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A Biochemical Method to Identify Irradiated Semi-dried Anchovies *Stolephorus commersonii*

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Gamma-irradiated (5kGy) semi-dried Anchovies (*Stolephorus commersonii*) along with unirradiated samples stored at room temperature for a period of 2 and 5 months were subjected to microbiological analysis to examine spoilage indices such as total volatile acids (TVA) and total volatile base nitrogen (TVBN) after inoculation of the fish homogenates with natural microflora, *Aeromonas hydrophila, Bacillus megaterium, Pseudomonas marinoglutinosa* and *Salmonella typhimurium*. The incubation period for the determination of TVA and TVBN was 18 h at 37°C. In comparison to control fish, irradiated fish showed consistently lower TVBN and TVA values. These results suggested that determination of TVBN and TVA values could serve as an index to identify irradiated and unirradiated semi-dried anchovies.

Fish is a highly perishable commodity and since it is available in abundance only during a peak season, there is a need to preserve them. Drying is one of the most prevalent methods of preserving fish. Non availability of proper infrastructure for drying in some tropical countries force them to adopt sun-drying. Owing to the environmental contamination prevailing during sun-drying, the final product is of poor microbial quality and has less storage stability. Therefore, alternative methods of preservation of fish have been developed. In our laboratory dehydro-irradiation process has been standardized for Bombay duck (*Harpodon nehereus*) (Doke *et al.*, 1978). Shrimps (*Penaeus indicus*) (Gore *et al.*, 1970) and white pomfret (*Pampus argenteus*) (Agarwal *et al.*, 1972) using combination of heat and gamma irradiation to stabilize seafoods at ambient temperature.

The method of gamma irradiation preservation could be applied to semi-dried fish to control microbial contamination as well as disinfestation by exposing them to 1 to 2 kGy of gamma radiation (Doke, 1990; Brinjolfsson, 1986; Farkas, 1989). However, for successful trade of irradiated semi-dried product, there is a need to develop a simple method to distinguish irradiated fish from control. Of late, international bodies have urged the governments to consider development of suitable method to detect irradiation to facilitate free trade of irradiated products among countries. Several laboratories in the world are engaged in developing a method to detect irradiated products; by ESR signals (Desrosiers *et al.*, 1990), microbiological enumeration of *Moraxella* in irradiated fish (Van Spreckens & Toepoel, 1978) and detection of free radicals in irradiated spice by ESR method (Dodd *et al.*, 1988, 1989).

In the present communication, we have attempted to standardise an identification method for irradiated semi-dried anchovies based on the measurement of volatile acids (TVA) and volatile bases (TVBN) (Alur *et al.*, 1991, 1992).

Materials and Methods

Semi-dried non-irradiated anchovies *Stolephorus commersonii*) were obtained from local market. Half of the samples were exposed to gamma - radiation dose of 5 kGy in a Co^{60} package irradiator (dose rate, 0.05 kGy/min) at ambient temperature. Both non

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- irradiated and irradiated samples were stored at ambient temperature (26°C) for 2 and 5 months in polyethylene sealed bags (200 gauge).

Spoilage organisms namely, *Aeromonas hydrophila, Bacillus megaterium* and *Pseudomonas marinoglutinosa* were isolated from Indian mackerel (*Rastrelliger kanagurta*) and identified in our laboratory, while *Salmonella typhimurium* was obtained from Haffkine Institute, Bombay, India.

Twentyfive g of non-irradiated or irradiated (5kGy) dried anchovies were placed in sterile 250 ml conical flask. An overnight culture grown in nutrient broth (Difco) of the above mentioned organisms was serially diluted in sterile saline and the appropriately diluted cell suspensions were transferred to flask containing the fish so as to give a final cell concentration of 10^5/g. Inoculated samples were incubated at 37°C overnight. To 25 g of samples 225 ml of distilled water was added and blended using Sorval Omnimixer for 2 min (5,000 rpm). The homogenates (10%) thus obtained were used to determine TVA and TVBN.

To 30 ml homogenate, 5 ml sulphuric acid (1 N) and 5 ml phospho-tungstic acid (15%) were added. 5 ml filtrate obtained after filtering through Whatman No. 1 filter paper was steam distilled and the distillate (30 ml) was titrated against 0.01 N Sodium hydroxide using 0.1% phenolphthalein as indicator as described by Venugopal *et al.*, (1981).

To 10 ml homogenate, an equal volume of trichloroacetic acid (10%) was added and filtered through Whatman No. 1 filter paper. 1 ml of TCA filtrate was used to determine TVBN by the Conway microdiffusion technique (Farber & Ferrow, 1956).

Results and Discussion

Pattern of the formation of TVA and TVBN in non-irradiated and irradiated (5 kGy) semi-dried anchovies stored for 2 months is depicted in Fig. 1. An incubation period of 18 h was selected as it was observed to be the optimum period required to produce maximum amount of TVA and TVBN (Alur *et al.*, 1991). It may be noted that amount of TVBN produced by inoculated organisms namely, *A. hydrophila, B. megaterium, P. marinoglutinosa, S. typhimurium* and natural microflora of anchovies after 18 h incubation at 37°C ranged from 550 to 900 mg% in controls. Spoilage organisms did not exhibit any significant difference in their ability to produce volatile bases. On the other hand, irradiated semi-dried anchovies after storage at ambient temperature for two months produced less than 100 mg% TVBN under identical condition. Thus, it is clear that there was a 5-fold decrease in the formation of volatile bases in irradiated semi-dried anchovies in comparison to unirradiated counterparts.

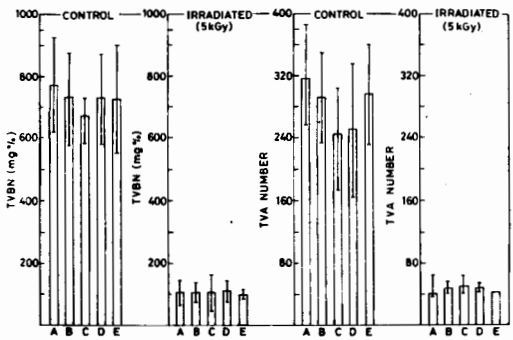

Fig. 1. Formation of volatile acids (TVA) and volatile bases (TVBN) in non irradiated and irradiated (5 kGy), ambient temperature stored (2 months) semi-dried anchovies inoculated with A. Natural microflora of Anchovies; B. *A. hydrophila;* C. *B. megaterium;* D. *P. marinoglutinosa;* and E. *S. typhimurium;* followed by incubation at 37°C for 18 h.

Fig. 1 also depicts the pattern of TVA formed in non-irradiated and irradiated semi-dried anchovies after incubation at 37°C for 18 h with the spoilage organisms. It can be seen that irradiated anchovies showed a TVA value of 40 in comparison to the value of 160-240 observed in control

sample. Apart from that, irradiated anchovies were found to be less susceptible to bacterial spoilage and also showed no significant differences among samples while non-irradiated samples exhibited differing amounts of TVA depending upon the type of organism inoculated. Thus, *B. megaterium* and *P. marinoglutinosa* produced 160-300 TVA number, while natural microflora and other bacteria produced 240-300 TVA number. Thus, there seemed to be some difference among bacteria in their ability to produce volatile acids although they exhibited no differences in producing volatile bases.

Volatile acids and bases content of stored (5 months at ambient temperature) semi-dried anchovies inoculated with spoilage microflora are shown in Fig. 2. Prolonged storage resulted in an additional increase in TVBN formation by 100 mg% in non-irradiated semi-dried fish irrespective of the type of organisms inoculated. Similarly amounts of TVBN were also doubled (200 mg%) in 5 month stored irradiated semi-dried anchovies as against 2 month old samples. However, it was of interest to observe that when incubated with spoilage organisms amounts of TVA did not change in both non-irradiated and irradiated samples after prolonged storage (5 months) in comparison to 2 month stored samples.

Food irradiation is an emerging technology which is employed to reduce the growth of spoilage organisms, thus extending the shelf life of flesh foods (Urbain, 1982), to decontaminate flesh foods (Alur & Lewis, 1984, Kamat *et al.*, 1991; Nerkar & Bandekar, 1990) and to disinfest dry fish (WHO, 1981). Although IAEA/FAO/WHO have cleared irradiated foods for human consumption as early as in 1981, there is considerable delay in implementing food irradiation commercially as there is no fool-proof method to distinguish irradiated food from unirradiated ones. Thus, there is need to develop a simple and rapid method to identify irradiated foods. Some attempts have been made to use ortho-tyrosine method to detect irradiated chicken meat but without success (Karam & Simic, 1986), because ortho-tyrosine has been reported to be present in unirradiated samples also. The method based on ESR signals produced in irradiated flesh foods requires not only sophisticated and expensive instrumentation but also the presence of bone in meat which gives the signals (Dodd *et al*, 1989; Desrosiers, 1989). Microbiological method based on enumeration of *Moraxella* in irradiated fish and shrimps required a lengthy incubation period of 4-5 days (Van Sprecken & Toepoel, 1978).

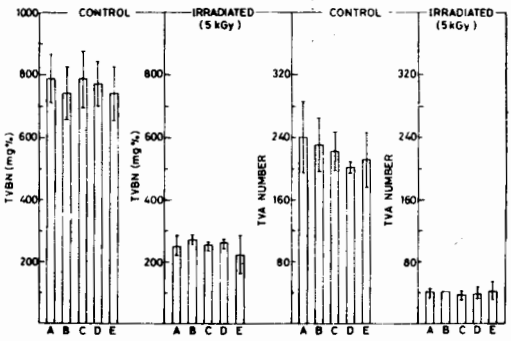

Fig. 2. Formation of volatile acids (TVA) and volatile bases (TVBN) in non-irradiated and irradiated (5 kGy), ambient temperature stored (5 months) semi-dried Anchovies inoculated with A. Natural microflora of Anchovies; B. *A. hydrophila;* C. *B. megaterium;* D. *P. marinoglutinosa;* and E. *S. typhimurium* followed by incubation at 37°C for 18 h.

Recently, in our laboratory we have developed a simple microbiological method based on differential spoilage profiles of bacteria in producing TVA and TVBN in irradiated and non-irradiated fresh fish and meat (Alur *et al.*, 1991; 1992). The method is unaffected by moisture content and initial bacterial load of the sample (*Ibid.*). It is also not affected by presence of preservatives (sorbate or propionate) in the dried fish or even when a lower dose of 1 kGy is used (data not presented).

The present investigation advocates for the first time a simple method to distinguish irradiated semi-dried fish having a shelf life

of 5 months at room temperature based on their lower susceptibility to bacterial spoilage. Formation of volatile acids and bases is reduced by 4-fold in irradiated samples in comparison to non-irradiated counterparts.

The rapid method described above shows that lower values of TVBN and TVA in irradiated dried fish in comparison to control can be taken as an index for the identification of irradiated dried ancovies.

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