Suppression of Spoilage Rates of Bombay Duck (*Harpodon nehereus*) Homogenates by Gamma Radiation

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The spoilage rates of unirradiated and irradiated (0.1 Mrad) Bombay duck (*Harpodon nehereus*) homogenates stored at 0°C, 10°C and 30°C in terms of organoleptic score (OS) total bacterial count (TBC), trimethylamine content (TMA), and total volatile basic nitrogen (TVBN) followed a linear response with increase in temperature. Unirradiated Bombay duck stored at 0°C were found to spoil faster than those irradiated at 0.1 Mrad; the spoilage rates derived for TBC, TMA, TVBN and OS were 0.42, 0.67, 1.93 and -0.86 respectively for unirradiated samples as compared with corresponding values of 0.31, 0.55, 1.19 and -0.19 for the irradiated samples. The spoilage rates of unirradiated Bombay duck stored at 10°C and 30°C measured as a function of OS, TMA and TVBN were consistently higher than their irradiated (0.1 Mrad) counterparts. In the case of irradiated samples, TBC was not a satisfactory index of spoilage.

The two important parameters in the radurization process are the radiation dose administered¹⁻³⁾ and the temperature during storage of irradiated sea-foods.⁴⁾ The main disadvantages of using high radurization doses are (i) spoilage microflora are inactivated¹⁾ thereby increasing the probability that such processed food could harbour toxigenic *Clostridium botulinum* since there would be less inhibitory influence on their growth, and (ii) loss of organoleptic attributes like color, flavor and texture. Current research is therefore aimed at using lower doses (0.1 Mrad ~0.2 Mrad) for limited extension in shelf-life of sea-foods, which treatment will not significantly alter the spoilage pattern from that of unirradiated samples.

Irradiation thus offers tremendous scope for transportation of radurized fish from the processing plant to distant areas, provided the temperature during transport is maintained between 0°C~3°C for control of *Cl. botulinum*.^{2,5)} In view of the stipulated temperature requirement, it is important to define a suitable scale in terms of freshness indices to determine fluctuations in temperature of the sea-foods which may occur during transporta-

tion.

Spencer and Baines⁶⁾ have formulated a mathematical correlation between spoilage rates and storage temperature of fish. The validity of this formula for irradiated Bombay duck (*Harpodon nehereus*) has been reported.⁷⁾

This paper provides detailed data for the recommendation of the formula of Spencer and Baines⁶⁾ to be applicable to low dose irradiated sea-foods.

MATERIALS AND METHODS

Fresh Bombay duck obtained from the local market were washed, eviscerated and filleted. 10 g samples were homogenised for 2 min with 90 ml sterile normal saline in omni-mixer cups. The fish homogenate was aseptically transferred in 10 ml portions into sterile test tubes, which were immediately exposed to gamma radiation at a dose level of 0.1 Mrad.

Irradiation. Irradiation was carried out in a Co⁶⁰-gamma cell 220 (Atomic Energy Canada Ltd.) at a dose rate of 11,100 rads/min. Unirradiated samples served as controls. For calculation of spoilage rates both the controls and irradiated samples were held at 3 different storage temperatures viz. 0°, 10° and 30°C. Representative samples were analysed for organoleptic, bacteriological and biochemical tests, at pre-determined intervals.

Organoleptic evaluation. Sensory scoring was done according to the Miyauchi *et al.*,⁸⁾ reference scale which is based upon changes in odors.

Chemical analysis. TMA and TVBN were determined in TCA extracts according to the methods adopted previously for Bombay duck.⁹⁾

Bacteriological examination. The total aerobic bacterial count (TBC) was assessed by the pour plate method using Tryptone-Glucose-Yeast-Extract agar (TGY agar). Appropriate dilutions were plated in triplicate. Plates were incubated for 48 hr at 27~28°C.

Spoilage units. Spoilage rates of the samples in terms of TBC, TVBN and organoleptic ratings were determined by the least square method. TMA content in fish muscle was converted into a TMA-index, defined as $10 \times \log (1 + \text{TMA content})$, for determining the spoilage units. 'C', the linear temperature response of unirradiated and irradiated homogenates was derived from the spoilage rates using Spencer and Baines' formula: $C = (U - V) / (V \times \theta)$, where C = linear temperature response; U = spoilage rate at $\theta^\circ\text{C}$; V = spoilage rate at 0°C and θ = specific storage temperature.

RESULTS AND DISCUSSION

Storage properties of unirradiated homogenate

Unirradiated samples incubated at 30°C showed precipitous decline in organoleptic acceptability. From the nature and intensity of putrid odors, a score of 5 was assigned to samples incubating for 7~8 hr (Fig. 1). However, samples similarly stored at 10° and 0°C showed signs of spoilage only after 5 and 7 days of storage respectively (Figs. 2 and 3). The storage temperature also influenced the nature of spoilage odors of the homogenates at the terminal spoilage. Thus, recognisable putrid odors were encountered at the terminal spoilage in the homogenates stored either at 10° or 30°C , as compared with samples stored at 0°C which developed unacceptable sweetish odors.

An inverse relationship is observed between rise in TBC, TMA, TVBN and fall in organoleptic scores during storage of the unirradiated homogenates. Total bacterial counts of $10^8 \sim 10^9/\text{g}$ accompanied by large TVBN values of 100 mg N% and 20 mg N% at 30°C and 10°C respectively are concurrent

FIG. 1. Status of Freshness Indices in Unirradiated and Irradiated Bombay Duck Homogenates (incubation temperature 30°C).

FIG. 2. Status of Freshness Indices in Bombay Duck Homogenates (storage temperature 10°C).

with the rapid decline in the organoleptic acceptability of the homogenates.

FIG. 3. Status of Freshness Indices in Unirradiated and Irradiated Bombay Duck Homogenates (storage temperature 0°C).

The extension in keeping quality of samples stored at low temperature also reflects on the trend of the quality indices. Thus, samples stored at 30°C show high values for TBC (10^8 /g), TMA (6 mg N%) and TVBN (100 mg N%) within 24 hr of storage. However, homogenates stored at 10°C reach these levels only after 5~7 days. Suppression in the rate of formation of TMA and TVBN is noteworthy in the samples stored at 0°C. Low values of TMA (2 mg N%) and TVBN (20 mg N%) were observed on the 7th day of storage

when the samples were rated organoleptically unacceptable.

Storage properties of irradiated (0.1 Mrad) homogenates

An irradiation dose of 0.1 Mrad was found to extend the storage life of the homogenates; irradiated samples were in acceptable condition for 30 hr, and for 10 and 19 days storage at 30°C, 10° and 0°C (Figs. 1, 2 and 3) respectively.

Radiation dose of 0.1 Mrad brought about a 70% reduction in TBC. During subsequent storage, however, there was a rapid increase in TBC of samples stored both at 10° and 30°C. As seen in Fig. 3, samples stored at 0°C were characterised by a prolonged lag phase of about 14 days. It was interesting to note that the total bacterial counts of 10^8 /g or more in the irradiated samples did not indicate spoilage, since characteristic putrid odors were absent in these samples.

Formation of TVBN was suppressed initially by irradiation; TVBN contents of samples stored at 30°C and 10°C for 30 hr and 14 days respectively did not exceed 50 mg N%. Likewise, TMA formation was suppressed by irradiation, the TMA contents of samples stored for 30 hr at 30°C being 2 mg N%.

Spoilage rate in terms of organoleptic score and TBC for Bombay duck homogenates stored at 10°C

As seen in Fig. 4, an inverse relationship is

FIG. 4. Retardation in Spoilage Rates in Irradiated Bombay Duck Homogenates at 10°C-TBC and OS.

FIG. 5. Retardation in Spoilage Rates in Irradiated Bombay Duck Homogenates at 0°, 10° and 30°C.

found to exist between TBC and OS. In the unirradiated samples, the rate of decline in acceptability (OS) is much more rapid than for irradiated samples as indicated by the steeper slope. Although irradiated samples had high TBC values, similar to those of the unirradiated samples, they were still acceptable up to 14 days storage at 10°C as compared with the unirradiated samples which were unacceptable after 4 days storage. These findings indicate that in the case of irradiated samples, TBC values are a poor index of spoilage. Thus a 4-fold reduction in spoilage units in terms of OS of irradiated samples indicates corresponding extension in the shelf

life over the unirradiated controls.

Straight line trends for TVBN in Bombay duck homogenates during storage at 0°C, 10° and 30°C

The data shown in Fig. 5 establishes that the rate of formation of TVBN varies directly with the temperature of storage as observed from the slopes of the trends. The results also show that radiation suppressed the rate of formation of TVBN, and the temperature response to a certain extent for irradiated samples stored at 0°, 10° and 30°C. This is evident from the increasing values of slope viz., 1.19, 2.48 and 19.68 respectively at these temperatures.

Spoilage units for chemical, microbiological and organoleptic indices

Table I indicates that spoilage rates for sensory scores of all the samples are negative, concurrent with decrease in organoleptic acceptability of the samples during storage. Spoilage rates expressed in terms of units/day increase with rise in temperature. Spoilage rates calculated for TBC at 30° and 10°C, are respectively 13-fold and 2-fold as compared with samples stored at 0°C. TMA and TVBN formation also showed similar trends in samples stored at 30°C and 10°C as compared with a storage temperature of 0°C.

Spoilage rates in terms of organoleptic score are reduced by 3~4 fold in irradiated samples. The spoilage units of TVBN and

TABLE I. SPOILAGE RATES OF UNIRRADIATED AND IRRADIATED BOMBAY DUCK HOMOGENATES

Spoilage units/day were calculated from the storage data on TBC, TVBN, TMA index and OS for unirradiated and irradiated (0.1 Mrad) Bombay duck using the equation $U = V(1 + C\theta)$, where U = spoilage units/day at $\theta^\circ\text{C}$, V = spoilage units/day at 0°C , θ = Temp. $^\circ\text{C}$; and C = linear temperature response.

Treatment	Temperature	Spoilage units/day			
		OS	TMA-index	TVBN	Log bact. nos.
Unirradiated	0°C	-0.86	0.67	1.93	0.42
Irradiated (0.1 Mrad)	0°C	-0.19	0.55	1.19	0.31
Unirradiated	10°C	-1.68	2.00	8.10	0.71
Irradiated (0.1 Mrad)	10°C	-0.37	0.58	2.48	0.35
Unirradiated	30°C	-9.12	7.20	93.60	5.42
Irradiated (0.1 Mrad)	30°C	-2.4	0.96	19.68	6.20

TMA showed 4~5 fold decrease in irradiated samples stored at 0° and 10°C. However, such significant differences in the rates of formation of TVBN and TMA were not observed between irradiated and control samples held at 0°C.

Derivation of linear temperature response

As seen in Table II, 'C' values for TMA, TVBN and TBC for irradiated homogenates are found to be 3.5 times lower than corresponding values for unirradiated samples. 'C' values when calculated from the differences for 0°C~30°C, are much higher than for 0°C~10°C, thereby indicating the abrupt increase in the spoilage rate at higher temperatures of storage.

An inverse relationship between fall in organoleptic score and rise in TBC and chemical indices, appears to be the general pattern for unirradiated fishery products stored at melting ice temperature. It has been consistently reported^{9~11)} that irradiation alters this relationship. Thus despite high TBC values, irradiated samples show low TVBN content and are organoleptically acceptable indicating that microorganisms which produce TVBN and putrid odors may have been suppressed. Radiation-induced alterations in microbial patterns have been reported for different varieties of sea-foods.^{12~15)}

Spoilage rates

Spencer and Baines⁶⁾ reported that the rate of increase of log TBC for Hull and Aberdeen fish stored at 0°C ranged from 0.10~0.25 and 0.16~0.23 respectively. In Bombay duck, bacterial growth occurs more rapidly, the daily

rate of increase being 0.42 log/day. The rapid rise in TBC also reflects on the organoleptic properties of Bombay duck. Thus, the spoilage rate for Bombay duck in terms of organoleptic score is 0.86, in contrast to spoilage units of 0.23~0.30 observed for Hull and Aberdeen fish. These differences in the spoilage rates may be ascribed to the differences in chemical composition and types of bacteria of the fishery products. *Pseudomonas* which is considered to be one of the predominant spoilage groups in fishery products¹⁰⁾ could not be detected in unirradiated spoiling Bombay duck.^{12,17)}

Spoilage rates for irradiated Bombay duck are comparatively lower than those of unirradiated samples. Retardation in spoilage rates of irradiated samples may be attributed to survival of relatively weak spoilers.^{10,18)} It is also significant to note that the linear temperature response is reduced for 0° to 10°C as compared with 0° to 30°C, demonstrating that spoilage rates are essentially temperature dependent. These findings clearly indicate that the storage properties of fishery products are primarily governed by the physiological requirements of bacteria and by metabolic variations in the predominant organisms.¹⁹⁾ It has been recommended²⁰⁾ that temperatures in the range of 0°~3°C should be adopted for preventing outgrowth of pathogenic *Clostridium* organisms. At this temperature range, Spencer and Baines equation and a sensory score of 5 taken as a critical level of spoilage, permits calculation of storage life of radurized Bombay duck as 26 days as against 6 days for unirradiated samples similarly stored.⁷⁾

TABLE II. LINEAR TEMPERATURE RESPONSE (c) FOR IRRADIATED BOMBAY DUCK HOMOGENATES

Linear temperature response (c) was calculated for temperature ranges of 0°C~10°C and 0°C~30°C for TBC, OS, TMA-index and TVBN using the equation given in Table I.

Treatment	Temperature range	Organoleptic scores	Bact. log nos.	TMA-index	TVBN mg %
Unirradiated	0°C~10°C	0.30	0.05	0.20	0.32
Irradiated (0.1 Mrad)		0.29	0.01	0.05	0.11
Unirradiated	0°C~30°C	0.38	0.40	0.32	1.6
Irradiated (0.1 Mrad)		0.36	0.63	0.03	0.55

Hence, any temperature fluctuation resulting from abuse of radurized products during commercial transport, would give higher spoilage rates and thus would indicate that these samples may not qualify for consumer distribution.

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