THE COMBINED EFFECTS OF HEAT AND IRRADIATION ON THE SURVIVAL OF THE SPORES OF *CLOSTRIDIUM BOTULINUM*, TYPE A5, IN RAW GROUND BEEF

by

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INTRODUCTION

Subjecting foods to ionizing radiation is not a new idea in food preservation. The lethal properties of X-rays and various radio-active materials have been realized since their first discovery and isolation. In fact, in 1929, a patent was issued in France which suggested the use of radiation for food processing.

During and immediately after World War II, food processing by ionizing radiation gained impetus because of the many sources of radio-active isotopes available, and the tremendous advances in ion-acceleration devices. This field possessed a great appeal to the food industry, since it was the first new technic suggested since thermal processing.

Since many foods are appreciably changed in color, odor, texture and flavor by thermal processing, researchers hoped that this new "cold sterilization" method would be applicable to all foods. Predicting commercial use of radiation processing within a decade, early investigators and the lay press were highly optomistic. However, as investigation proceeded, it was found that at the extremely high radiation levels required for sterilization and/or enzyme inactivation

the foods were altered to varying extents with regards to flavor, odor, color and texture (23, p. 439-445).

Most current research is aimed at methods of reducing sterilization dosages so that the irradiated products will remain unchanged within the limits of consumer acceptance.

A portion of this problem is being considered in this thesis. Specifically, this study was undertaken to evaluate the use of mild heat treatments as a method of lowering the irradiation dosage required to sterilize raw ground beef inoculated with spores of Clostridium botulinum, type A5.

REVIEW OF LITERATURE

The bacterium, Clostridium botulinum, is characterized by Dack (5, p. 60-61) as follows:

> "The organism.--Clostridium botulinum is a name applied to a group of rod-shaped, spore-forming, anaerobic bacteria producing exotoxins which may be absorbed from the intestinal tract and which give rise to a common train of symptoms. Five types have been distinguished on the basis of the specific nature of their toxins. These are designated as Cl. botulinum or parabotulinum, Types, A, B, C, D, and E. The proteolytic and cultural properties are used by some workers in designating the species as botulinum (nonproteolytic) or parabotulinum (proteolytic).....Human botulism has been reported for Types A, B, and more recently, E. Types C and D have not been reported as causing human outbreaks of botulism.
>
> Type A and, to a certain extent, Type B of Cl. botulinum are proteolytic and digest coagulated egg white, whereas Types C, D, and E do not. Gelatin is liquefied by strains of all types. Many of the European Type B strains are not proteolytic.
>
> In general, the cells of all the types are large gram-positive rods. The spores are usually subterminal but are terminal in Type C Cl. botulinum. The vegetative cells contain flagella (peritrichate) and are motile under suitable anaerobic conditions."

The extreme toxicity of Cl. botulinum combined with its spore forming ability has made it an organism of perennial concern in the food processing industry. While it is not the most heat resistant of the spore formers, it shows a high degree of resistance to thermal processing (27, p. 96, 99). Dack (5, p. 89), suggests

the following time at temperature relationships for total destruction of Cl. botulinum spores:

Time (min.)	Temperature (°C.)
360	100
120	105
36	110
12	115
4	120

This would constitute a severe thermal processing treatment, producing the undesirable organoleptic changes mentioned earlier. Meat, one of the most susceptible foods to botulinum poisoning, is also one of the foods most changed by heat treatment.

Cl. botulinum has to date proven to be the most radiation resistant, spore-forming organism. Considerable study has been conducted to determine the limit of resistance of Cl. species to irradiation (14, p. 332; 19, p. 357-366; 21, p. 149-152; 22, p. 378). Niven (20, p. 514-516) has summarized the findings concerning the radiation resistance of Cl. botulinum spores. He states, in substance, that 0.8 - 1.3 x 10^6 rad of gamma radiation is required to inactivate 99% of the spores present. He further states that the calculated dose required to sterilize a product containing Cl. botulinum spores is 4.5 x 10^6 rad. One should note that these extremely high doses are well over the dose (2 x 10^6 rad) considered the threshold of serious off-flavor and off-color

production in meats (12, p. 360-361; 24, p. 47; 25, p. 238).

In the treatment of meat with ionizing radiations, the extreme resistance of proteolytic enzymes present in the meat has been noted (6, p. 16; 7, p. 23; 32, p. 389-400). It is suggested that these autolytic enzymes are as much as ten times more resistant to ionizing radiation than the most resistant bacteria. However, investigation shows that relatively little heat is required to inactivate these enzymes. Chiambalero, et al. (2, p. 782-784) studied the use of heat with irradiation applied to the inactivation of proteolytic enzymes in beef and pork. The following data were given for complete inactivation of enzymes in beef (2, p. 783):

Temperature (°F.)	Time-at Temperature (min.)
140	23.0
150	6.0
155	3.5
160	1.5
165	0.65
170	0.28

Observation showed that no differences were noted whether the heat treatment was applied before or after irradiation.

The fact, that in most cases, heat would have to be used for enzyme inactivation in conjunction with any radiation treatment, led to investigations as to the

effectiveness of heat and irradiation as a combined treatment. There have been several approaches to this problem.

Wood (30, p. 157-174) and Stapleton (26, p. 229-230), working independently, found that elevated temperatures during irradiation increased the effectiveness of the treatment on vegetative cells. They both suggested that the increased lethality was due to an increase in the diffusion factor of the radiation poisons, (H^+), (OH^-), and H_2O_2. Wood (30, p. 167) found that heat applied before or after irradiation also increased the lethality. He concluded (30, p. 169), "all that can be said with certainty is that each of these two lethal agents, heat and X-rays, 'softens' the cell for the action of the other, the degree of interaction depending on the sequence of treatment."

Erdmand and Watts (9, p. 352) found that cured meats given a heat treatment of 70° C. after radiation treatments ranging from 2×10^5 to 2×10^6 showed no spoilage after 170 days. No microbiological examination was made.

Kempe and his co-workers conducted a series of experiments to determine the effectiveness of heat plus irradiation treatments on spores of Cl. botulinum types 213B and 62A in a phosphate buffer medium. He found (15, p. 352) that heat treatments of 99° C. prior to

irradiation had little effect on the lethality of subsequent gamma irradiation. However, a treatment of approximately 1.3 megarep, prior to heating, reduced the heat treatment required for sterilization four-fold in a phosphate buffer medium. Subsequent experiments with cooked and raw beef (16, p. 108-113; 17, p. 292-295; 18, p. 261-263) showed a lowering of the sterilizing heat treatment three-to-four fold, when a treatment of 1.0 megarep of gamma radiation was applied prior to heating. The results were the same for both the cooked and raw beef.

Kan and his colleagues concluded that irradiation sensitizes spores to subsequent heat by altering some protein constituent and thereby making it more heat sensitive (13, p. 518).

Bridges and Horne suggested (1, p. 110) that a great reduction in sterilizing dose might be obtained if a reliable method could be devised for inducing the germination of bacterial spores just prior to irradiation.

Evans and Curran in 1948 found that sublethal heat treatments ranging from 65-95° C. accelerated the germination of bacterial spores (10, p. 47-48). This technic would be applicable in the method suggested by Bridges and Horn.

In a review of the work in the field of radiation sterilization, Fuld, *et al.* (11, p. 43) stated, "Different bacterial species vary widely in degree of radiation resistance. The resistance of a species varies with the conditions before, during and after irradiation. Thus, the exact dose for any food must be determined experimentally under the exact conditions of commercial processing."

This briefly summarizes the status of the research in food irradiation pertaining to *Cl. botulinum*. While some interesting and significant results have been obtained, the problem remains to carefully test and apply these theses to each product and organism involved.

METHODS AND MATERIALS

Plan of Experiment

This experiment was designed, keeping in mind all the principles mentioned in the literature review, as an attempt to devise a minimal heat-irradiation treatment that would sterilize raw beef inoculated with Cl. botulinum, type A5, spores.

The basic plan is shown on Table 1. Preliminary experiments in this laboratory indicated that three megarads was approximately the threshold of lethality for Cl. botulinum, type A5, consequently this was chosen as the top limit of irradiation. The temperatures chosen represent those which will arrest enzyme action but are sublethal for unirradiated spores of Cl. botulinum. The other variable was the time at which the heat could be applied most effectively, either before, during or after irradiation.

A special experiment designed to test the effects of intermittent irradiation plus heat treatments on the survival of Cl. botulinum, type A5, was carried out. This experiment will be referred to as 1-2. The inoculated samples received 1.0 megarads of gamma irradiation along with the varying heat treatments. The samples were then incubated four to six hours at 90° F. and then

Table 1

BASIC PLAN FOR HEAT-IRRADIATION TREATMENT OF Cl. botulinum (A-5)*

	Heat Treatment (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5**	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
2.0	5	5	5	5	5	5	5	5	5	5
2.5	5	5	5	5	5	5	5	5	5	5
3.0	5	5	5	5	5	5	5	5	5	5

*This plan was repeated for each heat experiment; heat applied before, during and after irradiation. Complete experiment totaled 950 samples.

**Number of replications required for statistical significance.

were given a second 1.0 megarad dose of radiation, this time at ambient temperature. This treatment was designed to check the stimulatory effect of sublethal doses of heat and irradiation on spore germination.

Spores

The stock strain of Cl. botulinum, type A5, was obtained from the Quartermaster Food and Container Institute for the Armed Forces, 1819 Pershing Road, Chicago, Illinois. The large number of spores necessary for this experiment was grown and harvested as described below.

The inoculum to be used was heat shocked for fifteen minutes at a temperature of 80° C. (176° F.) (4, p. 437; 28, p. 82). Immediately after heat shocking, a 0.5% inoculum was introduced into freshly steamed trypticase-thioglycollate broth (Trypticase, 5.00%; Bacto-peptone, 1.50%; Glucose, 0.01%; Sodium thioglycollate, 0.1%; pH 7.0). The inoculated flasks were then incubated at 37° C. (98.5° F.) for 7-8 days, to allow the formation of a maximum number of spores.

After the 7-8 day growth period the spores were separated from the medium by centrifugation for 45 minutes at 2100 revolutions per minute. This was followed by two washings with a M/15 phosphate buffer

solution adjusted to pH 7.0. Finally, the spores were combined and stored in 120 milliliters of buffer solution (titer 1 x 10^8 spores/ml) at 4° C. (39° F.) until needed. Prior to being used, the titer of viable spores was checked by inoculating tubes of trypticase-thioglycollate broth with appropriate heat shocked dilutions of the spore suspension (most probable numbers).

Meat Packs

The meat used in this experiment was a high quality (85% lean and 15% fat and fiber) fresh ground beef round. It was obtained from a locally operated, government inspected, meat packing plant. The meat was weighed in 1000 gram portions and inoculated with a quantity of heat shocked type A5 spores, so that the total count would be approximately 1 x 10^6 spores per gram, evenly distributed throughout the meat.

The meat thus inoculated was packed into baby food (202 x 204) cans, (3, p. 495) 100 grams per can, using five replicates per treatment (see Table 1). The cans were then sealed using a modified Model No. 612.5147 Maid of Honor Automatic Can Sealer. After sealing, the cans were rinsed in a chlorinated (1000 ppm Cl) detergent solution and packed in wet ice for shipment to the

Gamma Test Facilities, Arco, Idaho. All cans were held at wet ice temperature until treated.

Heat Source

The concurrent heat treatment was provided by the equipment shown in Figures 1 through 5. The hot water source was provided by installing immersion heating elements (total 3300 watts) into each of two 25-gallon garbage cans. The temperature was kept constant, to within $\pm$ 0.5° F., by a NAPCO Safety-stat. This hot water supply served as the come-up tank and the reservoir for the submersible bath. A pump was used to circulate the water through the submersible bath and back to the reservoir.

The temperature in the submersible bath was determined experimentally by inserting a thermocouple in the inner chamber. This temperature was approximately 10° F. less than the temperature in the hot water reservoir. Temperatures of the reservoir and those recorded by the thermocouple were read at the temperature levels to be used in the experiment (see Table 1), while the water was being circulated under the conditions of actual operation. During irradiation the submersible bath was immersed in 16 feet of water (fuel canal). The water temperature in the canal containing the fuel rods was 68°F.

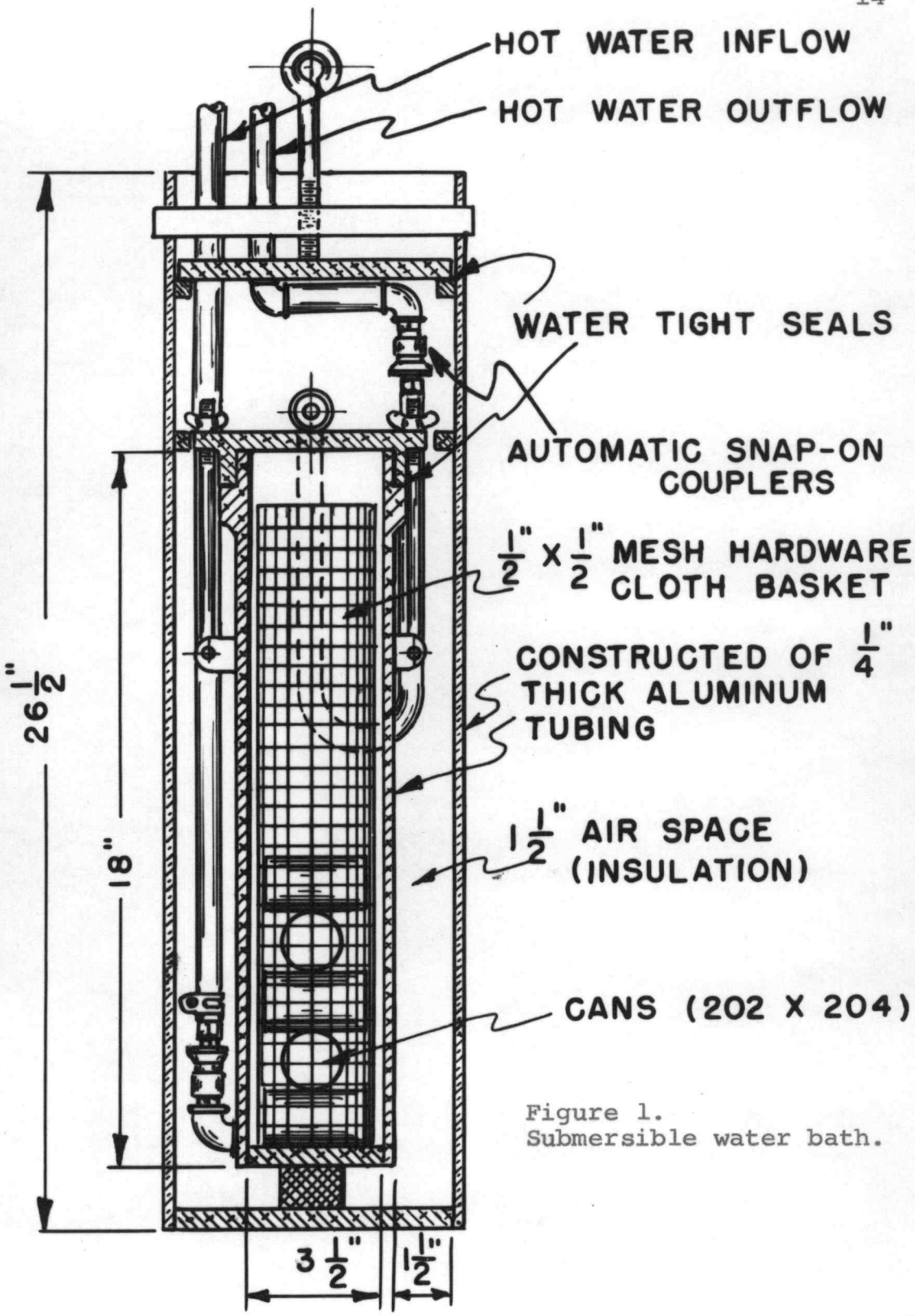

Figure 1.
Submersible water bath.

Figure 2. External view, submersible water bath.

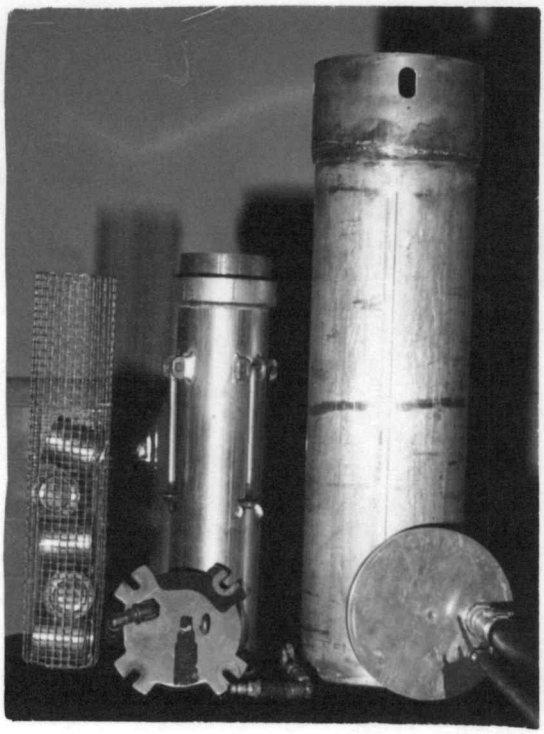

Figure 3. Components of submersible water bath.

Figure 4. Constant temperature chamber, showing hoses for the in- and out-flow of hot water.

Figure 5. Constant temperature chamber, showing position of wire basket and cans.

The garbage cans previously mentioned were also used to heat those cans heated either prior to or following irradiation. One garbage can was used as a come-up tank, and the second as the holding tank.

Come-up Times

The come-up times for the meat packs were determined experimentally by inserting thermocouples in the center of the cans, (8, p. 231-233) then the cans were filled with meat. The cans were immersed in water 10° F. higher than the temperature desired. The come-up curve was recorded by a Honeywell thermocouple recording instrument connected to the thermocouple inside the can. A typical come-up curve is shown in Figure 6. The come-up times for the other temperatures showed similar curves. A standard come-up time of 35 minutes was adopted for all temperatures, since this was the maximum time required for any one of the temperatures to reach the desired level. (Minimum come-up time 32.5 minutes.)

Radiation Source

All cans in this experiment were irradiated at the Gamma Test Facilities, Arco, Idaho.

The dosimetry was checked every eight hours of operation by ceric sulfate bottles (29, p. 28-31).

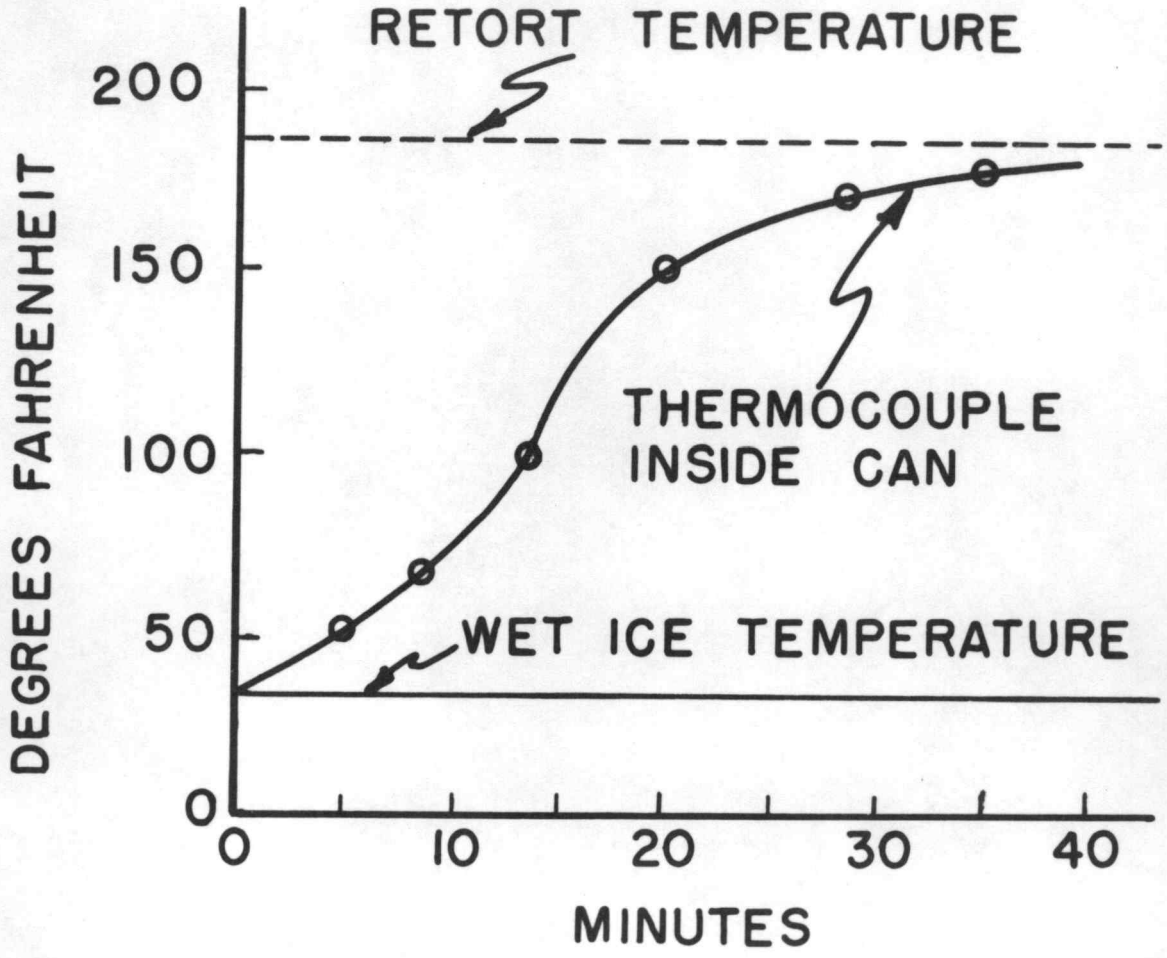

Figure 6. Typical come-up curve.

These bottles were located inside the submersible bath in positions corresponding to the centers of the top, bottom, and center cans as they fit inside the inner chamber of the submersible bath (Figure 1). Table 2 shows a typical set of dosimetry readings, plus the calculated time-dose relationships based on these readings.

Treatment

The total treatment of the samples in this experiment was determined by keeping three factors in mind. First, that the come-up time for all temperatures was 35 minutes; second, that the maximum flux rate would be approximately 4×10^6 rad/hour (therefore, about 45 minutes would be needed to obtain the highest dose, 3×10^6 rad); third, that all cans should be held at the specific temperatures for a constant length of time. A few minutes were allowed for handling. Based on the above consideration the following treatment was decided upon:

1. Come-up time 35 minutes.
2. Time at temperature 50 minutes. This included maximum irradiation time and five minutes for handling.

Table 2

DOSIMETRY AND CALCULATED DOSAGES

DOSIMETRY READINGS--6/14/60

Mid-plane------4.28×10^6 rad/hr.

3" above-------4.14×10^6 rad/hr.

3" below-------4.01×10^6 rad/hr.

Average--------4.14×10^6 rad/hr.

CALCULATED DOSAGE

Dose-10^6 rad	Time in Min.
1.0	14.5
1.5	21.5
2.0	29.9
2.5	36.0
3.0	43.0

3. The cans were held the additional time required in an auxilary water bath when irradiation time was less than 45 minutes.
4. Cans heated before or after irradiation were held at temperature for 50 minutes.

The cans treated before irradiation were heat treated prior to being shipped to Arco.

Cans treated after irradiation were heated upon their return from Arco. During transporting and/or storage the cans were held at wet-ice temperature.

Assay for Spore Survival

After irradiation the samples were returned to Oregon State College. Upon arrival these cans were placed in a 37° C. (98.5° F.) incubator. Incubation continued until the cans developed a hard swell, then these cans were assayed. If spoilage was not evident within 30 days, one of the group of cans was removed and assayed, the others remaining in the incubator. After the first 30-day period this procedure was repeated at 15-day intervals until all cans were assayed.

After spoilage, or a specific time lapse, the cans were aseptically opened. Five grams of meat was removed from the center of the sample and placed in a screw-cap test tube. Five milliliters of freshly steamed

trypticase-thioglycollate broth was added to each tube. (31, p. 64). The tubes were then incubated for 48 hours at 37° C. (98.5° F.).

After incubation, the subcultures were centrifuged for 45 minutes at 2100 revolutions per minute. The supernatant was separated, one portion was transferred to a test tube and fitted with a serum bottle stopper; the remaining supernatant was poured into a second test tube and boiled for 10 minutes. The boiling inactivated any Cl. botulinum toxin and boiled off any other toxic substances such as ammonia, thus served as a control (5, p. 81, 98-99; 14, p. 331; 31, p. 62). This material after boiling was again centrifuged and prepared for injection as was the unboiled supernatant.

The inoculum thus prepared was next injected into 25-30 gram white mice. Each mouse received 0.20 ml. For each sample two mice received the unboiled inoculum and one mouse received the boiled inoculum. If the mice receiving the unboiled material died and the mouse receiving the boiled sample survived, one assumed that toxin was present. This indicated that some spores did survive the particular heat-irradiation treatment.

As a further check on the survival of the spores, the residue of the subculture was examined microscopically for the presence of typical Cl. botulinum cells.

This procedure provided three opportunities to detect spore survival: from the spoilage of the incubated cans, from the microscopic examination of the subcultures, and from the mouse toxicity tests of the subcultures.

EXPERIMENTAL RESULTS

The three phases of this experiment--heat treatment applied before, during, and after irradiation--will be considered separately in reporting the results. In the discussion all three phases will be summarized and compared.

Heat Treatment Before Irradiation

As mentioned earlier, each sample was examined for spore survival by observing visible spoilage, by mouse toxicity tests, and by microscopic examination.

Visible Spoilage: Referring to Table 3, irradiation levels up to and including 1.5 megarads, all showed visible spoilage. At 2.0 megarads significantly fewer cans showed spoilage for heat treatments of 180 and 190° F. Most heat treatments at the 2.5 megarad level corresponded to ambient temperature; however a marked increase in visible spoilage was noted at 140, 175 and 185° F. At 3.0 megarads only those cans heated at temperatures of 165, 175 and 185° F. showed visible spoilage.

Toxicity Test: The results of the toxicity test expressed as mouse lethality is shown in Table 4. Samples at all irradiation levels through 1.5 megarads were 100% lethal for mice. This indicated definite spore survival.

Table 3

VISIBLE SPOILAGE OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment before irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
2.0	5	5	5	5	5	4	4	2	5	2
2.5	1	4	1	1	0	1	4	0	3	0
3.0	0	0	0	0	2	0	4	0	4	0

*Number of cans showing visible spoilage out of five replications. Incubated at 37° C. (98.5° F.) for 90 days.

Table 4

MOUSE LETHALITY OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment before irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
2.0	5	5	5	5	5	4	5	2	5	2
2.5	1	4	1	1	0	0	5	0	3	2
3.0	0	0	1	1	2	1	5	0	5	1

*Number of cans lethal out of five replications using mouse assay. Incubated at 37° C. (98.5° F.) for 90 days.

Heat treatments of 180 and 190° F. showed a marked reduction of spore survival at 2.0 megarads. At the 2.5 megarad level an increase in spore survival was noted at heat treatments of 140, 175 and 185° F. as compared to ambient temperature. Survival of spores was evidenced at 3.0 megarads at heat treatments of 150, 160, 170 and 190° F. with a marked increase at 165, 175 and 185° F.

Table 5 shows the number of samples that proved lethal even though they showed no signs of visible spoilage.

Microscopic Examination: In all cases, cells morphologically similar to Cl. botulinum were observed in samples that demonstrated toxicity.

Concurrent Heat and Irradiation

Visible Spoilage: In Table 6 it is noted that all samples receiving 1.5 megarads or less developed a hard swell. For the irradiation dose 1-2 (see page 9 and Table 6) a significant reduction of spoilage was noted for the following heat treatments: 150, 170, 175, 180, 185 and 190° F. Two megarads reduced spoilage at all heat treatments except 68, 140 and 160° F. Temperature difference showed little effect on spoilage of cans receiving 2.5 megarads, with the possible exception of

Table 5

SAMPLES SHOWING TOXICITY BUT NO VISIBLE SPOILAGE,

GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment before irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0	0	0	0
2.5	0	0	0	1*	0	0	1	0	0	2
3.0	0	0	1	1	0	1	1	0	1	1

*Number of cans lethal but not showing spoilage.
Incubated at 37° C. (98.5° F.) for 90 days.

Table 6

VISIBLE SPOILAGE OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Concurrent heat and irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
1-2**	5	4	3	5	5	1	2	1	1	0
2.0	5	5	3	5	2	2	2	2	1	1
2.5	1	4	1	1	0	0	1	0	0	0
3.0	0	0	0	0	0	0	0	0	0	0

*Number of cans showing visible spoilage out of five replications. Incubated at 37° C. (98.5° F.) for 90 days.

**Total dose, 2.0 megarads.

an increase in spoilage at 140° F. No spoilage was noted at the 3.0 megarad level.

Toxicity Tests: Referring to Table 7, all cans receiving an irradiation dose of 1.5 megarads or less were toxic to laboratory mice. Toxicity was reduced at the 1-2 level by heat treatments of 170, 180, 185 and 190° F. Those cans receiving 2.0 megarads showed a reduction in spore survival (expressed as toxicity) at heat treatments of 185 and 190° F. Only the heat treatment of 140° F. showed an influence on spore survival at the 2.5 megarad level. Toxicity tests indicated spore survival at 3.0 megarads irradiation and heat treatments of 150, 160 and 170° F.

Table 8 shows a considerable discrepancy in spoilage and toxicity as indications of spore survival. This will be considered further in the discussion of the results.

Microscopic Examination: Again, cells similar in morphology to Cl. botulinum were observed in the subcultures of all toxic samples.

Heat Treatment After Irradiation

Visible Spoilage: Table 9 shows that all samples receiving an irradiation dose of 1.5 megarads or less visibly spoiled when incubated at 37° C. (98.5° F.) A significant reduction was noted at the 2.0 megarad

Table 7

MOUSE LETHALITY OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replications per condition)

	Concurrent heat and irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
1-2**	5	5	5	5	5	3	4	2	3	1
2.0	5	5	5	5	4	5	4	4	3	3
2.5	1	5	1	2	0	2	2	1	0	2
3.0	0	0	1	1	0	1	0	0	0	0

*Number of cans lethal out of five replications using mouse assay. Incubated at 37° C. (98.5° F.) for 90 days.

**Total dose, 2.0 megarads.

Table 8

SAMPLES SHOWING TOXICITY BUT NO VISIBLE SPOILAGE,

GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Concurrent heat and irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
1-2**	0	1*	2	0	0	2	2	1	2	1
2.0	0	0	2	0	2	3	2	2	2	2
2.5	0	0	1	0	0	2	1	1	0	2
3.0	0	0	1	1	0	1	0	0	0	0

*Number of cans lethal but not showing spoilage. Incubated at 37° C. (98.5° F.) for 90 days.

**Total dose, 2.0 megarads.

Table 9

VISIBLE SPOILAGE OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment after irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
2.0	5	5	5	5	3	4	1	5	0	1
2.5	1	1	1	1	0	0	0	1	0	0
3.0	0	0	0	0	0	0	0	0	0	0

*Number of cans showing visible spoilage out of five replications. Incubated at 37° C. (98.5° F.) for 90 days.

level, particularly at heat treatments of 165, 175, 185, and 190° F. Two and one-half megarads showed no spoilage at the following heat treatments: 165, 170, 175, 185 and 190° F. No spoilage was noted at the 3.0 megarad level.

Toxicity Tests: Toxin was demonstrated in all samples receiving 1.5 megarads or less as shown in Table 10. A significant reduction of spore survival is indicated by combined treatments of 2.0 megarads and temperatures of 165, 175, 185 and 190° F. No toxicity was observed at 2.5 megarads and heat treatments of 150, 165, 170 and 185° F. At the 3.0 megarad level toxin was demonstrated only on samples receiving heat treatments of 165 and 190° F.

Table 11 shows the samples that proved toxic even though no signs of spoilage were observed.

Microscopic Examination: As in the two previous heat treatments (before and concurrent), spore survival was confirmed by observation of cells similar in morphology to Cl. botulinum in the toxic subcultures.

General Observations

Enzyme Activity: No evidence of autolytic enzyme activity was observed in any of the samples in this experiment. This evidence confirms the data of

Table 10

MOUSE LETHALITY OF GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment after irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	5*	5	5	5	5	5	5	5	5	5
1.0	5	5	5	5	5	5	5	5	5	5
1.5	5	5	5	5	5	5	5	5	5	5
2.0	5	5	5	5	3	5	1	5	1	1
2.5	1	1	0	1	0	0	1	1	0	1
3.0	0	0	0	0	1	0	0	0	0	1

*Number of cans lethal out of five replications using mouse assay. Incubated at 37° C. (98.5° F.) for 90 days.

Table 11

SAMPLES SHOWING TOXICITY BUT NO VISIBLE SPOILAGE,

GROUND ROUND INOCULATED WITH Cl. botulinum (A-5)

(5 Replicates per treatment)

	Heat treatment after irradiation (° F.)									
Megarad	68	140	150	160	165	170	175	180	185	190
0.0	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	1*	0	0	1	0
2.5	0	0	0	0	0	0	1	0	0	1
3.0	0	0	0	0	1	0	0	0	0	1

*Number of cans lethal but not showing spoilage.
Incubated at 37° C. (98.5° F.) for 90 days.

Chiambalero, et al. (2, p. 783) as quoted on page 4 of this paper.

Organoleptic Changes: The heat treatments above 140° F. were sufficient to cause shrinkage and firmness in the meat. A bright cherry red color was noted in all samples receiving at least 2.0 megarads, but the color faded after exposure to the air for a few minutes, and a burnt cereal smell was noticed in all unspoiled samples. These observations corroborate the work of Shultz, et al. (25, p. 238).

DISCUSSION

A brief restatement of the objectives of this experiment may aid in an evaluation of the results. The objectives were:

1. To determine the effects of enzyme deactivating heat treatments on the radiation resistance of Cl. botulinum, type A5, spores in a ground beef round medium.
2. To determine when such heat treatments can most effectively be applied; before, during, or after irradiation.
3. To determine whether a combined heat-irradiation treatment can be used to lower the sterilizing irradiation dose for Cl. botulinum spores below 2.0 megarads (threshold for undesirable organoleptic changes).

The results indicated (Tables 3 through 5) that the heat treatments applied prior to irradiation did not reduce the radiation resistance of the spores of Cl. botulinum, type A5. Kempe arrived at similar conclusions using types 213B and 62A spores in a phosphate buffer medium (15, p. 352). This also corroborated the work of Kan et al. (13, p. 518) with P. A. 3679 spores in a ham menstra medium. Therefore, one can state with reasonable sureness that heating prior to irradiation

would not be useful in reducing the sterilizing dose of irradiation required for Cl. botulinum, type A5, spores in beef.

It should be noted that pre-irradiation heat treatments of 175 and 185° F. appeared to markedly increase the resistance of the type A5 spores. A preliminary experiment carried out in this laboratory produced similar data. This experiment suggested an interesting line of investigation into the mechanism producing this increased radiation resistance. The author might suggest two possibilities which deserve further exploration.

First, Evans and Curran (10, p. 47-48) and others have found that sublethal heat treatments stimulate the germination of bacterial spores. This could lead to the conclusion that the increased radiation resistance, noted above, could be related to the state of the enzyme systems of the spores during the pre-germination state induced by the sublethal heat treatments.

Second, the effect of the heat treatments on the substrate could conceivably give mechanical protection to the spores. The coagulation and shrinkage of the meat protein (greatly reducing the dispersed water in the beef tissue) would certainly reduce the diffusion of the lethal ions produced by irradiation. It is also conceivable that the lipid materials rendered by the

heating could suspend the spores and protect them from the radiation poisons. This could afford a double protection; exclusion of water from the spore surface and absorption of the lethal ions by the unsaturated bonds of the lipid substances.

It is surprising that the same protection is not indicated by the 180° F. heat treatment at 2.0 megarads (Tables 3 and 4). This is perhaps an artifact and should be examined further.

Concurrent heat treatments (Tables 6 through 8) showed no promise in reducing sterilizing radiation doses for Cl. botulinum, type A5, spores. Spore survival for most heat treatments corresponded to spore survival at ambient temperature. Some reduction of spoilage and toxicity was noted at radiation levels of 2 and 1-2 (Tables 7 and 8). It should be noted that the 1-2 (two 1.0 megarad doses with an intervening incubation period) treatment appeared slightly more effective than a single dose of 2.0 megarads. Perhaps the incubation period did allow some germination which would account for the increased destruction of spores. Concurrent heat treatments were not effective in reducing radiation resistance of spores even though they have proved quite effective in enhancing the destruction of vegetative cells by irradiation (26, p. 229-230; 30, p. 157-174).

This might be explained in terms of the mechanical protection due to changes in the substrate, as suggested for the "prior" heat treatments.

Of the three types of heat treatments, the application of heat after irradiation showed the most promise of reducing the required sterilizing irradiation dose for Cl. botulinum, type A5, spores. Heat treatments of 165, 175, 185 and 190° F. appeared quite effective in destroying type A5 spores which had been pre-irradiated with a dose of 2.0 megarads of gamma irradiation. This corroborated the work of Kempe, et al. (16, p. 108-113; 17, p. 292-295; 18, p. 261-263) who found that pre-irradiation lowered the heat processing necessary to sterilize beef inoculated with Cl. botulinum spores.

Further work could conceivably develop a sterilizing dose of 2.0 megarads combined with a mild heat treatment after irradiation. This would destroy the spores, deactivate the autolytic meat enzymes, and produce minimal organoleptic changes in the beef.

Referring to Tables 5, 8, and 11, it was noted that a number of samples proved toxic without showing visible signs of spoilage. This may indicate a level of residual radiation poisons (30, p. 157-174) sufficient to inhibit germination but not sufficient to completely deactivate the spores. The subculture technic would favor the

germination of these spores by reducing the concentration of the radiation poisons to an insignificant level. However, this is not a startling observation. For many years it has been known that Cl. botulinum types A and B can grow and produce toxin without producing gas and other signs of spoilage. Dack (5, p. 80) states that bacteriological and toxicity tests are necessary to prove the presence or absence of Cl. botulinum. If toxicity tests had not been run on all samples in this experiment, much more optimistic results would have been obtained, particularly for the concurrent heat treatments.

The author emphasises this point to cast doubt on the validity of some of the earlier work on the combined effects of heat and irradiation on Cl. botulinum spores. The author suggests that a proper toxin assay would have appreciably modified the results.

SUMMARY

A study of the combined effects of heat and irradiation on the survival of spores of Clostridium botulinum was undertaken with the following objectives:

1. To determine the effects of mild enzyme deactivating heat treatments on the radiation resistance of Cl. botulinum, type A5, spores in a raw ground beef substrate.
2. To determine when such heat treatments could be most effectively applied; prior to, during, or after irradiation.
3. To determine whether a combined heat-irradiation treatment could be used to effectively lower the irradiation dose required to sterilize beef inoculated with A5 spores below 2.0 megarads (threshold for undesirable organoleptic changes).

Pertinent results from this study may be summarized as follows:

1. Heat treatments prior to and during irradiation did not decrease the radiation resistance of the type A5 spores in a raw beef substrate.

2. Heat treatments of 175 and 185° F. applied prior to irradiation appeared to increase the resistance of the spores to ionizing irradiation.
3. Heat treatments after irradiation appeared to significantly lower the radiation resistance of the type A5 spores, particularly at temperatures of 165, 175, 185 and 190° F.

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