Effect of $\gamma$-Irradiation on the Microflora of Freshwater Fish

I. Microbial Load, Lag Period, and Rate of Growth on Yellow Perch (Perca flavescent) Fillets

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Abstract

KAZANAS, N. (U.S. Bureau of Commercial Fisheries Technological Laboratory, Ann Arbor, Mich.), J. A. EMERSON, H. L. SEAGRAN, AND L. L. KEMPE. Effect of $\gamma$-irradiation on the microflora of freshwater fish. I. Microbial load, lag period, and rate of growth on yellow perch (Perca flavescent) fillets. Appl. Microbiol. 14:261–266. 1966.—Microbial flora were compared in irradiated and nonirradiated yellow perch fillets. These studies included effects of irradiation on the total microbial population, the lag phase, and rate of growth in this freshwater fishery product. The work was conducted concurrently with sensory and chemical evaluation, and constituted part of an investigation designed to evaluate the effect of substerilization doses (0.3 and 0.6 Mrad) of Co$^{60} \gamma$ rays on the storage life of yellow perch fillets at 1.0 or 6.0 C. In five storage tests, total plate counts prior to irradiation did not exceed $8.7 \times 10^5$ per gram of sample; this count was reduced nearly 100% by irradiation with either 0.3 or 0.6 Mrad. Progressively lower maximal bacterial populations and lengthened lag phases were obtained as more radiation was used. The growth rate of the population did not appear to decrease significantly. Microbial data obtained in these studies confirmed the sensory and chemical studies, by indicating that irradiation can significantly extend the refrigerated shelf life of freshwater fish.

Microbial growth, which is the principal cause of fish spoilage, can be effectively controlled by application of sufficiently high levels of ionizing radiation (2). Unfortunately, sterilization levels of radiation reduce the commercial acceptability of most fishery products due to the development of undesirable "irradiation flavors" (9). The feasibility of significantly extending the refrigerated storage life of marine fishery products, while still avoiding objectionable flavor and odor changes, has been accomplished by the application of substerilization doses of Co$^{60} \gamma$ rays to the raw product (8). Thus, pasteurization doses of radiation in conjunction with refrigerated storage permit an increase in shelf life of raw fishery products. This is caused by a reduction in the initial microbial population and an increase in the lag period during their subsequent growth.

Results of studies in the United States and the United Kingdom in which pasteurization levels of radiation were applied to a variety of fishery products of marine origin have been reviewed (1). This and subsequent papers continue to substantiate the earlier conclusion that appropriate selection of radiation and refrigeration conditions permits retention of the desirable characteristics of many fresh seafoods over long periods of time. Some important differences concerning the relative dose tolerance and suitability to radiation have been recognized for different species of fish and seafoods. Furthermore, investigations reported to date have been concerned only with fishery products of marine origin. To our knowledge, no studies have been reported concerning the practicability of controlling microbial spoilage of freshwater fishery products by irradiation. The purpose of the current investigation is to evaluate the use of Co$^{60} \gamma$ rays for preserving products of significance to the commercial fisheries of the Great Lakes.

This paper is confined to the microbiological data obtained from five storage tests of commercially processed fillets of yellow perch (Perca flavescent) irradiated at 0.3 and 0.6 Mrad. These studies were conducted during February, March, April, and September. The irradiated fish were
stored at 1 or 6 C. These irradiation levels were found to be appropriate in a preliminary investigation (3). Total microbial counts were made before and after irradiation, and during refrigerated storage. The total microbial population, the lag period, and the rate of growth of the gross aerobic-facultative microbial population of the yellow perch fillets were obtained from these counts. These data were used for interpreting any shelf life extension determined by sensory evaluation. A detailed comparison of chemical tests and total bacterial counts in relation to sensory evaluation will be reported separately (Emerson et al., Food Technol., in press).

**Nonirradiated Control Samples**

*Source and treatment of sample.* The samples of yellow perch used in these studies were fresh, hand-cut butterfly fillets obtained during February, March, April, and September from commercial processors of Lake Michigan and Lake Erie. The fillets were transported in ice to the laboratory, where they were packaged on the 2nd day after capture of the fish. The butterfly fillets were taken at random from fillet stock for the entire storage test, and were then split into single fillets. Two single fillets, each from a different fish, were then placed in a bag (Cryovac type S3-4 clear plastic); the bag was sealed with a metal clip, and the total weight was recorded. Each bag usually contained from 50 to 60 g of fish. The fish were evaluated at various times during storage at 1 and 6 C by sensory, chemical, and microbiological examination. The latter included total aerobic and facultative microbial plate counts, as well as yeast and mold plate counts. Nonirradiated control samples were evaluated initially for the native flora plus contaminants, and then routinely for increases in gross population during the storage interval. Irradiated samples were evaluated immediately after irradiation to determine the reduction in total count, and twice a week while in storage.

*Microbiological assay.* The contents of a bag were transferred quantitatively and aseptically to a sterile blender jar (Osterizer model 403) that was prechilled to 3 C. A cold, sterile 0.1% peptone solution (4, 7, 10) was next added to the jar to give a 1:4 dilution. The sample was then blended for 1.5 min at the high setting; if blended longer, the homogenate tended to coagulate. Premixed peptone diluent was added to 40 g of the resulting homogenate, to make a 1:5 dilution. This provided a total dilution of 1:20. Homogenization was continued for 1.5 min. Subsequent 10-fold dilutions were prepared by pipetting 10 ml of the 1:20 homogenate into 90 ml of the peptone solution. Final dilutions as low as 1:60 were prepared for counting during the first 2 weeks of storage of the irradiated sample.

Four plates were prepared for total aerobic-facultative microbial counts. The TPY medium (Trypticase-phytone-yeast extract-agar) had a final pH of 7.2. This pH was found to yield the highest average macrocolony counts (7). Malt-agar medium acidified to pH 4.5 with sterile 10% lactic acid (3) was used for total yeast and mold counts. Both TPY and malt-agar media were maintained at 42 C in a water bath prior to pouring the plates. Triplicate plates were prepared for each dilution, each medium, and each incubation temperature. Plates were incubated at 20 and 3 C. Counts from plates incubated at 20 C were made on the 5th day of incubation, and plates incubated at 3 C were counted between the 15th and 20th days of incubation.

*Results.* Microbiological data obtained on control samples stored at 1 and 6 C are presented in Fig. 1, 2, and 3. For the five storage tests representing different seasons of the year, initial total counts ranged from 0.93 \times 10^4 to 8.62 \times 10^6 per gram. Since the yellow perch were commercially filleted by hand, this microflora was composed both of contaminants and indigenous organisms. During the first days of storage, the counts obtained at 20 C were from 1.5 to 10 times greater than corresponding counts at 3 C. By the end of the storage test, however, the counts became similar. Samples stored at 1 C attained a total count of 10^6 per gram by the 5th to 6th day of storage, and similar samples stored at 6 C reached 10^6 per gram by the 4th day of storage. Sensory evaluation indicated shelf lives of 8 to 13 days at 1 C and 6 days at 6 C for these samples.

The initial numbers of yeasts and molds on the nonirradiated fillets never exceeded a total count of 1.88 \times 10^4 when incubated at 20 C. Initial yeast and mold counts were always lower when incubated at 3 C. Total yeast and mold counts for plates incubated at either 20 or 3 C did not increase significantly during 9 days of storage of the samples at either 1 or 6 C.

**Samples Irradiated at 0.3 Mrad**

*Procedure.* Except for irradiation, the irradiated samples were treated in the same manner as the unirradiated ones.

The fillets were irradiated to 0.3 Mrad with an average dose rate of 0.052 Mrad per hr in the Co\(^{60}\) source at the Phoenix Memorial Laboratory of The University of Michigan. The samples were iced to maintain the product temperature at 1 C during irradiation.

*Results.* Total microbial and total yeast and
EFFECT OF \(\gamma\)-IRRADIATION ON PERCH FILLETS

Fig. 1. Total microbial counts (20°C) of unirradiated and irradiated yellow perch fillets stored at 1°C. Microbial (a) and yeast and mold (b) growth curves of a storage test from perch caught in Lake Erie (September 1963). Microbial (c) and (d) yeast and mold growth curves of two storage tests from perch caught in Lake Michigan (February and September 1964).

Fig. 2. Microbial counts (20°C) of unirradiated and irradiated yellow perch fillets stored at 6°C. The fish were caught in Lake Michigan, March and April 1964.

mold counts of fillets irradiated to 0.3 Mrad and stored at 1°C are presented in Fig. 1 and 3. Figure 2 presents total microbial counts of fillets irradiated at the same dose level but stored at 6°C.

A dose of 0.3 Mrad generally reduced the total plate count 99.4 to 99.9%. In numbers, this represented a reduction from an average of \(3.7 \times 10^8\) to approximately \(10^6\) per gram. Total plate counts corresponding to the initial values were not reached until the 13th to 15th day of storage at 1°C. These counts increased progressively, reaching \(10^8\) per gram by the 33rd to 42nd day. For samples stored at 6°C, the initial control values were attained by the 6th day. Total microbial counts increased to \(10^8\) per gram by the 14th to 15th day. Samples were organoleptically acceptable through 26 to 54 and 15 to 21 days of storage at 1 and 6°C, respectively.

At 0.3 Mrad, the yeasts and molds in samples from perch caught in Lake Michigan were reduced initially by approximately 96% in the storage test conducted during September 1964 (Fig. 1d); subsequent storage did not show any significant increase.

In tests made during February (Fig. 1d), March, and April, irradiation reduced the yeast and mold counts to a level where they were not detectable by procedures used in the experiment. During subsequent storage, counts reached \(10^5\) to \(10^6\) per gram at the time the samples were scored unacceptable by sensory evaluation. In the September 1963 storage test of samples from perch caught in Lake Erie (Fig. 1b), the reduction of the yeast and mold population was small, being only about 22.5%. Before irradiation, the initial yeast and mold population was only one-eighth of...
the total microbial plate count. During storage at 1°C (Fig. 1), however, the yeast and mold population increased rapidly and eventually comprised nearly the entire microbial population developed during storage at 6°C.

**Samples Irradiated at 0.6 Mrad**

Procedure. Except for irradiation to 0.6 Mrad, the samples being presently considered were handled in the same way as the control samples and those irradiated to 0.3 Mrad.
Results. Total microbial and total yeast and mold counts of yellow perch fillets irradiated to 0.6 megarad and stored at 1 C are presented in Fig. 1 and 3. Figure 2 presents total microbial counts of fillets irradiated at the same dose level but stored at 6 C.

Irradiation at this level produced nearly a 100% reduction of the total flora; there was little increase in this minimal population during the first week of storage at either temperature. Then the total microbial counts increased slowly and progressively, reaching a value of 10⁶ per gram by the 51st to 57th day of storage at 1 C and by the 24th to 32nd day at 6 C. Psychrophilic microbial counts changed similarly. Samples, independently evaluated by a sensory panel, were scored as unacceptable by the 41st to 57th day and 20th to 21st day of storage at 1 and 6 C, respectively.

Yeasts and molds were reduced nearly 100% by irradiation, and remained at low values for about 20 to 26 days at 1 C (Fig. 1a, 1d) and 8 days at 6 C. Very little increase in numbers occurred throughout subsequent storage of Lake Michigan perch fillets. Data for the September 1963 storage test (Fig. 1b), which employed yellow perch from Lake Erie, showed a somewhat different pattern. Irradiation of these Lake Erie samples to 0.6 Mrad reduced the yeasts and molds by nearly 100% immediately after irradiation. The counts remained at insignificant levels for 23 days, and then the yeast and mold population increased rapidly and soon comprised essentially the entire microbial population of the fillets. It can be observed in Fig. 1b and Fig. 3 that maximal yeast and mold populations of 10⁷ were reached by the 34th day of storage and then remained stationary. Only by the 51st day of storage did bacteria contribute significantly to the total count.

Discussion

The initial microbial population of commercially hand-filleted yellow perch fillets averaged 3.5 × 10⁶ bacteria per gram of fish. Irradiation to 0.6 and 0.3 Mrad, while at 1 C, produced approximately 3.5 and 2.3 logarithm reductions, respectively. Progressively lower maximal bacterial populations and lengthened lag phases were obtained as more radiation was used. Increases in dosage did not appear to decrease significantly the growth rate of the microbial population. These observations duplicate in principle the results reported by Kempe et al. (5) for the growth of psychrophilic bacteria during refrigerated storage of irradiation-pasteurized ground beef and mesophilic organisms in irradiated barley malt (6).

The "irradiated" samples generally attained microbial counts of 10⁶ per gram several days before the product became organoleptically unacceptable. The shelf life of nonirradiated fillets at 1 C was found to be approximately 9 to 13 days; this was extended 3.6- and 5-fold by doses of 0.3 and 0.6 Mrad, respectively. Increasing the storage temperature to 6 C decreased the lag phase (Fig. 2) and greatly accelerated spoilage when compared with irradiated fillets stored at 1 C. The shelf life of nonirradiated samples stored at 6 C was approximately 6 days; this was extended 3- and 5-fold by irradiation to 0.3 and 0.6 Mrad, respectively.

Variations in the microbiological flora of different catches of yellow perch after irradiation can be attributed to quantitative and qualitative differences in the initial microbial population and its sensitivity to irradiation. Furthermore, differences in the initial microbial population can vary with the season of the year, geographical site of sampling, and plant sanitation. Such variations are illustrated by results obtained for samples examined in September 1963 and September 1964, which came from Lake Erie and Lake Michigan, respectively. When the irradiated fillets of perch from Lake Michigan became unacceptable, the microbial population consisted mostly of bacteria. Yeasts were present in lower amounts. On the other hand, yeasts predominated on fillets of perch taken from Lake Erie. Figure 1a illustrates the step-growth curves developed by microbial populations of irradiated perch fillets from Lake Erie. This growth pattern was not observed in Lake Michigan samples (Fig. 1c). The non-irradiated fillets of perch from both lakes had populations primarily composed of bacteria. A comparison of the number of microorganisms obtained at 20 and 3 C incubation temperature from perch caught in the aforementioned lakes is presented in Fig. 3.

A better understanding of the bacteriology of freshwater fishery products would permit a more definitive and meaningful evaluation of the effectiveness of irradiation treatments and subsequent storage procedures for preservation of freshwater fish.

Microbial data obtained in these studies have indicated that irradiation can significantly extend the refrigerated shelf life of freshwater fish.

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**LITERATURE CITED**


