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Progress Report

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

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SUMMARY

Based upon limited data, it appears that the preservative chemicals in pork luncheon meat potentiate the lethal action of gamma radiation for anaerobic bacterial spores. This tentative finding should be tested by further studies since it appears possible that commercial sterility may be attained in pork luncheon meat at dosages not very much greater than one megarad of gamma radiation when inocula of 1,500,000 C. botulinum spores are used per can. Studies are continuing on the radiation sterilization dosage required for canned ground beef inoculated with approximately one million anaerobic bacterial spores per gram.

Investigation has begun of the possible presence of latent bacterial spores or botulinus toxin in canned meat that was radiation-sterilized some years ago and incubated at room temperature during the interval.

PHASE I

EFFECT OF PREIRRADIATION OF CANNED GREEN PEAS ON THE F_o SUBSEQUENTLY REQUIRED FOR STERILIZATION

A paper covering this phase of the work was submitted to the QMC during this reporting interval for publication clearance.

PHASE II

DETERMINATION OF RADIATION STERILIZATION DOSE FOR CANNED MEAT

This was discussed in detail in the previous report and has not changed materially since that time.

PHASE III

DETERMINATION OF COMBINED IRRADIATION-HEAT PROCESSING TREATMENTS REQUIRED TO STERILIZE CANNED PORT LUNCHEON MEAT

The refrigerated storage lives of pork luncheon meat, bacon, etc., are considerably greater than those of the fresh meats from which they are made. The preserving chemicals, used in their preparation, appear to be reasonably efficient in this regard even when pork luncheon meat is incubated at 85°F. This study was undertaken to learn whether the presence of preserving chemicals in pork luncheon meat, together with small dosages of gamma radiation, would permit reliable room-temperature storage of the meat.

MATERIALS AND METHODS

Pork luncheon meat was obtained from Swift and Company through the courtesy of Dr. W. M. Urbain. The product was furnished from one batch and was reported to have the following composition:

	Percent by Weight
Pork	90.9
Salt	3.6
Sucrose	2.7
Sodium nitrate	0.014
Sodium nitrite	0.007
Spice	0.028
Water	2.7

The meat was packed in 6-lb square tins marked as a perishable product and labeled "Savor-tite Pure Pork Luncheon Meats." The unopened cans were kept at 40°F until used.

Our re-preparation of the meat for canning varied because difficulty was encountered in getting the PA 3679 spores to grow and develop gas during incubation at 85°F. In the beginning this difficulty was observed even with the inoculated controls. Following a suggestion of Dr. Urbain of Swift and Company, we took increasingly rigorous precautions to remove dissolved oxygen. Also, since heating the meat, as well as the alternate process of evacuation combined with nitrogen flushing, tended to remove moisture, each can was inoculated with spores suspended in 10 ml rather than in 1 ml of distilled water. A detailed description of the meat preparation follows:

Run LPA 1.—The luncheon meat was removed from the refrigerator, taken from the cans and ground in a commercial-type grinder. This ground meat was then packed in No. 1 picnic tin cans, which were covered loosely with covers and autoclaved at 121°C for 1 hr. Individual cans were removed from the autoclave, inoculated at the geometrical center of the meat with 1 ml of a spore suspension containing 300 PA 3679 spores per ml, sealed in a commercial-type closing machine, and plunged into cold running water. Here they cooled to about 65°F in a few minutes and were then further cooled to 33°F in a refrigerator. Following this the cans were either heat-processed or incubated at 85°F as indicated.

Run LPA 2.—This was the same as LPA 1 except that 10,000 PA 3679 spores were used per ml of spore suspension.

Run LPA 4.—The meat was ground as for previous runs but was packed directly into cans. These were inoculated with 1 ml of a spore suspension containing 10,000 PA 3679 spores per ml, and then sealed in a commercial-type, vacuum closing machine at a vacuum of 29 in. of Hg. The meat remained cold throughout this treatment. Following this, the cans were processed, and refrigerated at 33°F or immediately incubated at 85°F, as indicated.

Run LPA 5.—Cold ground pork luncheon meat was spread into shallow enamalled-ware pans to about a 2-in. depth and then placed in an evacuation chamber. Here the pressure was reduced to 25 in. of Hg and then nitrogen was introduced to restore atmospheric pressure. The nitrogen was allowed to remain in contact with the meat for five minutes. This was repeated three times. Next the ground beef was packed into No. 1 picnic tin cans and three more cycles of the evacuation and nitrogen replacement process were applied. Following this, 10,000 PA 3679 spores were injected into the geometrical center of the meat in each can. These spores were suspended in 10 ml of distilled water. Covers were then positioned and the cans were sealed at 29 in. of Hg vacuum, using a commercial-type vacuum closing machine. For processing, the canned pork luncheon meat was irradiated in the centerwell of the large cobalt-60 source at The University of Michigan where the dosage at the center of the cans was 125,000 rep per hr at this time. Irradiation was followed by heat processing or immediate incubation as required.

Run LPA 6.—This was the same as LPA 5.

Run LPA 7.—This was also the same as LPA 5 with one exception; after each evacuation, sufficient nitrogen gas was released into the chamber to develop a pressure of 15 psig. The meat remained under this nitrogen pressure for 5 mintues as part of each of the total of six evacuation cycles.

Runs LPA 8 and 9.—These were the same as LPA 7.

Run LB 1.—Same as LPA 7 except that an inoculum of 1,000,000 C. botulinum 213B spores was used per can of meat.

RESULTS AND DISCUSSION

Putrefactive anaerobic bacterial spores do not develop cultures easily in pork luncheon meat even when incubated at 85°F. This is to be expected since such meat contains salt, nitrites, etc., that are added as preservatives. However, by removing dissolved oxygen, bacterial growth was regularly obtained in control cans of Run LPA 5 et seq.

The results must be considered preliminary and, indeed, fragmentary. However, the data, based on approximately six months' incubation, suggest sterilization limits for pork luncheon meat, inoculated with 10,000 PA 3679 spores per No. 1 picnic can as shown in the following table.

COMBINED IRRADIATION-HEAT PROCESSING TREATMENT
REQUIRED TO STERILIZE PORK LUNCHEON MEAT

Run No.	Pre-Irradiation Megarad	F_o Range
a) Inoculated with 10,000 PA 3679 spores per can		
LPA 2	none	1.1-3.2
LPA 5	none	< 0.86
LPA 7	1.68	< 0.17
LPA 8	< 0.93	none
LPA 9	none	1.1-3.5
b) Inoculated with 1,530,000 C. botulinum 213B spores per can		
LB 1	>0.744	none

It appears from these results that the preservative chemicals lower the amount of irradiation or heat or the severity of combined irradiation-heat processing that is necessary to produce commercial sterility. Provided further incubation does not alter this conclusion, it seem desirable to pursue this lead with further study. Certainly something is accomplished in the preservative process for pork luncheon meat that potentiates the lethality of irradiation as a commercial sterilizing agent. Such a study would require considerable time, however, because long incubations are needed before conclusions can be reached.

COMBINED IRRADIATION-HEAT PROCESS REQUIRED TO STERILIZE CANNED PORK LUNCHEON MEAT INOCULATED WITH ANAEROBIC BACTERIAL SPORES

Run No.: LPA 1
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 300 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.75	5	-
	6	-
	7	-
1.18	1	-
	2	-
	3	-
	4	-
3.33	11	-
	12	-
	13	-
5.44	8	-
	9	-
	10	-
Controls:		
Noninoculated,	N1H 1	-
Unprocessed	N1H 2	-
	N1H 3	-
	N1H 4	-
Inoculated beef		
not heated	1	-

Conclusion: PA 3679 spores did not grow when inoculated into canned pork luncheon meat even though canned under the vacuum caused by steam exhaustion.

Run No.: LPA 2
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
1.09	5	-
	6	-
	7	-
	8	59
3.20	1	-
	2	-
	3	-
	4	-
5.09	9	-
	10	-
	11	-
	12	-
Controls:		
Nonheated,	INC 1	98
Inoculated	INC 2	210
	INC 3	-
	INC 4	-

Conclusion: Growth of PA 3679 is not assured under the packing conditions used. However, under these conditions the pork luncheon meat was sterilized with an F_o between 1.1, and 3.2 when sterility is defined by lack of gas production in the cans.

Run No.: LPA 4
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.74	1	-
	2	-
	3	-
	4	-
1.70	5	-
	6	-
	7	-
	8	-
3.29	9	-
	10	-
	11	-
	12	-
8.20	13	-
	14	-
	15	-
	16	-
Controls:		
Noninoculated	N1C 1	19
	N1C 2	26
	N1C 3	-
	N1C 4	-
Inoculated	INC 1	-
	INC 2	-

Conclusion: None.

Run No.: LPA 5
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.86	1	-
	2	-
	3	-
	4	-
1.71	5	-
	6	-
	7	-
	8	-
3.47	9	-
	10	-
	11	-
	12	-
6.52	13	-
	14	-
	15	-
Controls:		
Noninoculated	N1C 1	26
	N1C 2	15
	N1C 3	46
Inoculated	INC 1	11
	INC 2	14
	INC 3	15
	INC 4	34

Conclusion: Under these conditions, pork luncheon meat was sterilized with an F_o of 0.86 or less.

Run No. LPA 6
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: 0.930 megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.53	1	-
	2	-
	3	-
	4	-
0.88	9	-
	10	-
	11	-
	12	-
1.60	13	-
	14	-
	15	-
	16	-
3.12	5	-
	6	-
	7	-
	8	-
Controls:		
Noninoculated	N1C 1	-
Inoculated	INC 1	-

Conclusion: None.

Run No.: LPA 7
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: 1.68 megarad
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
0.17	13	-
	14	-
	15	-
0.38	9	-
	10	-
	11	-
	12	-
0.82	5	-
	6	-
	7	-
	8	-
1.51	1	-
	2	-
	3	-
	4	-
Controls:		
Noninoculated	N1C 1	6
	N1C 2	8
	N1C 3	8
Inoculated	INC 1	5
	INC 2	5
	INC 3	5
	INC 4	6

Conclusion: Under these conditions, pork luncheon meat was sterilized by an F_o of 0.17 or less following irradiation with 1.68 megarad of gamma radiation.

Run No.: LPA 8
Can Size: No. 1 Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: As indicated
Processing Temperature: None
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation
0.930	9	-
	10	-
	11	-
	12	-
1.86	1	-
	2	-
	3	-
	4	-
2.79	5	-
	6	-
	7	-
	8	-
3.72	13	-
	14	-
	15	-
	16	-
Controls:		
Noninoculated	N1C 1	5
	N1C 2	6
	N1C 3	6
	N1C 4	6
Inoculated	INC 1	5
	INC 2	5
	INC 3	5
	INC 4	6

Conclusion: Under these conditions, pork luncheon meat was sterilized with 0.93 megarad of gamma radiation or less.

Run No.: LPA 9
Can Size: No.1Picnic (211 x 400)
Product: Pork luncheon meat
Inoculum: 10,000 PA 3679 spores per can
Irradiation: None
Processing Temperature: 230°F
Incubation Temperature: 85°F

F_o	Can No.	Days-to-Gas Formation
1.11	1	56
	2	-
	3	42
	4	-
3.45	5	-
	6	-
	7	-
	8	-
8.15	9	-
	10	-
	11	-
	12	-
Controls:		
Noninoculated	N1C 1	-
	N1C 2	-
Inoculated	INC 1	12
	INC 2	12
	INC 3	6
	INC 4	6

Conclusion: Under these conditions pork luncheon meat was sterilized by an F_o between 1.11 and 3.45.

Run No.: LB 1
Can Size: Mushroom (202 x 202)
Product: Pork luncheon meat
Inoculum: 1,530,000 C. botulinum 213B spores per can
Irradiation: As indicated
Processing Temperature: None
Incubation Temperature: 85°F

Megarad	Can No.	Days-to-Gas Formation	Toxicity
0.186	16	62	*0/3
	17	54	
	18	66	
	19	74	
0.372	11	75	
	12	68	
	13	87	
	14	82	
	15	-	
0.558	1	-	
	2	69	
	3	-	
	4	84	
0.744	7	-	
	8	85	
	9	72	
	10	-	

*The meat in Can No. 16 had a slightly putrid smell, a slimy surface, and the color of the original pork luncheon meat. It contained Gram positive rods and a few cocci.

Conclusion: Under these conditions, pork luncheon meat was not sterilized by 0.744 megarad of gamma radiation.

PHASE IV

EXAMINATION OF IRRADIATION-STERILIZED CANNED FOODS FOR POSSIBLE TOXIN OR VIABLE BACTERIA

The possibility exists that food containing viable toxicogenic spores of bacteria could become toxic without developing sufficient gas to distend vacuum-packed cans or even without the presence of viable bacterial cells when the cans are opened after incubation. This could come about as a result of the aborted germination of irradiated spores followed by a few cycles of vegetative cell growth. Since relatively few vegetative cells of C. botulinum are needed to produce dangerous quantities of toxin, this potentiality of toxin development is being studied.

MATERIALS AND METHODS

Unopened cans of meat, and other foods that were sterilized by irradiation, have been kept for a number of years in this laboratory. These cans have been and are stored at room temperature. Occasionally they are examined for spoilage, which occurs very rarely after one month and almost never after 1-1/2 years.

When a can has been selected for study, it is aseptically opened. This process involves washing the cover with 95% alcohol, which is followed by flaming the alcohol. A pledget of sterile cotton in gauze is then placed over the can and the cover is punctured and cut with a sterile can opener. Approximately 15 gm of meat, or other food, are then removed with a sterile glass tube-plunger apparatus and placed in a tube of gas-exhausted liver infusion media for culture at 30°C. Another similar sample is placed in a sterile test tube and mixed with an equal volume of physiological saline. This mixture is filtered through glass wool to remove gross particles and further clarified by centrifugation. Finally, 1/2 ml of the supernatent fluid is injected intraperitoneally into each of 4 mice. Toxin production would manifest itself by death of the mice within three days. In this event, another portion of the meat would be prepared and tested for C. botulinum toxin by toxin-neutralization studies.

This work has just begun but so far tests of the dozen or so cans that have proceeded far enough for tentative conclusions to be drawn have not shown the presence either of viable bacteria or of toxin.

Approximately 90% of the contract research on the basic contract is complete, and it is expected that present funds are adequate for its completion. The extension to the contract, covered by Phase IV, has only begun.