content of $C_{16:0}$ which is the predominant fatty acid, was also not altered by irradiation. The index value varied between 0.43 for irradiated scad to 0.56 for control Bombay duck, thus showing the high relative content of the n-3 PUFAs in both the fishes.

Studies from this laboratory have already shown that irradiation of dried Bombay duck and Vietnamese scad at 3 kGy is effective in preventing insect infestation and mould growth and extending shelf life (Vinh *et al.*, 1993 a, b). The results in this study now show that the use of irradiation to preserve semi-dried Vietnamese scad and Bombay duck does not affect the total lipid content and the fatty acid profile in general. Except for the reduction in $C_{22:6}$ content of Vietnamese scad, the n-3 PUFA content was also not altered by irradiation. These results show that irradiated semi-dried Vietnamese scad and Bombay duck can still form a good source of the two n-3 polyunsaturated fatty acids, namely, $C_{20:5}$ and $C_{22:6}$, useful for their therapeutic value in the prevention and treatment of cardiovascular disease.

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