

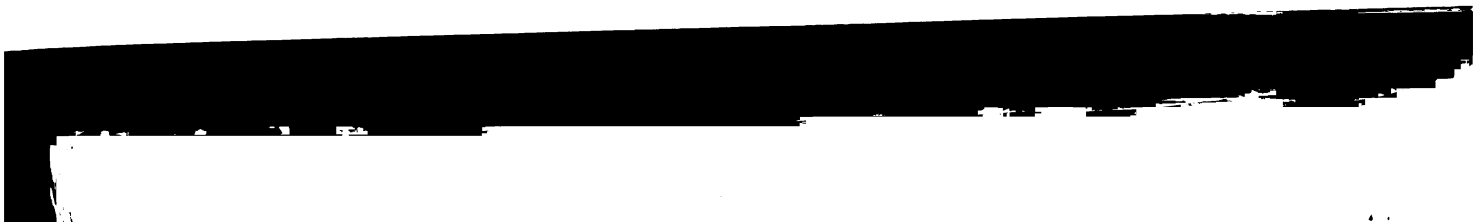
SIMULTANEOUS HEATING
AND IRRADIATION OF
CLOSTRIDIUM SPOROGENES SPORES
IN RAW GROUND BEEF

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
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ABSTRACT

SIMULTANEOUS HEATING AND IRRADIATION OF CLOSTRIDIUM SPOROGENES SPORES IN RAW GROUND BEEF

By

Usana Navanugraha

Sterilization by heat produces undesirable changes in the color, flavor, texture and causes loss of heat labile vitamins in many foods. Sterilization by radiation also produces undesirable flavor changes in some foods. Efforts have been made to use a combination process of heat and gamma radiation to sterilize foods without producing the undesirable changes associated with either treatment alone.

Preliminary studies were carried out to determine if heat and radiation applied simultaneously were complementary to each other and if it is so, whether the combination was additive or synergistic. Raw ground beef was used as the food substance and Clostridium sporogenes P.A. 3679, as the test organism. Inoculated beef in thermal death time cans was subjected to heating and irradiation separately and to simultaneous heating and irradiation. The D-values, based on partial spoilage

data, were determined using most probable number techniques. D-values at the temperatures of 25, 80, 91, and 95°C of irradiation, determined from the experimental data were designated "experimental" D-values. D-values designated as "calculated" were calculated from the effects of heat alone and of radiation alone. Comparisons of the "calculated" and "experimental" D-values were made. A synergistic effect was assumed to occur when the calculated D-values were significantly higher than the experimental values; otherwise, the effect was assumed to be only additive.

As the irradiation temperatures increased, the radiation D-values progressively decreased which suggested a complementary effect of heat and radiation. A more rapid decrease of D-value was found from 80 to 95°C with a very rapid decrease at 95°C. When the temperatures of irradiation were 80 to 91°C, the effect of simultaneous heating and irradiation appears to be synergistic, while at 95°C, the combined treatment appears to be additive.

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Usana Navanugraha

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INTRODUCTION

Ionizing radiation for food preservation has been extensively studied and appears feasible for treating a number of foods. Sterilizing doses, however, produce undesirable flavor changes in many foods. Attempts have been made to minimize the undesirable flavor changes associated with radiation-sterilized foods, and yet maintain the stability of the products. Curing agents, irradiation at refrigeration temperature, heat and irradiation, and many other methods have been used to overcome this difficulty. Among these methods, the use of mild heat together with low doses of irradiation appears promising and might be practical for radiation sterilization (Kempe et al., 1959; Zeeuw, 1971).

Heating and irradiation as performed by many researchers have been two step processes, either heat first and then irradiate or vice versa. Many works have been reported that pre-irradiation sensitized bacterial spores to subsequent heat treatment (Kan et al., 1957; Kempe et al., 1957; Kempe, 1959; Anderson et al., 1967; Grecz et al., 1967). Some workers, however, found that heating

just prior to electron radiation, was more effective in destroying the microorganisms and the flavor changes of the food were only slightly below that of the un-irradiated fresh control (Huber et al., 1953). Very little work has been reported on the effect of simultaneous heating and irradiation. While there is evidence that the radiation resistance of bacterial spores decreased with the increasing temperatures of irradiation (Grecz et al., 1971), reports where simultaneous heating and irradiation increased the radiation resistance of spores also exist (Anderson et al., 1967). Grecz et al. (1971) reported that treating Clostridium botulinum strain 33A spores at different temperatures while exposed to gamma radiation resulted in a decrease of radiation resistance of the spores as the temperature increased. At 95°C there was a very rapid decrease of radiation resistance which showed the complementary effect of heat and radiation. Raynolds and Garst (1970) observed the synergistic effect of dry heat at 60 to 105°C and gamma radiation on spores of Bacillus subtilis variety niger. The decrease of spore resistance to gamma radiation at higher temperatures, however, did not follow the same pattern as that reported by Grecz and his coworkers (1971).

The objectives of this research were to:

1. Determine whether heat and radiation applied simultaneously are complementary to each other.

2. Determine the radiation D-values of Clostridium sporogenes P.A. 3679 spores in raw ground beef at 25°C (radiation only) and at 80, 91, and 95°C (simultaneous heating and irradiation). The D-values obtained would be designated the experimental D-values.
3. Compute the "calculated" D-values of simultaneous heating and irradiation. In order to accomplish this, the thermal D-values at 80, 91, and 95°C and the radiation D-values at 25°C were to be determined. The amount of spore destruction in one minute by heat only ($\frac{1}{D_H}$) and by radiation only ($\frac{1}{D_{HR}}$) were added to give the amount of spore destruction in one minute by the simultaneous heating and irradiation process ($\frac{1}{D_{HR}}$) assuming that the effect of heating and irradiation was additive.
4. Compare the experimental D-values and the calculated D-values.
5. Conclude whether the effect of heating and irradiation simultaneously was additive or synergistic.

LITERATURE REVIEW

Various methods of food preservation such as curing, salting, refrigerating, dehydrating, canning, etc., have been widely used for a long time without the mechanisms involved in them being fully understood. Their use was accepted largely because experience showed that foods preserved in these ways were safe. No method of preservation, however, is perfect in every respect. Canning, for example, induces losses of heat-labile vitamins, and causes changes in texture, flavor, and odor.

Irradiation offers an opportunity to preserve foods through control of spoilage microorganisms. Enzymes which are responsible for biological actions in the living system are usually radiation resistant but can be easily controlled by heat (Cain and Anglemier, 1969). Food irradiation is designed to destroy either (a) all organisms in food, or (b) only specific pathogenic organisms, or (c) certain spoilage organisms. Three types of treatments have been defined (Anon., 1970) and named as follows:

- a. Radappertization: the name was derived from canning "appertization." It is the application of

doses of ionizing radiation sufficient to reduce the number and/or activity of viable organisms to such an extent that very few, if any, are detectable in the foods (viruses being excepted). In the absence of contamination after irradiation, no microbial spoilage nor toxicity from the microorganisms should develop. Radappertized food can be kept indefinitely without refrigeration.

- b. Radacidation: is the application of doses of ionizing radiation sufficient to virtually eliminate specific non-spore forming pathogenic microorganisms (other than viruses) whose presence is a significant health hazard.
- c. Radurization: is the application of doses of ionizing radiation sufficient to reduce the number of viable spoilage microorganisms, and thus enhance the keeping quality of the food.

Radurization, a low dose treatment, maintains the keeping quality of foods under refrigeration. For example, after treatment with gamma radiation at about 100 Krad, fresh beef and poultry containing condensed phosphates stores in a controlled atmosphere at 40°F, can be kept in a salable condition for about three weeks. In contrast, the salable life of the untreated meat, such as a cut of beef under good refrigeration, is a maximum of about three days (Urbain and Giddings, 1972). Radappertization,

which can be used to preserve food indefinitely without refrigeration, usually requires high doses of radiation to destroy both the pathogenic and food spoilage micro-organisms. Undesirable changes, especially flavor changes, are more pronounced with the required radappertizing dose.

The index of radiation bactericidal action is the D-value which is the dose required to destroy 90% of a bacterial population. In thermal processing, the D notation with the temperature subscribed is used and defined as the time usually in minutes required to destroy 90% of a bacterial population. The 12 D concept concerning a safe process for the sterilization of food originated from the studies of Esty and Meyer (1922) on heat resistance of spores of Bacillus botulinus (Clostridium botulinum). Over the years, the 12 D thermal process has provided safe canned foods and therefore, has found general acceptance.

When radiation sterilization of foods was introduced, it was stated that the new food sterilization process should provide a minimum sterilizing effect equivalent in safety to the accepted 12 D thermal process. By employing the best available data at the time, Schmidt (1960) calculated that radiation processing to provide the same degree of safety as conventional thermal processing would require 4.45 Mrad, i.e., $D = 0.37 \text{ Mrad}$. The radappertization dose of 4.5 Mrad is generally accepted.

Recently, questions have been raised concerning the validity of the 12 D concept in radiation sterilization. Anellis et al. (1967) reported that the 12 D doses for some C. botulinum strains (62 A, 12885 A, 41 B, 51 B, and 53 B) were lower than their respective minimal experimental sterilizing doses in diced cure ham.

The amount of radiation required to sterilize many foods produces undesirable sensory changes, the most significant of which is a flavor change (Huber et al., 1953; Kempe, 1959). In thermal processing of some foods such as meat, cream-style corn, etc., excessive cooking is required due to the low heat conductivity of the products. Attempts have been made to minimize the undesirable changes associated with both treatments.

Heat and gamma radiation are synergistic when used for killing the bacterial spores (Raynolds and Garst, 1970). The combination of heat and radiation could result in milder processing treatments which may yield an improvement in product quality (Kempe et al., 1957; Kan et al., 1957). The combination processes that have been studied were usually performed as 2-step processes with either heat and then irradiation or irradiation and then heat (Anderson et al., 1967; Grecz et al., 1967; Kempe, 1959; and Kan et al., 1957). The first step was intended to destroy vegetative cells and sensitize the spores. The second step was designed to destroy the sensitized spores and germinated spores with a rather moderate treatment. By

using mild radiation and heat treatments, a less severe total process is obtained and the sensory characteristics may be improved.

Radiation affects biological cells either by direct or indirect action. Direct absorption of the energy in a molecule, which is necessary for the survival of the cell, may cause a chemical change which will lead to cell death. When a covalent bond is broken in such a way that electrons are distributed unevenly between the two fragments, two ions, one positive and one negative, are formed. On the other hand, when the electrons are distributed evenly between the two fragments, two free radicals are formed. Free radicals are usually highly reactive and may have either a short life or a relatively long life depending upon their chemical nature and the physical conditions in the medium such as temperature, solid or liquid state, etc. Being a physical process, direct action does not depend on the temperature or the phase (solid or liquid) of the medium (Grecz et al., 1967).

The indirect effect of radiation occurs when the reactive chemical fragments formed by direct action of radiation further react with other molecules, ions or free radicals present in the substance. This is a true chemical reaction and is affected by the temperature during irradiation, by the composition of the medium, and by the phase (solid or liquid) in which the cells are irradiated. The

reaction rate increases with increasing temperature. Certain molecules, such as sulfhydryl compounds and oxygen, may react with ions or free radicals and thus interfere with the normal indirect effect of radiation. Ion and free radical diffusion in the liquid medium is greater than in the solid medium.

Spores of bacteria do not respond to heat and radiation in the same manner. Sublethal treatment of heat activates dormant spores to germinate while a sub-lethal dose of radiation may or may not activate spores to germinate, depending on species of bacteria. Spore activation by gamma radiation has been observed in some strains of C. botulinum, in C. bifermentans and C. sporogenes (Roberts, 1968).

Kempe (1959) and Kempe et al. (1959), reported that C. botulinum spores and P.A. 3679 spores were easily destroyed with heat following irradiation, and the complementary effect increased with the radiation dose.

Anderson et al. (1967), by applying heat before, during and after irradiation, concluded that mild heat treatment (75-87.5°C) following irradiation reduced the survival of spores of C. botulinum strain A-5 appreciably. In addition, heat applied before and during the irradiation actually increased the radiation resistance of the spores, especially in the temperature range 80-87.5°C.

Huber et al. (1953) showed the synergistic effect of heat and electron radiation in milk. In this case,

heating just prior to irradiation seemed to be considerably more effective than heating after irradiation. They obtained sterile milk with 30 seconds of heating at 73.5°C followed by irradiation of 500,000 rep. The flavor of the sterile milk was only slightly below that of an uninoculated fresh control.

Grecz et al. (1967) studied the differences in radiation resistance of spores at different irradiation temperatures from -196 to 95°C. They found that the radiation resistance of spores of C. botulinum strain 33A was strongly affected by the temperature during irradiation. Very low radiation resistance was consistently observed at 0°C. Below 0°C the resistance of spores increased. The solidly-frozen medium causes a decrease in diffusion of molecular fragments and free radicals and thus the indirect bactericidal effect of radiation is decreased. Astonishingly, as the temperature increased above 0°C the resistance of the spores also increased and was highest at 70 to 75°C. Above 80°C the resistance dropped sharply. However, Grecz et al. (1971) statistically computed a linear change in D values for spores of C. botulinum 33A in canned ground beef with irradiation temperatures from -196 to 95°C. As the temperature of irradiation was increased from -196 to 65°C, the corresponding radiation D-value decreased linearly. From 65 to 85°C there were more rapid drop in D-values with a very sharp drop at temperature range 85

to 95°C. Whether the drop in radiation resistance of spores was caused by a synergistic effect of heat and radiation, or by a simple additive effect of the two parameters, was not determined.

Attempts are made to minimize the heating of canned, cured meats, since overheating results in a decrease in quality and excessive shrinkage of the product. Ideally, the amount of heat used should be small enough to avoid quality degradation yet sufficient to render the product safe and stable. Since heat conductivity in meat is low, the use of gamma radiation to replace part of the heat treatment might overcome this difficulty (Zeeuw, 1971). Initial work, however, indicated that irradiated hams were inferior in flavor to the unirradiated control (Hansen, 1966).

Unpleasant flavors and aromas developed in food sterilized by irradiation are largely caused by indirect action (Harlan et al., 1967). Many substances are involved with the typical irradiated flavor (Urbain, 1971). Several laboratories have reported that the intensity of irradiation flavor is increased with increasing temperature when the dose of radiation is kept constant. The undesirable flavor change is believed to be caused by the volatile compounds produced by the action of ionizing radiation on the protein and lipid components of the food (Merritt et al., 1967). Wick et al. (1967) proposed that methional,

1-nonanal and phenyl-acetaldehyde in the proportion of 20:2:1 were the most important contributors to irradiation flavor and odor. Wierbicki et al. (1970) showed that irradiation of food in the frozen state (temperatures of -30°C and below) resulted in improvement of product flavor over ambient temperature, at the same irradiation dose. However, as temperatures are lowered, higher irradiation doses are required to achieve the same degree of bactericidal effect. Heating appears promising to be used with radiation to sterilize many foods. By using mild heat treatment together with low dose of radiation, the undesirable changes associated with either treatment alone can be minimized and the product quality might be improved (Kempe, 1957).

MATERIALS AND METHODS

Preparation of Beef Samples

Fresh raw ground beef was used as the experimental food substance for this research. Fresh beef was obtained from the Michigan State University Food Stores. An "eye of round" steak was trimmed to remove as much fat as possible and ground through a plate containing holes 2 mm. in diameter.

A 16 ± 0.2 gm. portion of chilled raw ground beef was filled into each thermal death time (TDT) can, and spread into a uniform layer with a flat spatula. Each TDT can was inoculated with 0.3 ml. of a diluted spore suspension of Clostridium sporogenes which gave an initial spore count of 1.5×10^6 spores per can. No attempt was made to mix the spore suspension with the beef. The inoculated cans were immediately closed using a closing machine and stored at 0°C in an ice-water bath until used for the experiments. The time of storage between inoculation of the samples and the experimental treatments varied from 10 to 60 minutes. Some uninoculated cans were also closed and stored likewise at 0°C to be used as controls with the inoculated samples.

Preparation of Spores

Spore Harvest

Clostridium sporogenes (P.A. 3679, NCA 7955) was obtained from the American Type Culture Collection. The spore suspension was produced by the method of Uehara et al. (1965) with the following modifications:

1. An incubation temperature of 37°C was used throughout the experiment.
2. The spores were washed in sterile distilled water 4 times instead of 20 times.

After the final wash, the spores were resuspended with small amount of sterile distilled water and stored at 4.4°C until needed.

Spore Count

The concentrated spore suspension in a 500 ml. screwcap flask was shaken with glass beads to disperse the spore clumps. A wet mount of the spore suspension was examined under the phase contrast optics to make sure that the spores were singly distributed rather than forming clumps.

One ml. of the thoroughly shaken spore suspension was diluted with 3 ml. of 30 percent glycerol solution to reduce Brownian movement of the spores which created difficulty in counting. A Petroff-Hausser chamber was used for counting the spores. Only totally refractile

spores, which indicated heat resistance, were counted. The average spore count in the original spore suspension was 5×10^8 spores per ml.

The concentrated spore suspension was kept at 4.4°C and the appropriate dilutions were made just prior to each experiment.

Radiation Dose-Rate Measurement

Irradiation was carried out in the Department of Food Science and Human Nutrition, Michigan State University. The Cobalt-60 Research Irradiator source consists of 24 BNL standard source strips, doubly encapsulated in stainless steel sheaths; arranged to form a vertical cylindrical configuration of about 30 cm. diameter. The specific activity at the time of installation (June, 1967) was 32 curies per gram, and the total activity was about 50,000 curies.

The radiation dose rates in air at the time of these experiments were available in the Department. However it was considered appropriate to measure the dose rate under the actual experimental conditions because irradiation was carried out in metal cans in a propylene glycol bath. The cupric-ferrous sulphate dosimetry as described by Jarrett (1967) was used.

Twelve glass vials, 1.5 cm. diameter and 6.0 cm. in height, were filled with the cupric-ferrous sulphate solution. Each glass vial was attached to the bottom of a

thermal death time can with masking tape. The cans with the glass vials were placed upright along the wall of a 4,000 ml. glass beaker at the same locations as used for irradiating the ground beef samples (Figure 1). One set of 6 vials was placed at a height of 17 cm. from the floor equally spaced along the wall of the beaker at a distance of 7 cm. from the center of the center well of the irradiator. Another set of 6 vials was similarly placed at a height of 23 cm. from the floor.

The beaker was filled with propylene glycol and the vials were irradiated for 30 minutes at 26°C. The color change due to irradiation was measured as absorbance at 304 nanometers in a Beckman DB spectrophotometer. The dose rate was calculated using the following formula.

$$\text{Dose (Rads)} = A \times 6.43 \times 10^5 \text{ (Jarrett, 1967)}$$

Where A is the difference in absorbance of the irradiated and the unirradiated sample.

The average dose rate was found to be 0.879 Mrad per hour at 17 cm. height and 0.775 Mrad per hour at 23 cm. height from the floor.

Experimental Setup

Heat

Heating was done in a 4,000 ml. glass beaker containing about 3,500 ml. pure propylene glycol as the heating medium. Propylene glycol was used as the heating

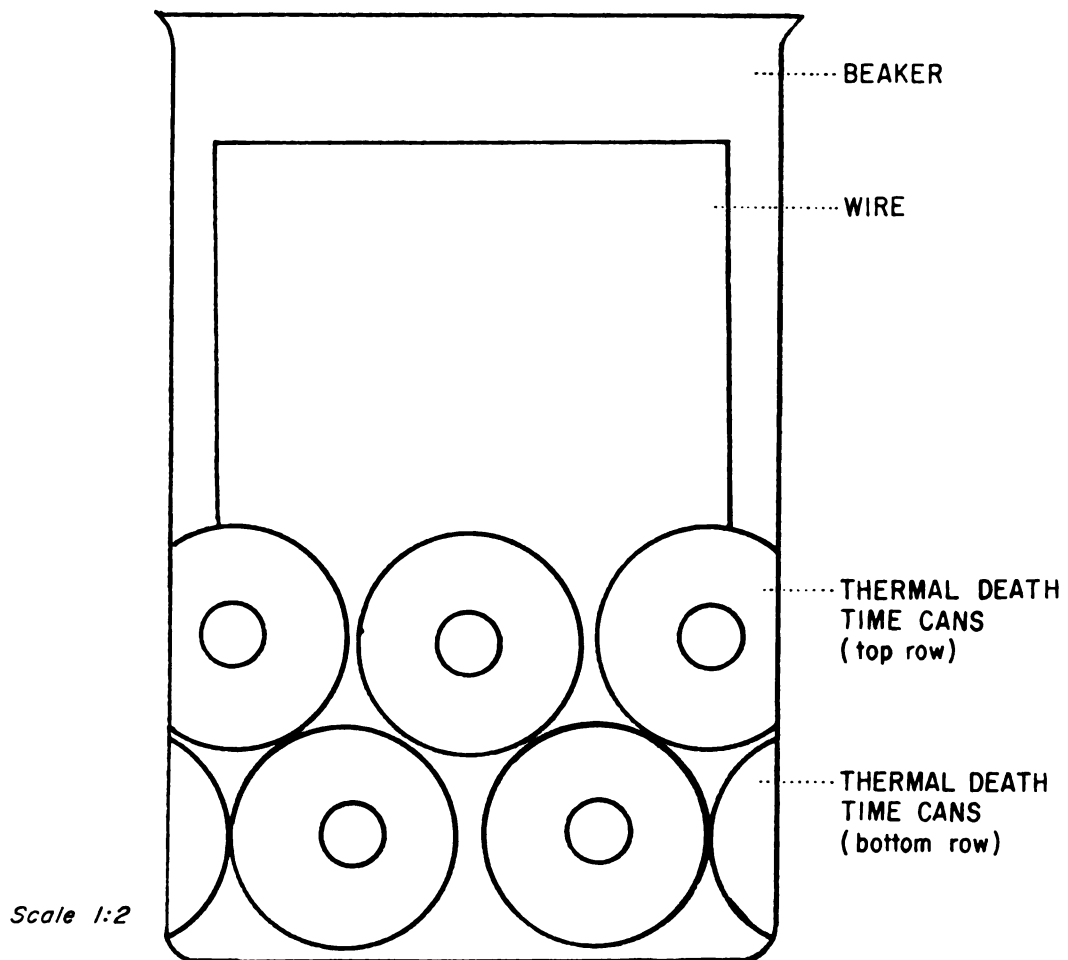


Figure 1. Experimental setup for irradiation of raw ground beef.

medium instead of water because it had a higher boiling point (189°C) than water and did not evaporate as fast as water. An immersion-type heater, thermostatically controlled to within $\pm 1^\circ\text{C}$, was used to maintain the desired constant temperature.

When the thermal death time cans containing the ground beef at 0°C were put in the heated bath, the bath temperature dropped by about 1 to 2°C and returned to the set temperature in about 3 minutes. At the end of the desired heating period, the heating action was stopped by putting the cans immediately into a bucket containing about 5,000 ml. cold tap water. The cans were then dried, labeled and incubated at 37°C .

The heating and cooling lag of the product were measured to determine whether the correction of the process time should be made. The temperature change of the ground beef during heating and cooling was recorded on a Honeywell temperature recorder using copper constantan thermocouples. The end of the thermocouple was surrounded by a piece of plastic tubing to make sure that the thermocouple was centrally located and did not touch the metal surface. The drill hole was sealed with epoxy resin and allowed to harden overnight. The can was then filled with ground beef and closed. The can was put in the propylene glycol bath, preheated to the desired temperature, and the rising temperatures were recorded. The time to heat the ground

beef to the desired temperature was approximately 4 minutes. At the end of the heating period, the can was immersed in cold tap water and the decreasing temperatures were recorded. The time to cool the product to room temperature was approximately 2 minutes. It was concluded that the heating lag correction and cooling lag correction were not necessary for this research. The process time was measured from the time the temperature of the propylene glycol bath, which has dropped after putting the can in, started to read the set temperature again, up to the time the can was taken out from the heating medium.

Irradiation

The samples were irradiated in a propylene glycol bath at room temperature, so that the results could be compared with samples subjected to simultaneous heating and irradiation treatments where propylene glycol was used as the heating medium.

The cans were immersed in the bath in an upright position with the bottom side of the cans facing the wall of the beaker. The cans were placed in the same locations as the vials in the dose rate measurement. The cans were held in an upright position by using a piece of metal screen bent in the form of a cylinder, which held the cans against the wall of the beaker (Figure 1). The cans were equally spaced along the wall of the beaker. The beaker was then centrally placed in the center well of the

irradiator. At the end of the desired irradiation dose, the cans were removed from the bath, dried, labeled and incubated at 37°C.

Simultaneous Heating and Irradiation

Simultaneous heating and irradiation was done in the same manner as irradiation only, except that the propylene glycol bath was preheated to the desired temperature. The time elapsed between placing of cans in the heated bath and start of irradiation was about one minute. Likewise, the time elapsed between stopping of irradiation and placing of cans in cold water was about one minute.

D-Value Calculations

Thermal D-Value

The method used to estimate the thermal D-value was that described by Stumbo et al. (1950). The equation used allowed the computation of the D-value when partial spoilage was observed.

$$\text{D-value (minutes)} = \frac{\text{Process time (minutes)}}{\log M - \log [2.303n (\log \frac{n}{q})]}$$

The process time was measured from the time the bath, with samples, rose to preset temperature until the samples were removed from the bath. M is the total inoculum (organisms per sample units times number of replicates); N is the

total number of replicates per treatment; and q is the number of negative sample units per one treatment.

The negative sample is the number of flat cans without growth and gas production after one month incubation at 37°C. These flat cans were assumed to contain no survival of spores after treatment.

The positive sample is the number of swollen cans which show growth and gas production of the survival spores after one month incubation at 37°C.

Radiation D-Value

Experimental D-value.--The method used for computing D-values of radiation was analogous to the equation for computing thermal D-value by Stumbo et al. (1950) except that the thermal process time was replaced by the radiation dose in Mrad (Anellis and Werkowski, 1968) and the equation becomes:

$$D \text{ (Mrad)} = \frac{\text{radiation dose (Mrad)}}{\log M - \log \left[2.303n \left(\log \frac{n}{q} \right) \right]}$$

Where M is the total inoculum (organisms per sample unit times number of replicates); n is the total number of replicates per dose; and q is the number of negative sample units per dose. The experimental radiation D-value equation was used when irradiation was performed at room and at higher temperatures.

Since the D-values by heat, irradiation and simultaneous heating and irradiation were to be compared to study the complementary effect when heat and irradiation were performed at the same time, it was necessary that the D-values be in the same units. D-values in Mrad, therefore, were changed to the corresponding time of radiation, thus represented radiation resistance as a function of time of exposure. This comparison is valid only when the dose rate is constant. The D-value becomes:

$$D \text{ (minutes)} = \frac{\text{radiation time (minutes)}}{\log M - \log [2.303n (\log \frac{n}{q})]}$$

This equation was used where irradiation was performed at room temperature and at higher temperatures. The D-value calculated in this manner applies only to the dose-rate used in this experiment.

Calculated D-value.--Let D_H be the time in minutes at a given temperature to reduce the number of spores by 1 log cycle by heat only. Therefore, $\frac{1}{D_H}$, is equal to the amount of spore destruction in 1 minute by heat.

Let D_R be the time in minutes to reduce the number of spores by 1 log cycle by radiation only (assuming a fixed, constant radiation dose-rate). Therefore, $\frac{1}{D_R}$, is equal to the amount of spore destruction in 1 minute by radiation.

Let D_{HR} be the time in minutes to reduce the number of spores by 1 log cycle by the combination treatment (at a given temperature and a fixed constant dose rate).

Therefore, $\frac{1}{D_{HR}}$, is equal to the amount of spore destruction in 1 minute by the combination treatment.

If the effect of heat and radiation performed simultaneously is additive, the spore destruction in 1 minute by the combined treatment will be equal to the sum of the spore destruction in 1 minute by heat only and by radiation only. Thus:

$$\frac{1}{D_{HR}} = \frac{1}{D_H} + \frac{1}{D_R}$$

D_{HR} represents the calculated D-value of simultaneous heating and irradiation in terms of time.

RESULTS AND DISCUSSION

The preliminary study of this research was to establish the range of temperatures and radiation doses for detailed later study. Since a combination treatment of heat and radiation was found from some literature to be at least additive (Grecz et al., 1971), if not synergistic, less severe heat treatment and radiation doses than are normally used for sterilization of foods were used. Temperatures of 80, 90, and 100°C were selected for the preliminary experiment for the following reasons:

1. Temperatures around 121°C are normally used in thermal processing. In the combination process, temperatures to be selected should be lethal temperatures below 121°C.
2. Grecz et al. (1971) reported that radiation D-values of C. botulinum strain 33A spores varied inversely with temperatures from -196 to 65°C. Above 65°C, there was more rapid drop in D-values with a very sharp drop at 85 to 95°C was caused by a synergistic effect of heat and radiation, or by

an additive effect, was not determined by these investigators.

For these two reasons, the temperatures of 80, 90, and 100°C were selected for the preliminary experiments.

Radiation doses selection was arrived at as follows:

1. Radiation sterilizing dose that gives an equivalent sterility as a 12D thermal process is 4.45 Mrad as was calculated by Schmidt (1960). Therefore, doses below this sterilizing dose should be used in the combined treatment.
2. Radiation D-value of P.A. 3679, strain S-2 in phosphate buffer was reported to be 0.209 Mrad (Anellis and Koch, 1962). The inoculum in the order of 10^5 per gm. of meat (1.5×10^6 spores per can) which was used throughout to study the resistance of spores in raw ground beef would then require about 1.2 to 1.4 Mrad to give partial spoilage data in 10 replicates. Therefore, radiation doses of 1, 2, and 3 Mrad were selected for the preliminary experiments.

Preliminary Experiments

First Trial

Results are shown in Table 1.

TABLE 1.--Preliminary results on the survival of C. sporogenes spores in raw ground beef subjected to heating; irradiation; and simultaneous heating and irradiation treatments.

Radiation Dose (Mrad)	Heating and/or Radiation Time (Minute)	Positive Cans per 10 Cans Total			
		Room Temperature	80°C	90°C	100°C
0	77.4	. .	10	10	0
0	154.8	. .	10	10	0
0	232.2	. .	10	10	0
1	77.4	10	10	0	0
2	154.8	0	0	0	0
3	232.2	0	0	0	0

Note: Initial spore count = 1.5×10^6 spores per can.

Heat.--Heating was performed for the same length of time as the time of radiation to allow comparison when heating and radiation were done simultaneously. Heating the inoculated ground beef samples at 80 and 90°C for 77.4, 154.8, and 232.2 minutes (time to achieve 1, 2, and 3 Mrad respectively) resulted in all positive cans while heating at 100°C for the same length of time gave all negative cans. The uninoculated controls at all time-temperature combinations were all negative. This demonstrated that the positive cans at 80 and 90°C were the result of the inoculation with C. sporogenes spores. The negative cans at 100°C indicated that the spores might have low heat resistance or the heated spores did not germinate. To clarify this, the heating time at 100°C in the next experiment was reduced to half (38.7 minutes) of the shortest time used here.

Irradiation.--As expected, a radiation dose of 1 Mrad was not enough to destroy the spore inoculum and all 10 cans were positive. Radiation doses of 2 and 3 Mrad were enough to destroy the spore inoculum thus yielding all negative cans. The uninoculated control cans exposed to 1, 2, and 3 Mrad of radiation were all negative which indicated that any organisms previously present in raw ground beef did not survive the radiation treatment. The positive cans that survived the treatment were considered to be from the inoculated C. sporogenes spores only.

From the all negative cans at 2 Mrad and all positive cans at 1 Mrad, the next experiment was designed to investigate the spore survival at 1.5 Mrad.

Simultaneous Heating and Irradiation.--At 80°C, a radiation dose of 1 Mrad was not enough to destroy the spore inoculum and all 10 cans were positive. Either heat alone for 77.4 minutes (time to achieve 1 Mrad of radiation) or radiation alone at room temperature of 1 Mrad was not enough to destroy the spores. Therefore, heat alone, radiation alone and heat plus radiation at this level were not enough. A more severe treatment, radiation at 80°C for 1.5 Mrad, was employed in the next experiment.

At 90°C radiation doses of 1, 2, and 3 Mrad gave all negative cans which indicated total destruction of the inoculated spores. Heat treatment alone even at the longest time was not enough to give negative results. However, radiation at 2 and 3 Mrad at room temperature gave negative results which showed that heat treatment was not necessary at these doses. Therefore, a combination treatment of 90°C-1 Mrad was only significant. At this combination, there was no growth of bacteria even after 5 months incubation at 37°C. This showed that heat and radiation were complementary to each other but whether the effect was additive or synergistic could not be concluded at this stage.

Second Trial

Results are shown in Table 2.

Heat.--Heating at 80°C from 38.7 minutes to 232.2 minutes gave all positive cans. Heating at 90°C for the same length of time gave the same result as at 80°C except that at 232.2 minutes only partial spoilage was obtained. Cans heated at 90°C for 232.2 minutes gave positive results in 4 cans out of 10 cans. The decimal reduction time (D-value) from this partial spoilage data was determined to be 35.9 minutes. At 100° there was no survival of bacteria even at the shortest time of 38.7 minutes. This indicated that the spores had low heat resistance. In the next experiment, temperature of 95°C was also included to study the bacterial heat resistance.

Irradiation.--Radiation at room temperature at the doses of 0.5 and 1.0 Mrad gave all positive cans, while doses of 1.5, 2.0, and 3.0 Mrad gave all negative cans. Partial spoilage data between 1 to 1.5 Mrad were desired to allow calculation of D-value for radiation.

From the results of only radiation and only heat, temperature of 100°C and radiation dose above 1.5 Mrad gave all negative cans by either treatment alone. The combination treatments of heat and radiation beyond these would not be of value but were included to confirm the

TABLE 2.--Survival of C. sporogenes spores in raw ground beef subjected to heating; irradiation; and simultaneous heating and irradiation treatments.

Radiation Dose (Mrad)	Heating and/or Radiation Time (Minute)	Positive Cans Out of 10			
		Room Temperature	80°C	90°C	100°C
0	38.7	. .	10	10	0
0	77.4	. .	10	10	0
0	116.1	. .	10	10	0
0	154.8	. .	10	10	0
0	232.2	. .	10	4	0
0.5	38.7	10	10	10	0
1.0	77.4	10	10	0	0
1.5	116.1	0	0	0	0
2.0	154.8	0	0	0	0
3.0	232.2	0	0	0	0

Note: Initial spore count = 1.5×10^6 spores per can.

results obtained and to study the reproducibility of the method when using raw beef from a different batch. Moreover, a negative result obtained when a more severe treatment was used might be due to retardation of germination. A negative result should be confirmed by allowing more time for incubation and/or by replication of the experiment.

Simultaneous heating and irradiation.--At 80°C with radiation, the combinations of 80°C-0.5 Mrad and 80°C-1.0 Mrad gave all positive cans. The result at 80°C-1 Mrad was the same as in the first trial and the positive result was supported by a less severe treatment, 80°C-0.5 Mrad.

Negative cans which showed a complementary effect of heat and radiation were consistently observed at 90°C-1 Mrad. Radiation dose of 0.5 Mrad at this temperature resulted in all positive cans. Radiation dose between 0.5 and 1.0 at 90°C should give partial spoilage data which would allow the calculation of D-value using most probable number approach.

The combined treatments at 100°C were not of value, since heat alone at this temperature was shown to be enough to destroy the spore inoculum. Radiation treatment, therefore, was not needed at this temperature.

Third Trial

Results are shown in Table 3.

Heat.--A thermal resistance study of the organism in raw ground beef at 80 and 90°C was carried out at the two longest time periods, namely 154.8 and 232.2 minutes. Results were positive in all cans. At 100°C, the three shortest time, 38.7, 77.4, and 116.1 minutes were used and all negative results were obtained. Heating at 90°C for 38.7 and 77.4 gave all positive cans while 1 positive can was obtained at 116.1 minutes. The D-value at 90°C was determined to be 16.2 minutes.

Irradiation.--At room temperature and a radiation dose of 0.5 Mrad, all 10 cans were positive, as was found in the second trial. At 1 and 1.5 Mrad there were 9 and 1 positive cans respectively. The D-values determined from these two conditions were 0.172 and 0.210 Mrad with the average of 0.191 Mrad.

Simultaneous heating and irradiation.--The number of negative and positive cans at a certain temperature-dose relationship in the third trial was somewhat different from the first and the second. At 80°C, with radiation, 0.5 Mrad was not enough to provide sterility, and all 10 cans were found to be positive. With the higher dose of radiation, 1 Mrad, 8 cans were positive.

TABLE 3.--Confirmatory results on the survival of C. sporogenes spores in raw ground beef subjected to heating; irradiation; and simultaneous heating and irradiation treatments.

Radiation Dose (Mrad)	Heating and/or Radiation Time (Minutes)	Positive Cans Out of 10				
		Room Temperature	80°C	90°C	95°C	100°C
0	38.7	. .	. .	. .	10	0
0	77.4	. .	. .	. .	10	0
0	116.1	. .	. .	. .	1	0
0	154.8	. .	10	10	0	. .
0	232.2	. .	10	10	0	. .
0.5	38.7	10	10	10	. .	0
1.0	77.4	9	8	0	. .	0
1.5	116.1	1	0	0	. .	0
2.0	154.8	0	0	0	. .	0
3.0	232.2	0	0	0	. .	0

Note: Initial spore count = 1.5×10^6 spores per can.

The radiation D-value was derived from the experimental data to be 0.168 Mrad, which was equivalent to 13.0 minutes of radiation at this dose rate. Further increasing of the radiation dose resulted in all negative cans at 1.5, 2.0, and 3.0 Mrad. At 90°C, 0.5 Mrad gave all 10 positive cans while none was positive at higher doses of radiation. No partial spoilage was observed. At 100°C with radiation, the combined treatments of heat and radiation were enough to give only negative cans at all doses applied.

Conclusion of the Preliminary Experiments

Heat.--Heat treatment alone at 80°C for as long as 232.2 minutes was not enough to destroy the spore inoculum of 1.5×10^6 spores per can and the initial flora in raw ground beef. Heat treatment at 90°C for as long as 232.2 minutes was not enough to destroy the same spore inoculum and the initial flora in raw ground beef except in one case where partial spoilage occurred and the resulting D-value was 35.9 minutes. Heat treatment at 95°C gave partial destruction at 116.1 minutes and the D-value was 16.2 minutes. At 100°C, however, no bacterial growth was found even after the shortest time, 38.7 minutes.

Irradiation.--A radiation dose of 1 Mrad at room temperature was not enough to destroy the spore inoculum

and the initial flora in raw ground beef. At 1.5 Mrad there were total destruction in two trials out of three and in the other one, partial destruction was observed. The radiation D-value was 0.191 Mrad. Doses between 1 and 1.5 Mrad, therefore, gave partial spoilage data. The doses at 2 Mrad and above were enough to destroy the microorganisms.

Simultaneous Heating and Irradiation.---The D-value obtained from irradiation at 80°C (0.168 Mrad) was lower than that found at room temperature (0.191 Mrad). At 90°C-1 Mrad, the complementary effect of heat and radiation was consistently observed in all three trials. However, the radiation D-value at 90°C could not yet be determined. At 100°C, heat only was enough to destroy the spore inoculum. Therefore, complementary effect of heat and radiation in raw ground beef should be studied at temperatures between 80°C and 100°C.

Resistance of Spores in Distilled Water

Thermal Resistance

As reported in the preliminary experiments, the heat resistance of the C. sporogenes spores in beef was found to be low. This low heat resistance could be due to some biochemical changes in beef which inhibited the germination of surviving spores or increased the heat

sensitivity of the spores. In this connection the heat resistance of spores in distilled water was of interest and was, therefore, determined.

One ml. of diluted spore suspension containing 5×10^5 spores per ml. was aseptically transferred into each presterilized glass ampule using a sterile syringe. The ampules were sealed in a gas burner flame and were stored at 4.4°C until used for experiments. Ten sealed ampules were subjected to different time-temperature combinations in a miniature retort. At the end of the heating time, the necks of the ampules were flamed before breaking, and about 5 ml. of freshly prepared trypticase broth (BBL; Uehara et al., 1965) was added to each ampule. The open necks of the ampules were closed with presterilized cotton plugs. The ampules were incubated at 37°C in a nitrogen atmosphere for 7 days. At the end of the incubation period, the growth of surviving spores was observed. The D-value at each temperature was determined from partial spoilage data using most probable number approach (Stumbo, 1950). The thermal resistance data of C. sporogenes spores in distilled water are shown in Table 4.

The average D-values at 104.4, 107.2, 110.0, and 115.6°C were found to be 4.33, 3.14, 0.98, and 0.29 minutes respectively. The thermal death time curve was plotted on

TABLE 4.--Thermal resistance of C. sporogenes spores in distilled water.

Heating Temperature (°C)	Heating Time (Minutes)	Positive Tubes Per 10 Tubes Total	D-Value (Minutes)
104.4	20.0	10	. .
	22.5	10	. .
	25.0	10	. .
	27.5	2	4.33
	30.0	0	. .
	32.5	0	. .
			<u>4.33</u> average
107.2	15.5	10	. .
	17.0	9	3.19
	18.5	4	3.09
	20.0	0	. .
			<u>3.14</u> average
110	4.5	7	0.80
	5.1	9	0.96
	5.7	8	1.04
	6.3	1	0.94
	6.9	1	1.03
	7.5	1	1.12
	8.1	0	. .
			<u>0.98</u> average
115.6	1.8	1	0.27
	2.0	1	0.30
	2.2	0	. .
			<u>0.29</u> average

Note: Initial spore count = 5.0×10^5 spores per can.

a semilog paper and is shown in Figure 2. A straight line was obtained which showed that the death rate of C. sporogenes spores was logarithmic. The straight line allowed the extrapolation of the curve and the thermal resistance D-values at different temperatures could be obtained directly from the line. $D_{121^{\circ}\text{C}}$ ($D_{250^{\circ}\text{F}}$) was found to be 0.08 minute and the resistance parameter, Z-value, 9.7°C . Stumbo (1950) reported the D-value of C. sporogenes P.A. 3679 in distilled water at 121°C to be 0.8 minute and the Z value of 9.8°C (17.6°F). It is clear that the heat resistance of this spore crop was lower than what Stumbo reported but the slope of the thermal death time curve was approximately the same.

Radiation Resistance

One ml. of diluted spore suspension in distilled water containing 5×10^5 spores was transferred aseptically to a screw-cap test tube (pyrex, 15 x 1.8 cm.) using a syringe. Ten replicates were used at each dose. The radiation was performed in the center well of the irradiator; no propylene glycol bath was used. The bath was not used because of the difficulty in maintaining the tubes in position in a liquid medium. The spores were irradiated in the center well at the floor level. The dose rate at the time of irradiation was measured and calculated to be 0.972 Mrad per hour using the method described by Jarrett (1967). After irradiation, approximately 10 ml. of

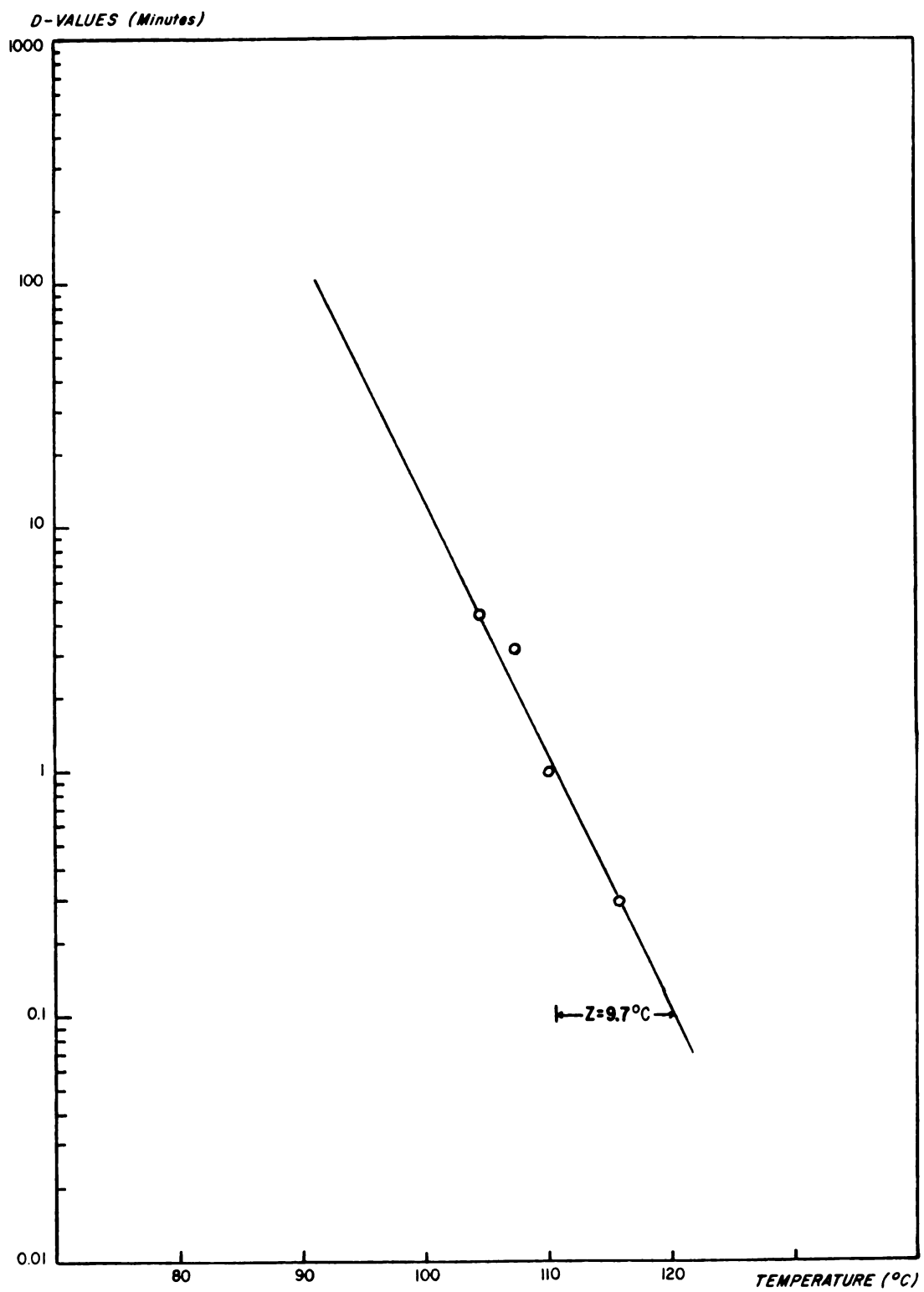


Figure 2. Thermal resistance of *C. sporogenes* spores in distilled water.

trypticase glucose broth (BBL, Uehara et al., 1965) were aseptically transferred to each irradiated sample. The tubes were incubated at 37°C in a nitrogen atmosphere for 7 days. Results are shown in Table 5.

The average D-value of C. sporogenes spores in distilled water was found to be 0.216 Mrad. The results were in agreement with those reported by others. Anellis and Koch (1962) reported a D-value of C. sporogenes P.A. 3679 strain S-2 to be 0.209 Mrad in phosphate buffer.

From the results of the resistance of spores in distilled water it was clearly confirmed that this spore crop had low heat resistance compared with that reported by others. The radiation resistance was approximately the same as that found by others.

The thermal D-value is expressed as time in minutes at a certain temperature to destroy the bacterial population by 90 percent. Based on the same degree of destruction, the radiation D-value is expressed in terms of radiation dose. At a constant dose rate, the radiation resistance of bacterial spores can be expressed either in Megarads or in minutes. It should be noted that the comparison of radiation D-values as irradiation time in minutes can be used only at the same radiation dose rate. Since the length of time in this study was short compared to the half-life of cobalt, 5.26 years, the radiation dose rate at room temperature measured at the beginning of the experiment was used throughout.

TABLE 5.--Radiation resistance of C. sporogenes spores in distilled water at room temperature.

Radiation Dose (Mrad)	Radiation Time (Minutes)	Positive Tubes out of 10 Total	D-Value (Mrad)
1.00	61.7	10	. .
1.10	67.9	9	0.206
1.15	70.0	10	. .
1.20	74.0	7	0.214
1.25	77.1	8	0.228
1.30	80.2	3	0.212
1.35	83.3	3	0.220
1.40	87.4	0	. .
1.45	90.5	0	. .
1.50	93.6	0	. .

Note: Initial spore count = 5×10^5 spores per tube.

Resistance of Spores in
Raw Ground Beef

Heat

Results are shown in Table 6. The heat resistance of the spores was determined in the same manner as in the preliminary experiments. Three different temperatures 90, 95, and 100°C were selected. Heating at temperatures below 90°C was not included because it required very long exposure time. The time used was selected based on the preliminary results.

At 90°C, heating times from 225 to 270 minutes at 5 minute intervals were used. At the three shortest times, the heating effect was not enough to destroy the spore inoculum and all 10 cans were positive. From 240 to 255 minutes, there was partial spoilage and the D-value at each time-temperature combination was found to be 40.2, 40.2, 40.2, and 39.4 minutes respectively. The average thermal D-value at 90°C was 40.0 minutes. The difference between the minimum and maximum D-values was 0.8 minute. The D-value of 90°C, therefore, probably is 40.0 ± 0.4 minutes. Thermal process times of 260 and 270 minutes resulted in all negative cans.

At 95°C, heating times from 72 minutes to 86 minutes were used at 2 minute intervals. The thermal process times of 72 and 74 minutes were too short for any partial spoilage to develop and all 10 cans at each

TABLE 6.--Thermal resistance of C. sporogenes spores in raw ground beef.

Heating Temperature (°C)	Heating Time (Minutes)	Positive Cans Per 10 Cans Total	D-Value (Minutes)
90	225	10	. .
	230	10	. .
	235	10	. .
	240	8	40.2
	245	7	40.2
	250	6	40.2
	255	4	39.4
	260	0	. .
	270	0	. .
95	72	10	. .
	74	10	. .
	76	9	13.1
	78	2	11.4
	80	1	11.2
	82	0	. .
	84	0	. .
	86	0	. .
100	20	9	2.8
	21	9	2.9
	22	4	3.4
	23	0	. .
	24	0	. .
	25	0	. .

Note: Initial spore count = 1.5×10^6 spores per can.

treatment were positive. At longer times, namely 76, 78, and 80 minutes, however, partial spoilage occurred and the corresponding D-values were 13.1, 11.4, and 11.2 minutes respectively. Heating times of 82, 84, and 86 minutes were enough to destroy the spore inoculum and the 10 cans at each of these time-temperature combination were negative. The average thermal D-value at 95°C was 11.9 minutes. The difference between the minimum and the maximum D-values was 1.9 minutes. The D-value at 95°C, therefore, probably is 11.9 ± 0.95 minutes.

At 100°C, thermal process times of 20 minutes to 25 minutes with 1 minute intervals were used. At 20, 21, and 22 minutes, the cans showed partial spoilage. The D-values at these 3 process times were calculated to be 2.8, 2.94, and 3.4 minutes respectively, with 3.1 minutes average. The difference between the minimum and the maximum D-values was 0.6 minute. The D-value at 100°C, therefore, probably is 3.1 ± 0.3 minutes.

The thermal death time curve of C. sporogenes spores in raw ground beef was obtained by plotting on a semilog paper. The D-values at different temperatures in minute were plotted on the logarithmic scale and the temperatures in degrees Celcius were on the linear scale (Figure 3). A straight line which indicated logarithmic destruction of the spores was obtained with the Z-value 9.4°C. By assuming that the bacterial destruction was logarithmic,

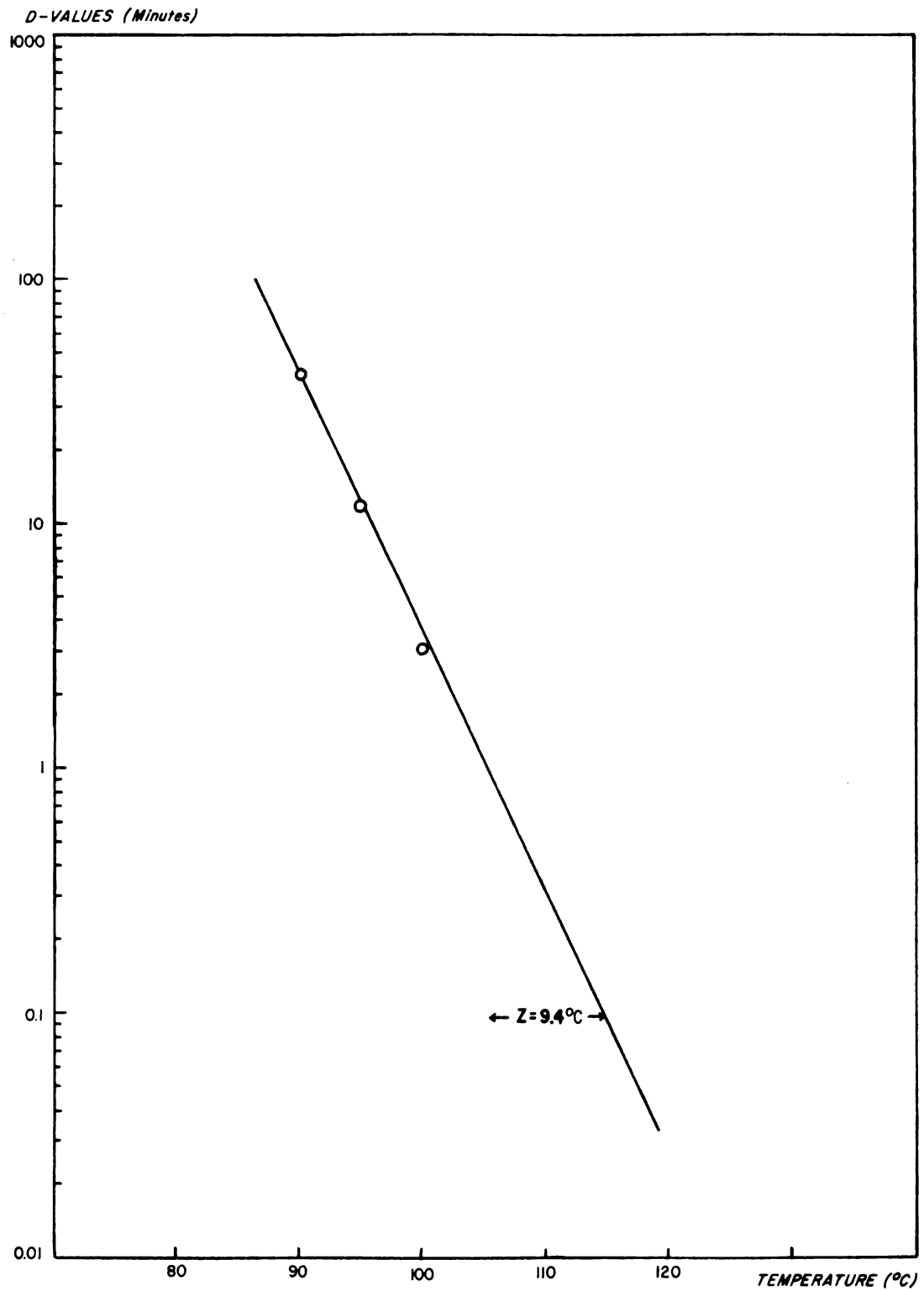


Figure 3. Thermal resistance of C. sporogenes spores in raw ground beef.

the D-values at temperatures other than those used in the experiments may be obtained by extrapolation of the curve.

Irradiation

The results of radiation resistance study are shown in Table 7. To study the radiation resistance, doses of 1.0 to 1.5 Mrad were used with 0.1 Mrad intervals. From 1 to 1.2 Mrad all cans were positive. At higher doses, 1.3 and 1.4 Mrad, partial spoilage occurred with 6 and 3 positive cans respectively. The corresponding D-values were 0.209 and 0.211 Mrad. The average D-value was 0.210 Mrad. The difference between the minimum and the maximum D-values was 0.002 Mrad. The radiation D-value at room temperature, therefore, probably is 0.210 ± 0.001 Mrad. Assuming constant dose rate, the D-value of radiation expressed as time in minute could also be obtained. At 1.3 Mrad (100.6 minutes radiation time), the D-value was 16.2 minutes and at 1.4 Mrad (108.4 minutes radiation time), the D-value was 16.4 minutes. The average D-value at room temperature was 16.3 minutes. The difference between the minimum and the maximum D-values was 0.2 minutes. The average radiation D-value in minute probably is 16.3 ± 0.1 minutes.

Simultaneous Heating and Irradiation

Simultaneous heating and irradiation was performed at three different temperatures 80, 91, and 95°C. Results

TABLE 7.--Radiation resistance of C. sporogenes spores in raw ground beef, at room temperature.

Radiation Dose (Mrad)	Radiation Time (Minutes)	Positive Cans Out of 10 Cans Total	D-Value (Mrad)
1.0	77.4	10	. .
1.1	85.1	10	. .
1.2	92.9	10	. .
1.3	100.6	6	0.209
1.4	108.4	3	0.211
1.5	116.1	0	. .

Note: Initial spore count = 1.5×10^6 spores per can.

of radiation at 80, 91, and 95°C are shown in Tables 8, 9, and 10 respectively.

At 80°C-1 Mrad radiation, 8 out of 10 cans were positive and the D-value in Megarad was 0.168. Assuming constant dose rate and the exposure time of 77.4 minutes to obtain 1 Mrad, the radiation D-value in minutes was 13.0.

As the temperature of radiation increased, the D-values expressed in Megarads and in minutes decreased. At higher temperatures, lower doses of radiation were needed to achieve the same bactericidal effect. At 91°C-0.5 Mrad and 91°C-0.6 Mrad, all treated cans were positive and the combination treatments were not enough to sterilize

TABLE 8.--Radiation resistance of C. sporogenes spores in raw ground beef, at 80°C.

Radiation Dose (Mrad)	Radiation Time (Minutes)	Positive Cans Out of 10 Cans Total	D-Value (Mrad)
0.5	38.7	10	. .
1.0	77.4	8	0.168
1.5	116.1	0	. .

Note: Initial spore count = 1.5×10^6 spores per can.

TABLE 9.--Radiation resistance of C. sporogenes spores in raw ground beef, at 91°C.

Radiation Dose (Mrad)	Radiation Time (Minutes)	Positive Cans Out of 10 Cans Total	D-Value (Mrad)
0.5	38.7	10	. .
0.6	46.4	10	. .
0.7	54.2	7	0.115
0.8	61.9	4	0.124
0.9	69.7	0	. .
1.0	77.4	0	. .

Note: Initial spore count = 1.5×10^6 spores per can.

TABLE 10.--Radiation resistance of C. sporogenes spores in raw ground beef, at 95°C.

Radiation Dose (Mrad)	Radiation Time (Minutes)	Positive Cans Out of 10 Cans Total	D-Value (Mrad)
0.30	23.2	10	. .
0.35	27.1	10	. .
0.40	31.0	10	. .
0.45	34.9	10	. .
0.50	38.7	10	. .
0.55	42.6	5	0.087
0.60	46.4	0	. .

Note: Initial spore count = 1.5×10^6 spores per can.

the inoculated beef sample. Irradiation of the spores at 91°C-0.7 Mrad, 7 cans were positive and the D-value was 0.115 Mrad. By using the same assumption, i.e., the dose rate was constant, the radiation D-value expressed in time was shown to be 8.9 minutes. Similarly, at 91°C-0.8 Mrad, the D-value was 0.124 Mrad with the corresponding D-value in minutes as 9.6. The average D-value at 91°C of radiation was 0.119 Mrad or 9.2 minutes. The difference between the minimum and the maximum D-values was 0.009 Mrad which was equal to irradiation time of 0.7 minute. The average radiation D-value at 91°C, therefore, probably is 0.119 ± 0.005 Mrad or 9.2 ± 0.35 minutes.

Radiation D-values at 95°C were lower than the D-values obtained at lower temperature of radiation. At 95°C, radiation doses of 0.3 to 0.6 Mrad at intervals of 0.05 Mrad were used to find the D-value. From 0.3 to 0.5 Mrad, the treated cans at each time-temperature combination were all positive indicating inadequate treatment. At 0.55 Mrad of radiation, however, there was 5 positive cans out of 10 cans total. The D-values expressed as the radiation dose and as the radiation time were 0.087 Mrad and 6.7 minutes respectively.

Too few data was obtained for the radiation D-values at 80 and 95°C to estimate the possible accuracy of these values. The data, however, showed a clear trend that the radiation resistance of the spores progressively decreased as the temperatures of irradiation increased.

The experimental radiation D-values at different temperatures of irradiation, expressed as time in minutes, were plotted on a semilog paper. The D-values were plotted on the logarithmic scale and the temperatures of radiation on the linear scale (Figure 4). The radiation D-values decreased as the temperatures of radiation increased. From 25 to 80°C, the D-values decreased slowly. From 80 to 95°C, the D-values of C. sporogenes decreased very rapidly. The radiation D-value curve became very steep and almost joined the thermal death time curve at 95°C. At this temperature, the D-values of the combination

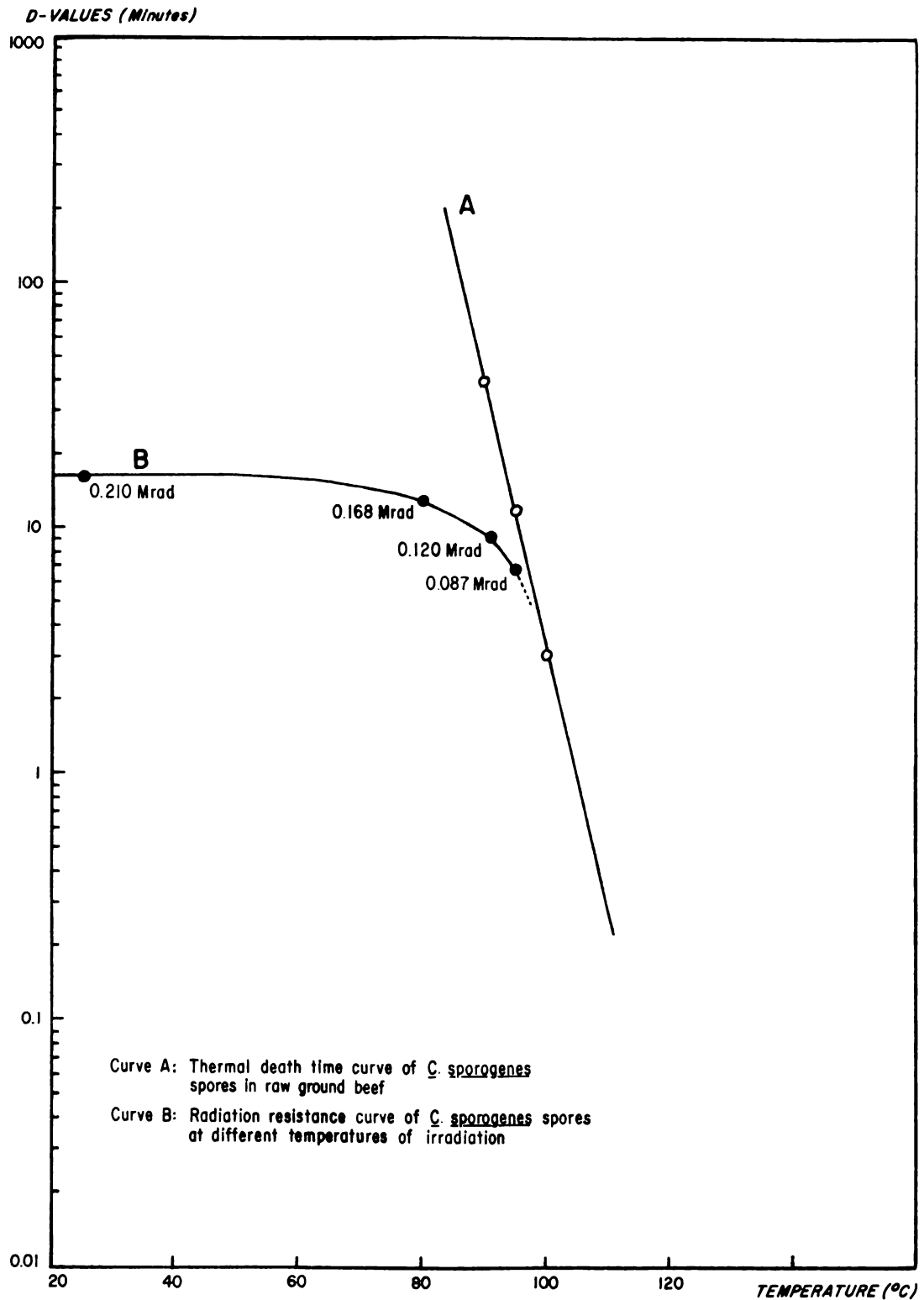


Figure 4. Radiation resistance of *C. sporogenes* spores at different temperatures of irradiation.

treatment and that of heating only were very close, which indicated that the effect of heat was more pronounced than that of radiation.

The progressive decrease in D-value with increasing irradiation temperatures was in excellent agreement with that of Grecz et al. (1971) regardless of many different factors in the two sets of experiment. They reported the linear change in D-values of C. botulinum strain 33A spores in ground beef with change of irradiation temperatures from -196 to 65°C. From 65 to 85°C, the D-values decreased more rapidly. The decline became very steep from 85 to 95°C. They did not, however, report that the decrease in resistance at 85 to 95°C was caused by a synergistic effect of heat and radiation, or by an additive effect of the two parameters.

A similar decrease in radiation D-values with increasing temperatures of radiation has been found when dry heat was applied. Reynolds and Garst (1970) observed the synergistic effect of dry heat and gamma radiation on spores of Bacillus subtilis variety niger. The thermal D-value at 105°C was 4.5 hours and the radiation D-value was 105 Mrad (approximately 13 hours radiation time). The D-value of the simultaneous heating (at 105°C) and irradiation was 1.5 hours. In this 1.5 hours period, the lethality by heat alone was 30 percent and that of radiation alone was 10.4 percent, which would have given only

40.4 percent decrease in bacterial count instead of 90 percent (1 log cycle). They concluded that the rapid decrease in D-value resulted from the synergistic effect of the combined heat and irradiation treatment, and the synergistic effect was found at a temperature as low as 60°C. The decrease of radiation D-value as the irradiation temperature increased did not follow the same pattern as those observed by Grecz and his coworkers and by author. In Reynolds and Garst's paper, the change of radiation D-values (logarithmic scale) with temperatures (linear scale) followed a straight line from 25 to 90°C. At higher irradiation temperatures, from 90 to 105°C, a more progressively rapid decrease in radiation D-values was found. The rapid decrease in D-values at higher irradiation temperatures might have resulted from the use of dry heat which is less effective in sterilization than wet heat.

Whether the effect of simultaneous heating and irradiation was synergistic or additive was the second objective of this research. To obtain the answer, comparison of the "calculated D-values" and the "experimental" D-values was made.

Comparison of the "Calculated" and
"Experimental" Radiation D-Values
of Simultaneous Heating
and Irradiation

The "experimental" D-values of the simultaneous heating and irradiation were obtained by computing the

D-values based on the actual dose used in the experiment that exhibited partial spoilage data. The equation for computing the D-values was that described by Anellis and Werkowski (1968). The "calculated" D-values were the D-values of the combined treatment calculated from the thermal D-values and radiation D-values. The calculated D-values represented the simultaneous heating and irradiation D-values assuming the effect of heat and radiation to be additive. A comparison of the experimental D-values and calculated D-values at the same irradiation temperature was made. The difference between the two kinds of D-values expressed as the percentage of the experimental D-value is shown in Table 11. In all combinations, the calculated D-values were higher than the experimental D-values which suggested that the effect of heat and radiation was at least additive. The difference between the two kinds of D-value was 21.1 percent at 80°C with radiation, and progressively decreased as the temperatures of irradiation increased to 91 and 95°C to be 16.2 and 4.2 percent respectively.

An effort has been made to assign the contribution of heat and radiation individually and when used in combination. In order to do this the following relationship which was derived from the definition of D-value was employed.

TABLE 11.--Comparison of the "calculated" and "experimental" radiation D-values of simultaneous heating and irradiation.

	D-Value (Minutes)				% Difference		
	Room Temperature	80°C	91°C	95°C	80°C	91°C	95°C
Experimental	16.3	13.0	9.2	6.7			
Calculated	. .	15.7	10.7	6.9	21.1	16.2	4.2

Note: % Difference = $\frac{\text{Calculated D-value} - \text{Experimental D-value}}{\text{Experimental D-value}} \times 100$

$$\frac{1}{D_H} + \frac{1}{D_R} = \frac{1}{D_{HR}}$$

or

$$\frac{D_{HR}}{D_H} + \frac{D_{HR}}{D_R} = 1$$

$\frac{D_{HR}}{D_H}$ is the ratio of the D-value of simultaneous heating and irradiation to the D-value of heat alone and is equal to the amount of bacterial destruction contributed by heat only during the time D_{HR} . Since during the time D_H minute, the amount of bacterial destruction is 90 percent by heat only, during the time D_{HR} , the percentage of destruction is equal to $\frac{D_{HR}}{D_H} \times 90$ percent. The percentage of destruction cannot be expressed as the total destruction of 100 percent because by definition of the D-value, only 90 percent destruction can be obtained. Similarly $\frac{D_{HR}}{D_R}$ is the ratio

of the D-value of simultaneous heating and irradiation to the D-value of radiation alone and is equal to the amount of bacterial destruction contributed by radiation only during the time D_{HR} . The percentage of destruction is equal to $\frac{D_{HR}}{D_R} \times 90$ percent. The percentage of destruction cannot be expressed as the total destruction of 100 percent because by definition of the D-value, only 90 percent destruction can be obtained. The percentage of destruction due to heat and to radiation alone or in combination at 80, 91, and 95°C were obtained from the data of Table 11 and D-values extrapolated from Figure 4 and are shown in Table 12. Were the combination effect due to simply the additive action of each individual agent, the sum of these percentages would equal 90 percent. On the other hand, were the combination effect due to the synergistic action of each individual agent, the sum of these percentages would be less than 90 percent.

At 80°C with radiation, the bacterial destruction was approximately 2.5 percent from heat and 71.3 percent from radiation. The combined effect, if additive, would have resulted in only 73.8 percent destruction instead of 90 percent (1 log cycle) as was found experimentally. The remaining 16.2 percent of destruction, therefore, might have resulted from the synergistic effect of heating at 80°C and of radiation. Similarly at 91°C with radiation, the bacterial destruction was approximately 26.4 percent

TABLE 12.--The percentage of spore destruction by heating only and irradiation only as calculated from the experimental D-values of simultaneous heating and irradiation.

Temperature of Radiation (°C)	Percentage of Spore Destruction			
	Heat	Radiation	Heat Plus Radiation	Difference of Spore Destruction
80	2.5	71.3	73.8	16.2
91	26.4	51.0	77.4	12.6
95	50.8	37.2	88.0	2.0

Note:

1. Percentage of spore destruction =

$$\frac{\text{Experimental D-value of heating and radiation}}{\text{D-value from heat}} \times 90$$
2. Percentage of spore destruction by radiation =

$$\frac{\text{Experimental D-value of heating and radiation}}{\text{D-value from radiation}} \times 90$$
3. The difference of spore destruction = 90% -
 (percentage of spore destruction from heat plus radiation).

from heat and 51.0 percent from radiation. The remaining 12.6 percent out of 90 percent destruction resulted from the combined effect of heat and irradiation. The effect of heat and irradiation when the combination treatments were performed at 80 and 91°C is more than additive and appears to be slightly synergistic. The apparent synergistic effect at 80°C is higher than at 91°C.

At 95°C with radiation, the percentage of destruction from heat, unlike at 80 and 91°C, was higher than that from radiation. The bacterial destruction caused by heat only was 50.8 percent and that from radiation was 37.2 percent. The sum of the destruction, if the two treatments were additive, would have been 88 percent and only 2 percent from the effect of the combined treatment. This 2 percent difference was small enough to be considered within experimental error. It was therefore, concluded that the effect of heat and radiation appears to be additive when heating at 95°C and radiation in the combined treatment was performed. The synergistic effect at 80 and 91°C, however, is small and has not been statistically evaluated in this experiment. More data are required to show whether the apparent synergistic action observed at 80 and 91°C is statistically significant.

It has been clearly demonstrated in this research that the bactericidal effect of heat and of radiation on C. sporogenes spores are interchangeable. The thermal

energy can be replaced by the radiation energy and vice versa. Heat and radiation applied at the same time do not increase the resistance of bacterial spores as was previously reported by some authors (Anderson et al., 1967; Grecz et al., 1967). The combination treatment of heat and radiation appears to be synergistic when the irradiation temperatures are at 80 and 91°C. At 95°C with radiation, the combination treatment appears to be only additive.

SUMMARY AND CONCLUSIONS

The radiation resistance of Clostridium sporogenes spores (P.A. 3679, NCA 7955) inoculated in raw ground beef has been studied using most probable number technique (Stumbo et al., 1950; Anellis and Werkoski, 1968). The temperatures of radiation were 25, 80, 91, and 95°C. The spore inoculum of 1.5×10^6 spores per one thermal death time can or 1.5×10^7 spores per treatment (10 cans) was used.

At 25°C with radiation, the spore destruction was from radiation only and the radiation resistance was highest with the D-value 0.210 Mrad. As the temperatures increased, the percentage of destruction from heat increased while that of radiation decreased. The radiation D-values decreased progressively with increasing temperatures and were observed to be 0.168, 0.119, 0.087 Mrad at 80, 91, and 95°C respectively.

At 80°C with radiation, the percentage of spore destruction was 2.5 percent from heat and 71.3 percent from radiation. The difference between the calculated

D-value and the experimental D-value was 16.2 percent. The effect of simultaneous heating and irradiation appears to be synergistic.

At 91°C with radiation, the percentage of spore destruction was 26.4 percent from heat and 51.0 percent from radiation. The difference between the calculated D-value and experimental D-value was 12.6 percent. The effect of simultaneous heating and irradiation appears to be less synergistic than at 80°C.

At 95°C with radiation, the percentage of spore destruction was 50.8 percent from heat and 37.2 percent from radiation. The difference between the calculated D-value and the experimental D-value was 2 percent. The effect of simultaneous heating and irradiation appears to be additive.

The difference between the calculated and the experimental D-values was lowest when either radiation (at 25°C of radiation temperature) or heat was predominate. From 80 to 95°C, the difference between the two values progressively decreased with the increasing temperatures.

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