Combined Irradiation-Heat Processing of Canned Foods

I. Cooked Ground Beef Inoculated with Clostridium botulinum Spores

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The quantity of ionizing radiations needed to sterilize canned foods generally impairs both their nutritive qualities and palatability (Hannah, 1955; Schultz et al., 1956). However, thermal processes also lower these qualities in canned foods (Stumbo, 1949). In both instances, the extent of damage increases with the severity of the treatments. Consequently, the heat processing schedules that are used industrially are based upon the minimum levels required to kill those microorganisms that might endanger the health of consumers or which would cause the food to spoil during storage (Stumbo, 1949). For these reasons, new sterilizing techniques that will retain or improve the organoleptic properties of canned foods without lowering their safety or nutritional qualities are constantly being sought. Since it has recently been shown that heat and gamma radiation are synergistic when used together for killing the spores of anaerobic bacteria (Morgan and Reed, 1954; Kempe, 1955), it is possible that combined irradiation-heat processing of canned foods could result in an improved procedure. The present study is concerned with establishing combined irradiation-heat processing schedules for previously heat sterilized ground beef that was then inoculated with Clostridium botulinum 213B spores before the cans were closed. Future studies will deal with experiments utilizing putrefactive anaerobe (PA)3679 spores.

Materials and Methods

A. Packing

Lean ground beef was purchased locally from the University of Michigan food stores. The meat was placed in shallow pans and autoclaved at 15-psig steam pressure for 1/4 hr. Excess liquid was poured off, and approximately 300 g of hot meat was packed into each of 28 no. 1 picnic tin cans: 4 of these cans had previously been fitted with O. F. Ecklund (1949) thermocouples. The covers were then set loosely on the cans of meat which were placed in an autoclave where they were processed at 17-psig steam pressure for 1 hr. Next, each can was removed individually from the hot autoclave and the meat was inoculated with 2 ml of a spore suspension. The cans were then sealed in a commercial-type closing machine, immersed in cold tap water for about 20 min, and finally placed in ice water for 1 hr. Next the experimental cans were either irradiated or temporarily stored in a refrigerator. The 8 controls were placed in an incubator immediately: the experimental cans were incubated after thermal processing but were first cooled by immersion in cold water. Incubation was carried out at 99 F or 84 F as indicated.

B. Irradiation

The canned meat was irradiated in the center well of the large cobalt-60 gamma radiation source in the Fission Products Laboratory at the University of Michigan (Lewis et al., 1954). In this center well the radiation field had an essentially constant intensity which was measured by ferrous-ferric sulfate dosimetry as previously described (Kempe et al., 1954). During the period of this investigation, a total dosage of 1.08 megarep required about 6 hr irradiation because the dosage rate averaged approximately 180,000 rep/hr in the center of the cans. For this reason, the meat was kept at a temperature below 40 F during irradiation. However, artificial cooling was not needed since the cobalt-60 source is located in an unheated concrete vault, this work was done during midwinter and the meat was precooled to less than 40 F as part of the packing process.

C. Heat Processing

Following irradiation, the cans were either immediately heat processed or were temporarily stored in a refrigerator. Control experiments indicated that refrigerated storage for 2 days did not affect the results.

For heat processing, six cans of meat, two of which contained thermocouples, were placed in a 3 gal pail that was half-filled with water at 180 F and which was positioned in the upper part of an autoclave. Steam was then introduced at the rate necessary to maintain a water temperature of 180 F until the thermocouples in the cans of meat showed identical temperatures of 170 F or more.

Processing was begun by raising the water bath temperature to 230 F. When sufficient time had elapsed to provide the desired F value, the autoclave was quickly opened and the cans were plunged into cold water. Four sets of cans were autoclaved for each run.

1 One rep is a dose of ionizing radiation capable of producing energy absorption of 93 ergs per g of tissue.
Following heat processing, those cans inoculated with approximately 5,000,000 spores per can were incubated at 99°F, while those receiving 300 spores per can were incubated at 84°F. \( F_0 \) values were calculated by Schultz's graphical modification of Ball's General Method (Schultz and Olson, 1940), using the special graph paper constructed for a Z value of 18 and the general equation, for example:

\[
F_0 = \frac{m\Delta}{10^d}
\]

\( F_0 \) = the number of minutes required to sterilize the can of meat at 250°F when the Z value equals 18

\( m \) = number of min represented by 1 in. on the time scale

\( \Delta \) = area under the curve in square in.

\( n \) = the number of 18°F changes that must be made to change the number of the top line from 250 to the desired number (\( n \) is positive if the top line is numbered less than 250 and negative if greater than 250)

\( d \) = number of in. from the zero line to the top line.

### D. Spores

The spores of anaerobic bacteria used in these studies were prepared and used according to techniques described in previously published work from this laboratory (Kempe et al., 1954).

### E. Controls

Each run included eight control cans. Four of these were uninoculated, and four were selected at random.

#### TABLE 1. Combined irradiation-heat processing treatments required to sterilize ground beef packed in no. 1 picnic tin cans and inoculated with Clostridium botulinum 213B spores

<table>
<thead>
<tr>
<th>Run No.</th>
<th>No. of Spores per Can</th>
<th>Preirradiation</th>
<th>( F_0 ) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. Series I</strong>—approx. 5,000,000 spores per can; incubated at 99°F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 2</td>
<td>7,200,000</td>
<td>0</td>
<td>0.75-1.3</td>
</tr>
<tr>
<td>C 3</td>
<td>10,700,000</td>
<td>0</td>
<td>0.36-0.93</td>
</tr>
<tr>
<td>CB 10</td>
<td>6,000,000</td>
<td>0.500</td>
<td>0.77-1.1</td>
</tr>
<tr>
<td>CB 5</td>
<td>6,300,000</td>
<td>0.675</td>
<td>&gt;0.58</td>
</tr>
<tr>
<td>CB 6</td>
<td>6,000,000</td>
<td>1.000</td>
<td>0.41-0.80</td>
</tr>
<tr>
<td>CB 7</td>
<td>3,800,000</td>
<td>1.200</td>
<td>0.09-0.29</td>
</tr>
<tr>
<td>CB 4</td>
<td>5,200,000</td>
<td>1.350</td>
<td>0.11-0.21</td>
</tr>
<tr>
<td>CB 12</td>
<td>5,000,000</td>
<td>1.500</td>
<td>0.063-0.27</td>
</tr>
<tr>
<td>CB 15</td>
<td>5,000,000</td>
<td>3.420-3.960</td>
<td>0</td>
</tr>
<tr>
<td><strong>b. Series II</strong>—approx. 300 spores per can; incubated at 84°F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 24</td>
<td>300</td>
<td>0</td>
<td>0.33-0.47</td>
</tr>
<tr>
<td>CB 25</td>
<td>300</td>
<td>0.500</td>
<td>0.31-0.51</td>
</tr>
<tr>
<td>CB 26</td>
<td>300</td>
<td>0.750</td>
<td>0.22-0.34</td>
</tr>
<tr>
<td>CB 23</td>
<td>300</td>
<td>1.000</td>
<td>0.086-0.18</td>
</tr>
<tr>
<td>CB 27</td>
<td>300</td>
<td>1.640-1.920</td>
<td>0</td>
</tr>
<tr>
<td>CB 21</td>
<td>300</td>
<td>1.360-1.800</td>
<td>0</td>
</tr>
</tbody>
</table>

Two series of tests were conducted. In the first series, shown in Table 1 and Figure 1, approximately 5,000,000 spores from the 24 inoculated cans. All eight cans were then incubated. If no gas pressure developed in the uninoculated controls, the meat in the experimental cans was presumed to contain only those spores that had been purposely introduced. Gas development in the inoculated control cans demonstrated viability of the culture. In addition, occasional cans were selected at random from among those producing gas for verification of the culture's identity. Toxin production, as indicated by mouse inoculation tests (Kempe et al., 1954), was used for this purpose.

### Results

![Figure 1. \( F_0 \) required to sterilize ground beef packed in no. 1 picnic tin cans, inoculated with 5,000,000 Clostridium botulinum 213B spores per can, and irradiated with gamma rays from cobalt-60 before heat processing.](http://aem.asm.org/)

The results are shown in Figure 1 and Table 1. The \( F_0 \) values were calculated using the general equation:

\[
F_0 = \frac{m\Delta}{10^d}
\]

Where:

- \( m \) is the number of minutes required to sterilize the can of meat at 250°F when the Z value equals 18.
- \( \Delta \) is the area under the curve in square in.
- \( n \) is the number of 18°F changes that must be made to change the number of the top line from 250 to the desired number (positive if the top line is numbered less than 250 and negative if greater than 250).
- \( d \) is the number of inches from the zero line to the top line.

The spores of anaerobic bacteria used in these studies were prepared and used according to techniques described in previously published work from this laboratory (Kempe et al., 1954).
C. botulinum 213B spores were used per can of meat and the incubation was carried out at 99 F. In the second series, shown in table 1 and figure 2, 300 such spores were used per can of meat and the incubation temperature was 84 F. The data in both series of runs showed that when irradiation and heat were used together the amount of each form of energy required to produce sterile canned meat was less than that required when either form was used alone. Also, approximately 1.0 megarep of gamma radiation was required before the accompanying heat-processing treatment could be appreciably reduced and still produce sterile meat. These data further indicate that the initial spore concentration in the meat significantly affected the severity of the combined irradiation-heat processing treatment required to produce sterility. For example, with 5,000,000 spores per can, an $F_o$ of more than 0.6 was needed to produce sterility following treatment with 1.0 megarep of gamma radiation, but, with 300 spores per can, an $F_o$ of less than 0.2 was required after a like amount of radiation.

**Discussion**

Besides the previously observed (Kempe, 1955; Morgan and Reed, 1954) synergistic action of gamma radiation and heat for killing anaerobic bacterial spores, other considerations indicate the desirability of a combined irradiation-heat processing treatment for sterilizing canned foods. For example, the dosage of 2.0 megarep that is commonly suggested (Schultz et al., 1956) for sterilizing foods will not completely inactive enzymes (Hannan, 1955), pathogenic viruses (Jordon and Kempe, 1956), certain micrococci (Anderson et al., 1956), or botulinus toxin (Hannan, 1955). On the other hand, viruses, micrococci, botulinus toxins (Dack, 1943) and all but a few enzymes are quickly destroyed by moist heat at 212 F. Since all these should be inactive in canned foods, a combined irradiation-heat processing treatment that is designed to kill C. botulinum spores should produce safe canned foods because any such process would involve heating the food to temperatures of 230 F or above. However, since C. botulinum spores are less resistant to heat than certain other anaerobic bacterial spores found in foods, prevention of spoilage will likely require combined irradiation-heat processes of greater severity than those reported here. The combined irradiation-heat processing treatments required to sterilize canned ground beef inoculated with PA3679 spores will be described in a future paper.

**Acknowledgments**

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**Summary**

Mild heat treatments are known to inactivate botulinus toxin, most enzymes, and pathogenic viruses as well as vegetative bacteria that could become radiation resistant. Since all these must be inactive in properly canned foods, combined irradiation-heat processing offers other advantages besides those related to the improved food quality and nutritive value that
may result from the reduced quantity of each of these forms of energy required when they are used together for food sterilization.

Ground beef packed in no. 1 picnic tin cans and inoculated with Clostridium botulinum 213B spores was sterilized by combined irradiation-heat processing. Using 5,000,000 such spores per can, an $F_o$ of more than 0.6 was required to produce sterility following 1.0 megarep of gamma radiation; with 300 such spores per can, an $F_o$ of less than 0.2 was sufficient for this purpose after the same amount or radiation. Without pre-irradiation, $F_o$ values of approximately 1.0 and 0.4 were required in the first and second instances, respectively. Preirradiation with less than 1.0 megarep of gamma radiation did not appreciably reduce the $F_o$ value subsequently required in either instance.

REFERENCES


Schultz, O. T. and Olson, F. C. W. 1940 Thermal processing of canned foods in tin containers. Food Research, 5, 56-64.


Studies in the Recovery of Viable Cells of Freeze-Dried
Serratia marcescens

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Increased use of the freeze-drying technique to preserve bacterial stock cultures has emphasized the problem of the recovery of viable cells from such preparations. Bacterial viability is an expression of the interrelationship of several independent functions but, as determined by the standard bacteriological plating technique, is a measure only of the cellular function of division. Exposure of an organism to unfavorable environmental conditions, either physical or chemical, may result in physiological injury which is not immediately lethal. Subsequent treatment of such a cell, by supplying the proper nutrients or a more suitable environment, may afford the cell an opportunity of overcoming the injury, and thus of continuing as a viable organism.

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2 A Laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Streptomycin injury to Escherichia coli was reversed by a variety of salt solutions (Wasserman et al., 1954). Lethal injury induced by several bactericidal chemicals was circumvented by the exposure of cells to metabolic intermediates (Heinmetz et al., 1954). The number of nonviable cells produced by UV irradiation was reduced by supplying the cell with coenzymes and metabolism intermediates whose action or formation is presumably inhibited by the treatment (Heinmetz and Lehman, 1955). The reversal of “death by unbalanced growth” in a thymineless mutant of E. coli and in strains of E. coli treated with UV irradiation have been reported (Cohen and Barner, 1954; Barner and Cohen, 1956). This paper reports on the increased recovery rate of viable organisms from preparations of freeze-dried Serratia marcescens restored in a variety of salt solutions as compared to the detrimental effects of restoration in water.