Microbiological Analyses of *Merluccius merluccius hubbsi* (Marini, 1933) Fillets Treated with Co-60 Ionizing Radiation[1]

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Fillets of a hake, **Merluccius merluccius hubbsi,** *were exposed to 2 kGy, 6 kGy, and 10 kGy of ionizing radiation from a Cobalt 60 source and were subsequently stored frozen at 0°C. Both these irradiated samples and control samples were examined to determine their bacterial counts and organoleptic qualities (texture, elasticity, odor, color, and drip loss) for a period of seven weeks following the irradiation. The irradiated fillets showed an initial marked decrease in the total number of bacteria—amounting to roughly one order of magnitude for those exposed to 2 kGy and roughly three orders of magnitude for those exposed to 6 kGy and 10 kGy as compared to the controls. The samples irradiated with 6 and 10 kGy had bacterial counts well below the minimum acceptable level of 0.8 x 10^6 bacteria per gram for the entire seven-week study period. Regarding organoleptic quality, the fillets exposed to 2 kGy and 6 kGy were found to retain acceptable qualities for an average of about six weeks, somewhat longer than the controls and other irradiated samples. Considering both bacterial counts and organoleptic qualities, the most effective of the three radiation doses employed was 6 kGy.*

Irradiation can be used to considerably prolong the storage life of certain foods, both at room temperature and under refrigeration, if appropriate packaging is used that prevents the foods from becoming contaminated (*1–3*).

In 1896, one year after Roentgen discovered the X ray and the same year that Becquerel discovered radioactivity, a work was published with the title "Study of the Effect of Roentgen Rays on Bacteria and the Possibility of their Eventual Use" (*4*).

Nevertheless, the work initially undertaken in this field proceeded slowly, because only low-intensity emitters were available. It was not until the mid-1940s that sources of radiation became available that had the intensity and energy needed for preserving foods. Since then, a considerable bibliography has accumulated on food irradiation (*5–6*).

In 1980, a mixed WHO/FAO/IAEA committee of experts declared that consumption of foods irradiated with an average of 10 kilograys (kGy) was not dangerous (*7*).

The aim of the work reported here, performed in mid-1981, was to determine how much bacterial decay might be reduced by exposing fillets of a hake (*Merluccius merluccius hubbsi*) packed and frozen at −18°C to Cobalt 60 ionizing radiation if the fish were subsequently stored frozen at 0°C. Counts of the total bacteria, total coliforms, and fecal coliforms present were made for both the

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control and irradiated samples, and various organoleptic qualities of the samples were assessed.

MATERIALS AND METHODS

Methodologic and economic criteria were applied in choosing the fish (*8*). That is, a low-fat species was chosen so as to minimize organoleptic changes in the fillets that would result from oxidation of lipids; and the species selected was also one of commercial importance.

Fillets of *Merluccius merluccius hubbsi* were provided by the A.C.E.R. fishing company of Uruguay. The area of capture was along the fortieth parallel of the Uruguayan Maritime Front. After capture, the fish were stored for 40 days in a refrigeration chamber at −18°C. Those destined for irradiation were then packed in bags of 30-micron polyethylene that had been presterilized with a dose of 54 kGy.

The radiation device used was a Gamacell 4000 type belonging to the Nuclear Research Center of the University of Uruguay (Centro de Investigaciones Nucleares, Universidad de la República, Uruguay). This device, which has a cylindrical chamber 14 cm in diameter and 20 cm tall, employs an electromechanical system and an elevator to provide the operator with access through the top of the unit. The sample-holder weighs 800 kg. Irradiation is provided by 48 Cobalt 60 rods laid out in a cylindrical pattern, and these in turn are surrounded by four tons of lead shielding.

In order to irradiate samples under isothermic conditions at −78.5°C, we used thermal insulation consisting of 2 cm polystyrene foam shaped to the maximum dimensions allowed by the irradiation chamber. The samples were placed inside this insulation and were surrounded by pieces of dry ice. After irradiation, it was verified that part of the dry ice was still present, thereby confirming that isothermic conditions had been maintained.

The radiation doses used were 2 kGy, 6 kGy, and 10 kGy. Since the radiation dose was constant (0.8 kGy per hour), the exposure times of different samples varied. Control samples were subjected to −78.5°C for the length of time that the samples receiving the highest dose were irradiated. The storage temperature after irradiation for both the control and irradiated samples was 0°C, the samples being kept in a frozen state.

Microbiologic analyses of the irradiated and control samples were performed at the Fish Research Institute (School of Veterinary Medicine) and the Institute of Hygiene (Medical School) of the University of Uruguay.

To estimate the total bacteria present, total plate counts were made using Aerobic Plate Count Agar (Difco), the pour plate method, and a surface count, with the plates being incubated at 25°C for 72 hours (*9*). These counts were made in triplicate at the end of the first seven weeks following irradiation.

To estimate total coliforms, the technique of counting dilutions to determine the most probable number (MPN) was used. The bacteria were grown on Brilliant Green Lactose Broth with 2% bile (Difco) and were incubated for 48 hours at 37°C (*10*).

To estimate fecal coliforms, we used the McKenzie, Taylor, and Gilbert technique, growing the coliforms on Brilliant Green Lactose Broth with 2% bile (Difco) and Tryptone Broth (Difco) incubated at 44.5°C for 48 hours (*10*). Both total coliform and fecal coliform counts were made in triplicate at one week following irradiation.

Organoleptic analyses were performed on the raw control and irradiated fillets by a panel at Uruguay University's Nuclear Research Center in Montevideo.

The organoleptic qualities assessed were texture, elasticity, odor, drip loss, and color. This examination employed a scale from zero to five as described elsewhere (*11*), the score for the best organoleptic state being five and for the greatest spoilage being zero. Average values for the analyses, which were done in quintuplicate, were graphed as a function of time (weeks of study), considering two to be the lowest score of samples still fit to eat.

RESULTS AND DISCUSSION

Bacterial Counts

The total bacterial counts found for the control and irradiated samples indicated that the bacterial populations in the irradiated samples had been markedly reduced. The average apparent reduction was greatest in the samples receiving the higher doses of radiation.

As shown in Table 1, one week after irradiation the average apparent reduction was around one order of magnitude in the samples irradiated with 2 kGy and roughly three orders of magnitude in the samples irradiated with 6 kGy and 10 kGy.

The limit value in fish flesh otherwise fit for consumption is considered to be 0.8×10^6 bacteria per gram. Quantities of bacteria greater than this are felt to have a critical impact upon freshness.

By the third week the control fillets had developed over 10^6 bacteria per gram, and by the fourth week this number had increased another order of magnitude. Further microbiologic analyses were not performed, since the fillets had clearly become unfit for consumption.

Samples irradiated with 2 kGy developed roughly 10^6 bacteria per gram during the seventh week following irradiation. Samples irradiated with 6 kGy and 10 kGy had less than 10^6 bacteria per gram throughout the seven weeks of the study period.

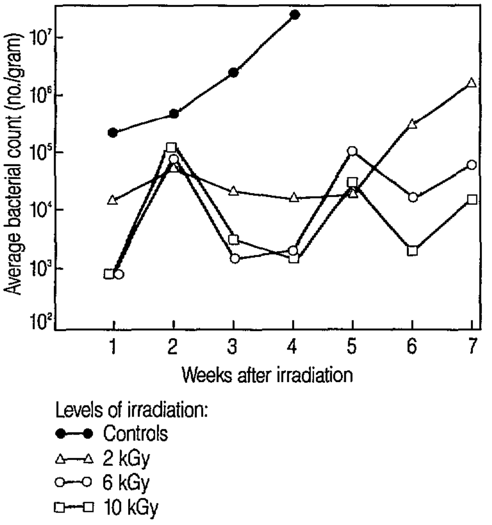
As Figure 1 shows, the control samples and those irradiated with 2 kGy showed stasis or fairly steady growth of bacterial counts during the study period, while those irradiated with 6 kGy and 10 kGy showed fluctuations in these counts. These observations appear reasonable, since bacterial growth after irradiation is affected by many circumstances—creation of mutant bacteria with differential growth rates among those that survive the irradiation process and factors influencing competitiveness when certain numbers and types of bacteria disappear (factors including upsets in biologic equilibria when certain types of associations are interrupted as well as modification of prevailing antagonistic and symbiotic relationships—*12, 13*).

The *Merluccius merluccius hubbsi* fillets irradiated with 2 kGy showed a conside-

Table 1. Average counts of total bacteria per gram in control and irradiated samples during the seven weeks following irradiation.

Time (weeks following irradiation)	Average no. of bacteria per gram of sample			
	Control samples	Samples irradiated with: 2 kGy	6 kGy	10 kGy
1	3.2×10^5	1.6×10^4	8.5×10^2	8.5×10^2
2	6.0×10^5	7.4×10^4	8.5×10^4	9.4×10^4
3	3.3×10^6	2.9×10^4	1.1×10^3	4.5×10^3
4	3.0×10^7	1.6×10^4	2.1×10^3	1.6×10^3
5	$>10^7$	2.3×10^4	9.6×10^4	3.5×10^4
6		4.3×10^5	1.2×10^4	2.0×10^3
7		1.5×10^6	6.5×10^4	1.0×10^4

Figure 1. Average counts of total bacteria per gram in controls and in samples irradiated with 2 kGy, 6 kGy, and 10 kGy at one to seven weeks after irradiation.



rable initial drop in the total number of bacteria relative to the controls. However, since the competitive relationships among the microorganisms present were not fundamentally altered (*14*), the bacterial population tended to maintain itself and then increase steadily over time.

In contrast, 6 kGy and 10 kGy doses have been found to affect the relationships among bacteria by eliminating certain types. This accounts for the apparent lack of steady microbial growth after irradiation, and for fluctuations in the bacterial counts obtained (*15–18*).

Regarding coliform bacteria, counts in the range of 50 to 100 bacteria per gram are low enough to ensure good control of hygienic conditions during production and marketing. Studies of total coliforms and fecal coliforms were performed at one week after irradiation in order to assess relationships between these bacterial populations and the doses of irradiation applied (*19*). Table 2 shows the results obtained. Doses of 6 and 10 kGy are lethal for *Escherichia coli* and most other entero-

Table 2. Total coliforms and fecal coliforms per gram in controls and in irradiated samples one week after irradiation with 2 kGy, 6 kGy, and 10 kGy.

Samples	Total coliforms per gram	Fecal coliforms per gram
Controls	43	4
2 kGy	36	1
6 kGy	15	0
10 kGy	9	0

bacterial populations (*7*); hence, it seems reasonable that no fecal coliforms were found in the samples irradiated with these doses.

Organoleptic Qualities

The results of the organoleptic analyses are shown in Figure 2. The control fillets fell below the acceptable values for elasticity and texture in the fifth and sixth weeks, respectively, and fell below the acceptable values for odor, drip loss, and color between the sixth and seventh weeks.

The fillets irradiated with 2 kGy fell below the acceptable values for elasticity and texture around week seven, but exceeded acceptable limits for odor and drip loss somewhat earlier (between weeks six and seven). These fillets retained acceptable color values throughout the study period.

The fillets irradiated with 6 kGy fell below acceptable values for elasticity and color between weeks six and seven but retained acceptable odor and drip loss scores until week seven and maintained an acceptable texture throughout the study period.

The fillets irradiated with 10 kGy retained acceptable texture and elasticity throughout the study period. However, odor became unacceptable between weeks six and seven, and color and drip loss values fell below acceptable levels between weeks five and six.

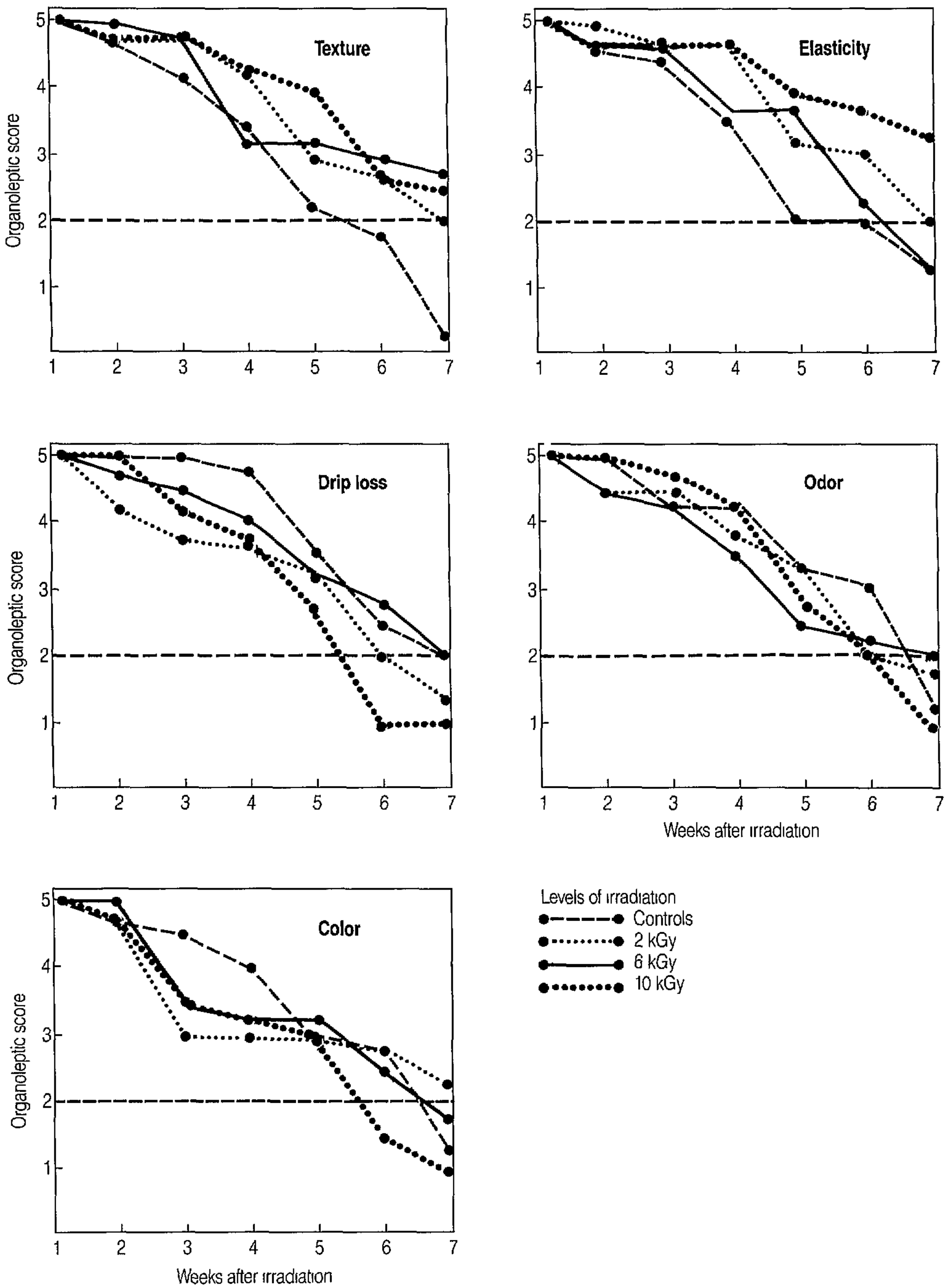


Figure 2. Average organoleptic scores of controls and of samples irradiated with 2 kGy, 6 kGy, and 10 kGy at one to seven weeks after irradiation. The different charts show scores of raw fillets in terms of texture, elasticity, drip loss, odor, and color. A score below two indicates a sample unfit for consumption.

CONCLUSIONS

The work reported here evaluated irradiation of *Merluccius merluccius hubbsi* fillets with doses of 2 kGy, 6 kGy, and 10 kGy. All of these doses bring about considerable reductions in the total number of bacteria capable of causing spoilage and decay.

Comparative study of the three doses applied to the fillets shows that the microbial load present in the *Merluccius merluccius hubbsi* fillets diminished as the dose of radiation applied increased. However, consideration of both the bacterial counts and the organoleptic qualities of the samples over time suggests that the most effective dose for sound preservation of this fish is 6 kGy.

The study also serves to demonstrate, in terms of both bacterial content and the organoleptic qualities assessed, that the combined processes of Cobalt 60 irradiation and frozen storage at 0°C have synergistic effects and extend the time over which the fish studied can be maintained in an acceptable condition for consumption. Employment of these two processes in combination can thus be expected to make a significant contribution to human health.

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Horticulture and Human Health Symposium

The American Society for Horticultural Science (ASHS) is sponsoring the Second International Symposium on Horticulture and Human Health: Contribution of Fruits and Vegetables, to be held 2-5 November 1989 in Alexandria, Virginia, USA. The objective of the symposium is to provide a forum for horticultural scientists, nutritionists, and physicians to exchange the latest information on the relationship of horticultural products to human health, with the additional goal of disseminating that information to other interested scientists, newswriters, public health officials, and the lay public.

The scientific program will include discussion of the following topics: biotechnology, delayed senescence, and modified atmospheric packaging for enhanced food quality and nutritive retention; safety of biotechnology-derived food ingredients; role of fruit and vegetable dietary fiber and pectin in lowering cholesterol; global crop diversification process; home vegetable gardens and nutrition in Third World countries; role of selected vitamins and minerals from fruits and vegetables in disease control; and pests, pesticides, and irradiation as they affect consumer perceptions of produce quality and safety.

For further information contact either Bruno Quebedeaux (General Chairperson of the Symposium), Department of Horticulture, University of Maryland, College Park, MD 20742; Hamdy Eisa (Scientific Program Committee Chairperson), World Bank, 1818 H Street, N.W., Washington, D.C. 20433; or Skip McAfee, ASHS, 701 North Saint Asaph Street, Alexandria, VA 22314-1998, telephone (703) 836-4606.