[Reprinted from Food Technology, 1960, Vol. XIV, No. 10, Pages 483–487]

Thermal Resistance of Microorganisms to Dry Heat: Design of Apparatus, Operational Problems and Preliminary Results[a, b]

I. J. Pflug

Food Science Department, Michigan State University, East Lansing, Michigan

(Manuscript received May 21, 1960)

The nature of the atmosphere surrounding microorganisms during heat sterilization influences the effectiveness of the process; therefore, it is necessary to distinguish between a sterilization process where the cells are surrounded by a medium or atmosphere containing liquid water and one where the atmosphere surrounding the cells does not contain water in the liquid state. The terms "wet heat" and "dry heat" are used to describe these two sterilization processes. Wet-heat sterilization conditions exist when microorganisms are subjected directly to saturated steam in an autoclave or heated in a closed vessel, such as a can of food, that contains an appreciable amount of water. Dry-heat sterilization conditions exist when the microorganisms are exposed to superheated steam or air.

Interest in dry heat sterilization has been stimulated by the Dole aseptic canning system *(5)*, which uses superheated steam as a source of dry heat for sterilizing the containers and covers prior to aseptically canning the sterile food product.

The literature contains considerable data relative to the wet-heat resistance of microorganisms; however, there are comparatively few data regarding dry-heat resistance. Both Walter *(11)* and Perkins *(6)* discuss the use of dry heat for the sterilization of bacteriological and hospital supplies and recommend one hour at 320° F (160° C) for glassware and cutting instruments. Tanner and Dack *(10)*, studying the dry heat resistance of spores of *Cl botulinum*, reported that the time required to kill *Cl botulinum* spores varied from 15 to 60 minutes at 284° F (140° C) and from 5 to 15 minutes in the range 320° to 356° F (160° to 180° C). Collier and Townsend *(3)* reported thermal resistance data for spores of three organisms subjected to superheated steam: *B. stearotheormophilus* (1518) $F_{350} = 0.708$, $z = 26°$ F; *B. polymyxa* (PSO) $F_{350} = 0.667$ and $z = 28°$ F and PA 3679 $F_{350} = 0.625$ and $z = 60°$ F. (F_{350} is the minutes at 350° F to destroy the organism under specified conditions; z is a measure of the slope of the thermal death time or thermal resistance curve.) Schmidt *(8)*, reviewing the resistance of microorganisms to dry heat, states, "In general, most of the methods used for measuring resistance to dry heat in the temperature range above 212° F have not had a very high degree of precision. . . ."

The primary purpose of this paper is to describe precision equipment developed for determining the dry-heat resistance of microorganisms. In addition, some problems encountered in dry-heat testing, the evaluation of the lag correction factor, and preliminary dry-heat resistance data for one species of microorganism to superheated steam are presented.

EXPERIMENTAL

Development considerations. Precision control and measurement of time and temperature was the principal design consideration in developing this apparatus for dry heat thermal resistance testing. An adequate temperature range, flexibility as to heating medium (superheated steam, air, saturated steam), asepsis, and the need to test replicate samples were also important considerations. Mechanical operation, convenience, and economy were given secondary consideration.

A basic problem of both dry-heat and wet-heat testing apparatus is moving the samples in and out of a controlled temperature and pressure chamber. This problem of movement and leakage was given extensive consideration. The fact that under dry-heat conditions the heating medium could not be expected to lubricate any moving parts was also considered.

A double-seal, two-step operation was developed instead of using a sliding plate *(9)* or double pistons with rings *(7)* to obtain a more positive seal and eliminate possible lubrication problems. In this design a shoulder supporting an "O" ring near the end of the sample-carrying pistons seals the exposure chamber while the samples are being heated, and a seal-bar located inside the chamber closes the sample entry ports the remainder of the time. A single, double-acting pneumatic cylinder located in the center rear of the machine operates the seal bar. Two double-acting pneumatic cylinders, one located on each side of the apparatus, move the samples from the loading position to the exposure position and, after heating, return them to the dumping or subculturing position. The sample cups are identical to those used by Pflug and Esselen *(7)* and are supported by two 1/16-inch diameter stainless steel wires which are welded into and operated by a 1/2-inch trip shaft located inside the sample-carrying pistons. The trip mechanism is located to the rear of the pistons and is cam operated. Leakage past the cup supporting wires is prevented by an "O" ring on the trip shaft.

Figure 1 is a photograph of the complete apparatus; Figure 2 is a cut-away view showing the exposure chamber and related parts.

The time control system was designed so that the entire operation will proceed automatically through the operational sequence when the start button is actuated.

The temperature control system for dry heat consists of a temperature recording pneumatic controller (thermocouple sensing element), a pneumatic needle valve, an electrically-operated superheater with a variable transformer input control and heaters and variable transformers for maintaining the thermoresistometer wall temperatures. A steam reservoir main-

[a] Journal Paper No. 2624 of the Michigan Agricultural Experiment Station.

[b] Presented before the Nineteenth Annual Meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania, May 19, 1959.

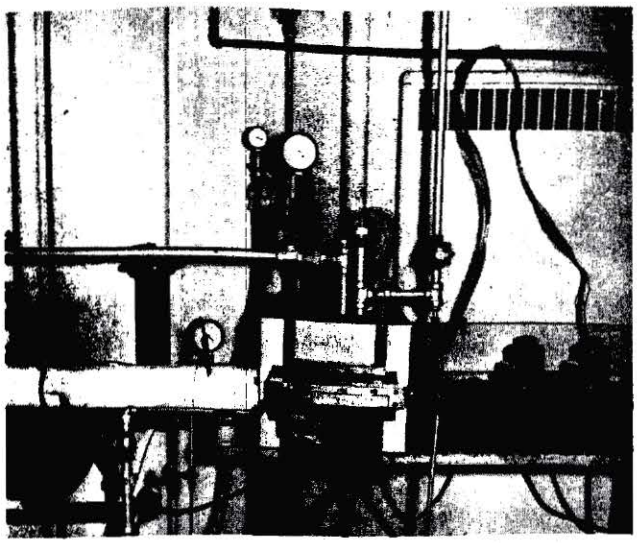

Figure 1. Front view of dry heat thermoresistometer.

tained at constant temperature by a pressure controller supplies the saturated steam to the superheater. The hot gas temperature controller modulates the flow of gas to maintain a constant temperature.

Operational problems. Two types of problems were encountered in putting this apparatus into operation: (1) controlling the temperature change of the gas as it flows through the apparatus and (2) obtaining uniform sample cup temperatures.

The temperature drop problem is a result of the low heat capacity of the gas. It was calculated that when the flow rate was 2.5 cfm, the removal of 1 BTU/min or 60 BTU/hr will reduce the temperature of superheated steam 26.1° F and air 32.3° F. When the thermoresistometer is operated so that the outlet temperature is only 5° F below the inlet temperature, the heat loss from the exposure chamber must be less than 0.2 BTU/min (12 BTU/hr) for superheated steam and less than 0.16 BTU/min (9.6 BTU/hr) for air. Therefore, the wall temperature of the exposure chamber is critical. The top, bottom, and front of the apparatus were fitted with electrical heaters to maintain the wall temperatures; thermocouples were attached to the inside of these walls so that the wall temperature could be measured and then controlled. During operation it is necessary to maintain the top, bottom, and front of the apparatus about 5° F above the exposure temperature to obtain the desired temperature control.

In hot-gas heating the gas stream must be distributed so that each cup is exposed to gas where the over-all heating effect of temperature and velocity is the same.

Cup temperatures were measured using 30-gage thermocouples soldered to the inside of the cup bottom. Thermocouple wires were looped away from the cup (Figure 3) and then brought out through the center of the pistons. This insured an isothermal wire length of about 1.0 inch, eliminating, from a practical standpoint, temperature errors resulting from the conduction of heat along the wires. It was necessary to remove the cup supporting mechanism in order that the thermocouple wire could be threaded through these pistons; ears were soldered onto the 5 sample cups so that they could be hung on the cup guide wire (Figure 3). Through trial and error design of the gas manifold, it was possible to have all 5 cups within a temperature range of less than 1.0° F; however, it was impossible to find a reliable correlation between cup temperature and inlet or wall temperatures. Figure 4 shows the interior of the exposure chamber and the distributing manifold. The direction of flow of the gas from the inlet manifold has a definite effect on the temperature pattern of the 5 cups.

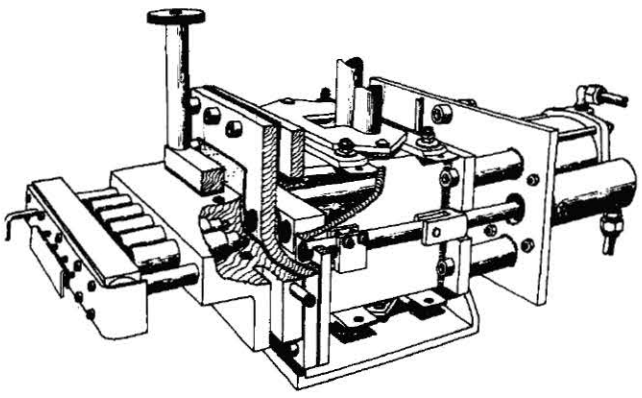

Figure 2. Cut-away drawing of the dry heat thermoresistometer showing the relation of the pistons and inside seal bar.

Wall temperatures are controlled by 3 manually-operated variable transformers that supply energy to the electric heaters. Wall temperatures are measured by thermocouples attached to the inside walls of the apparatus. Wall temperature input requirement varies with exposure temperature, room temperature, sample heating time, and temperature difference between wall temperatures and gas control temperature.

The problem of determining the temperature of the cups during actual testing was solved by reducing the number of cups per test from 5 to 4 and leaving one cup, fitted with a thermocouple similar to those described above, in the machine at all times. This thermocouple, connected to a temperature recording potentiometer, gives a complete temperature record of this one cup. Since the range of variation between cups has previously been established to be less than 1.0° F, all cups will always be within ± 1.0° F of the temperature of the cup with the thermocouple.

Lag correction factor. Heating characteristics of sample cups were determined in both air and superheated steam for gas flow rates ranging from TRP's (thermoresistometer pressure) of 5 to 20 in of water; the normal operating TRP is 10 to 15 in of water. TRP, used as a measure of flow for convenience, is the inches of water pressure necessary to force the gas flow volume through the discharge orifice. The relationship of TRP with flow rate of air under standard conditions is illustrated in Figure 5. Cups in all 5 positions were tested and each test was replicated 3 times for each variable. Data were plotted and the heating rate (f_h) and time to reach 1.0° F below heating medium (B) determined. [Terms are defined and the method of plotting data given in Ball and Olsen *(2)*]. Initial temperature of the cups was 90 ±3° F below heating medium temperature.

Values of f_h and B were plotted as a function of TRP and a straight line was drawn by eye through each set of data. Values of f_h and B for a TRP of 5 and 20 obtained from these curves are listed in Table 1.

TABLE 1

Average heating rates of sample cups in air and superheated steam

	TRP	f_h	B
	in of water	*min*	*min*
Air	5	0.39	0.68
	20	0.26	0.48
Superheated steam	5	0.36	0.62
	20	0.24	0.42

The lethality accumulated during heating, U, to 1.0° F below the heating medium temperature was calculated, using the table of functions of Hicks *(4)* for z values of 30, 40, 50, and 60° F assuming a straight line semi-logarithmic heating rate and a logarithmic rate of bacterial destruction. The heating correcion is B minus U.

The cooling correction is based on the assumption that no appreciable cooling occurs until the cups touch the recovery liquid, whereupon the surface temperature is instantly reduced to a point at which the lethal effect is negligible. The correction was calculated to be 0.015 min, the time required for the samples to be withdrawn from the heating chamber and dropped into the recovery medium and be quenched. The cooling correction is the same for all values of z.

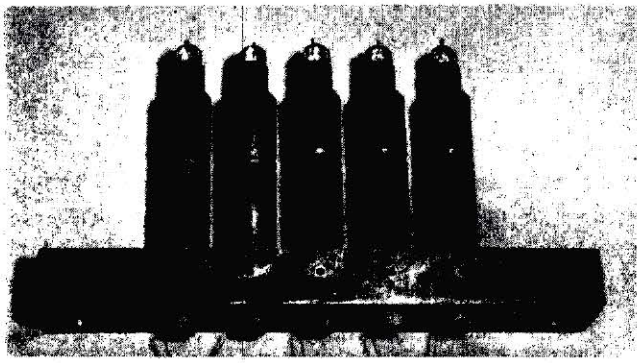

Figure 3. Sample carrying pistons and operating bar (this unit can be removed from machine in less than 5 min). Special sample cups fitted with thermocouples are in position at the end of the pistons.

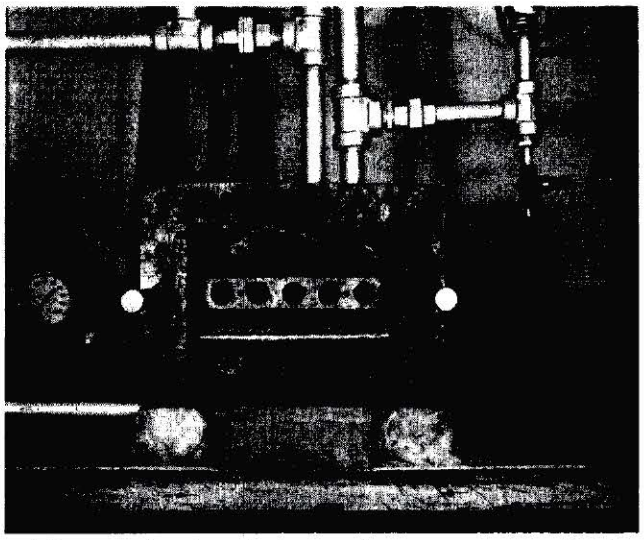

Figure 4. Interior of thermoresistometer exposure chamber showing gas distributing manifold, wall thermocouples and seal bar.

The total lag correction, algebraic sum of the heating and cooling corrections, is shown graphically in Figure 6 as a function of z and TRP.

Dry heat thermal resistance studies. Bacterial spores of a Curran's strain 15 μ of the facultative mesophile *Bacillus subtilis* [c] was used as the test organism. The identification number 5230 used by Schmidt was retained. Cultures were grown on slants of nutrient agar containing 1 ppm manganese; the slants were incubated at 99° F (37° C) for 5 to 7 days before harvesting the spores. The washed and filtered spores were suspended in M/15 phosphate buffer (pH of 7.0) and put in sterile 8-oz bottles with glass beads. The spores were counted by plating on Dextrose-Tryptone-Starch Agar after a 15 minute heat shock at 212° F (100° C). The inoculating suspension was prepared by diluting the original spore suspension with M/15 phosphate buffer to give approximately 10^6 spores per ml (actual count 1.1 x 10^6 spores per ml). Cleaned tin plate TDT cups were placed in a glass petri dish, the cups and dish sterilized in hot air, after which 0.01 ml of the inoculating suspension was placed in each cup, using a micropipette. Samples were dried to remove the free water. Initially a vacuum oven operated at a temperature of 95° F (35° C) was used; however, the petri dishes containing the loaded cups were placed in a desiccator with a drying agent in later studies. The samples remained in the desiccator, which was stored in a refrigerator at 40.0° F (4.4° C) until needed.

[c] Obtained from C. F. Schmidt, Continental Can Co., Inc., Chicago.

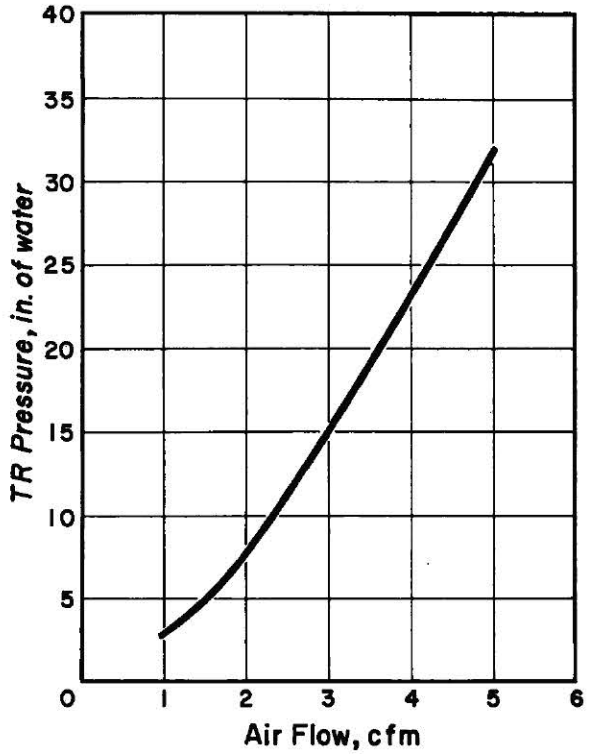

Figure 5. Relationship of exposure chamber pressure and gas flow rate.

Dextrose-Tryptone-Starch broth with 0.04% BCP indicator was used as the subculture medium. Positive tubes were those containing acid as evidenced by a color change and the characteristic pellicle. The subculture tubes were incubated at 99° F (37° C) for 4 weeks.

Only 4 cups per test were used in the several preliminary dry-heat studies (including the one reported below); however, 8 cups (2 runs of 4 cups each) are being used in studies now in progress.

Data were evaluated using the probability method of Schmidt *(8)*; both LD50 and D values were calculated. (LD50 is defined as the time in minutes when 50% of the samples

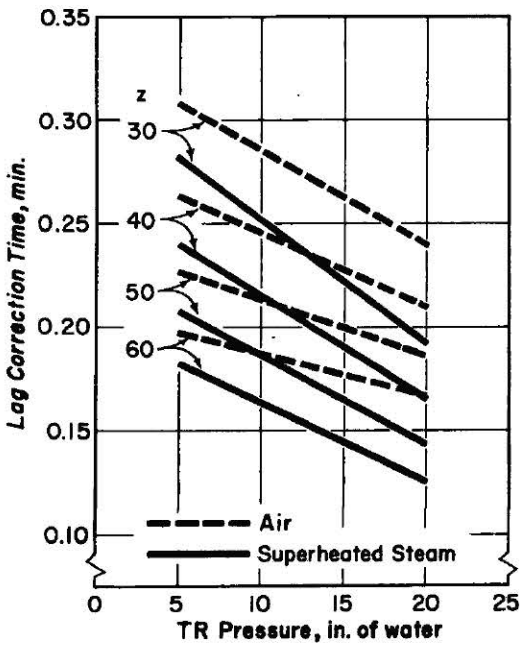

Figure 6. Lag correction time as a function of the exposure chamber pressure.

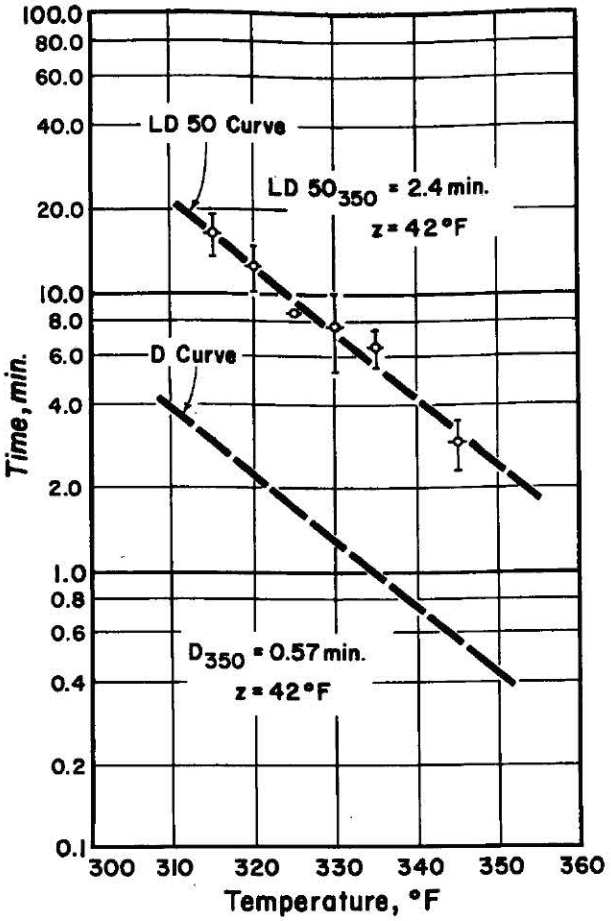

Figure 7. Thermal resistance curve for 5230 in superheated steam.

tested would be negative; D value is defined as the time in minutes for a 90% reduction in the number of organisms at a given temperature.) The 95% confidence interval was calculated where sufficient data were available. Use of LD50 values [suggested by Schmidt *(8)*] is appropriate for these data since it is desirable to obtain an accurate comparison of the values at the different temperatures without having to resort to D values.

Wet-heat thermal resistance studies. Wet-heat (saturated steam) thermal resistance studies were made over the temperature range of 241 to 272° F (116 to 133° C) with the same spore suspension and recovery medium as used in the dry heat studies. Studies made by Anderson *(1)* indicated that reliable wet-heat results can be obtained with dried spores. A total of 10 cups was exposed at each time-temperature interval (2 runs of 5 cups each) using the apparatus described above.

Data were analyzed using the probability method described by Schmidt *(8)* and the results were expressed as D values.

RESULTS AND DISCUSSION

Results of thermal resistance tests of 5230 in superheated steam as LD50 points, their 95% confidence limits, and the corresponding D values are presented in Figure 7. A straight line was drawn to represent these data since it appears that the logarithm of time varies linearly with temperature. The LD50 line has a z of 42° F and a $LD50_{350}$ of 2.4 min; the D_{350} is 0.57 min. The 95% confidence limits of the LD50 points permit z values from 36° to 48° F. Dotted lines are used in Figure 7 to indicate (a) that there are not sufficient data available at this time to substantiate that the relationship is linear and (b) that justification in the form of death rate data to calculate meaningful D values is not possible at present. The D curve is illustrated to show the range of values that can be expected and for comparison with the wet-heat data in Figure 8.

Results of thermal resistance tests of 5230 in wet heat are presented in Figure 8. The D curve has a z of 14.8° F and a D_{250} of 0.35 min.

Variation in LD50 values and the wide confidence interval appear to be normal for dry heat in contrast to the behavior of wet-heat data illustrated in Figure 8. It is acknowledged that use of 8 or 12 cups per test is more desirable and could reduce the confidence interval.

The operation of the equipment, in use since December 1957, has been satisfactory. Dry heat is more difficult to use than wet-heat due to the more complicated temperature control and the necessity of allowing the equipment to reach steady state conditions before testing can begin.

SUMMARY

An apparatus is described that makes possible the study of the dry-heat resistance of microorganisms under controlled conditions of time and temperature in the range 300 to 380° F (149 to 193° C). The accuracy of the temperature control system is ± 1.0° F and time ± 0.1 minutes. The lag correction factor of the sample cups has been evaluated as a

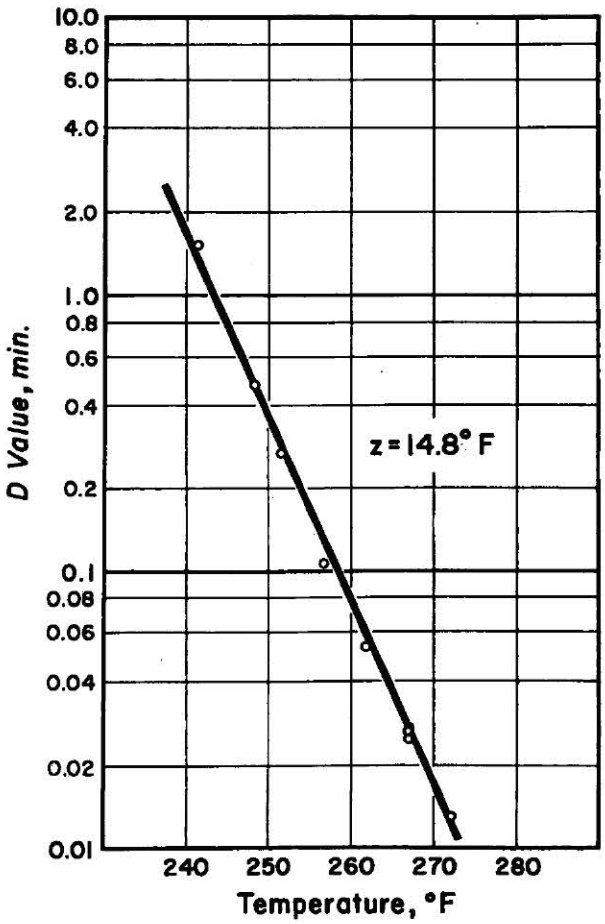

Figure 8. Thermal resistance curve for 5230 in saturated steam.

function of gas flow for both air and superheated steam. The dry-heat resistance of spores of organism 5230 (similar to *B. subtilis*) to superheated steam has been determined and it was found to have an $LD50_{350}$ of 2.4 min and a z of 42° F.

Acknowledgment

The author wishes to acknowledge and to extend his thanks to Dr. C. F. Schmidt and J. H. Bock of the Metal Division, Research and Development, Continental Can Co. Inc. for their inspiration, assistance, and tolerance over the several years that we have worked together in this effort; to Mr. L. T. Deason of the Adams Tool Company, Lansing, who made valuable suggestions in the design of the machine; and also to Dr. R. N. Costilow, Mr. T. E. Anderson, Mr. R. H. Wilkinson and Mr. Allen Stewart of Michigan State University, who have helped on this project.

The financial assistance of the Continental Can Co., Inc. in making this project a reality is gratefully acknowledged.

LITERATURE CITED

1. Anderson, T. E. Some factors affecting the thermal resistance values of bacterial spores as determined with a thermoresistometer. (Thesis for the M.S. degree, Michigan State University, 1959.)
2. Ball, C. O., and Olson, F. C. W. *Sterilization in Food Technology.* 1957. McGraw-Hill Book Company, New York, N. Y.
3. Collier, C. P., and Townsend, C. T. The resistance of bacterial spores to superheated steam. *Food Technol.*, 10, 477–481 (1956).
4. Hicks, E. W. A revised table of the P_s function of Ball and Olson. *Food Research*, 23, 396–400 (1958).
5. Martin, W. M. U. S. Patent 2,549,216, April 17, 1951.
6. Perkins, J. J. Principles and methods of sterilization. *Manual of Sterilization.* Volume 12, 1953. American Sterilizer Company, Erie, Pa.
7. Pflug, I. J., and Esselen, W. B. Development and application of an apparatus for study of thermal resistance of bacterial spores and thiamine at temperatures above 250° F. *Food Technol.*, 7, 237–241 (1953).
8. Schmidt, C. F. Thermal resistance of microorganisms. Chapter 32 in Reddish, C. F. (Ed.), *Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization.* 2nd ed. 1957. Lea and Febiger, Philadelphia, Pa.
9. Stumbo, C. R. A technique for studying resistance of bacterial spores to temperatures in the higher range. *Food Technol.*, 2, 228–240 (1948).
10. Tanner, F. W., and Dack, G. M. *Clostridium botulinum. J. Infectious Diseases*, 31, 92–100 (1922).
11. Walter, C. W. Aseptic technique. *The Cyclopedia of Medicine, Surgery and Specialties.* 1954. F. W. Davis Company, Philadelphia, Pa. Volume 13, 383–409.