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Radiation Sterilization of Prototype Military Foods: Low-Temperature Irradiation of Codfish Cake, Corned Beef, and Pork Sausage[1]

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"Screening" packs comprising 10 lots each of codfish cake, corned beef, and pork sausage, each lot containing about 10^6 spores of a different strain (five type A and five type B) of *Clostridium botulinum* per can, were irradiated at -30 ± 10 C with a series of increasing doses (20 replicate cans/dose) of ^{60}Co gamma rays. The cans were incubated for 3 months at 30 C and examined for swelling, toxin, and recoverable botulinal cells. Based on the latter criterion of spoilage, median lethal dose (LD_{50}) and *D* values were estimated for each strain in each food. The most resistant strain in codfish cake, corned beef, and pork sausage was, respectively, 53B, 77A, and 41B. There was no clear-cut trend in the comparative order of resistance between the two antigenic types among the three foods. LD_{50} values gave essentially the same order of resistances as the *D* values and may be used interchangeably with the latter for the 10 test organisms. "Clearance" packs consisting of the most resistant strain (about 10^7 spores/can) with its respective food were irradiated with a variety of doses at -30 ± 10 C, using 100 replicate cans/dose (about 10^9 spores/dose). These packs were incubated for 6 months at 30 C and assayed for the three types of spoilage. Based on recoverable cells, the experimental sterilizing doses (ESD) for codfish cake, corned beef, and pork sausage were $2.5 < \text{ESD} \leq 3.0$, $2.0 < \text{ESD} \leq 2.5$, and $1.5 < \text{ESD} \leq 2.0$ Mrad, in that order. Assuming exponential spore death, the 12*D* values, or minimal radiation doses (MRD), were 3.24, 2.44, and 2.65 Mrad, respectively. Estimation of the MRD values by a method which assumes that spore death in the cans follows a normal distribution, yielded 3.09, 2.57, and 2.39 Mrad, respectively. Weibull analyis of the pooled 10-strain viable cell spoilage data of the screening packs for codfish cake or corned beef suggested that spore death in the cans follows a normal distribution yielded 3.09, 2.57, pooled data were not amenable to such analysis. Sublethal doses (0.5, 0.75 Mrad) increased the visible spoilage rate of corned beef over that of unirradiated controls. Apparently radiation-injured spores of *C. botulinum* were sensitized to the presence of food additives such as curing salts, NaCl, and spices.

The U.S. Army has been engaged in a long-range research and development program for the preservation of food products by ionizing radiation. Prototype radiation processes have been reported for bacon (5), ham (3), and pork loin (4). All these foods had been irradiated at ambient temperature, i.e., the center of the can contents, which had an initial temperature of 2 to 5 C, were not permitted to rise above 25 C during irradiation. Numerous investigators (8-10, 12, 13, 23-27, 31, 35, 38, 47, 49) had observed that foods irradiated at freezing temperatures gave higher acceptance scores than foods irradiated at higher temperatures. Although the radiation resistance of the index organism, *Clostridium botulinum*, also increased with a decrease in irradiation temperature (2, 14-22, 28, 44, 45), in-house experience indicated that a reasonable balance between sterilizing dose, organoleptic quality, and process costs was attained at a radiation temperature of about -30 ± 10 C. Hence we concentrated our efforts on the preservation of foods by irradiation at this cryogenic temperature. Codfish cake, corned beef, and pork sausage were the food products initially selected

[1] This paper is no. IV of a series, "Radiation Sterilization of Prototype Military Foods."

for study because of the indicative moderate sterilizing doses, even at low temperatures, with relatively high acceptance characteristics.

Very little information is available on high-dose irradiation of seafood, although doses under 1.0 Mrad have been used extensively to increase their shelf-life (radurization). Data on irradiation of codfish with doses ≥ 1.0 Mrad are even more scarce and of little use for our purpose. Wheaton et al. (48) irradiated codfish inoculated with various strains of *C. botulinum* spores with only 0.85 and 1.7 Mrad. The fish was initially frozen to −29 C and was kept cold in the spent fuel rod source with cold ethanol during irradiation. Instead of incubating the irradiated pack they made survival counts. Their most resistant strain, 12885A, decreased from 5.8×10^6 spores/g to 8.5×10^3/g at the higher dose.

The other investigators of codfish used uninoculated samples. Nickerson et al. (37) reported that they obtained "commercial" sterility with 1.5 Mrep (1.46 Mrad) cathode rays; the irradiation temperature was not indicated. Miyauchi (36) used doses of 0 to 1.86 Mrad and followed survival counts with storage time at 0 to 1.7 C, but he did not give data at doses above 0.7 Mrad. Sinnhuber et al. (41–43) subjected codfish to 4.5 Mrad at ambient temperature for organoleptic quality studies; the only microbiological test they performed was the verification of the absence of *C. botulinum* toxin by the mouse neutralization test.

The available information on the irradiation of pork sausage is also scanty and involved uninoculated samples. Licciardello et al. (34) found that "commercial" sterility was obtained when the food was irradiated to 3.5 Mrep (3.4 Mrad) with cathode rays at −23 C. In a second study, Licciardello et al. (33) exposed samples to 1.08 Mrad and could not recover any bacteria. Although Coleby et al. (11) irradiated the food to 1, 2, 3, and 5 Mrad at ambient temperature and Kirn et al. (30) exposed samples to 1.45 and 2.0 Mrep (1.35 and 1.86 Mrad, respectively) at 1 and at −29 C, no microbiological examinations were included in their studies.

The only irradiation data on corned beef which could be found in the scientific literature was reported by Kirn et al. (30). Again, they were not concerned with the microbiological aspects.

Consequently, practically nothing is known about the radiation resistance of *C. botulinum* spores in these three foods. This paper therefore reports on a prototype radiation sterilization process at −30 ± 10 C for codfish cake, corned beef, and pork sausage using *C. botulinum* type A and B spores as the index of microbiological safety.

MATERIALS AND METHODS

Food preparation. Cod fillet was ground and mixed with 4% white corn meal, 1.5% gelatin, and 0.5% NaCl (noniodized). Approximately 2,268-g (5 lb) lots of the mixture were stuffed into casings (Visking, 3.5 C), clip-sealed, cooked in boiling water to a minimal internal temperature of 71.1 C (about 30 min) to inactivate food enzymes, wrapped in clean wax paper, and chilled overnight at 3 to 5 C.

Raw brisket (corned beef) was pumped to 110% green weight with an aqueous solution of 25.10% NaCl (noniodized), 0.35% $NaNO_3$, and 0.13% $NaNO_2$, then was covered with this pickle cure for 24 hr, autoclaved at 104 C to the desired tenderness (about 90 min), wrapped in clean wax paper, and chilled overnight at 3 to 5 C.

Pork was ground and mixed with 1.88% NaCl (noniodized), 0.22% black pepper, 0.094% ginger, 0.31% sage (well rubbed), 0.38% sucrose, and 3% chipped ice, stuffed in natural sheep casing to make 28.35-g (1 oz) links, baked at 204.4 C to a minimal internal temperature of 71.1 C, wrapped in clean wax paper, and chilled overnight at 3 to 5 C.

The three foods were diced (after removing the casings), packed in 45 ± 5 g quantities into 211 by 101.5 C-enamel metal cans, and loosely closed with lids, leaving a 0.6-cm (¼ inch) headspace per can. Sanitary precautions were followed throughout the handling operations, including the prior autoclaving of the cans, lids, and other food handling equipment for 10 min at 5 psi.

Proximate chemical analysis on uninoculated unirradiated random cans of food were performed. Each food product consisted of 10 samples, four cans per sample, and was assayed in duplicate. Average results are tabulated in Table 1.

Test organisms, inoculation, can sealing. The 10 strains of *C. botulinum* spores, the method of inoculating the foods, and the sealing of the cans were previously described (3). The sealed cans were then placed in a −23 C room to await irradiation.

Irradiation. A "screening" and a "clearance" inoculated pack were prepared for each food product. The first pack consisted of 20 replicate cans per dose per strain and was irradiated in the range 0.5 to 4.0 Mrad in increments of 0.25 Mrad. This pack selected the most radiation resistant strain of the 10 tested. The second pack contained 100 replicate cans/dose inoculated with the most resistant strain. Codfish cake was irradiated in the range 1.0 to 4.5 Mrad, corned beef from 1.0 to 4.0 Mrad, and pork sausage 0.5 to 3.5 Mrad in units of 0.5 Mrad. The latter pack provided partial spoilage data from which the "12*D*", or minimal radiation dose (MRD), was estimated.

Irradiation was conducted with ^{60}Co gamma rays at −30 ± 10 C. The irradiation temperature was

TABLE 1. *Chemical analysis[a] of some enzyme-inactivated food products used for irradiation studies*

Food product[b]	Constituent							
	Protein (%)	Fat (%)	Moisture (%)	NaCl (%)	Brine[c] (%)	$NaNO_3$ (ppm)	$NaNO_2$ (ppm)	pH
Codfish cake	20.36	0.23	75.11	0.60	0.79			6.8
Corned beef	14.10	23.19	59.05	2.78	4.50	207	6.58	6.3
Pork sausage	18.31	35.22	40.59	2.42	5.63			6.1

[a] In accordance with *Official Methods of Analysis*, Association of Official Agriculture Chemists, 1965.
[b] Duplicate determinations on 10 random uninoculated, unirradiated samples. Each sample consisted of the entire pooled contents of four replicate cans.
[c] Per cent brine = (per cent NaCl/ per cent NaCl + per cent moisture) × 100.

controlled and monitored continuously as described by Jarrett (29).

Assay for spoilage. The screening packs were incubated for 3 months and the clearance peaks for 6 months at 30 C and examined for swelling and for the presence of *C. botulinum* toxin and viable cells as cited earlier (3).

Calculation of radiation resistance. The 12*D* or its equivalent (MRD) was estimated by three independent statistical methods. (i) Assuming exponential spore death in the cans, *D* values were computed both by the conventional Schmidt-Nank formula (40):

$$D = \frac{\text{radiation dose (Mrad)}}{\log M - \log S}$$

and by the Spearman-Kärber equation (6):

$$LD_{50} = t_u + \frac{d}{2} - d \sum_{i=1}^{u} P_i$$

followed by (39):

$$\frac{LD_{50}}{D = \log A - \log 0.69}$$

The MRD was calculated as $D \times 12$. (ii) Assuming, on the other hand, that the spore death rate follows a normal distribution, the 12*D* equivalent was found by the Anellis-Werkowski equation (7):

$$x_{12D} = \bar{x} + SZ_\alpha$$

(iii) Also, whenever spoilage data was adequate, the MRD was computed by the Wiebull expression (6):

$$F(x) = 1 - \exp\left[-\left(\frac{x-\alpha}{\eta}\right)^\beta\right]$$

which makes no assumption about the shape of the death rate curve.

RESULTS

Comparative radiation resistance of *C. botulinum* strains. The severest criterion of spoilage of irradiated cans of food is the presence of recoverable *C. botulinum* cells. Swollen cans always had toxin and viable cells; flat cans frequently contained both toxin and recoverable cells; nonswollen nontoxic cans occasionally harbored dormant survivors, whereas sterile cans were always flat and nontoxic. Hence, Table 2 provides only viable cell spoilage data for the three foods with each of the 10 strains at all radiation levels, as well as Schmidt-Nank *D* values based upon these data. Interestingly, the *D* values increased with increasing dosage in each food. This deviation from a nonlinear *D* value response was also observed with irradiated pork (4) and several other foods (6).

This preliminary pack also indicated that the experimental sterilizing dose (ESD) for codfish cake was 2.75 < ESD ≤ 3.0 Mrad, for corned beef was 2.0 < ESD ≤ 2.25 Mrad and for pork sausage was 1.63 < ESD ≤ 1.90 Mrad.

Information in Table 2 was used to determine the comparative resistances of the 10 botulinal strains in the three foods. Table 3 lists the LD_{50} and *D* values, and their confidence intervals, of each strain estimated by the Spearman-Kärber (6) and Schmidt (39) expressions, respectively. The Schmidt-Nank formula was not employed due to the dose-dependent *D* values mentioned above. Spores of strain 53B were the most radiation resistant in codfish cake ($D = 0.331 \pm 0.019$), 77A in corned beef ($D = 0.262 \pm 0.011$), and 41B in pork sausage ($D = 0.123 \pm 0.012$). The order of decreasing resistances of the 10 strains in the three foods is indicated in Table 4.

MRD. Clearance pack data reflecting the three types of spoilage for each food are presented in Table 5. For codfish cake, the sterilizing doses were identical for the three

TABLE 2. *Effect of* ^{60}*Co irradiation on spoilage of some food products inoculated with Clostridium botulinum spores*

Strain no.	Radiation dose (Mrad)	No. of cans out of 20 with recoverable *C. botulinum*					
		Codfish cake		Corned beef		Pork sausage[a]	
		Spoiled cans	*D* value[b]	Spoiled cans	*D* value	Spoiled cans	*D* value
33A	0	20		18		20	
	0.5	20		17	0.078	13	0.091
	0.75	18	0.126	9	0.112	5	0.127
	1.0	13	0.163	10	0.150	0	
	1.25	17	0.208	1	0.164	0	
	1.5	0		0		0	
	1.75	1	0.242	0		0	
	2.0–4.0[c]	0		0		0	
36A	0	20		20		20	
	0.5	20		20		8	0.085
	0.75	20		18	0.120	3	0.119
	1.0	20		17	0.159	0	
	1.25	14	0.204	3	0.177	0	
	1.5	3	0.220	0		0	
	1.75	1	0.240	0		0	
	2.0–4.0	0		0		0	
62A	0	20		20		20	
	0.5	20		20		1	0.076
	0.75	20		20		0	
	1.0	19	0.163	20		0	
	1.25	4	0.184	20		0	
	1.5	0		13	0.224	0	
	1.75	0		3	0.238	0	
	2.0–4.0	0		0		0	
77A	0	20		20		20	
	0.5	20		20		6	0.083
	0.75	20		20		3	0.118
	1.0	20		20		4	0.162
	1.25	19	0.203	20		1	0.185
	1.5	10	0.233	16	0.230	1	0.221
	1.75	1	0.235	11	0.262	0	
	2.0	0		1	0.259	0	
	2.25–4.0	0		0		0	
12885A	0	20		20		20	
	0.5	20		20		1	0.073
	0.75	20		20		0	
	1.0	20		17	0.157	0	
	1.25	13	0.197	4	0.178	0	
	1.5	2	0.209	0		0	
	1.75	1	0.234	0		0	
	2.0–4.0	0		0		0	
9B	0	20		20		20	
	0.5	20		20		10	0.088
	0.75	20		20		0	
	1.0	19	0.163	20		0	

[a] Due to accidental improper handling of the cans in the irradiation source, the doses delivered were in increments of 0.27 Mrad from an initial dose of 0.55 Mrad.

[b] Computed by the Schmidt-Nank equation D = dose/(log M − log S) (40).

[c] Doses increase in 0.25 Mrad increments.

TABLE 2—*Continued*

Strain no.	Radiation dose (Mrad)	No. of cans out of 20 with recoverable *C. botulinum*					
		Codfish cake		Corned beef		Pork sausage[a]	
		Spoiled cans	*D* value[b]	Spoiled cans	*D* value	Spoiled cans	*D* value
	1.25	8	0.192	19	0.207	0	
	1.5	1	0.202	11	0.239	0	
	1.75	1	0.236	0		0	
	2.0–4.0	0		0		0	
40B	0	20		12		3	
	0.5	20		17	0.078	1	0.077
	0.75	20		20		0	
	1.0	20		14	0.153	0	
	1.25	11	0.198	2	0.170	0	
	1.5	5	0.225	0		0	
	1.75	2	0.248	0		0	
	2.0	3	0.290	0		0	
	2.25–4.0	0		0		0	
41B	0	20		20		20	
	0.5	20		20		18	0.088
	0.75	20		19	0.120	17	0.131
	1.0	20		18	0.160	18	0.176
	1.25	20		11	0.193	2	0.190
	1.5	13	0.238	0		1	0.218
	1.75–4.0	0		0		0	
51B	0	20		20		20	
	0.5	6	0.073	17	0.078	16	0.090
	0.75	0		2	0.102	6	0.125
	1.0	0		1	0.130	3	0.161
	1.25	0		1	0.163	0	
	1.5–4.0	0		0		0	
53B	0	20		20		2	
	0.5	20		20		0	
	0.75	20		20		1	0.117
	1.0	20		14	0.154	1	0.157
	1.25	20		11	0.190	0	
	1.5	17	0.240	0		0	
	1.75	12	0.273	0		0	
	2.0	10	0.308	0		0	
	2.25	7	0.339	0		0	
	2.5	7	0.376	0		0	
	2.75	5	0.405	0		0	
	3.0–4.0	0		0		0	

spoilage criteria (2.5 < ESD ≤ 3.0 Mrad). At 2.5 Mrad, the 99 flat cans were also nontoxic and sterile, but at 2.0 Mrad 57 cans contained recoverable botulinal cells, whereas only 44 cans were swollen and toxic.

The sterilizing dose for corned beef was 2.0 < ESD < 2.5 Mrad; at 2.0 Mrad, none of the 100 cans were swollen or toxic, but one can harbored viable *C. botulinum*. At 1.5 Mrad, 53 cans yielded the three kinds of spoilage.

A population of 2.75×10^9 spores of strain 41B in pork sausage per dose was destroyed by only 1.5 < ESD ≤ 2.0 Mrad. At 1.5 Mrad, 80 cans exhibited the three spoilage criteria, whereas an additional six flat nontoxic cans contained viable but dormant botulinal cells.

According to the most stringent spoilage criterion, the MRD for the three food products was estimated both on the basis of exponential and normal distribution death kinetics (Table 6). Codfish cake had an MRD of 3.24 and 3.09 Mrad, corned beef had an MRD of 2.44 and 2.57 Mrad, and pork sausage had an MRD of 2.65 and 2.39 Mrad, respectively.

TABLE 3. *Comparative radiation resistance of strains of Clostridium botulinum spores inoculated in some food products*

Strain no.	Radiation values based on recoverable *C. botulinum* from					
	Codfish cake		Corned beef		Pork sausage	
	LD_{50}[a]	D value[b]	LD_{50}	D value	LD_{50}	D value
33A	1.237 ± 0.077	0.203 ± 0.013	0.837 ± 0.090	0.129 ± 0.014	0.658 ± 0.071	0.109 ± 0.012
36A	1.350 ± 0.068	0.220 ± 0.011	1.100 ± 0.064	0.172 ± 0.010	0.563 ± 0.037	0.090 ± 0.006
62A	1.162 ± 0.050	0.186 ± 0.008	1.575 ± 0.065	0.236 ± 0.010	0.428 ± 0.024	0.070 ± 0.004
77A	1.500 ± 0.064	0.238 ± 0.010	1.725 ± 0.074	0.262 ± 0.011	0.617 ± 0.091	0.098 ± 0.013
12885A	1.325 ± 0.066	0.209 ± 0.010	1.137 ± 0.059	0.176 ± 0.009	0.428 ± 0.024	0.066 ± 0.004
9B	1.362 ± 0.068	0.217 ± 0.011	1.500 ± 0.060	0.243 ± 0.009	0.550 ± 0.055	0.090 ± 0.090
40B	1.388 ± 0.089	0.223 ± 0.014	0.737 ± 0.072	0.120 ± 0.011	0.428 ± 0.026	0.071 ± 0.004
41B	1.538 ± 0.052	0.245 ± 0.008	1.225 ± 0.070	0.192 ± 0.011	1.171 ± 0.083	0.184 ± 0.013
51B	0.450 ± 0.050	0.070 ± 0.012	0.637 ± 0.061	0.098 ± 0.009	0.752 ± 0.079	0.122 ± 0.013
53B	2.100 ± 0.123	0.331 ± 0.019	1.187 ± 0.074	0.183 ± 0.011	0.442 ± 0.036	0.076 ± 0.006

[a] Computed by the Spearman-Karber equation $LD_{50} = t_u + d/2 - d \sum_{i=1}^{u} P_i$ (6).
[b] Computed by the Schmidt equation $D = LD_{50}/(\log A - \log 0.69)$ (39).

TABLE 4. *Order of radiation resistance of strains of Clostridium botulinum spores inoculated in some food products*

Order of decreasing resistance	Codfish cake		Corned beef		Pork sausage	
	LD_{50}	D	LD_{50}	D	LD_{50}	D
1	53B	53B	77A	77A	41B	41B
2	41B	41B	62A	9B	51B	51B
3	77A	77A	9B	62A	33A	33A
4	40B	40B	41B	41B	77A	77A
5	9B	36A	53B	53B	36A	36A, 9B
6	36A	9B	12885A	12885A	9B	—
7	12885A	12885A	36A	36A	53B	53B
8	33A	33A	33A	33A	62A, 12885A, 40B	62A, 40B
9	62A	62A	40B	40B	—	—
10	51B	51B	51B	51B	—	12885A

Effect of sublethal doses on spoilage. Irradiation of the corned beef screening pack to 0.5 Mrad evoked a remarkable increase in the rate of visible spoilage over the unirradiated controls (Fig. 1). The effect manifested itself within 2 weeks of incubation of cans containing strains 62A, 77A, 12885A, 9B, 40B, and 41B. It required 8 to 12 weeks of incubation for the controls to equal the number of spoiled cans produced by 0.5 Mrad; however, control cans with strain 40B never did overtake the 0.5-Mrad cans. A dose of 0.75 Mrad caused an even more notable increase in the spoilage rate of cans inoculated with strains 40B and 41B than when irradiated with 0.5 Mrad; the remainder of the test organisms (33A, 36A, 51B, 53B) were unaffected by these low doses. At doses above 0.75 Mrad, unirradiated cans equaled or exceeded the irradiated spoilage response. Codfish cake and pork sausage did not undergo this phenomenon.

DISCUSSION

It was observed earlier that a cured (3) and an uncured pork product (4) yielded D values which increased with increasing radiation doses when estimated by the Schmidt-Nank method. Similar findings were made in this investigation with an uncured fish product of low brine content (0.79%), with corned beef of high brine concentration (4.50%) and containing curing salts, and with pork sausage which had a higher level of brine (5.63%) and containing spices instead of curing salts (Table 2). Thus, the types of irradiated foods which gave dose-dependent D values are sufficiently diverse as to make one wary of the Schmidt-Nank statistical procedure. The substitute D value calculation employed herein has no such defect.

D values are commonly used to compare resistances between organisms subjected to identical conditions of physical or chemical stress. Table 4 indicates that LD_{50} values may be employed as an alternative technique to determine comparative radiation resistances between strains of *C. botulinum* spores in different foods. The order of decreasing resistances obtained for the 10 strains by the LD_{50} and *D* value estimates are almost identical in the three foods. Since the *D* value assumes exponential death kinetics, and the Spearman-Kärber LD_{50} does not assume the shape of the dose-response data (6), we find the latter more attractive for ascertaining relative resistances between organisms. Lewis (32) and Vas (46) also evinced a preference for the LD_{50} (or ED_{50}), which they used for evaluating thermal resistance processes.

TABLE 5. *Experimental sterilizing dose (ESD) for some food products inoculated with spores of Clostridium botulinum*

Strain	Spores/ dose[a] (× 10⁹)	Radiation dose[b] (Mrad)	No. of cans out of 100		
			Swollen	With toxin	With recoverable *C. botulinum*
		Codfish cake			
53B	2.48	1.0	100	100	100
		1.5	100	100	100
		2.0	44	44	57
		2.5	1	1	1
		3.0-4.5[c]	0	0	0
		Corned beef			
77A	2.47	1.0	54	54	100
		1.5	53	53	53
		2.0	0	0	1
		2.5-4.0	0	0	0
		Pork sausage			
41B	2.75	0.5	100	100	100
		1.0	100	100	100
		1.5	80	80	86
		2.0-3.5	0	0	0

[a] Spores/can × no. of cans/dose.
[b] Dose rate 3.00×10^4 rads/min.
[c] Doses increase in 0.5-Mrad increments.

None of the inoculated packs provided sufficient data for analysis by the Weibull distribution. Nevertheless, to determine whether additional useful information may be extracted from the spoilage data at hand, the screening pack viable cell results obtained with each strain in a food were pooled as if every can subjected to a specific dose reflected a mixture of all 10 strains (Table 7). The resulting data were amenable to statistical treatments by the Weibull, normal and exponential distribution methods (Table 8), and indeed had prediction value for the outcome of the codfish cake and corned beef clearance packs, but not for pork sausage.

The ESD of the pooled screening (Table 8) and the clearance (Table 6) packs for the three foods were remarkably close. The MRD computed by the exponential procedure for the accumulated screening, and clearance, packs did not agree, but the MRD values estimated by the normal distribution were surprisingly close for both codfish cake and corned beef (3.32 and 3.09, and 2.75 and 2.57 Mrad, respectively). The Weibull MRD values for these two foods agreed very closely with the normal calculations. The Weibull technique could not be used with the pork sausage pooled data since the plotted points were all above the 50% sterility level.

The Weibull equations for the combined codfish cake and corned beef data are

$$F(x) = 1 - \exp\left[-\left(\frac{x}{1.5}\right)^{3.1}\right]$$

and

$$F(x) = 1 - \exp\left[-\left(\frac{x + 4.5}{1.8}\right)^{4.8}\right]$$

TABLE 6. *Minimal radiation dose (MRD) for some food products inoculated with Clostridium botulinum spores*

Food product	Strain	Experimental sterilizing dose (ESD)	Method of estimating 12D dose[a]	
			Exponential distribution[b]	Normal distribution[c]
Codfish cake	53B	$2.5 < ESD \leq 3.0$	3.24	3.09
Corned beef	77A	$2.0 < ESD \leq 2.5$	2.44	2.57
Pork sausage	41B	$1.5 < ESD \leq 2.0$	2.65	2.39

[a] Based on recoverable cells.
[b] Computation of LD_{50} followed by $12[D = LD_{50}/(\log A - \log 0.69)]$.
[c] Computation by $x_{12D} = \bar{x} + SZ_{\alpha}$.

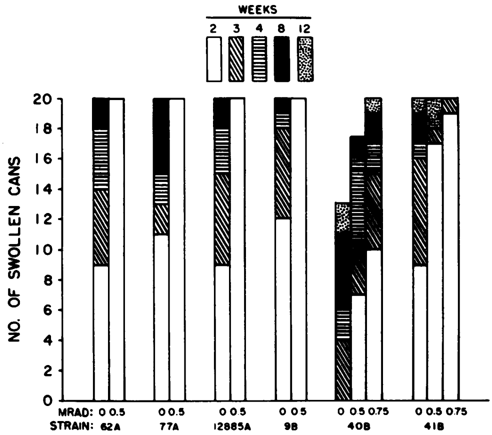

FIG. 1. *Effect of sublethal radiation doses on swelling of cans of corned beef inoculated with Clostridium botulinum. Results represent 3 months of incubation at 30 C.*

TABLE 7. *Cumulative viable cell spoilage data of irradiated food products inoculated with Clostridium botulinum spores*

Radiation dose (Mrad)	No. of cans of spoiled food[a]		
	Codfish cake[b]	Corned beef[c]	Pork sausage[d]
0	200	190	165
0.5	186	191	74
0.75	178	168	35
1.0	171	151	26
1.25	126	92	3
1.5	51	40	2
1.75	19	14	0
2.0	13	1	0
2.25	7	0	0
2.5	7	0	0
2.75	5	0	0
3.0–4.0[e]	0	0	0

[a] The spoiled cans with all 10 strains in each food were pooled.

[b] 1.13×10^7 pooled spores/can, or 2.26×10^9 spores/dose.

[c] 2.24×10^7 pooled spores/can, or 4.48×10^9 spores/dose.

[d] 1.09×10^7 pooled spores/can, or 2.18×10^9 spores/dose. The lowest dose is 0.55 Mrad and then increases in 0.27-Mrad increments (see Table 2, footnote *a*).

[e] Doses increase in 0.25-Mrad levels except for pork sausage, which increase 0.27-Mrad steps.

respectively. The shape parameter β (3.1, 4.8) indicates that the death rate of the spores in the two foods reflects a normal, or related, type of distribution, and is quite remote from exponential death whose β is near 1.0. Perhaps that is the reason why the predicted exponential MRD values (Table 8) did not agree with the exponential MRD values of the clearance packs (Table 6).

Of the three foods, only corned beef exhibited accelerated spoilage with low dose (0.5, 0.75 Mrad) irradiation (Fig. 1). High brine content did not seem to be the primary cause of this activity, since pork sausage was higher in brine concentration (5.63%) than the corned beef (4.50%). Apparently the curing salts ($NaNO_2$, $NaNO_3$), either in the presence or absence of NaCl, were the causative agents in the increased rate of spoilage. Rowley et al. (Bacteriol. Proc., p. 64, 1971) also observed enhanced recovery of irradiated *C. botulinum* spores (strain 62A) in the presence of $NaNO_2$. This phenomenon also occurred in irradiated cured ham (3).

The influence of food substrate on the radiation resistance of bacteria is demonstrated in Table 4. Whereas strain 53B was the most resistant of the 10 organisms tested in codfish cake, it was fifth in the order of decreasing resistance in corned beef and seventh in pork sausage. On the other hand, strain 77A was the most resistant in corned beef and strain 41B was the hardiest in pork sausage. These results emphasize the need to screen a number of test organisms in each food prior to determining a prototype radiation process, rather than use an arbitrarily selected single index organism.

Codfish cake, the only food product of the three tested which had no chemical additives of significance (Table 1), had the highest MRD. The two foods which contained additives (curing salts, spices, relatively high brine levels) had notably lower MRD values than the fish (Tables 6, 8). That radiation- or heat-injured bacteria are more sensitive to curing agents and NaCl is well documented. That certain spices also enhance the radiation death of *C. botulinum* spores was observed by Anderson et al. (1), but this phenomenon is not as widely recognized. Although Huber et al. (26) found that spices protected foods against deleterious radiation changes by acting as chemical interceptors of free radicals, they did not investigate the ability of spices to sensitize bacteria. Our data indicate that the proper combination of spices with high brine content were at least as effective, if not more so, in reducing the MRD of pork sausage as were the curing agents with NaCl in lowering the MRD of corned beef (Tables 6, 8). Investigations involving spices and NaCl individually, and in combination, would help elucidate the role of these additives on *C. botulinum* in the radiation process.

TABLE 8. *Predicted minimal radiation dose (MRD) for some food products inoculated with Clostridium botulinum spores*[a]

Food product	Experimental sterilizing dose (ESD)	Method of estimating 12*D* dose		
		Exponential distribution	Normal distribution	Weibull distribution
Codfish cake	2.75 < ESD ≤ 3.00	2.21	3.32	3.29
Corned beef	2.00 < ESD ≤ 2.25	1.95	2.75	2.50
Pork sausage	1.63 < ESD ≤ 1.90	0.93	1.89	—[b]

[a] Based on recoverable cells from the screening packs. The data from all 10 strains/food were pooled.
[b] Insufficient data for computation.

The prototype radiation process, based upon the computation of a 12*D* equivalent by the normal distribution method (for reasons discussed above), using the clearance pack data (Table 6), is 3.1, 2.6 and 2.4 Mrad for codfish cake, corned beef, and pork sausage, respectively, when irradiated at −30 ± 10 C.

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