Gamma Ray Sterilization of Canned Meat Previously Inoculated with Anaerobic Bacterial Spores[1]

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Before gamma irradiation can be evaluated as a technique for preserving food in tin cans, it is necessary that the amount of radiation required for sterilization be known. Although information is available concerning the sensitivity of bacterial spores in buffers (Proctor and Goldblith, 1951; Lawrence *et al.*, 1953), such conditions are quite different from those existing in tin cans. It therefore seemed desirable to study the effectiveness of gamma radiation for killing the spores of anaerobic, food-spoilage bacteria that were inoculated into meat packed in No. 2 tin cans; data presented here correlate the quantity of gamma radiation from cobalt-60 required to produce sterility with the numbers of such spores present in the meat.

MATERIALS AND METHODS

Three anaerobic, spore-forming bacteria were used in this study. Putrefactive anaerobe no. 3679 came from the National Canner's Association Research Laboratory, while *Clostridium botulinum*, strains 62A and 213B, were furnished by the Hooper Foundation for Medical Research at the University of California. Spore suspensions were prepared according to procedures described by Reed *et al.* (1951); putrefactive anaerobe no. 3679 was grown in pork infusion broth, but Difco bacto-casitone was substituted for casein digest in the medium, specified by these workers, for clostridia. Stock spore suspensions were suspended in sterile distilled water and stored at 4 C. Samples of these suspensions were tested for heat resistance by C. W. Bohrer of the National Canner's Association using the technique of Esty and Williams (1924). The results of his tests indicated that the spores were typical of those used to determine adequate heat processes for canned foods. Appropriate dilutions for inoculation into canned meat were prepared after counting the viable spores present in the stock suspensions (Reed *et al.*, 1951). For this purpose, 0.1 per cent soluble starch was incorporated into the pork agar medium to aid germination of the spores (Wynne and Foster, 1948).

All samples for irradiation were prepared from frozen ground beef. The meat was defrosted, placed in shallow pans, and cooked for 30 min at 15 pounds steam pressure. No. 2 tin cans[2] were then filled within ¼ in of the top with hot meat, covered loosely by can lids, and sterilized at 121 C for 60 min. Following this, individual cans were removed from the autoclave as needed; their covers were aseptically lifted, and 1 ml of a properly diluted spore suspension was injected into the geometrical center of the meat. This method of inoculation, of course, did not result in uniform spore distribution throughout the meat but rather concentrated them in the center of the can. Finally, the cans were sealed in a Western Type closing machine. Since the meat was still at a temp of about 95 C, the cans were immersed in running tap water for 30 min, which cooled the meat to an average temp of 20 C and produced a vacuum of about 28 in of mercury in the cans. The canned meat then continued to cool to the radiation room temp of 5 C.

For irradiation, one can was placed on top of a second, and the two were taped together at the junction. These pairs of cans were then placed on turntables that rotated at 1 rpm and which were set around the periphery of the cobalt-60 radiation source in such positions that the central axes of the cans were 2½ in from the outside shield. When these experiments were initiated, the radiation field delivered a max dosage of 85,500 rep[3] per hr to meat in the center of the cans; thus, a dosage of 2,000,000 rep required an exposure of 23.4 hr. However, the intensity of the field decayed somewhat during the course of the work.

Radiation equipment and calibration data used in this study were described by Nehemias *et al.* (1954), and Lewis *et al.* (1954) respectively. For routine purposes, the dosage of gamma radiation was calculated as a function of can position in the radiation field. The calculation was based on a value of 10.5 in for the half-thickness of meat in a tin can and the data of Lewis, *et al.* (1954) for this source of cobalt-60 gamma radiation. The half-thickness value had previously been measured in this laboratory. In addition, dosages of gamma radiation actually delivered to the meat inside the cans were checked using ferrous sulfate dosimetry

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[2] The outside dimensions of the cans are 3 7/16 by 4 9/16 inches.

[3] One rep unit is a dose of ionizing radiation capable of producing energy absorption of 93 ergs per gram of tissue.

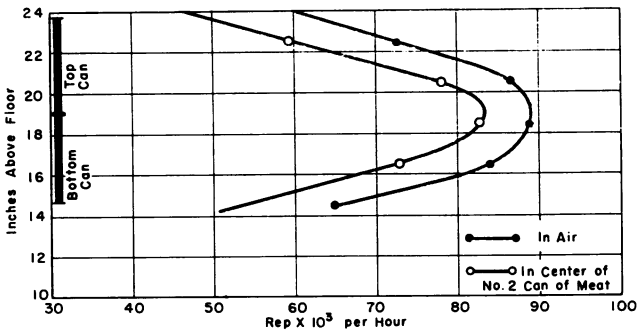

FIG. 1. Ferrous sulfate dosimetry values in air and canned hamburger at various elevations and at a distance of 2½ in. from the outside shield of the cobalt-60 radiation source.

techniques (Weiss, 1952). For this purpose, glass vials 2 in high, ½ in outside diameter, and about 1/32 in thick were filled with a ferrous ammonium sulfate solution. The tubes were wrapped in cellophane and positioned in the meat on the vertical axes of the cans which in turn were placed on turntables set around the cobalt-60 source as previously described. After irradiation for 15 min, the vials were removed, and the solution was analyzed for ferric iron with a spectrophotometer. Dosimetry values were obtained in air by locating vials of ferrous ammonium sulfate solution vertically, one above the other, also at a distance of 2½ in from the outside shield. Results of a calibration, including both kinds of data, are shown in figure 1. It will be noted that the intensity of the radiation varied with elevation along the vertical center line of the two cans of meat which were stacked, one on top of the other. Although the sterilizing values reported in this paper are max levels reaching the cans in question, spores were injected into the geometrical center of the meat where the dosage of gamma radiation was about 7 per cent below the max; min dosages received in the cans were considerably lower.

Following irradiation, the cans of meat were incubated at 37 C. Some of those that swelled were covered with a cotton pad, punched, and the odor of the escaping gas was noted. Other swelled cans were aseptically opened and subcultured. For this purpose, approximately 5 g samples were removed and transferred to each of three test tubes of veal infusion medium (Cameron, 1937) containing 0.1 per cent soluble starch. Confirmation included a second transfer to veal infusion medium and, in some cases, to pork infusion agar; the latter was overlayed with 2 per cent plain agar and this, in turn, with petrolatum. A similar procedure was followed for cans that did not swell except that they were removed at intervals after storage.

It was of interest to know whether the Clostridia that grew in the meat produced toxin. For this purpose, 50 g of meat were taken from a swollen can and placed along with 100 ml of physiological saline in a sterile 250-ml Erlenmeyer flask containing glass beads and sand. These were agitated together on a shaker that rotated at 350 rpm on a ½ in radius. The extract was centrifuged, the supernatant liquid passed through a Seitz filter, and 0.1 ml of the filtrate injected intraperitoneally into each of 5 mice. For controls, a portion of the filtrate was boiled 2 min before it was similarly injected into 5 mice.

RESULTS AND DISCUSSION

Tables 1 and 2 show the fractions of groups of cans that swelled during incubated storage at 37 C following inoculation with anaerobic spores and irradiation with gamma rays from cobalt-60. On being opened, those cans that swelled liberated putrid odors, and the meat

TABLE 1. *Development of gas in No. 2 cans of meat following inoculation with various bacteria and irradiation*

Gamma Ray Treatment* REP × 10⁶	Inoculum†		
	Clostridium botulinum, strain 62 A	*Clostridium botulinum*, strain 213 B	Putrefactive anaerobe no. 3679
	Fraction of cans developing gas‡		
Control B§	0/4	0/2	0/5
Control H¶	5/5	2/2	2/2
2.0	2/2		2/2
2.5	2/2	2/2	0/4
3.0	3/4	1/2	0/4
3.5	2/4	0/4	0/2
4.0	0/4	0/2	
4.5	0/4	0/2	
5.0	0/2	0/2	

* Irradiation with cobalt-60.
† Inoculated with 40,000 bacterial spores per gram.
‡ Incubation at 37 C for a period of at least 1 month.
§ Control B = Uninoculated and unirradiated.
¶ Control H = Inoculated but not irradiated.

TABLE 2. *Development of gas in No. 2 cans of meat following inoculation with different amounts of spores of* Clostridium botulinum *62A and irradiation.*

Gamma Ray Treatment*†† REP × 10⁶	Number of Spores of *Clostridium botulinum* 62A per Gram of Meat					
	0.4	4	40	400	4,000	40,000
	Fraction of cans developing gas†					
Control B‡	0/3	0/4	0/5	0/2	0/4	0/5
Control H§	3/3	5/5	3/3	4/4	3/4	2/2
0.5	2/2					
1.0	2/2	2/2				
1.5	4/4	2/2	4/4			
2.0	1/4	1/4	2/2	2/2	4/4	2/2
2.5	0/4	0/4	4/6	2/2	2/4	2/2
3.0	0/2	0/2	0/2	2/6	1/4	3/4
3.5	0/4	0/4	0/4	0/2	0/4	2/4
4.0		0/2	0/4	0/4	0/4	0/4
4.5				0/4	0/2	0/4
5.0					0/2	0/2

*†† Irradiation with cobalt-60.
† Incubation at 37 C for a period of at least 1 month.
‡ Control B = Uninoculated and unirradiated.
§ Control H = Inoculated but not irradiated.

was foamy. When the meat was subcultured, the organisms recovered in every instance were the same as those originally inoculated into the meat. The controls showed that the meat was sterile before inoculation.

Those cans that did not swell were still under vacuum when removed from the incubator and sampled. The meat had the normal appearance of cooked hamburger but had a slight odor that is common to cooked meat that has been subsequently irradiated. Sampling of these unswollen cans has been carried out over a period of 6 months but has not yet been finished. The extended incubation period was designed to detect delayed germination if it should appear. Subcultures made from the meat indicated that those cans were sterile which had not swollen after 1 month's incubation at 37 C. Although an occasional contaminant was found in one of the three tubes of veal infusion medium inoculated from each can, the kind of organism originally inoculated into the heat-sterilized meat was never recovered from subculture tubes of cans that did not swell.

Spores of *C. botulinum*, strain 62A, are slightly more resistant to gamma radiation than those of *C. botulinum*, strain 213B, (table 1). Also, spores of putrefactive anaerobe no. 3679 are more easily killed than those of either strain of the toxin-producing clostridia.

Table 2 and figure 2 show that the amount of gamma radiation required to sterilize meat varies linearly with the log of the spore concentration. Thus, 2,500,000 rep were required to sterilize meat in which 0.4 *C. botulinum*, strain 62A, spores were present per g of meat, while 4,000,000 rep were needed when the concentration was 40,000 such spores per g of meat.

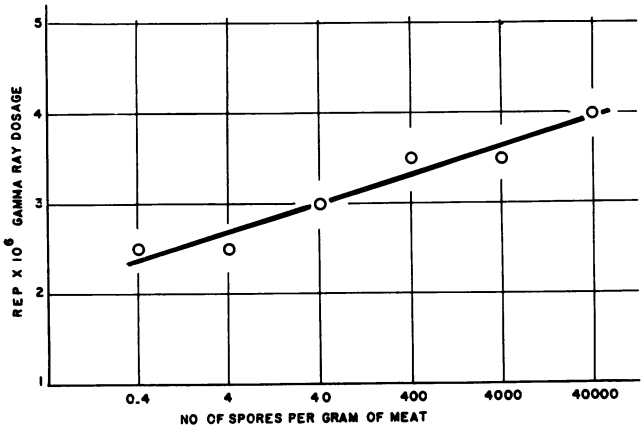

FIG. 2. The dosage of gamma radiation from cobalt-60 required to sterilize canned hamburger containing spores of *Clostridium botulinum*, strain 62A.

Toxin production was established for both strains of *C. botulinum* by the mouse inoculation test using cans which were selected at random from among those that developed gas during regular experiments.

All the irradiation experiments reported in this paper were carried out under refrigeration since the work was done during the winter when the radiation room temp was about 5 C. In this manner, the possibility of spore germination during irradiation was eliminated as a factor that might otherwise have affected our results.

SUMMARY

The sterility dosage for canned beef increased from 2,500,000 to 4,000,000 rep of cobalt-60 gamma radiation as the concentration of spores of *Clostridium botulinum*, strain 62A, increased from 0.4 to 40,000 per g of meat; using 40,000 spores of *C. botulinum*, strain 213B or an equal no. of putrefactive anaerobe no. 3679 spores per g of meat, sterilization occurred at 3,500,000 and 2,500,000 rep respectively.

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