Effects of Substerilization Doses of Co^{60} Gamma Radiation on the Cold-Storage Life Extension of Shucked Soft-Shelled Clams and Haddock Fillets

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Abstract

Masurovsky, E. B. (Massachusetts Institute of Technology, Cambridge), S. A. Goldblith, and J. T. R. Nickerson. Effects of substerilization doses of Co^{60} gamma radiation on the cold-storage life extension of shucked soft-shelled clams and haddock fillets. Appl. Microbiol. **11**:220–228. 1963.—Total aerobic-facultative and anaerobic (clostridia) macrocolony count data are presented, with analyses and interpretation, for both haddock fillets and shucked soft-shelled clams which received doses of from 50,000 to 800,000 rad of Co^{60} gamma rays. These data indicated that haddock fillets may be maintained in good condition at refrigeration temperatures above freezing for about 1 week at 6 C, and approximately 2 weeks at 0 C, when treated with from 50,000 to 150,000 rad of ionizing radiation. In the dose range from 200,000 to 350,000 rad, the storage life may be extended up to some 2 weeks at 6 C, and 3 weeks at 0 C. Treatments in the dose range from 400,000 to 500,000 rad may defer spoilage for about 1 month, and doses of 550,000 to 650,000 rad afford protection against bacterial spoilage up to approximately 1.5 months. At the high substerilization doses of 700,000 to 800,000 rad, haddock fillets may be held for from 2 to 3 months in refrigerated storage before becoming unfit for marketing and consumption. Shucked soft-shelled clams can be held for about 2.5 weeks at 0 C and close to 12 days at 6 C, when given low substerilization doses of from 50,000 to 150,000 rad of ionizing radiation. At doses of from 200,000 to 350,000 rad, the clams may be preserved effectively for periods up to 3 weeks at 0 or 6 C, and some 6 weeks at these temperatures with doses of about 450,000 rad. With treatments of 500,000 to 600,000 rad, the storage life may be extended for some 2 months, and at doses of 650,000 to 800,000 rad the shucked clams remain in a good state of preservation for up to 3 months at temperatures of 0 to 6 C. Thus, it would appear that shucked soft-shelled clams may be maintained for significantly longer periods in refrigerated storage than haddock fillets when the same radiation treatments are applied to each product. Clostridia levels in both products were relatively low initially, and were reduced significantly by the gamma rays at the doses studied. Moreover, those clostridia that survived the radiation treatments were found to remain at safe, low levels during the various periods in refrigerated storage employed for these products, a very encouraging result from the public health, as well as commercial, standpoint.

The fisheries industry has long cherished the hope of supplying inaccessible, inland markets with "fresh" or "freshlike" sea food products, and of balancing the supply and demand of marine products at coastal markets over substantially longer periods of time than present commercial practices permit. In recent years, with the great strides made in harnessing the power of the atom for peaceful purposes, the means for transforming this hope into reality appears to have excellent possibilities.

The pioneering work that demonstrated the feasibility of extending the storage life of fresh sea foods by treatment with substerilization doses of atomic radiation was carried out by Nickerson and Goldblith (1950), Nickerson et al. (1954), and Shewan and Liston (1958). Since the principal cause of sea food spoilage, at storage temperatures above freezing, is microbial in origin, and since potentially pathogenic microbes, such as *Clostridium botulinum* type E (Dolman, 1957), *Staphylococcus aureus* (Nickerson et al., 1962), and certain members of the *Enterobacteriaceae* (Raj, Wiebe, and Liston, 1961), are occasionally encountered in some marine foodstuffs, a major research program has been undertaken at the Department of Nutrition and Food Science, Massachusetts Institute of Technology, to study the effects of ionizing radiation on the inactivation of these microorganisms *in situ*.

In this communication, data are presented from investigations concerned with the total numbers of aerobic-facultative and obligately anaerobic microorganisms obtained from two commercially important sea foods, shucked soft-shelled clams and haddock fillets, before and at various periods of time after irradiation and storage at 0 and 6 C. The attendant changes in the constitution of the microflora of these marine products, and the deleterious effects (spoilage, etc.) attributable to those changes, are reported in the second paper of this series (Masurovsky,

Voss, and Goldblith, 1963). These studies constitute the first phases of an integrated program intended to bring to realization the potentialities for commercial preservation of sea foods by ionizing radiation.

Materials and Methods

General. Macrocolony counts of the aerobic-facultative microorganisms associated with these foodstuffs were made from samples plated out on both distilled water and synthetic sea water culture media. Preliminary investigations with over a score of different culture media (Department of Nutrition and Food Science, Massachusetts Institute of Technology, 1961) had shown that, although maximal plate counts often could be obtained from these sea foods on a suitable distilled water culture medium, the generic constitution of the microflora was subject to rather wide fluctuations, thus indicating the prudence of employing a synthetic sea water culture medium as well.

Anaerobic (clostridia) macrocolony counts were made with the medium of Mossel et al. (1955), as modified by Angelotti and Hall (1961).

Irradiation of the haddock and clams was carried out in a Mark I Co^{60} Food Irradiator, designed as a prototype commercial unit by the U.S. Atomic Energy Commission and the Brookhaven National Laboratories, and installed in the facilities of the Department of Nutrition and Food Science of the Massachusetts Institute of Technology. The source contained some 32,000 c of Co^{60} at the time of installation, and had a food-irradiation capacity of about 320 lb per day at an average dose level of 1 Mrad. The studies reported herein were conducted in the substerilization dose range from 50,000 to 800,000 rad.

Irradiated and control samples of both sea foods to be stored for different periods of time were placed in metal containers maintained at the temperature of melting ice (0 C) and at 6.0 $\pm$ 0.5 C. These temperatures were selected as representative of the lower and higher ranges of storage temperature employed in regular commercial food channels.

Microbiological assay of irradiated and stored haddock fillets and shucked soft-shelled clams: sampling procedure. Haddock fillets and shucked soft-shelled clams were taken from the processing lines of a filleting and a shucking plant in the greater Boston area known by a previous survey of fishery establishments (Nickerson, Goldblith, and Masurovsky, J. Milk and Food Technol., *in press*) to provide quality products. These samples were packed in clean containers surrounded by crushed ice and quickly transported to the laboratory for assaying or radiation processing, or both.

Preparation of samples for irradiation. Samples (50 g), selected at random from a given lot of haddock fillets and shucked soft-shelled clams, were aseptically cut, weighed, and transferred into radiation-sterilized ($\geqslant 2 \times 10^6$ rad) polyethylene film envelopes. These envelopes were heat-sealed and over-wrapped and sealed with a second layer of polyethylene film. The finished package unit was approximately 7.5 by 7.5 by 2.0 cm. Five replicate packages of each sea food were prepared in this manner for each irradiation dose, storage temperature, and storage time. Unirradiated control samples were prepared in the same manner as the units which were to be irradiated. All samples were then cooled to about 5 C.

Samples designated for irradiation were secured to a specially designed rack (made entirely of wood to minimize gamma photon attenuation and scatter) which was positioned in the center of the middle insulated irradiation chamber. In this position, the samples received as uniform a dose of radiation as was possible according to the geometry of the Co^{60} rods in the source.

Microbiological assay procedure. The irradiated or control packages were carefully opened, the polyethylene film envelopes containing the haddock or clam sections were aseptically opened in turn, and the 50 g of sea food were aseptically transferred to a sterile, precooled Waring Blendor cup. The envelopes were next rinsed out three times with sterile, refrigerated diluent into the same Waring Blendor cup. A sample plating diluent contained (g/liter of distilled water): Trypticase (BBL), 1.0; KH_2PO_4, 0.0425; and Antifoam AF (Dow Corning Corp.), 0.01. The final pH of the emulsion was 7.2 $\pm$ 0.1. A total of 450 ml of diluent was put into a given blender cup together with the sea food sample, and blended for 3 min at 17,000 rev/min.

Aerobic-facultative plating procedure. Appropriate decimal dilutions were made from the blended samples by use of sterile, precooled diluent; 0.1 ml from these dilutions was aseptically pipetted onto the surface of previously poured agar culture dishes of medium B(DW) and medium B(SSW).

Medium B(DW) was that medium found in previous investigations (Department of Nutrition and Food Science, Massachusetts Institute of Technology, 1961) to give the highest average macrocolony counts for both haddock fillets and shucked soft-shelled clams. It was prepared with distilled water (DW). Medium B(SSW) contained the same basal complex organic nutrient and energy sources as medium B(DW), but was prepared with a solution of inorganic salts of such a composition and concentration, as recommended by MacLeod and Onofrey (1956), to provide an optimal ionic environment for those microorganisms of marine origin which require some of the elements present in sea water.

The surface of the agar in these culture dishes had been dried at 37 C for 24 hr and cooled to 20 C prior to the above inoculation. The 0.1-ml inoculum was next spread over the surface of the surface-dried agar with silicone (Siliclad)-treated glass rods, by the method of Buck and Cleverdon (1960), to distribute the organisms as homogeneously as possible, and to provide optimal aerobic conditions. Three replicate culture plates were prepared for each dilution and each medium. Separate glass rods

were employed for spreading the inoculum in each dish. These petri-dish cultures were incubated for 5 days at 20 C before observation and enumeration of the macrocolonies which developed.

Anaerobic tube procedure. The anaerobic microflora of these same samples were cultured and scored in the sulfite-polymixin-sulfadiazine agar of Angelotti and Hall (1961), using Pyrex no. 9200 oval tubes with a thioglycolate agar overlay. Three replicate tubes were made from the 1:10 dilution of each sample, and incubated at 30 and 37 C for 40 hr. Black zone-forming macrocolonies were analyzed for catalase activity, morphology, and spore formation subsequent to enumeration as a *Clostridium* sp.

RESULTS AND DISCUSSION

Haddock fillets. Total aerobic-facultative and anaerobic (clostridia) macrocolony count data for haddock fillets irradiated at doses of from 50,000 to 350,000 rad are presented in Tables 1 and 2.

The aerobic-facultative plate counts were less than 25×10^3 viable microorganisms per g of haddock tissue immediately after irradiation for all the aforementioned doses, which was equivalent to the inactivation of approximately 96% of the original commensal and contaminating microflora.

Results from storage studies with the irradiated fillets indicated that aerobic-facultative counts remained below 110×10^4/g at 6 C and 40×10^3/g at 0 C during the first week after treatments with doses of 200,000 to 350,000 rad of Co^{60} gamma rays. In the dose range from 50,000 to 150,000 rad, the counts rose to over 1 million per g at 6 C and into the tens of thousands per g at 0 C, after about 1 week in storage.

By the 12th day of storage at 6 C, macrocolony counts of well into the millions per g of haddock tissue were obtained from the entire series of doses from 50,000 to 350,000 rad. However, fillets receiving doses of 200,000 to 350,000 rad, when stored at 0 C, did not show aerobic-

TABLE 1. *Aerobic-facultative macrocolony counts obtained from haddock fillets before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for 24 days*[a]

Treatment	Storage temp	Medium	Absorbed dose (rad) 50,000	100,000	150,000	200,000	250,000	300,000	350,000
	C								
Before irradiation		DW[b]	595×10^3	294×10^3	294×10^3	336×10^3	336×10^3	445×10^3	445×10^3
		SSW[c]	199×10^3	204×10^3	204×10^3	197×10^3	197×10^3	341×10^3	341×10^3
Immediately after irradiation		DW	228×10^2	169×10^2	56.0×10^2	12.6×10^2	55.5×10^1	49.1×10^1	30.5×10^1
		SSW	173×10^2	127×10^2	56.5×10^2	9.27×10^2	52.5×10^1	36.5×10^1	21.5×10^1
After 3 days of refrigerated storage	6	DW	149×10^4	42.1×10^4	18.1×10^4	63.6×10^2	41.8×10^2	45.2×10^2	22.9×10^2
		SSW	107×10^4	37.5×10^4	15.7×10^4	51.2×10^2	23.6×10^2	28.5×10^2	17.9×10^2
	0	DW	426×10^2	115×10^2	73.5×10^2	20.7×10^2	21.3×10^2	23.7×10^2	14.7×10^2
		SSW	278×10^2	103×10^2	56.9×10^2	14.1×10^2	13.7×10^2	15.3×10^2	11.1×10^2
After 6 days of refrigerated storage	6	DW	112×10^6	78.8×10^5	73.1×10^5	109×10^4	70.1×10^4	73.9×10^4	—
		SSW	104×10^6	63.6×10^5	49.9×10^5	85.5×10^4	45.3×10^4	50.1×10^4	—
	0	DW	399×10^4	64.9×10^3	50.7×10^3	182×10^2	38.1×10^3	18.8×10^3	—
		SSW	279×10^4	60.5×10^3	38.6×10^3	159×10^2	22.0×10^3	12.8×10^3	—
After 9 days of refrigerated storage[d]	6	DW	$\gg 10^6$	148×10^6	103×10^6	127×10^5	66.5×10^5	37.0×10^5	30.2×10^5
		SSW		120×10^6	82.3×10^6	119×10^5	59.2×10^5	32.9×10^5	26.6×10^5
	0	DW	55.7×10^6	113×10^4	77.5×10^4	53.5×10^3	39.6×10^3	50.1×10^3	45.5×10^3
		SSW	50.1×10^6	103×10^4	67.1×10^4	41.3×10^3	26.9×10^3	46.8×10^3	38.5×10^3
After 12 days of refrigerated storage	6	DW		$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	—	58.5×10^6
		SSW						—	46.0×10^6
	0	DW		119×10^5	74.7×10^5	133×10^3	72.6×10^3	—	30.1×10^4
		SSW		109×10^5	52.9×10^5	120×10^3	54.0×10^3	—	17.6×10^4
After 15 days of refrigerated storage	6			$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	—
									—
	0	DW		83.8×10^6	553×10^6	205×10^4	126×10^4	89.1×10^4	—
		SSW		57.5×10^6	484×10^6	182×10^4	110×10^4	56.7×10^4	—
After 18 days of refrigerated storage	6					$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	$\gg 10^6$
	0	DW				30.7×10^6	204×10^5	128×10^5	413×10^4
		SSW				18.6×10^6	176×10^5	121×10^5	396×10^4
After 24 days of refrigerated storage	6					$\gg 10^6$	$\gg 10^6$	$\gg 10^6$	$\gg 10^6$
	0	DW				189×10^6	70.5×10^6	116×10^6	78.3×10^6
		SSW				179×10^6	64.5×10^6	97.2×10^6	63.1×10^6

[a] Average ($\bar{X}$) of five replicate samples plated in triplicate ($X \cdot = 15$) and incubated for 5 days at 20 C. Results expressed as count per g of medium.

[b] Distilled water culture medium.

[c] Synthetic sea water culture medium.

[d] Readings for 100,000, 150,000, 200,000, and 250,000 rad doses were taken after 8 days of storage.

TABLE 2. *Anaerobic (clostridia) macrocolony counts obtained from haddock fillets before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for 24 days*[a]

Treatment	Storage temp	Absorbed dose (rad)						
		50,000	100,000	150,000	200,000	250,000	300,000	350,000
	C							
Before irradiation		1.73	1.54	1.54	4.13	4.13	3.73	3.73
Immediately after irradiation		~1.00[b]	~1.00	~1.00	~1.00	~1.00	~1.00	<1.00[c]
After 3 days of refrigerated storage	6	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
	0	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
After 6 days of refrigerated storage	6	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	—
	0	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	—
After 9 days of refrigerated storage	6	—	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
	0	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
After 12 days of refrigerated storage	6		—	—	—	—	—	~1.00
	0		~1.00	~1.00	~1.00	~1.00	—	~1.00
After 15 days of refrigerated storage	6		—	—	—	—	—	—
	0		~1.00	~1.00	~1.00	~1.00	~1.00	—
After 18 days of refrigerated storage	6				—	—	—	—
	0				~1.00	~1.00	~1.00	~1.00
After 24 days of refrigerated storage	6				—	—	—	—
	0				~1.00	~1.00	~1.00	~1.00

[a] Average ($\bar{X}$) of five replicate samples tubed in triplicate ($X\cdot = 15$) and incubated at 30 and 37 C for 40 hr. Results expressed as (count × 10)/g.

[b] At ~1.00, several, but not all, replicate anaerobic culture tubes displayed at least one *Clostridium* macrocolony.

[c] At <1.00, no anaerobic culture tubes displayed a single *Clostridium* macrocolony.

[d] Counts at 100,000, 150,000, 200,000, and 250,000 rad levels were taken after 8 days of storage.

TABLE 3. *Aerobic-facultative macrocolony counts obtained from haddock fillets before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for 3 months*[a]

Treatment	Storage temp	Medium[b]	Absorbed dose (rad)							
			450,000	500,000	550,000	600,000	650,000	700,000	750,000	800,000
	C									
Before irradiation		DW	572×10^3	129×10^3	191×10^3	91.3×10^3	245×10^3	118×10^3	45.5×10^3	118×10^3
		SSW	325×10^3	81.5×10^3	88.8×10^3	57.7×10^3	127×10^3	49.9×10^3	18.7×10^3	49.9×10^3
Immediately after irradiation		DW	19.4×10^1	39.5×10^1	11.9×10^1	21.3×10^1	27.3×10^1	22.4×10^1	11.5×10^1	13.5×10^1
		SSW	17.5×10^1	24.3×10^1	11.2×10^1	18.0×10^1	14.6×10^1	18.6×10^1	7.46×10^1	9.26×10^1
After 1 month of refrigerated storage	6	DW	55.7×10^{4c}	23.4×10^{4c}	44.5×10^{5d}	46.8×10^5	283×10^4	129×10^3	108×10^3	155×10^3
		SSW	55.6×10^4	21.6×10^4	41.0×10^5	46.1×10^5	253×10^4	146×10^3	97.1×10^3	146×10^3
	0	DW	74.1×10^2	67.1×10^2	32.0×10^3	67.1×10^4	63.8×10^4	22.5×10^3	66.9×10^2	68.3×10^2
		SSW	70.4×10^2	65.4×10^2	29.1×10^3	60.3×10^4	58.5×10^4	18.7×10^3	51.0×10^2	68.2×10^2
After 2 months of refrigerated storage	6	DW	77.9×10^{6e}	50.3×10^{6e}	218×10^6	33.8×10^6	203×10^6	36.7×10^5	50.7×10^5	60.3×10^5
		SSW	73.2×10^6	46.0×10^6	210×10^6	32.8×10^6	184×10^6	32.9×10^5	43.6×10^5	56.8×10^5
	0	DW	33.1×10^5	27.0×10^6	60.3×10^6	169×10^5	48.5×10^5	98.6×10^3	52.5×10^4	27.2×10^4
		SSW	28.3×10^5	25.0×10^6	64.5×10^6	164×10^5	46.0×10^5	94.3×10^3	47.5×10^4	24.1×10^4
After 3 months of refrigerated storage	6	DW	—	—	—	—	—	198×10^6	222×10^6	199×10^6
		SSW	—	—	—	—	—	182×10^6	216×10^6	173×10^6
	0	DW	—	—	—	—	—	58.3×10^6	149×10^6	104×10^5
		SSW	—	—	—	—	—	54.3×10^6	137×10^6	99.3×10^5

[a] Average ($\bar{X}$) of five replicate samples plated in triplicate ($X\cdot = 15$) and incubated for 5 days at 20 C. Results expressed as count per g of medium.

[b] DW = distilled water culture medium; SSW = synthetic sea water culture medium.

[c] Readings taken after 2 weeks of storage.

[d] Readings taken after 3 weeks of storage.

[e] Readings taken after 1 month of storage.

TABLE 4. *Anaerobic (clostridia) macrocolony counts obtained from haddock fillets before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for 3 months*[a]

Treatment	Storage temp	Absorbed dose (rad)							
		450,000	500,000	550,000	600,000	650,000	700,000	750,000	800,000
	C								
Before irradiation		6.40	2.87	2.13	2.33	2.67	~1.00[b]	~1.00	~1.00
Immediately after irradiation		~1.00	~1.00	<1.00[c]	<1.00	<1.00	<1.00	<1.00	<1.00
After 1 month of refrigerated storage	6	1.00[d]	~1.00[d]	~1.00[e]	~1.00	~1.00	~1.00	~1.00	~1.00
	0	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
After 2 months of refrigerated storage	6	~1.00[f]	1.00[f]	1.00	~1.00	~1.00	~1.00	~1.00	~1.00
	0	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00	~1.00
After 3 months of refrigerated storage	6	—	—	—	—	—	1.53	1.53	1.26
	0	—	—	—	—	—	~1.00	~1.00	~1.00

[a] Average ($\bar{X}$) of five replicate samples tubed in triplicate ($X\cdot = 15$) and incubated at 30 and 37 C for 40 hr. Results expressed as (count × 10) per g.

[b] At ~1.00, several but not all replicate anaerobic culture tubes displayed at least one *Clostridium* macrocolony.

[c] At <1.00, no anaerobic culture tubes displayed a single *Clostridium* macrocolony.

[d] Counts taken after 2 weeks of storage.

[e] Counts taken after 3 weeks of storage.

[f] Counts taken after 1 month of storage.

TABLE 5. *Aerobic-facultative macrocolony counts obtained from haddock fillets treated with a 400,000-rad dose of Co^{60} gamma rays and subsequently stored at 0 or 6 C for some 2 weeks**

Medium†	At 0 C			At 6 C		
	Storage	Irradiated samples (4×10^5 rad)	Nonirradiated controls	Storage	Irradiated samples (4×10^5 rad)	Nonirradiated controls
	days			*days*		
DW	0	35.9×10^1	36.7×10^4	0	35.9×10^1	36.7×10^4
SSW		27.3×10^1	21.5×10^4		27.3×10^1	21.5×10^4
DW	5	25.5×10^2	46.4×10^5	4	29.2×10^2	28.4×10^5
SSW		15.8×10^2	36.3×10^5		20.3×10^2	18.3×10^5
DW	8	75.1×10^2	12.5×10^6	7	10.6×10^3	27.0×10^6
SSW		68.7×10^2	61.8×10^5		98.0×10^2	16.5×10^6
DW	13	61.7×10^3	—	12	64.3×10^4	24.3×10^7
SSW		56.0×10^3	—		51.9×10^4	17.2×10^7
DW	15	37.7×10^4	10.5×10^7	14	94.3×10^5	47.9×10^7
SSW		27.7×10^4	87.4×10^6		87.3×10^5	41.3×10^7

* Average ($\bar{X}$) of five replicate samples plated in triplicate ($X\cdot = 15$) and incubated for 5 days at 20 C. Results expressed as count/g of medium.

† DW = distilled water culture medium; SSW = synthetic sea water culture medium.

TABLE 6. *Anaerobic (clostridia) macrocolony counts obtained from haddock fillets treated with a 400,000-rad dose of Co^{60} gamma rays and subsequently stored at 0 or 6 C for some 2 weeks**

At 0 C			At 6 C		
Storage	Irradiated samples (4×10^5 rad)	Nonirradiated controls	Storage	Irradiated samples (4×10^5 rad)	Nonirradiated controls
days			*days*		
0	0	~1.00	0	0	~1.00
5	~1.00	~1.00	4	~1.00	1.50
8	~1.00	1.14	7	~1.00	1.70
13	~1.00	1.44	12	~1.00	1.90
15	~1.00	2.46	14	~1.00	2.31

* Average ($\bar{X}$) of five replicate samples tubed in triplicate ($X\cdot = 15$) and incubated at 30 and 37 C for 40 hr. Results expressed as count × 10 per g of medium.

facultative counts in the millions until over 1 week later. Thus, fillets treated at these dose levels, and subsequently stored at the temperature of melting ice, may be kept in a microbiologically satisfactory state of preservation for about 2 to 3 weeks. On the other hand, at doses of 50,000 to 150,000 rad, no more than 9 to 15 days of storage at 0 C may elapse before the microbial populations rise into the millions per g and cause serious spoilage of the product.

Turning to the higher substerilization dose range, from 400,000 to 800,000 rad, one finds significantly longer storage times to have passed before the bacterial growth in the product reached high population levels. Macrocolony count data for this dose range are presented in Tables 3 to 6.

Aerobic-facultative plate counts were generally less than 400 organisms per g shortly after irradiation over this entire dose range, or better than 99% of the original microflora was destroyed by the absorption of these levels of ionizing energy.

Data from storage studies with the fillets irradiated at doses of 700,000 to 800,000 rad showed that aerobic-facultative counts stayed below 1.5×10^5/g at 6 C and $<2.5 \times 10^4$/g at 0 C, up to and including 1 month of storage, but increased to about 6.0×10^6/g at 6 C and $<55 \times 10^4$/g at 0 C after 2 months of storage.

By contrast, the aerobic-facultative macrocolony counts of fillet sections treated with from 550,000 to 650,000 rad of ionizing radiation reached approximately 50×10^5/g at 6 C and $\leqslant 67 \times 10^4$/g at 0 C after only 1 month of

storage. After 2 months in storage, the counts rose to some 20 to 200 $\times 10^6$/g at 6 C, and 50 to 150 $\times 10^5$/g at 0 C.

It is noteworthy that haddock fillets irradiated in the dose range from 450,000 to 500,000 rad had counts of about 50 $\times 10^4$/g at 6 C, and $\sim$ 70 $\times 10^2$/g at 0 C after only 2 weeks in storage, then became unfit for marketing after 1 month in storage, with counts in the millions per g at both 6 and 0 C. Such results are not surprising, however, in light of the microbial population levels of $\sim$ 90 $\times 10^5$/g at 6 C and $\sim$ 30 $\times 10^4$/g at 0 C that were reached after about 2 weeks in storage with fillet sections given a dose of 400,000 rad of ionizing radiation. Nevertheless, it does indicate that in the dose range from 400,000 to 500,000 rad, the useful refrigerated-storage life of haddock fillets may be extended only from about 2 weeks to 1 month. In the dose range from 550,000 to 650,000 rad, the storage life is prolonged up to approximately 1.5 months, and at doses of 700,000 to 800,000 rad the fillets can be well maintained under refrigeration for from 2 to 3 months.

Anaerobic (clostridia) tubes were generally negative for the freshly irradiated samples over the entire dose range studied. The number of positive tubes began to increase after the samples had been stored for at least 1 week at 0 or 6 C. These counts were negligible for all practical purposes, a very promising result from the commercial and public health standpoints.

Shucked soft-shelled clams. In Tables 7 and 8 are presented total aerobic-facultative and anaerobic (clostridia) macrocolony count data for shucked soft-shelled clams

TABLE 7. *Aerobic-facultative macrocolony counts obtained from shucked soft-shelled clams before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for up to 24 days*[a]

Treatment	Storage temp	Medium[b]	Absorbed dose (rad)						
			50,000	100,000	150,000	200,000	250,000	300,000	350,000
	C								
Before irradiation		DW	37.6×10^3	37.6×10^3	78.3×10^3	193×10^3	89.0×10^3	89.0×10^3	123×10^3
		SSW	39.5×10^3	39.5×10^3	60.1×10^3	152×10^3	61.8×10^3	61.8×10^3	134×10^3
Immediately after irradiation		DW	78.6×10^2	35.4×10^2	87.0×10^2	38.5×10^2	20.9×10^2	15.4×10^2	131×10^1
		SSW	62.2×10^2	29.5×10^2	79.5×10^2	31.8×10^2	17.3×10^2	9.13×10^2	113×10^1
After 3 days of refrigerated storage	6	DW	82.5×10^4	263×10^3	478×10^2	69.3×10^2	45.6×10^2	33.0×10^2	21.5×10^2
		SSW	52.1×10^4	147×10^3	335×10^2	38.6×10^2	32.9×10^2	19.6×10^2	19.0×10^2
	0	DW	18.9×10^3	96.7×10^2	112×10^2	40.9×10^2	30.1×10^2	25.2×10^2	14.7×10^2
		SSW	12.2×10^3	58.4×10^2	105×10^2	36.7×10^2	22.9×10^2	19.3×10^2	13.4×10^2
After 6 days of refrigerated storage[c]	6	DW	89.0×10^5	57.9×10^5	30.7×10^5	134×10^4	38.5×10^3	78.9×10^2	72.3×10^2
		SSW	64.2×10^5	42.3×10^5	22.0×10^5	120×10^4	22.4×10^3	40.8×10^2	60.2×10^2
	0	DW	36.1×10^4	62.1×10^3	260×10^2	106×10^2	57.7×10^2	25.5×10^2	26.0×10^2
		SSW	22.8×10^4	46.7×10^3	239×10^2	88.1×10^2	40.1×10^2	13.3×10^2	12.5×10^2
After 9 days of refrigerated storage[d]	6	DW	537×10^6	374×10^6	129×10^5	80.7×10^5	13.8×10^4	73.1×10^3	93.0×10^3
		SSW	510×10^6	357×10^6	119×10^5	75.8×10^5	11.5×10^4	53.7×10^3	72.5×10^3
	0	DW	26.2×10^5	19.7×10^5	40.5×10^3	45.9×10^3	16.3×10^3	12.3×10^3	37.1×10^3
		SSW	17.9×10^5	15.0×10^5	30.7×10^3	44.9×10^3	11.1×10^3	7.20×10^3	34.5×10^3
After 12 days of refrigerated storage[e]	6	DW	$\gg 10^6$	$\gg 10^6$	124×10^6	—	52.9×10^4	—	13.0×10^4
		SSW			115×10^6	—	38.3×10^4	—	12.5×10^4
	0	DW	25.1×10^6	—	62.7×10^3	—	29.5×10^3	—	97.5×10^3
		SSW	15.1×10^6	—	25.1×10^3	—	15.3×10^3	—	94.5×10^3
After 15 days of refrigerated storage	6	DW		$\gg 10^6$	$\gg 10^6$	122×10^6	76.3×10^4	97.0×10^4	—
		SSW				107×10^6	62.7×10^4	81.7×10^4	—
	0	DW		66.3×10^5	164×10^4	106×10^3	32.8×10^3	63.9×10^3	—
		SSW		53.5×10^5	124×10^4	100×10^3	16.3×10^3	43.0×10^3	—
After 18 days of refrigerated storage[f]	6	DW		$\gg 10^6$	$\gg 10^6$	263×10^6	857×10^6	23.7×10^5	21.9×10^5
		SSW				249×10^6	796×10^6	22.9×10^5	20.5×10^5
	0	DW		178×10^6	64.5×10^6	52.6×10^5	24.0×10^4	111×10^3	59.5×10^4
		SSW		161×10^6	55.2×10^6	48.6×10^5	18.9×10^4	109×10^3	58.7×10^4
After 24 days of refrigerated storage[g]	6	DW				$\gg 10^6$	$\gg 10^6$	33.6×10^6	42.1×10^6
		SSW						26.8×10^6	31.8×10^6
	0	DW				28.7×10^6	19.1×10^6	25.1×10^5	19.9×10^6
		SSW				18.5×10^6	12.1×10^6	18.6×10^5	14.9×10^6

[a] Average ($\bar{X}$) of five replicate samples plated in triplicate ($X \cdot = 15$) and incubated for 5 days at 20 C. Results expressed as count per g of medium.

[b] DW, distilled water culture medium; SSW, synthetic sea water culture medium.

[c] Counts at 250,000 rad ware taken after 5 days of storage.

[d] Counts at 200,000 rad were taken after 10 days of storage.

[e] Counts at 50,000 rad were taken after 13 days of storage.

[f] Counts at 200,000 rad were taken after 20 days of storage.

[g] Counts at 300,000 rad were taken after 22 days of storage.

before and after treatment with Co^{60} gamma rays at doses of 50,000 to 350,000 rad and storage for 24 days at 0 and 6 C.

Approximately 98% of the initial commensal and contaminating aerobic-facultative microflora of this shellfish was inactivated at these moderate substerilization doses, just as in the case of the haddock fillet microflora. Shucked clams irradiated in the dose range from 200,000 to 350,000 rad had aerobic-facultative macrocolony counts below 150×10^4/g at 6 C and $< 100 \times 10^2$/g at 0 C after about 1 week in storage. At doses of 50,000 to 150,000 rad, the counts rose into the hundreds of thousands per g with samples stored at 6 C, and in the tens of thousands per g at 0 C during the first week of storage.

TABLE 8. *Anaerobic (clostridia) macrocolony counts obtained from shucked soft-shelled clams before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for up to 24 days*[a]

Treatment	Storage temp	Absorbed dose (rad)						
		50,000	100,000	150,000	200,000	250,000	300,000	350,000
	C							
Before irradiation		13.2	13.2	15.2	28.7	24.9	24.9	38.1
Immediately after irradiation		8.73	6.60	11.0	20.1	18.9	10.8	15.9
After 3 days of refrigerated storage	6	10.3	8.40	9.53	26.3	12.6	13.9	17.6
	0	7.93	6.60	7.13	17.1	9.20	8.47	16.6
After 6 days of refrigerated storage[b]	6	8.93	13.6	12.3	9.20	14.1	11.0	18.0
	0	6.33	8.73	8.47	7.87	9.06	6.86	16.2
After 9 days of refrigerated storage[c]	6	11.7	8.73	10.1	12.8	12.3	11.7	18.5
	0	6.87	5.93	8.27	6.33	7.86	9.40	15.7
After 12 days of refrigerated storage[d]	6	—	—	6.87	—	10.6	—	18.5
	0	5.73	—	5.87	—	7.80	—	15.3
After 15 days of refrigerated storage	6		—	9.00	21.9	8.13	9.73	—
	0		6.20	6.93	19.7	6.80	6.53	—
After 18 days of refrigerated storage[e]	6		—	—	16.8	8.27	12.7	25.1
	0		4.47	9.53	8.80	4.67	6.40	11.3
After 24 days of refrigerated storage[f]	6				—	—	10.5	12.6
	0				9.00	8.33	9.47	8.93

[a] Average ($\bar{X}$) of five replicate samples tubed in triplicate ($X \cdot = 15$) and incubated at 30 and 37 C for 40 hr. Results expressed as count × 10 per g.

[b] Counts at 250,000 rad were taken after 5 days of storage.

[c] Counts at 200,000 rad were taken after 10 days of storage.

[d] Counts at 50,000 rad were taken after 13 days of storage.

[e] Counts at 200,000 rad were taken after 20 days of storage.

[f] Counts at 300,000 rad were taken after 22 days of storage.

Counts of over 1 million microorganisms per g were obtained from clam meats, treated with doses of 200,000 to 350,000 rad of ionizing radiation, after the samples had been held at 6 C for some 18 days. Comparably high levels of microbial populations were found in clams irradiated at 50,000 to 150,000 rad by the 9th to 12th day of storage at 6 C. At 0 C, counts in the millions per g were not observed until after some 3 weeks in storage for samples irradiated with over 150,000 rad of gamma rays, whereas clams treated at $\leqslant$150,000 rad exhibited counts of about 1 million per g after some 2 weeks of storage at 0 C. These storage periods were significantly longer than for haddock fillets irradiated and stored under the same conditions.

Data from the high substerilization dose range from 400,000 to 800,000 rad are presented in Tables 9 and 10.

Once again it is to be noted that more than 99% of the original aerobic-facultative microflora of this mollusk was destroyed at these high substerilization doses, as with the haddock fillet microflora in this high radiation dose range. After 1 month in cold storage, the microbial populations in the clam samples treated at doses of from 650,000 to 800,000 rad remained below 50×10^4/g at 6 C and 30×10^4/g at 0 C.

After 2 months in storage, the counts on these clams increased to about 4.0 to 20.0×10^5/g at 6 C, and between 3 and 90×10^4/g at 0 C; after 3 months of storage, the counts went into the millions of microbes per g of product.

Results from storage studies of clams irradiated at doses of 600,000 rad and lower indicated that concentrations of some hundreds of thousands of microorganisms per g were present after no longer than 1 month in storage at both temperatures. Counts of over 1 million per g were obtained from samples kept in storage at 6 C for some 3 to 6 weeks, and at 0 C for from 1 to 2 months.

The foregoing data suggest that shucked soft-shelled clams may be effectively maintained in refrigerated storage for periods of up to 3 months when treated with from 650,000 to 800,000 rad of Co^{60} gamma rays. In the dose range of 500,000 to 600,000 rad, the storage life of this product may be extended for some 2 months, and with treatments of 400,000 to 450,000 rad the shucked clams might be held under refrigeration for 1 month to 6 weeks.

The high degree of inactivation of the aerobic-facultative microbial populations achieved with these high radiation doses was likewise reflected in the highly significant reduction brought about in the anaerobic (clostridia) microflora of this shellfish. At the moderate radia-

tion doses of 50,000 to 350,000 rad, more of these spore-forming, radiation-resistant microorganisms survived the irradiation treatments.

During storage, the samples irradiated with from 400,000 to 800,000 rad of Co^{60} gamma rays showed variably slight increases in number of clostridia present, and remained below 100 organisms per g at both 0 and 6 C, even after 3 months in storage. Shucked clams treated with doses of 50,000 to 350,000 rad exhibited a similar fluctuation in clostridia levels during storage, but stayed below 200 per g at 0 or 6 C, or both, up through 24 days in storage. These results are most encouraging from a public health stand-

TABLE 9. *Aerobic-facultative macrocolony counts obtained from shucked soft-shelled clams before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for up to 3 months*[a]

Treatment	Storage temp	Medium[b]	Absorbed dose (rad)								
			400,000	450,000	500,000	550,000	600,000	650,000	700,000	750,000	800,000
	C										
Before irradiation		DW	49.7×10^3	112×10^3	15.1×10^3	11.9×10^3	31.2×10^3	43.7×10^3	37.2×10^3	11.9×10^4	20.5×10^4
		SSW	20.9×10^3	106×10^3	6.93×10^3	10.7×10^3	20.6×10^3	23.4×10^3	26.6×10^3	65.3×10^3	12.3×10^4
Immediately after irradiation		DW	22.9×10^1	24.7×10^1	5.80×10^1	3.93×10^1	9.80×10^1	4.47×10^1	3.87×10^1	22.2×10^1	6.73×10^1
		SSW	41.9×10^1	17.5×10^1	3.00×10^1	2.87×10^1	5.33×10^1	1.87×10^1	2.35×10^1	12.7×10^1	6.53×10^1
After 1 month of refrigerated storage	6	DW	29.1×10^4[c]	78.1×10^3[c]	11.6×10^4	65.1×10^4	152×10^4	38.5×10^2	36.3×10^3	42.7×10^3	35.7×10^4
		SSW	26.5×10^4	76.2×10^3	11.2×10^4	60.1×10^4	141×10^4	30.6×10^2	28.0×10^3	36.9×10^3	31.2×10^4
	0	DW	21.7×10^3	61.7×10^2	33.6×10^3	50.8×10^3	86.3×10^4	25.5×10^2	38.7×10^2	32.0×10^3	22.3×10^4
		SSW	19.8×10^3	57.7×10^2	33.7×10^3	47.8×10^3	81.3×10^4	22.6×10^2	32.7×10^2	21.1×10^3	21.7×10^4
After 2 months of refrigerated storage	6	DW	16.6×10^6[d]	23.5×10^4[d]	46.9×10^5	11.1×10^5[e]	67.7×10^5[e]	39.6×10^3	30.3×10^4	30.1×10^4	21.5×10^5
		SSW	12.9×10^6	17.7×10^4	43.1×10^5	10.5×10^5	61.1×10^5	35.8×10^3	23.7×10^4	21.7×10^4	17.2×10^5
	0	DW	18.0×10^5	9.33×10^3	24.9×10^5	28.5×10^4	24.2×10^5	28.0×10^3	80.7×10^3	99.8×10^3	91.8×10^6
		SSW	14.3×10^5	6.53×10^3	21.4×10^5	24.4×10^4	21.1×10^5	26.9×10^3	69.2×10^3	79.9×10^3	78.5×10^6
After 3 months of refrigerated storage	6	DW	—	3.4×10^6[e]	—	1.9×10^6[f]	1.1×10^6[g]	41.8×10^5	16.6×10^5	15.5×10^5	64.6×10^6
		SSW	—	4.1×10^6	—	0.5×10^6	1.5×10^6	41.8×10^5	13.1×10^5	14.0×10^5	65.8×146
	0	DW	—	6.9×10^5	—	1.3×10^5	4.6×10^5	30.1×10^5	97.5×10^4	66.0×10^4	11.7×10^4
		SSW	—	6.5×10^5	—	2.1×10^5	1.4×10^5	25.1×10^5	90.8×10^4	61.6×10^4	10.4×100

[a] Average ($\bar{X}$) of five replicate samples plated in triplicate ($X\cdot = 15$) and incubated for 5 days at 20 C. Results expressed as count per g of medium.
[b] DW, distilled water culture medium; SSW, synthetic sea water culture medium.
[c] Count taken after 2 weeks of storage.
[d] Count taken after 3 weeks of storage.
[e] Count taken after 6 weeks of storage.
[f] Count taken after 8 weeks of storage.
[g] Count taken after 10 weeks of storage.

TABLE 10. *Anaerobic (clostridia) macrocolony counts obtained from shucked soft-shelled clams before and after treatment with Co^{60} gamma rays and storage at 6 or 0 C for up to 3 months*[a]

Treatment	Storage temp	Absorbed dose (rad)								
		400,000	450,000	500,000	550,000	600,000	650,000	700,000	750,000	800,000
	C									
Before irradiation		42.1	10.9	15.5	29.1	42.4	11.9	9.07	14.3	34.2
Immediately after irradiation		17.7	4.80	4.80	8.20	15.3	2.00	1.47	2.13	2.53
After 1 month of refrigerated storage	6	11.1[b]	8.87[b]	4.07	14.7	13.1	3.07	3.80	10.7	4.60
	0	9.53	5.87	5.20	9.87	8.07	2.33	3.27	5.33	4.10
After 2 months of refrigerated storage	6	16.5[c]	12.3[c]	7.93	12.2[d]	10.5[d]	4.58	4.33	8.00	5.00
	0	15.3	9.87	7.13	8.27	7.73	3.00	1.73	2.07	1.20
After 3 months of refrigerated storage	6	—	11.07[d]	—	10.5[e]	10.47[f]	2.33	1.47	3.47	6.73
	0	—	7.07	—	8.43	10.00	1.67	1.00	1.80	2.46

[a] Average ($\bar{X}$) of five replicate samples tubed in triplicate ($X\cdot = 15$) and incubated at 30 and 37 C for 40 hr. Results expressed as count $\times$ 10 per g of medium.
[b] Count taken after 2 weeks of storage.
[c] Count taken after 3 weeks of storage.
[d] Count taken after 6 weeks of storage.
[e] Count taken after 8 weeks of storage.
[f] Count taken after 10 weeks of storage.

point, for they indicate that those clostridia remaining after irradiation can be kept at safe, low levels during refrigerated storage.

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