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Variations in the Microbial Log Reduction Curves of Irradiated Cod Fillets, Shrimp, and Their Respective Homogenates

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When cod (*Gadus morhua morhua*) and headless white shrimp (*Penaeus setiferus*) were gamma irradiated with a series of low-ionizing radiation doses, a "shoulder(s)" was observed in the graph (log microbial counts versus dose) in the approximate range of 25 to 75 krads. When the microbiological survivors were differentiated into total counts, proteolytic and pseudomonad-type bacteria, it was observed that the pseudomonad-type bacteria were rapidly destroyed by 25 krads and that proteolytic bacteria were destroyed at a faster rate than the rest of the microorganisms. When cod fillets and shrimp were compared with their respective homogenates and irradiated at doses of 0, 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, and 300 krads, the homogenates did not exhibit the characteristic shoulders. A further experiment was designed to test surface versus uniform dispersion of microorganisms on/in gelatin disks subjected to low doses of irradiation. Differences were found that may explain the observed differences between solid food materials such as fish fillets and shrimp and their homogenates.

At the National Oceanic and Atmospheric Administration's National Marine Fisheries Service laboratory facility in Gloucester, Mass., a Marine Products Development Irradiator, a prototype of a commercial irradiator, has been use to explore the use of low doses of ionizing radiation of fishery products with gamma (^{60}Co source) radiation (4, 5, 8, 9). The purpose of low doses of ionizing radiation would be to reduce potential spoilage microorganisms and thus extend the shelf life of fresh market fish. During preliminary studies, when doses of 0, 100, 200, 300, etc., krads of gamma radiation were used, smooth, descending, parabolic curves were observed when the log numbers of the surviving viable microorganisms were plotted (1.7). These will henceforth be referred to as microbial log reduction curves.

When 50-krad doses of gamma radiation were introduced into these studies, a slight leveling or "shoulder" was observed in the otherwise smooth microbial log reduction curve. When 25-, 50-, and 75-krad doses were introduced into these studies, a pronounced shoulder was observed in the 25- to 75-krad dose range (3; J. H. Green and J. D. Kaylor, Bacteriol. Proc., A80, 1969). It was postulated that different groups of microorganisms might be surviving at different rates as expressed by the apparent shoulder. Further studies were performed in which the surviving microbes were differentiated into total, proteolytic, and pseudomonad-type microorganisms. It was observed that the pseudomonad-type microorganisms disappear at a faster rate than do other constituents of the microflora (3; Green and Kaylor, Bacteriol. Proc. A80, 1969).

This report is on a more detailed study, in which a greater number of different gamma radiation doses in the 0- to 100-krad range were used on headless white shrimp (*Penaeus setiferus*), pieces of cod (*Gadus morhua morhua*) fillets, and cod or shrimp homogenates. In addition, we used gelatin disks (petri plates) with diluted cod slime containing microbes smeared either on one disk surface of the gelatin or dispersed throughout the gelatin disk to explore the shoulder phenomenon. The data shown in the figures are of single experiments, but they are typical of several replicate experiments and are presented merely to demonstrate the shoulder effect.

MATERIALS AND METHODS

Fish preparations. (i) Cod fillets and homogenates. Fresh, medium-sized cod (less than 18 h out of the water) that had been landed in the Gloucester,

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Mass., fishing port were prepared into skinless fillets. The thin tail sections of the fillets were discarded, the remaining thicker sections of the fillets were cut into approximately 1- by 3-inch (2.54- by 7.62-cm) pieces, which were randomly distributed into plastic pouches (Scotchpack, 3M Co.) containing four to five fillet pieces, and the pouches were sealed.

From 1.0 to 1.5 liters of a cod fillet homogenate, 1:10 (wt/vol) dilution, was prepared in a Waring blender, and 40-ml portions were placed into plastic pouches and sealed. Sanitary techniques were used in preparing these samples and homogenates to eliminate introduction of other microbes.

Sealed pouches were held on ice (0°C) until irradiated. After irradiation experiments, the cod fillet samples were prepared into a homogenate, 1:10 (wt/vol) dilution, prior to microbiological assay procedures.

(ii) Shrimp and homogenates. Fresh, iced headed white shrimp shipped from Florida and less than 4 days out of the water were used in these experiments. From four to six shrimp, depending upon size, were placed in plastic pouches and sealed. From 1.0 to 1.6 liters of a shrimp homogenate, 1:10 (wt/vol) dilution, was aseptically prepared in a blender, and 40-ml portions were aseptically placed into plastic pouches and sealed.

Samples were kept on ice (0°C) until irradiated. After irradiation experiments, the shrimp samples were prepared into a homogenate, 1:10 (wt/vol) dilution, prior to microbiological assay procedures.

(iii) Dilute cod slime inoculum. Slime from the surface of unskinned whole cod was aseptically scraped off into a tared sterile petri dish and weighed, and sterile water was added to form a 1:5 (wt/vol) dilution for ease in pipetting. This inoculum preparation was used in the gelatin disk experiments described below.

Gelatin streak/pour plates. Melted, sterilized gelatin (BBL) medium, 15%, was prepared and maintained at 32°C (water bath) until used. About half of the gelatin was prepared into disks by pouring into blank, sterile petri plates and allowed to solidify at 4°C (refrigerator) for 1 to 2 h. These gelatin disks were aseptically streaked over the entire exposed surface with 0.1 ml of dilute cod slime inoculum by use of bent glass rods.

To an equal number of blank sterile petri plates, 0.1 ml of dilute cod slime inoculum was pipetted in, and melted gelatin was poured into these plates and well mixed by gentle swirling.

These gelatin streak/pour plates were refrigerated (0°C) until ready for use in the experiment described below. After irradiation, microorganisms were recovered from these gelatin plates by floating the petri plates in a water bath (32°C) for about 10 to 15 min until the gelatin disks melted. The melted gelatin was gently mixed with a sterile glass rod and microbiologically assayed.

Microbiological assay procedures. (i) Total plate counts. Total plate counts were prepared from decimal dilutions of the homogenates or melted gelatin disks by the pour plate technique, using Eugon agar (BBL) with NaCl (0.5%) and yeast extract (0.5%; Difco) added to the medium. The pour plates were incubated for 5 days at 20°C prior to counting.

(ii) Pseudomonad plate counts. Pseudomonad plate counts were prepared from the same decimal dilutions used for total plate counts by the pour plate technique, using Pseudosel agar (BBL) as the selective medium. The pour plates were incubated for 5 days at 20°C prior to counting. Any colony growth was interpreted as pseudomonad-like organisms.

(iii) Proteolytic plate counts. Proteolytic plate counts were done in the following manner, based on the lysis of gelatin. A 0.1-ml portion of a sample dilution was streaked onto the surface of a Eugon agar petri plate using a sterile bent glass rod. The surface was then overlaid with 2.5 ml (premeasured in tubes) of the following sterile, melted aqueous medium: gelatin (BBL), 10%; Eugon broth (BBL), 3%; NaCl, 0.5%; and yeast extract, 0.5%. The gelatin-overlaid petri plates were refrigerated (4°C) for 1 to 2 h to solidify the gelatin and then incubated for 5 days at 20°C. At the end of incubation, all colonies were counted.

A thin layer of 2 N HCl containing 15% $HgCl_2$ was poured over the gelatin surface, causing a white, cloudy appearance where the gelatin remained. Clear zones around developed colonies were interpreted as proteolytic activity. The percentage of proteolytic colonies was calculated and then multiplied by the corresponding total plate count (see above) to obtain an estimation of the number of surviving proteolytic microorganisms.

Dosimetry measurements. Measurement of absorbed dose was obtained by using Fricke dosimeter solution in sealed 5-ml glass ampoules positioned at the point of minimum dose absorption. The ampoules were just about the same size as the shrimp that were being irradiated and only slightly different in density, thus introducing the minimum amount of aberration.

Sample dosimeters (in duplicate) were used throughout the zone of absorbed dose so as to result in a gradient. The time of exposure, not including transit time, was set at 3 min in order to determine the average dose rate per minute. The minimum dose rate for this particular experiment was 8,280 rads/min, with a maximum to minimum ratio of 1.05. All samples, therefore, were run the same day, based upon a dose rate of 8,280 rads/min of exposure (3). The exact amount of rads received per sample was based on precise timing, using a stopwatch.

Irradiation procedure. Samples in plastic pouches or petri plates with solidified gelatin were irradiated in duplicate at each of the desired dose levels. The samples were centrally positioned (at the point of minimum absorbed dose) in a specially adapted household pressure cooker, which was lowered by a metal chain to the bottom of the Marine Products Development Irradiator pool at a point equidistant from three sets of ^{60}Co source plaques. The pressure cooker was rotated by an electric gear head motor at 2 rpm so as to obtain uniform dose distribution.

Irradiation experiments. Shrimp were irradiated at 0, 10, 25, 50, 75, 100 and 200 krads. Cod fillets and cod homogenate were irradiated simultaneously at 0, 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, and 300 krads. Shrimp and shrimp homogenate were irradiated simultaneously at 0, 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, and 300 krads. Gelatin streak and pour petri plates were irradiated simultaneously at 0, 10, 20, 30, 40, 50, 60, and 100 krads.

RESULTS

Typical microbial log reduction curves for shrimp are shown in Fig. 1, in which pseudomonad-like and proteolytic microorganisms have been differentiated from the total. The typical shoulder appears on the total count curve and also on the proteolytic curve, which is a fraction multiple of the total count curve.

When cod fillets and homogenates were simultaneously irradiated (Fig. 2), the typical shoulder appeared in the cod fillet total count microbial log reduction curve in the 25- to 60-krad dose range. Possibly another shoulder could be interpreted from the data between the 60- to 150-krad dose range. The cod homogenate exhibits a relatively smooth, parabolic microbial log reduction curve between 0- and 300-krad doses. Pseudomonad-like microorganisms in both cod fillets and homogenates disappear at about the same rate. Similar observations apply for the data obtained from the tests conducted on the shrimp and shrimp homogenates (see Fig. 3).

Because cod or shrimp homogenates exhibit nearly smooth total count microbial log reduction curves as compared with those of their respective cod fillet or headed shrimp (particularly in past experiments), it is postulated that the concentration of microbes on the surface, as

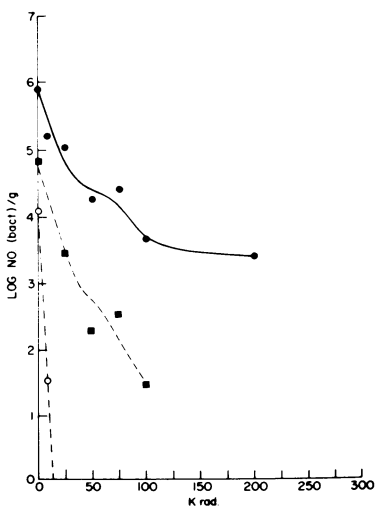

FIG. 1. *Log reduction curves; total, proteolytic, and pseudomonads: Florida white shrimp. See text for details. Symbols: ●, total count; ○, pseudomonads; ■, proteolytic.*

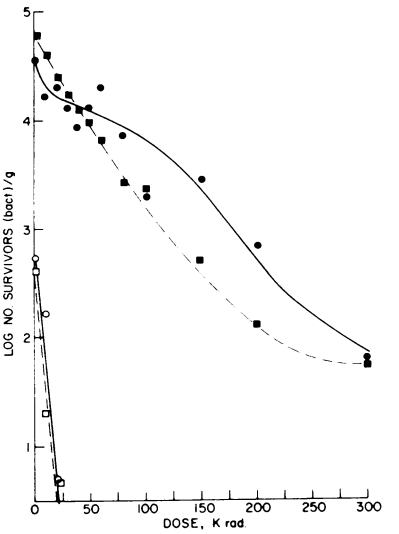

FIG. 2. *Log reduction of bacteria: irradiated cod fillets and homogenate. See text for details. Total plate count: ●, cod fillets; ■, cod homogenate. Pseudomonads: ○, cod fillets; □, cod homogenate.*

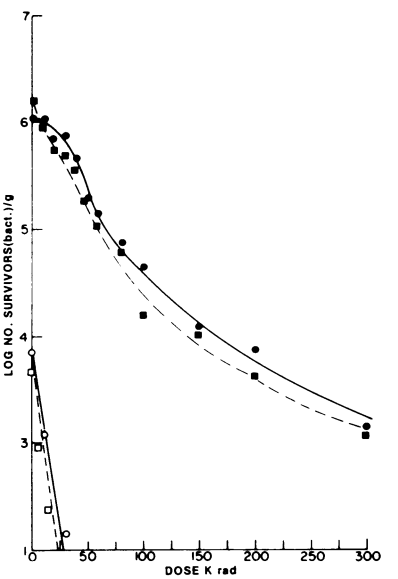

FIG. 3. *Log reduction of bacteria: irradiated shrimp and homogenate. See text for details. Total plate count: ●, shrimp; ■, shrimp homogenate. Pseudomonads: ○, shrimp; □, shrimp homogenate.*

opposed to evenly dispersed microbes, may account in part for the observed shoulder phenomenon.

The gelatin disk experiment was performed to demonstrate possible differences in microbial survival based on their location: surface versus evenly dispersed. Figure 4 shows the results of this gelatin experiment. Cod slime microbes spread on one surface of a gelatin disk exhibit a typical shoulder phenomenon, whereas when evenly dispersed they exhibit a smoother, slightly sigmoidal microbial log reduction curve.

DISCUSSION

The shoulder effect in the bacteriological data on fillets is believed to reflect, largely, the time period after the inactivation of the more radiation-susceptible constituents of the microflora. (Although the role of oxygen was not elucidated, we believe it is related to the phenomenon). Figure 1 shows the relatively low resistance of pseudomonads and, to some extent, that of the proteolytic bacteria. The relatively high radiation susceptibility of pseudomonads is adequately documented (6, 7, 10, 11). Additionally, the role of pseudomonads as the principal spoilage microorganisms in fresh fish has also been adequately documented (1, 6, 10). It might then be concluded from Fig. 1 and from known facts that a dose of less than 25 krads would be sufficient to extend the shelf life of refrigerated fish significantly. Certainly, doses in the range of 50 to 75 krads appear adequate for reducing the numbers of the spoilage bacteria to very low levels.

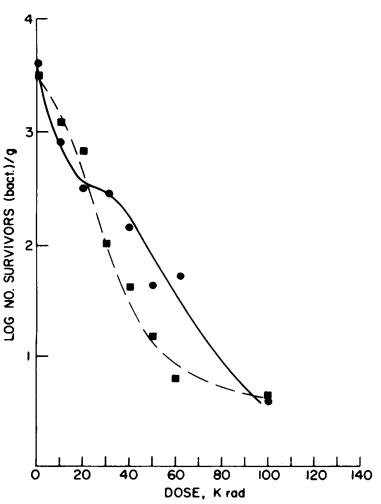

FIG. 4. *Irradiated gelatin petri plates: surface-spread and evenly dispersed bacteria. See text for details. Symbols: ●, surface; ■, dispersed.*

Because it has been demonstrated that the lethal effect of ionizing radiation on microbes is enhanced in the presence of oxygen, and because microbes in fish fillets are generally located on their surfaces, it was theorized that a slower, more uniform bacterial reduction curve would be obtained by causing the bacteria to be uniformly distributed in the fish where most of them would be remote from the surface. The data show that the rate of bacterial inactivation was indeed uniform. However, it was not slower; instead, the numbers were reduced more quickly (Fig. 2 and 3). The surface-inoculated versus evenly dispersed inoculated gelatin disks showed the same relationship (Fig. 4). Whereas it was originally believed that distributing the bacteria uniformly would create a protective environment in the bacteria below the surface, the data distinctly refute the theory. As a matter of fact, the process of mixing in a blender must incorporate air, and enough of it may be incorporated to enhance the lethal effect of the radiation throughout the mass. The faster rate of bacterial reduction in the homogenates remains unexplained, however, and an investigation of this was beyond the scope of this study, which focused mainly on the significance of the shoulder effect as an indicator of the optimum effective preserving dose.

One further implication of this study is that if irradiation is used to pasteurize minced fish flesh and other fish products in which the original surfaces are dispersed internally, there may be expected different microbial log reduction curves, at the low-dose range, from those obtained on the whole fish or on the fillets of the fish.

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