AD

TECHNICAL REPORT
FD-28

DETERMINATION OF EQUIVALENCE BETWEEN STERILIZATION PROCESS AND COMMERCIAL THERMAL PROCESS FOR CURED MEATS

by

R. A. GREENBERG
B. O. BLADEL
W. J. ZINGELMANN

SWIFT & COMPANY
Chicago, Illinois

Contract No. DA 19-129-QM-2008

October 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

AD

TECHNICAL REPORT
FD -28

DETERMINATION OF EQUIVALENCE BETWEEN RADIATION STERILIZATION PROCESS AND COMMERCIAL THERMAL PROCESS FOR CURED MEATS

by

R. A. Greenberg B. O. Bladel

W. J. Zingelmann

SWIFT & COMPANY
Chicago, Illinois

Contract No. DA 19-129-QM-2008

Project Reference:
7X84-01-002

October 1963

U. S. Army Materiel Command

U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

FOREWORD

The minimum thermal process for the destruction of C. botulinum is $F_0 = 2.5$ (corrected for thermal lag). Yet, for decades the public has consumed large quantities of shelf-stable, unrefrigerated cured meats which have received commercial thermal processes in the range of $F_0 = 0.08$ to 0.5 without contracting the deadly disease, botulism. The possible causes for this remarkable safety record may be attributed to: (1) extremely low initial incidence of the organism in meats, and (2) the curing salts in the meats provide a hostile environment for the development of the heat-injured organism.

It is reasonable to assume that cured meats could be made equally safe by relatively low levels of non-lethal radiation. This contract provides evidence that a commercial radioprocess can be developed which will yield the same degree of protection against C. botulinum as is obtained by a typical commercial thermal process.

The Project Officer of this contract was Mr. Abe Anellis, and his alternate was Mr. Morris Simon of the Quatermaster Food & Container Institute, Chicago Illinois.*

EDWARD S. JOSEPHSON, Ph.D.
Associate Director for Food Radiation
Food Division

APPROVED:

FERDINAND P. MEHRLICH, Ph.D.
Director, Food Division

DALE H. SIELING, Ph.D.
Scientific Director

W. W. VAUGHAN
Brigadier General, USA
Commanding

* Currently Microbiology Branch, Food Division, U.S. Army Natick Labs.

CONTENTS

LIST OF TABLES

SUMMARY

Chopped ham, inoculated with spores of Clostridium botulinum strains 33A and 41B, was canned and subjected to an enzyme-inactivating heat pasteurization. The cans were then irradiated with 0.5, 1.5, 2.5, and 3.5 Mrads in the Natick Cobalt-60 facility. A portion of the pack was not irradiated and received a commercial thermal process of F_o = 0.2. Toxic spoilage occurred in inoculated product at 0 and 0.5, but not at 1.5, 2.5, or 3.5 Mrad. Inoculum survivors were isolated from all product variables before and after six months incubation at 80-100°F. However, neither growth nor toxin was observed in unspoiled product. The "injury" phenomenon previously described in thermally processed cured meats (survival of botulinal spores without capacity of outgrowth and/or toxinogenesis) apparently occurs also in irradiated cured meats.

INTRODUCTION

The use of ionizing radiation as a means of preserving foods has been under investigation for more than a decade. Much research has been carried out on the radiation resistance of various strains of Clostridium botulinum in a number of environmental systems. The data obtained from this work has been, for good reason, extrapolated to establish minimum irradiation process values for guaranteeing the destruction of theoretically possible botulinal spore loads. This extrapolation is based upon the classic thermal process studies of Meyer and Esty.

The minimum radiation sterility dose is about 4.5 Mrad. Such treatment causes severe organoleptic damage in many foods.

Adoption of the "minimal health process" or "botulinum cook" (equivalent to 2.78 minutes at 250°F.) by the canning industry, has resulted in a perfect botulism-free history in commercially canned foods. Those botulism outbreaks which have originated with commercially canned products (i.e., tuna fish, Detroit, 1963; liver paté, Montreal, 1963) have been traceable to defective cans or gross underprocessing.

In reviewing the above information, one cannot help but be impressed by the fact that shelf-stable cured meat products, experimentally capable of supporting growth and toxin formation of C. botulinum organisms, have been marketed by the meat industry for many years. No reported incidence of botulism intoxication has occurred and this class of products remains unique as the only low acid canned unrefrigerated foods that characteristically do not receive a "minimal health process".

The perfect health history of non-refrigerated canned cured meats is thought to be due to two principal factors, - the extremely low incidence of botulinal spore contamination in meats and the spore-injuring effect of the mild heating process in the presence of the curing system. "Injury" is the term we have chosen to describe the inability of a processed survivor to proliferate in the product, even though its viability can be established by sub-culture in a suitable medium.

The purpose of this study is to determine the level of radiation required to impose the same degree of protection against botulinal toxin as is obtained by the typical commercial thermal process currently employed by industry.

METHODS

The work was divided into two phases. The first was the establishment of the inoculum level required to produce botulinal toxin in cured meat receiving a typical commercial thermal process. After a survey of the industry was made, the Project Officer indicated his selection of $F_o = 0.2$ as the thermal process to be used in the investigation. 230°F was selected as a retort temperature typically employed by industry in processing canned shelf stable cured meats. The actual process designed for the test was 35 minutes at 230°F., initial temperature = 30°F., can size 202 X 202. The calculated F_o for this set of conditions = 0.203.

Meat Emulsion Preparation:

Fifty pounds of green boneless ham was cut into small pieces and ground to ½-inch size in the grinder. This meat was then placed in a vacuum mixer. One pound of sugar, 1.5 pounds of salt, and 1.773 grams of sodium nitrite were then added to the meat and mixed for 3 minutes. 9.2 pounds of finely ground dry ice was then added and the meat mixed under vacuum for 5 minutes. The emulsion was then ground through a 3/16 inch plate, inoculated with C. botulinum spores, sufficient dry ice added to the mixture to result in an initial temperature of 28-30°F., packed in 202 X 202 cans, closed under 25 inches of vacuum, and held in ice water until completion of the packing operation. Two individual packs were prepared. The first, prepared on 12 July, 1962, was, by error, processed 40 minutes at 230°. The second, prepared on 30 July, 1962, received the desired process of 35 minutes at 230°. Chemical analyses of the Phase I products are listed in Table 1.

PHASE I (INOCULUM LEVEL)

Inoculum:

Strains 33A and 41B were used as inoculum. The spore suspensions were supplied by the QMFCI Microbiology Laboratory * Equal numbers of the two spore suspensions were used in sufficient quantity to give 24 cans each of product containing 2.5, 25, 250, 2500, and 25,000 spores per gram of meat in the July pack. The product prepared in August was inoculated in 24 can lots in decimal increments starting with 25,000 per gram.

Incubation:

All product was incubated six months in a room in which the temperature cycled between 80 and 100°F.

* Currently, Microbiology Branch, Food Division, U.S. Army Natick Laboratories.

Botulinal Toxin Evaluation:

A 25 gram sample of product was removed from each can, diluted with an equal volume of 0.0025 M phosphate buffer. Presumpti evidence of toxicity was established by means of intraperitoneal injection of 0.5 ml. of the sample suspension into Swiss strain white mice (15-20 gram). Suspensions causing death within four days were confirmed as containing botulinal toxin by protection testing against trivalent ABC anti-toxin (Fort Dodge Laboratories, Fort Dodge, Iowa).

Table 2 shows the results of the toxin assays. Botulinal toxin was found in product containing 24,400 and 288,000 spores per gram in product prepared for the August pack. On the basis of these data, the decision was made to employ 2,500 and 250,000 Clostridium botulinum spores per gram of product as inoculum levels for the secon phase of the study.

PHASE II (IRRADIATION EQUIVALENCE EXPERIMENT)

Product Preparation:

Fresh uncured boneless ham was ground and mixed with sodium chloride, dextrose, and sodium nitrate and handled in the same manner as was the product prepared for Phase I. Chemical analyses of the raw an cooked product are described in Table 4.

Inocula:

The procedures used for manufacture and standardization of spore stocks are described in Addendum A. A master inoculum was prepared containing 2×10^7 type 33A and 2.8×10^7 type 41B spores. Product containing 2,500 spores per gram was inoculated with 3 ml. of this inoculum per 100 pounds. Product containing 2,500 spores per gram received 300 ml. per 100 pounds.

Thermal Process:

The product was packed in 202 x 202 cans.

A. Industry Process:

70 cans each of uninoculated, 2500 per gram, and 250,000 per gram product, were given a typical commercial process of 35 minutes at 230°F. ($F_0 = 0.203$).

B. Irradiated

All product to receive irradiation was processed 25 minutes at 190°F. A center temperature of 165°F. was attained in these cans.

Irradiation and Handling:

Following thermal processing, the cans receiving irradiation were packed in ice cream shippers containing dry ice and shipped to Natick on April 12, 1963. The cans were thawed in 45°F. water on April 16, and irradiated by means of the Cobalt ^{60} source. 190 cans (50 uninoculated and 70 of each of the two inoculated series) were subjected to irradiation doses of 0.5, 1.5, 2.5 and 3.5 Mrad. Each irradiation "run" consisted of 100 cans. The second run of each irradiation code contained 90 product cans plus 10 dummy cans. Dose rate was calculated to be 74,700 rads per minute $\pm$ 2.5%. Irradiation information is detailed in Addendum C.

The irradiated product was packed without coolant and shipped from Natick to Swift & Co., arriving April 24. Twenty cans of each inoculated radiation code were placed at -15°C. for subsequent viable C. botulinum assay. The remaining 50 cans of each inoculated radiated code and 50 cans of all non-irradiated code products (which had been held at 2°C. since April 11) were incubated at 80-100°F. on April 24.

A complete listing of product codes, detailing inoculum level, thermal process, and irradiation dose, is presented in Table 3.

Toxin Assay:

Botulinal toxin was assayed in the same manner as described in Phase I.

Viable C. botulinum Assay

Twenty replicate cans from each irradiation treatment were examined quantitatively for putrefactive anaerobes and C. botulinum, with the exception of the uninoculated, irradiated cans. The spore assay was accomplished by means of a most probable number analysis in beef infusion agar. The formulation of beef infusion agar is presented in Addendum B.

After introduction of sample material into melted beef infusion agar, each tube was layered with sterile Stanolene. The tubes were incubated 7 days at 37°C. Any tube showing gas production was considered a positive putrefactive anaerobe. All positive tubes

were assayed for Clostridium botulinum toxin by emptying the tube contents into a sterile glass jar, mixing with an equal quantity of 0.0025 M phosphate buffer and using the resultant suspension in standard mouse toxin assay protocol. Table 5 lists the pre-incubation levels of both putrefactive anaerobes and Clostridium botulinum.

The radiation resistance of the inoculum in the chopped ham product is calculated in Table 6. The apparent average D value of these organisms in this experiment was 0.211 Mrad.

RESULTS

Detectable putrefactive anaerobic spore survivors were found in all inoculated product. (See Table 5). Confirmed viable Clostridium botulinum spores were isolated from commercially heat processed product at both inoculum levels (codes NAL-T and NAG-T). Toxicogenic botulinal spores were also found in high level inoculated product irradiated at all doses up to and including 3.5 Mrad. They were found in the low level inoculated product receiving 0, 0.5, 1.5, and 2.5 Mrad, but not in those cans receiving 3.5 Mrad. The lower limit of detection was less than 0.03 per gram. Although small numbers of putrefactive anaerobes were found in uninoculated product, botulinal spores were never recovered.

Table 7 lists the spoilage and toxicity results occurring during the 6-month incubation period at 80-100°F. Confirmed botulinal toxin was demonstrated in inoculated product irradiated at 0.5 Mrad, but not in product receiving 1.5, 2.5, or 3.5 Mrad. Radiation activation of the inoculum was evident in that more spoilage and toxin formation occurred in the product irradiated at 0.5 Mrad than in identical product receiving no irradiation treatment. During the incubation period, 32 of the 50 low inoculum cans receiving no irradiation, showed spoilage. Seven of these were confirmed toxic. Within 86 days, all 50 of the low inoculum cans irradiated at 0.5 Mrad had developed a swelled condition. Forty-seven of these were toxic. This trend continued in the high inoculum chopped ham. Unirradiated cans showed a total of 41 swellers out of the 50 incubated during the incubation period, with 17 toxics. High inoculum product irradiated at 0.5 Mrad. was 100% swelled within 70 days at 80 to 100°F. Forty-three of these cans proved to contain botulinal toxin.

No toxin was found in any of the cans receiving 1.5, 2.5 or 3.5 Mrad assayed at the termination of the 6-month incubation period. This included all of the uninoculated cans, as well.

The fate of process survivors during 6 months incubation in the product environment, can be seen in Table 10. No increase in population was observed in any product variable in which spoilage did not occur. Focusing on the inoculated 1.5, 2.5, and 3.5 Mrad. products (Table 8), one observes an apparent decrease in Clostridium botulinum population (as defined by ability to elaborate botulinal toxin in sub-culture) during incubation.

The inability of putrefactive anaerobe survivors to proliferate in irradiated cured meats during incubation is demonstrated by the data in Table 9. At 1.5 Mrad. and above, just as with the commercial thermal process, putrefactive anaerobe counts all averaged less than 1 per gram before and after incubation. As previously stated, toxin was found only in inoculated product receiving 0 and 0.5 Mrad.

DISCUSSION

The purpose of the Phase I portion of this study was to establish the inoculum level requirements for toxinogenesis in product receiving the commercial thermal process. The data suggested that 25,000 to 250,000 spores per gram were required. 250,000 spores per gram was utilized in the high inoculum series, while 2,500 was chosen as the other inoculum level to afford a series of product variables containing botulinal spores which could be expected to remain stable throughout incubation. Unfortunately, all product receiving the commercial thermal process in the Phase II work remained unspoiled and toxin-free at the 6-month cut-off. Perhaps this can be attributed to differences in handling of the inoculated products. Phase I was manufactured, processed, and placed into incubation during a single day. The irradiation requirements for the second phase product necessitated shipment from Chicago to Natick to Chicago with elapsed time of 10-12 days from manufacture to incubation. It is conceivable that sufficient germination could have occurred between formulation and thermal processing to reduce the spore population below the critical concentration.

If one plots from data in Table 5, a C. botulinum spore survival curve and extrapolates to extinction, the sterility point falls at about 4.5 Mrad in the high inoculum product. Thus, if sterility is the criterion required for safety in irradiated cured meats, these data add grist to the 12 D mill.

The data also support the hypothesis that organoleptically compatible radiation processes induce the same stability (and safety) in cured meats as do the non-sterilizing thermal processes currently employed by industry. If we are to support the 12 D concept in irradiated foods containing no inhibitory substances because of the trouble-free history of their thermally-processed counterparts, is it

not inconsistent to demand sterilizing radiation processes in those products which do not require thermal sterilization?

There is an obvious need for careful study of the physiology of "injured" spores in the cured meat environment. Knowledge of the specific intra-cellular damage imposed by sub-lethal irradiation and thermal treatment, as well as the capacity of injured organisms to overcome their infirmities, will help establish the irradiation process requirements necessary for cured meat safety and stability.

TABLE 1

CHEMICAL ANALYSES: PHASE I PRODUCT

	Emulsion 7/12/62	Emulsion 8/30/62
Moisture	61.1%	63.3%
Salt	2.74%	2.76%
Brine	4.29%	4.18%
Sodium nitrite	56 ppm	64 ppm

TABLE 2

PHASE I PRODUCT TOXICITY

Product Date	Spores Added Per Gram	Presumptively Toxic Cans	Confirmed Toxic
12 July 1962	2,784	0/21	-
30 Aug. 1962	24,400	5/24	1/5
12 July 1962	28,920	0/23	-
30 Aug. 1962	288,000	7/24	7/7

TABLE 3

PRODUCT CODING: IRRADIATION EXPERIMENT

Code	Inoculum level	Thermal Process	Irradiation Dose
NAO-T	0	35 min @ 230°F.*	0
NAO-0	0	25 min @ 190°F.**	0
NAO-0.5	0	25 min. @ 190°F.	0.5 MRAD
NAO-1.5	0	25 min. @ 190°F.	1.5 MRAD
NAO-2.5	0	25 min. @ 190°F.	2.5 MRAD
NAO-3.5	0	25 min. @ 190°F.	3.5 MRAD
NAL-T	2,500/gm	35 min. @ 230°F.	0
NAL-0	2,500/gm	25 min. @ 190°F.	0
NAL-0.5	2,500/gm	25 min. @ 190°F.	0.5 MRAD
NAL-1.5	2,500/gm	25 min. @ 190°F.	1.5 MRAD
NAL-2.5	2,500/gm	25 min. @ 190°F.	2.5 MRAD
NAL-3.5	2,500/gm	25 min. @ 190°F.	3.5 MRAD
NAH-T	250,000/gm	35 min. @ 230°F.	0
NAH-0	250,000/gm	25 min. @ 190°F.	0
NAH-0.5	250,000/gm	25 min. @ 190°F.	0.5 MRAD
NAH-1.5	250,000/gm	25 min. @ 190°F.	1.5 MRAD
NAH-2.5	250,000/gm	25 min. @ 190°F.	2.5 MRAD
NAH-3.5	250,000/gm	25 min. @ 190°F.	3.5 MRAD

* F_o = 0.203

** Center Temperature = 165°F.

TABLE 4

CHEMICAL ANALYSES: PHASE II PRODUCT

Product	Salt	Moisture	Brine	Na Nitrite
Raw ham	2.38%	61.4%	3.76%	210 ppm
Raw ham	2.20	63.3	3.38	250
NA-O Ham	2.36	60.3	3.76	40
NA-O Ham	2.41	59.3	3.90	180

TABLE 5

PRE-INCUBATION LEVELS OF CLOSTRIDIUM BOTULINUM

Code	Anaerobes MPN/gm		Confirmed Cl. Botulinum MPN/gm	
	Range	Log Mean	Range	Log Mean
NAH-0	360-23,000	2,670	360-23,000	2,670
NAH-0.5	0.43-43.0	6.10	0.43-43.0	6.10
NAH-1.5	0.11-9.3	0.42	0.11-93	0.392
NAH-2.5	0.073-2.3	0.44	<0.03-2.3	0.139
NAH-3.5	<0.03-0.23	0.038	<0.03-0.23	0.021
NAH-T	<0.03-4.3	0.158	<0.03-0.43	0.074
NAL-0	43-430	173	43-430	173
NAL-0.5	0.23-0.93	0.322	0.23-0.93	0.322
NAL-1.5	0.036-9.3	0.293	<0.03-9.3	0.227
NAL-2.5	<0.03-23	0.098	<0.03-23	0.090
NAL-3.5	<0.03-0.091	0.019	<0.03	
NAL-T	<0.03-0.036	0.020	<0.03-0.036	0.017
NA-0-0	<0.03	-	<0.03	-
NA-0-T	<0.03-0.021	0.021	<0.03	-

* For averaging purposes, <0.03 = 0.015

TABLE 6

RADIATION RESISTANCE OF INOCULUM IN PRODUCT

$$D = \frac{\text{Dose (megarad)}}{\log M - \log S}$$ Dose = 0.5

A. High level inoculum:

M = 2,670

S = 6.1

$$D = \frac{0.5}{3.426 - 0.786} = \frac{0.5}{2.64} = 0.197$$

B. Low level inoculum:

M = 173

S = 0.322

$$D = \frac{0.5}{2.24 - 0.05} = \frac{0.5}{2.19} = 0.226$$

$$\text{Average D} = \frac{0.423}{2} = 0.211$$

TABLE 7

CHOPPED HAM SPOILAGE AND TOXICITY
(6 MONTHS @ 80-100°F.)

Low Inoculum

Radiation Dose	Swelled Cans	Toxic Cans (Confirmed botulinal toxin)
0 MRAD	32/50	7
0.5	50/50 (86 days)	47
1.5 - 3.5	1/150	0

High Inoculum

Radiation Dose	Swelled Cans	Toxic Cans (Confirmed botulinal toxin)
0 MRAD	41/50	17
0.5	50/50 (70 days)	43
1.5-3.5	0/150	0

TABLE 8

SURVIVAL OF CLOSTRIDIUM BOTULINUM SPORES IN IRRADIATED CHOPPED HAM DURING INCUBATION

Treatment	Pre-Incubation	Post-Incubation (6 Months @ 80-100°F.)
1.5 MRAD	0.392	0.018
	0.227	0.016
2.5 MRAD	0.139	0.015*
	0.090	0.019
3.5 MRAD	0.021	0.019
	0.015*	0.016

* $<0.03 = 0.015$ for averaging purposes.

TABLE 9

RADIATION INJURY EFFECT ON P.A. SPORES IN CHOPPED HAM

Treatment	Pre-Incubation	Post-Incubation (6 Months @ 80-100°F.)
1.5 MRAD	0.42	0.72
	0.292	0.78
2.5 MRAD	0.44	0.069
	0.098	0.088
3.5 MRAD	0.038	0.164
	0.019	0.078
F_o = 0.2	0.158	0.020
	0.020	0.019

TABLE 10

POST-INCUBATION LEVELS OF CLOSTRIDIUM BOTULINUM

Code	Anaerobes (MPN/gm)		Confirmed C. botulinum	
	Pre-incubation	Post-incubation	Pre-incubation	Post-incubation
NAO-T	0.021*	0.017	< 0.03	< 0.03
NAO-0	< 0.03	0.016	< 0.03	< 0.03
NAO-0.5	--	0.022	--	< 0.03
NAO-1.5	--	0.016	--	< 0.03
NAO-2.5	--	0.059	--	< 0.03
NAO-3.5	--	0.025	--	< 0.03
NAL-T	0.020	0.019	0.017	< 0.03
NAL-0	173	Spoilage	173	Toxin
NAL-0.5	0.322	Spoilage	0.322	Toxin
NAL-1.5	0.292	0.720	0.227	0.016
NAL-2.5	0.098	0.088	0.090	0.019
NAL-3.5	0.019	0.078	< 0.03	0.016
NAH-T	0.158	0.020	0.074	< 0.03
NAH-0	2,670	Spoilage	2,670	Toxin
NAH-0.5	6.10	Spoilage	6.10	Toxin
NAH-1.5	0.420	0.720	0.392	0.018
NAH-2.5	0.440	0.069	0.139	< 0.03
NAH-3.5	0.038	0.164	0.021	0.019

* For averaging purposes - < 0.03 = 0.015

ADDENDUM A

PRODUCTION AND STANDARDIZATION OF Clostridium botulinum SPORE STOCKS

A. Medium:

Trypticase	40 gm
Sodium chloride	5 gm
K_2HPO_4	2 gm
Distilled H_2O	950 ml

Autoclave 15 minutes @ 121°C.
Add 50 ml Seitz filtered 2% sodium thioglycolate.
Final pH: 7.0

B. Growth and Spore Production:

1. Seed 200 ml media in a 250 erlenmeyer flask with 1 ml stock spores. Incubate 18-24 hours, 37°C.

2. Contents of (1) are added to a 2 liter-erlenmeyer flask containing about 1700 ml media and a magnetic stirring bar. Placed on magnetic stirrer, insulated with asbestos pad. Incubate 18-24 hours at 27-32°C.

3. Replace stopper on (2) with pre-sterilized gassing stopper. (2-hold rubber stopper fitted with glass tubing. One glass tube extending almost to bottom of flask for introduction of illuminating gas, the other serving as burn-off exhaust.) Gas for one hour, replace with sterile rubber stopper and return flask to magnetic stirrer.

4. 18-24 hours after gassing, check culture for presence of spores by any convenient method. If culture is well sporulated (90%+), replace stopper with fresh gassing stopper. Attach to house air line and bubble air through media at a rapid rate for 30 minutes. If culture is not well sporulated, continue incubation and examine periodically until sporulation is well advanced.

5. Spores are harvested by centrifugation in plastic bottles at 6500 RPM for 1 hour. The spores are resuspended in 0.0025M phosphate buffer (pH 7.2) and recentrifuged. This step is repeated and the twice-washed spores suspended finally in fresh buffer in screw capped glass bottles in 100 ml aliquots.

6. The washed spore suspensions are placed in a pre-heated 82°C. water bath and are held here until 30 minutes after the contents of a thermometer-equipped buffer blank bottle reaches 77°C.

C. Spore Count:

Aliquots of heat-shocked spore stocks (from B-6) are suitably diluted and quantified by MPN in 5-tube brain-heart infusion broth series and by trypticase soy agar deep tube contents. Direct count values are the average of 4 or more tubes.

D. Toxicity Verification:

15-20 gram Swiss strain white mice are inoculated intraperitoneally with 0.1 ml aliquots of cultures at the beginning of stages B-2 and B-4 and the supernatent liquid from the initial centrifugation step described in B-5.

In addition, 0.1 ml of culture derived from the highest positive dilution series of brain-heart infusion MPN tubes (C) are tested by I.P. mouse challenge. Protection tests are carried out simultaneously with challenged mice protected with trivalent (ABC) antitoxin (Fort Dodge Laboratories).

ADDENDUM B

BEEF INFUSION AGAR PREPARATION

A. Fresh, lean beef is boiled one hour in distilled water (one pound beef per liter of water). The meat is then removed and sufficient distilled water added to the broth to restore original volume.

B. To each liter of infusion A add:

peptone	5.0 grams
tryptone	1.6 grams
glucose	1.0 gram
soluble starch	1.0 gram
K_2HPO_4	1.25 grams
sodium thioglycollate	2.0 grams
agar	15.0 grams

pH 7.4

Bring to rolling boil, boil one minute. Tube 50 ml amounts in 1 x 8 screw capped test tubes. Autoclave 20 minutes @ 121°C.

ADDENDUM C

DOSE RATE CALCULATION
COBALT^{60} SOURCE, NATICK, APRIL 16, 1963

Special Ferrous Copper Dosimetry

Can Number / Row	1 Dose Rate Rads/Hr.	5 Dose Rate Rads/Hr.	10 Dose Rate Rads/Hr.
A	4.47	4.50	4.54
B	4.49×10^6	4.43	4.50
C	4.56	4.50	4.60
D	4.50	4.44	4.53
E	4.55	4.50	4.64
F	4.52	4.47	4.63
G	4.57	4.53	4.57
H	4.55	4.48	4.59
I	4.53	4.53	4.57
J	4.57	4.55	4.63
AVERAGE, Rows A, C, E, G, I	4.54	4.51	4.58
AVERAGE, Rows B, D, F, H, J	4.53	4.47	4.58

Average = $4.54 \times 10^6 \pm 2.3\%$

Dose rate = 4.48×10^6 Rads/Hour or 7.47×10^4 rads per minute.

Intended Dose	Exposure Time
0.5 MRAD	6.69 minutes
1.5 MRAD	20.08 minutes
2.5 MRAD	33.47 minutes
3.5 MRAD	46.85 minutes

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)
Swift & Company, Chicago Illinois

2a REPORT SECURITY CLASSIFICATION
Unclassified

2b GROUP

3 REPORT TITLE
DETERMINATION OF EQUIVALENCE BETWEEN RADIATION STERILIZATION PROCESS AND COMMERCIAL THERMAL PROCESS FOR CURED MEATS

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final report 25 June 1962 - 24 December 1963

5 AUTHOR(S) (Last name, first name, initial)
GREENBERG, R. A.
BLADEL, B. O.
ZINGELMANN, W. J.

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
October 1965	21	0

8a CONTRACT OR GRANT NO
DA 19-129-QM-2008

b PROJECT NO
7X84-01-002

c

d

9a ORIGINATOR'S REPORT NUMBER(S)

9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
FD-28

10 AVAILABILITY LIMITATION NOTICES
Available at DDC and CFSTI
Distribution of this document is unlimited

11 SUPPLEMENTARY NOTES

12 SPONSORING MILITARY ACTIVITY Radiation Sources Branch, Food Division, U. S. Army Natick Laboratories, Natick, Mass. 01762

13 ABSTRACT

Chopped ham, inoculated with spores of Clostridium botulinum strains 33A and 41B, was canned and subjected to an enzyme-inactivating heat pasteurization. The cans were then irradiated with 0.5, 1.5, 2.5, and 3.5 Mrads in the Natick cobalt-60 facility. A portion of the pack was not irradiated and received a commercial thermal process of F_o^*0.2. Toxic spoilage occurred in inoculated product at 0 and 0.5, but not at 1.5, 2.5, or 3.5 Mrad. Inoculum survivors were isolated from all product variables before and after six months incubation at 80-100°F. However, neither growth nor toxin was observed in unspoiled product. The "injury" phenomenon previously described in thermally processed cured meats (survival of botulinal spores without capacity of outgrowth and/or toxinogenesis) apparently occurs also in irradiated cured meats.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification

KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Destruction	8		4			
Injury	8		4			
Clostridium botulinum	1		3,4			
Meat	5,9		1,2			
Thermal processing	10		1			
Irradiation	10		1			
Pasteurized	0		0			
Canned	0		0			
Comparisons			8			
Ham			1,2			
Chopped			0			

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2*a*. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2*b*. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES. If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year, or month, year. If more than one date appears on the report, use date of publication.

7*a*. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7*b* NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8*a*. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8*b*, 8*c*, & 8*d*. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9*a* ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9*b* OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY LIMITATION NOTICES. Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES. Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY. Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13 ABSTRACT Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report If additional space is required, a continuation sheet shall be attached

It is highly desirable that the abstract of classified reports be unclassified Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS) (S) (C), or (U)*

There is no limitation on the length of the abstract However the suggested length is from 150 to 225 words

14 KEY WORDS Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report Key words must be selected so that no security classification is required Identifiers such as equipment model designation trade name military project code name geographic location may be used as key words but will be followed by an indication of technical context The assignment of links rules and weights is optional