

AD621921

AD

TECHNICAL REPORT
FD-24

**RESISTANCE OF MICROORGANISMS
TO
IONIZING RADIATION APPLIED TO FOODS**

CLEARINGHOUSE FOR FEDERAL SCIENCE AND TECHNICAL INFORMATION			
Hardcopy	Microfilm		
\$ 1.00	\$0.50	21	pp. <i>aw</i>
ARCHIVE COPY			

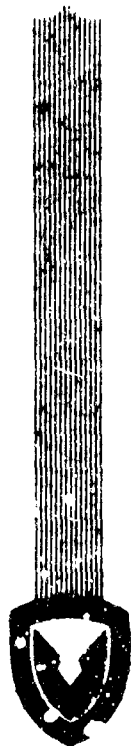
by

A. W. Anderson
OREGON STATE UNIVERSITY
Corvallis, Oregon

Contract No. DA 19-129-OM-2035

September, 1965

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



The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

Citation of trade names in this report does not constitute an official indorsement or approval of the use of such items.

DDC AVAILABILITY NOTICE

Qualified requesters may obtain copies of this report from Commanding Officer, Defense Documentation Center (DDC) (formerly ASTIA), Cameron Station, Alexandria, Virginia 22314.

Released to the Clearing House for Federal Scientific and Technical Information, Springfield, Virginia 22151, for sale to the public.

DISPOSITION INSTRUCTIONS

Destroy; do not return.

AD

TECHNICAL REPORT
FD-24

RESISTANCE OF MICROORGANISMS
TO IONIZING RADIATION APPLIED TO FOOD

by

A. W. Anderson
Oregon State University
Corvallis, Oregon

Contract No. DA19-129-QM-2035

Project Reference:
7-84-01-002

September 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts 01762

FOREWORD

Relatively high dose levels of ionizing radiation are needed to make foods sterile. These high dosages frequently produce deleterious organoleptic changes in the product. The degree of food spoilage, and the cost of the sterilization process, will vary directly with the quantity of ionizing energy applied. Hence, means must be sought to lower sterilizing doses.

This contract undertook a study on the synergistic lethal effects of food additives and radiation on spores of Clostridium botulinum in meat. Results are presented which indicate that certain combinations of simple edible chemicals (NaCl, NaNO₃, NaNO₂), in concentrations permitted by the FDA, effectively reduced the sterilizing dose of ground round beef, infected with massive numbers of Clostridium botulinum spores, by 0.5 to 1.0 Mrad.

The work covered by this report was performed by the Microbiology Department, Oregon State University, under Contract No. DA 19-129-QM-1517 during the period June 1962 to September 1963. A. W. Anderson was official investigator and P. R. Elliker, R. F. Cain, and K. L. Krabbenhoft were his collaborators.

The activities under this contract were monitored by the Microbiology Section, Q.M. Food & Container Institute*. Miss Dorothy A. Huber served as the Project Officer and Mr. Abe Anellis was Alternate Project Officer.

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

EDWARD S. JOSEPHSON, Ph.D.
Associate Director for 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

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION.....	1
MATERIALS AND METHODS.....	2
RESULTS AND DISCUSSION.....	4
REFERENCES.....	7

LIST OF TABLES

1	Spoilage of canned ground round beef inoculated with <u>Clostridium botulinum</u> (33A) plus test compound and irradiated at 2.0 megarad	8
2	Spoilage of canned ground round beef inoculated with <u>C. botulinum</u> (33A) plus NaNO_2 and irradiated at various dosage levels.....	9
3	Spoilage of canned ground round beef inoculated with <u>Clostridium botulinum</u> (33A) plus NaNO_3 and irradiated at various dosage levels.....	10
4	Spoilage of canned ground round beef inoculated with <u>Clostridium botulinum</u> (33A) plus NaCl and irradiated at 2.0 megarad.....	11
5	Spoilage of canned ground round beef inoculated with <u>Clostridium botulinum</u> (33A) plus test compounds and irradiated at various dosage levels.....	12
6	Spoilage of canned ground round beef inoculated with <u>Clostridium botulinum</u> (33A) plus test compounds and irradiated at various dosage levels.....	13

ABSTRACT

Spores of Clostridium botulinum (33A) were inoculated into canned ground round beef containing various additives and subsequently irradiated. Sodium nitrate (1000 ppm) plus sodium chloride (2.5%) completely inhibited spoilage for 120 days at 35°C when the samples were exposed to 2.0 Mrad, and no viable spores or toxin were recovered upon sub-culture. One hundred and twenty five cans were used per run. The use of either additive, with or without radiation, did not prevent spoilage unless higher levels of radiation such as 2.5 and 3.0 Mrad were used, with the concomitant production of undesirable odors.

Sodium nitrite (200 ppm) plus sodium chloride (2.5%) inhibited spoilage for 120 days but there was evidence of spore viability and toxin production in some of the unspoiled cans when the radiation level was 2.0 Mrad. Higher levels of radiation gave results similar to those obtained for sodium nitrate and sodium chloride.

INTRODUCTION

Clostridium botulinum has been reported to be one of the most radiation resistant food spoilage bacteria (Morgan, 1953, 1954; Kempe, 1954). In addition C. botulinum produces a highly potent toxin and is a heat resistant, spore-forming organism which survives in many non-acid foods. For these latter reasons particularly, it is highly desirable that adequate means be developed to inhibit the growth of this organism in those foods where acid and/or heat cannot be used.

The use of radiation for the preservation of foods inoculated with various bacterial species has been reviewed by Niven (1958). Riemann (1962) has also recently reviewed the use of other agents to control the growth of C. botulinum. Among these, sodium nitrate, sodium nitrite and sodium chloride have been successfully applied to cheese, canned meat and other foods (Jensen, 1954; Silliker, 1958; Kempe, 1962). However, the complete inhibitory effect of sodium chloride and sodium nitrate at concentrations which meet Federal Food and Drug Administration specifications has not been substantiated by other workers (Hansen, 1955). It is also well known that when radiation is used as the sterilizing agent, it frequently causes serious defects in odor, flavor, color and texture in food. This is particularly true when high radiation levels are used, such as those required to destroy botulinal spores. Consequently it would be highly desirable to determine if certain chemical compounds in combination with radiation can effectively destroy or inhibit germination of botulinal spores; and equally important, to determine the minimal levels of radiation and test compounds which will consistently destroy these spores in food. A previous report on a preliminary study by Anderson (1962) indicates that when NaNO_2 , NaNO_3 and NaCl are used in various combinations with irradiation (2.0 Mrad), there is inhibition of spoilage of canned ground round steak which has been inoculated with C. botulinum (type 5A) spores when incubated for 90 days or less at 35°C. However, viable spores were recovered in subculture and these produced a toxin lethal for mice. The present study was undertaken to determine which levels of certain test compounds and radiation will destroy botulinal spores when inoculated into canned ground beef. In addition, spores of type 33A will be used since recent studies (Anellis and Koch, 1962) have indicated that it is one of the most radiation resistant strains.

MATERIALS AND METHODS

Preparation of Spores. The stock strain of C. botulinum, type 33A, was obtained from the Quartermaster Food and Container Institute for the Armed Forces, 1819 Pershing Road, Chicago, Illinois*. The spores were grown and harvested according to the procedure described below.

The inoculum was heat shocked for 10 minutes at 80°C. Immediately after heat shocking a 0.5% inoculum was introduced into 400 cc of freshly steamed trypticase thioglycollate broth (trypticase, 5.0%; Bacto-peptone, 1.5%; glucose, 0.01%; sodium thioglycollate, 0.01%). The inoculated flasks were then incubated for 6 days at 35°C in an anaerobic atmosphere (97% nitrogen and 3% carbon dioxide) to allow for the formation of a maximum number of spores.

After the 6 day growth period, the spore suspension was centrifuged (20 minutes at 2200 RPM). This was followed by six washings with sterile phosphate buffer (0.15M) adjusted to pH 7.0. Finally, the spores (titer 10^8 spores/ml) were resuspended in the buffer and stored in screw cap tubes at 4-6°C.

For each experiment the refrigerated stock spore suspension was heat shocked and inoculated into 2500 cc of fresh trypticase-thioglycollate medium. After the 6 day growth period, the spores were centrifuged and washed (2 times in buffer) and resuspended in 200 ml of buffer. This latter suspension was used in making the viable spore count by the "most probably number" technique and was also the inoculum to be used in inoculating the meat prior to canning and irradiation. In all experiments the inoculum consisted of 1×10^6 spores per gram of ground round and was added just before the meat was canned.

Chemical Additives and Spore Inoculation. The following test compounds were used at the indicated levels in various combinations, with and without radiation:

<u>Test Compound</u>	<u>Levels</u>
NaNO ₂	100 & 200 ppm
NaNO ₃	500 & 1000 ppm
NaCl	1.5, 2.0 & 2.5%

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

The above substances were weighed out in flasks and sufficient water was added to make a heavy viscous suspension which was then added to the previously weighed meat sample (approx. 1000 gms). Following a thorough hand-mixing, the meat mixture was held at 4-6°C. for 18 hours to allow the added compound(s) to diffuse throughout the meat.

The meat was then inoculated with 1×10^6 viable, heat shocked spores per gram and thoroughly mixed. Approximately 100 gms. of the inoculated meat mixture was packed into baby food cans (202 x 204) and sealed. They were then packed in wet ice in an insulated ice chest and shipped to the Gamma Test Facilities, Arco, Idaho, for radiation. The cans were returned by the same method.

The meat used in these experiments was high-quality freshly ground round steak obtained from a locally operated government-inspected retail market.

Radiation Source. All cans in these experiments were irradiated at the Gamma Test Facilities, Arco, Idaho. The radiation levels used were 2.0, 2.5 and 3.0 Mrads.

Handling of Samples and Assay for Spore Survival. The irradiated samples returned to Oregon State University were incubated at 35°C. until a hard swell developed. They were then opened and examined microscopically to determine if spore growth had occurred. If no spoilage occurred within 120 days, the cans were opened and assayed.

The assay procedure consisted of opening the cans aseptically and removing a 10 gm. sample from various parts of the can with alcohol-flamed forceps. These samples were transferred to sterile screw cap tubes, covered with 7 ml. of steamed trypticase-thioglycollate broth and incubated anaerobically for 4 days at 35°C. The incubated subcultures were centrifuged at 2200 RPM for 20 minutes to separate the meat from the supernatant which contained the botulinum toxin. The supernatant was decanted off and divided into two aliquots. One portion was transferred to a sterile culture tube (15 x 150 mm) and served as the active preparation; the remaining portion was transferred into another culture tube and boiled for 15 minutes to inactivate any toxin present and to volatilize certain toxic substances such as ammonia. These latter tubes were again centrifuged and subsequently served as a control. Both sets of culture tubes were covered with rubber hooded stoppers and taped shut.

The liquid samples were then injected intraperitoneally into white mice (15-20 gms. each), using a dose of 0.20 ml. per mouse. Two mice received the unboiled inoculum and one received the boiled inoculum. Toxicity of the preparation was characterized by the typical symptoms of botulinal poisoning followed by death. Lethal effects were absent in the boiled control.

In order to ascertain the presence of viable spores in the toxin-containing samples, subcultures were made from the 10 gm. meat samples. These subcultures were then examined microscopically for presence of spores and toxin-producing ability.

RESULTS AND DISCUSSION

Results shown in Table 1 indicate that C. botulinum spores (33A) are not destroyed when inoculated into canned ground round and irradiated (2.0 Mrad) in the presence of various combinations of NaNO_2 (100 ppm), NaNO_3 (500 ppm) and NaCl (1.5%). Microbial spoilage was retarded for 30-55 days when the irradiated cans contained NaNO_3 or $\text{NaNO}_3 + \text{NaCl}$. In all other cases, spore germination occurred as rapidly in the treated samples as in the controls. The uninoculated controls remained in satisfactory condition during the 120 day incubation period (35°C).

When NaNO_2 was used in higher concentrations (200 ppm) with irradiation, the rate and extent of spoilage was decreased but not prevented entirely (Table 2). The highest level of radiation used (3.0 Mrad) in combination with NaNO_2 gave 16% spoilage (4 out of 25 cans) whereas the lower levels of irradiation (2.0 and 2.5 Mrad) resulted in 50% spoilage (12 out of 25 cans). The use of NaNO_2 or irradiation separately was ineffective. Viable spores and lethal toxin were recovered from 7 out of 50 non-spoiled cans.

In Table 3 NaNO_3 was used at the 1000 ppm level. The extent of spoilage was reduced to 4% (1 can out of 25) and there were no recoverable viable spores or toxin when irradiated to 3.0 Mrad. However, the can contents possessed a very objectionable, rancid odor which may be attributed to the high radiation level used. Lower levels of irradiation resulted in 20-40% spoilage (out of 25 cans), but viable spores and lethal toxin were found in the unspoiled samples.

Since NaCl has long been used as a food preservative, this compound was tested at concentrations of 2 and 2.5% in combination with radiation doses which do not produce undesirable odors in the meat. The results of Table 4 indicate there was 60% spoilage (out of 30 cans) and a high rate (40-100%) of recovery of viable spores and lethal toxin from the unspoiled cans.

A previous report (Anderson, 1962) indicated that nitrates and nitrites in combination with sodium chloride prevented the development of C. botulinum spores (5A and 115B) in irradiated canned meat during a 90 day incubation period. It was felt desirable to test these substances on spores of C. botulinum (33A). This is reported to be one of the most radiation resistant strains of botulinum when irradiated in phosphate buffer (Anellis and Koch, 1952).

When NaNO_2 (200 ppm) plus NaCl (2.5%) were added to the inoculated canned meat and irradiated at 2.5 or 3.0 Mrad, no spoilage, recoverable viable spores, or lethal toxin (out of 60 cans, total) was found after 120 days of incubation. Using the same combination of chemicals and a lower level of radiation (2.0 Mrad) resulted in 0.8% spoilage (1 can out of 125, total) but 5 of the remaining 124 cans contained viable spores and toxin (Table 5). It is conceivable that the one spoiled can was due to mishandling at the radiation source or to a localized high spore concentration in the can and, hence, may not be significant. However, the observation that 4% of the unspoiled cans contained lethal toxin is of considerable importance in evaluating this treatment. Kempe (1962) inoculated high levels of C. botulinum (62A) into canned ground meat followed by irradiation and incubation for 6 months to 5 years. He found no visible spoilage or viable spores but found ample evidence of botulinum toxin. He attributed the presence of toxin to the original inoculum of heat shocked spores which, by themselves, contained sufficient toxin to kill mice. In the present experiment, viable spores were recovered from all samples which contained toxin even though there was no apparent spoilage. Hence, the use of NaNO_2 and NaCl in conjunction with radiation will prevent spoilage of inoculated meat, but there is evidence of spore viability even after 120 days of storage.

When NaNO_3 (1000 ppm) plus NaCl (2.0 & 2.5%) were incorporated into inoculated canned ground round and subsequently irradiated, no spoilage over the 120 day incubation period and no viable spores or toxin were found (Table 6).

All levels of radiation proved equally effective. This observation is perhaps more significant than some previous reports, since a large number of samples was analyzed (125 total at the 2.0 Mrad exposure level), and the incubation period was extended to 120 days (vs. 90 days). In addition, there was little, if any, undesirable odor from the meat when irradiated at the lowest level, and the color was quite comparable to that of fresh ground round.

There are several areas which merit further study. Do the growth temperature and media of the inoculum subsequently alter the radiation resistance of the spores when introduced into meat? It should also be emphasized that the inoculum consisted of 1×10^6 spores per gram of meat. Since Kempe (1962), Niven (1958) and Riemann (1962) reported that the inoculum size is an important factor in determining the required radiation sterilization dosage, it is possible that the levels of additives and radiation used in this experiment could be appreciably reduced if the inoculum size was decreased. It would seem more practical to devise a method of sterilization wherein the inoculum size more nearly approaches that found in naturally contaminated foods. Also, considerable basic research remains to be done in explaining the mechanism whereby nitrates, nitrites and sodium chloride function synergistically in preventing spore development in irradiated substances.

In conclusion it was observed that inoculated canned ground round was effectively sterilized by radiation when NaNO_3 and NaCl were used in combination as additives. The use of NaNO_2 plus NaCl was less effective, and nitrite, nitrate, sodium chloride and radiation when used separately, proved to be quite ineffective in preventing spoilage.

REFERENCES

- Anderson, A. W., P. R. Elliker and R. F. Cain. 1962. Resistance of microorganisms to ionizing radiation applied to food. Report No. 9 (Final Report) of Quartermaster Food and Container Institute Project No. 7-84-01-002.
- Anellis, A. and R. B. Koch. 1962. Comparative resistance of strains of Clostridium botulinum to gamma rays. Appl. Microbiol., 10:326-330.
- Hansen, J. D. and M. D. Appleman. 1955. The effect of sorbic, propionic and caproic acids on the growth of certain clostridia. Food Research 20:92-96.
- Jensen, L. B. 1954. Microbiology of Meats. Third Ed., Garrard Press, Champaign, Illinois.
- Kempe, J. L. and J. T. Graikoski. 1962. Gamma-ray sterilization and residual toxicity studies of ground beef inoculated with spores of Clostridium botulinum. Appl. Microbiol. 10:31-36.
- Kempe, J. L., N. T. Graikoski and R. A. Gillies. 1954. Gamma ray sterilization of canned meat previously inoculated with anaerobic bacterial spores. Appl. Microbiol. 2:330-332.
- Morgan, B. H. and C. W. Bohrer. 1953. Sterilization by atomic radiation. Nat'l. Cannery Assoc. Information Letter no. 1426, Feb. 28, p. 1-4.
- Morgan, B. H. and J. M. Reed. 1954. Resistance of bacterial spores to gamma irradiation. Food Research 19:357-366.
- Niven, C. F. Jr. 1958. Microbiological aspects of radiation preservation of food. Ann. Rev. of Microbiol. 12:507-524.
- Riemann, H. 1962. Anaerobe toxins. In Chemical and Biological Hazards in Food. Edited by J. C. Ayres, A. A. Kroft, H. E. Snyder and H. W. Walker. Iowa State University Press, Ames, Iowa. pp 279-302.
- Silliker, J. H., R. A. Greenberg and W. R. Schack. 1958. Effect of individual curing ingredients on the shelf stability of canned comminuted meats. Food Technol. 12:551-554.

TABLE 1

Spoilage of canned ground round **beef inoculated with Clostridium botulinum (33A)** plus test compound and irradiated at 2.0 megarad*

Test Cmpd.	<u>C. botulinum</u> . Conc. Spores/gm.	Mrad.	Days of Incubation Before Spoilage	Per cent of Total Cans Spoiled**
None	-- 0	0	5	100%
None	-- 1 x 10 ⁶	0	5	100%
NaNO ₂	100 ppm 1 x 10 ⁶	0	5	100%
NaNO ₃	500 ppm 1 x 10 ⁶	0	5	100%
NaNO ₂ + NaCl	100 ppm 1.5% 1 x 10 ⁶	0	5	100%
NaNO ₃ + NaCl	500 ppm 1.5% 1 x 10 ⁶	0	5	100%
None	-- 0	2	120	0
None	-- 1 x 10 ⁶	2	5	100%
NaNO ₂	100 ppm 1 x 10 ⁶	2	5	100%
NaNO ₃	500 ppm 1 x 10 ⁶	2	55	100%
NaNO ₂ + NaCl	100 ppm 1.5% 1 x 10 ⁶	2	15	100%
NaNO ₃ + NaCl	500 ppm 1.5% 1 x 10 ⁶	2	30	100%

* Five cans were used per sample run

** Spoilage indicated by hard swell of the can and/or spoiled contents.

TABLE 2

Spoilage of canned ground round beef inoculated with C. botulinum (33A) plus NaNO₂ and irradiated at various dosage levels

Test Cmpd.	<u>C. botulinum</u> Conc.	Spores/gm.	Mrad.	No. of cans	Days of incubation before spoilage	% Total cans spoiled	% Unspoiled cans containing mouse-lethal toxin*
NaNO ₂	200 ppm	0	0	5	10	100%	---
NaNO ₂	200 ppm	1 x 10 ⁶	0	25	15	100%	---
None	---	1 x 10 ⁶	2	5	5	100%	---
NaNO ₂	200 ppm	1 x 10 ⁶	2	25	120	48%	0
None	---	1 x 10 ⁶	2.5	5	120	80%	0
NaNO ₂	200 ppm	1 x 10 ⁶	2.5	25	120	52%	25%
None	---	1 x 10 ⁶	3.0	5	120	40%	0
NaNO ₂	200 ppm	1 x 10 ⁶	3.0	25	120	16%	14%

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 3

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus NaNO₃ and irradiated at various dosage levels

Test Cmpd.	<u>C. botulinum</u> Conc.	Spores/gm.	Mrad.	No. of cans	Days of incuba- tion before spoilage	% Total cans spoiled	% Unspoiled cans containing mouse-lethal toxin*
NaNO ₃	1000 ppm	1 x 10 ⁶	0	25	5	100%	---
NaNO ₃	1000 ppm	0	0	5	5	100%	---
NaNO ₃	1000 ppm	1 x 10 ⁶	2	25	120	20%	10%
None	---	1 x 10 ⁶	2	5	30	100%	---
NaNO ₃	1000 ppm	1 x 10 ⁶	2.5	25	120	40%	20%
None	---	1 x 10 ⁶	2.5	5	30	100%	---
NaNO ₃	1000 ppm	1 x 10 ⁶	3.0	25	120	4%	0
None	---	1 x 10 ⁶	3.0	5	120	60%	0

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 4

Spoilage of canned ground round **beef inoculated with Clostridium botulinum** (33A) plus NaCl and irradiated at 2.0 megarad

Test Cmpd.	Conc.	<u>C. botulinum</u> . Spores/gm.	Mrad.	No. of cans	Days of incubation before spoilage	% Total cans spoiled	% Unspoiled cans containing mouse-lethal toxin*
NaCl	2.5%	0	0	5	5	100%	---
NaCl	2.5%	1×10^6	0	5	5	100%	---
NaCl	2.5%	1×10^6	2	25	120	60%	40%
NaCl	2.0%	1×10^6	2	5	120	60%	100%
None	--	1×10^6	2	5	20	100%	---

* All cans which did not spoil after 120 days incubation (35°C) were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

TABLE 5

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus test compounds and irradiated at various dosage levels

Test Cmpd.	<u>C. botulinum</u> . Conc. Spores/gm.	Mrad..	No. of cans	Days of incubation before spoilage	% Total cans spoiled	% Unspoiled cans containing mouse-lethal toxin*	
NaNO ₂ + NaCl	200 ppm + 2.5%	0	5	25	100%	---	
NaNO ₂ + NaCl	200 ppm + 2.5%	1 x 10 ⁶	25	80	100%	---	
None	---	1 x 10 ⁶	5	7	100%	---	
NaNO ₂ + NaCl	200 ppm + 2.5%	1 x 10 ⁶	2	a. 25** b. 100*** tot. 125	120	0.8%	4%
None	---	1 x 10 ⁶	5	15	100%	---	
NaNO ₂ + NaCl	200 ppm + 2.5%	1 x 10 ⁶	2.5	a. 25** b. 10** tot. 35	120	0	0
None	---	1 x 10 ⁶	5	120	80%	0	
NaNO ₂ + NaCl	200 ppm + 2.5%	1 x 10 ⁶	3.0	25	120	0	0
NaNO ₂ + NaCl	200 ppm + 2%	1 x 10 ⁶	2.0	10	120	10%	0

* All cans which did not spoil after 120 days were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

** Results of preliminary study.

*** Results of final experiment.

TABLE 6

Spoilage of canned ground round beef inoculated with Clostridium botulinum (33A) plus test compounds and irradiated at various dosage levels

Test Cmpd.	<u>C. botulinum</u> . Conc. Spores/gm.	Mrad.	No. of cans	Days of incuba- tion before spoilage	% Total cans spoiled	% Unspoiled cans containing mouse-lethal toxin*
NaNO ₃ + NaCl	1000 ppm 2.5%	0	5	120	80%	0
NaNO ₃ + NaCl	1000 ppm 2.5%	1 x 10 ⁶	25	30	100%	---
None	---	1 x 10 ⁶	5	85	100%	---
NaNO ₃ + NaCl	1000 ppm 2.5%	1 x 10 ⁶	2	a. 25** b. 100*** tot. 125	120	0
None	---	1 x 10 ⁶	5	120	80%	0
NaNO ₃ + NaCl	1000 ppm 2.5%	1 x 10 ⁶	25	120	0	0
None	---	1 x 10 ⁶	5	120	0	0
NaNO ₃ + NaCl	1000 ppm 2.5%	1 x 10 ⁶	25	120	0	0
NaNO ₃ + NaCl	1000 ppm 2.0%	1 x 10 ⁶	10	120	0	0

* Those cans which did not spoil after 120 days were checked for botulinum toxin. The presence of viable spores was indicated by microscopic examination of subcultures.

** Results of preliminary study.

*** Results of final experiment.

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)

OREGON STATE UNIVERSITY
Corvallis, Oregon

2a REPORT SECURITY CLASSIFICATION

Unclassified

2b GROUP

3 REPORT TITLE

RESISTANCE OF MICROORGANISMS TO IONIZING RADIATION APPLIED TO FOOD

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final. June 1962 - September 1963

5 AUTHOR(S) (Last name, first name, initial)

ANDERSON, A. W.

6 REPORT DATE

September 1965

7a TOTAL NO OF PAGES

13

7b NO OF REFS

11

8a CONTRACT OR GRANT NO

DA19-129-QM-2035

9a ORIGINATOR'S REPORT NUMBER S

b PROJECT NO

7-84-01-002

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

FD-24

10 AVAILABILITY LIMITATION NOTICES

Qualified requestors may obtain copies of this report from DDC. Release to Clearing House for Federal Scientific and Technical Information is authorized.

11 SUPPLEMENTARY NOTES

12 SPONSORING MILITARY ACTIVITY

Food Division, U. S. Army Natick
Laboratories, Natick, Mass. 01762

13 ABSTRACT

Spores of Clostridium botulinum (33A) were inoculated into canned ground beef containing various additives and subsequently irradiated. Sodium nitrate (1000 ppm) plus sodium chloride (2.5%) completely inhibited spoilage for 120 days at 35°C when samples were exposed to 2.0 Mrad, and no viable spores or toxin were recovered upon sub-culture. One hundred and twenty five cans were used per run. The use of either additive, with or without radiation, did not prevent spoilage unless higher levels of radiation such as 2.5 and 3.0 Mrad were used, with the concomitant production of undesirable odors.

Sodium nitrite (200 ppm) plus sodium chloride (2.5%) inhibited spoilage for 120 days but there was evidence of spore viability and toxin production in some of the unspoiled cans when the radiation level was 2.0 Mrad. Higher levels of radiation gave results similar to those obtained for sodium nitrate and sodium chloride.

Unclassified
Security Classification

KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Inoculation	8				6	
Clostridium botulinum	1		7		6	
Beef	1,2		7		7	
Canned	0		0		0	
Ground	0		0		0	
Round	0		0		0	
Irradiation					6	
Sodium nitrate			6		6	
Sodium chloride			6		6	
Sodium nitrite			6		6	
Inhibition			4		7	
Spores	2		4		7	
Viable	0		0		0	
Toxin	2		4		7	
Irradiated			0		0	
Non-irradiated			0		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.

2a. 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.

2b. GRDUP: 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.

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

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

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

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

9a. 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.

9b. 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.