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PERFORMANCE OF BACTERIAL SPORES IN A CARRIER SYSTEM IN MEASURING THE F_0-VALUE DELIVERED TO CANS OF FOOD HEATED IN A STERITORT

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ABSTRACT

Plastic rod biological indicator units (BIUs), filled with a suspension of *Bacillus stearothermophilus* spores and calibrated at 115.0, 121.1, and 127.0°C, were placed in 303 × 406 cans of corn and heated in a Steritort at 115.6, 121.1, and 127.8°C. The BIUs were assayed for surviving spores. Biological F-values were determined from the calibration curves and F_0 (BIO)-values calculated using appropriate z-value corrections. The F_0 (BIO)-values for the same number of survivors per BIU but using calibration charts at different temperatures were compared and found to be in general agreement. F_0 (PHY)-values were calculated using time-temperature data from thermocouple-equipped cans. The F_0 (BIO)- and F_0 (PHY)-values were compared. The results of this study indicate that BIUs can be used effectively to measure F_0-values delivered to cans of food heated in continuous and/or agitating processing machines.

INTRODUCTION

THIS IS THE REPORT of a series of experiments carried out to evaluate the performance of plastic rod biological indicator units (BIUs). These BIUs were used to measure the sterilization process delivered to cans of food processed in a Steritort. The Steritort is a process simulator for the FMC Sterilmatic food sterilization machine. The Sterilmatic is a commercial machine where the cans move through this apparatus continuously and undergo intermittent agitation. In the Sterilmatic machine, it is impossible to directly measure temperatures using thermocouples.

The use of bacterial spores to monitor sterilization processes is not new. Bacterial spores have been used to monitor sterilization processes in the pharmaceutical industry throughout most of this century. The use of bacterial spores in the testing of sterilizers has been described by Kelsey (1961). Brewer et al. (1956) described the use of bacterial spores in the control of sterilization processes. A review of the use of bacterial spores for monitoring sterilization processes in the hospital and in the pharmaceutical industry is presented by Perkins (1969).

Bacterial spores can be used in either a survivor curve or endpoint mode. When bacterial spores are used in a survivor curve mode, numbers of surviving organisms are determined, and this number is related through some type of spore calibration curve to yield a sterilization value. "Count reduction procedure" is the common name for the use of bacterial spores in a survivor curve mode. When bacterial spores are used in an endpoint mode, also called fraction-negative or quantal response, several replicate units are subjected to each test condition. After incubation, each unit is scored as either showing growth or no growth. The number of survivors is calculated from the quantal response. Bacterial spore strips used to evaluate hospital autoclaves and in the pharmaceutical industry to validate sterilization processes for steam and ethylene oxide are examples of the use of biological indicators in an endpoint mode.

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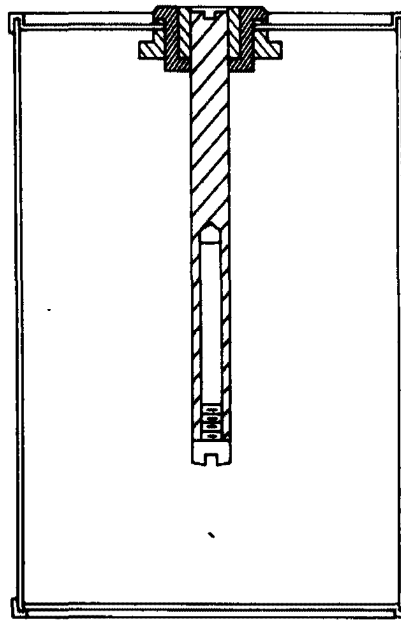

Fig. 1—Cross-section of a can containing a BIU.

Heat-resistant bacterial spores are used in the food industry in making inoculated packs (NCA, 1968). The inoculated pack is an endpoint method and has been used primarily to validate a calculated food sterilization process.

The late Dr. Stanley Yawger was responsible for much of the development and use of the count reduction procedure in the canning industry. Yawger (1967) describes the count reduction method for measuring the sterilization value delivered to cans of food when the spores are inoculated directly into the product. The count reduction procedure was widely used by Dr. Yawger in the validation of sterilization processes for foods sterilized in the FMC Sterilmatic processing machines.

The count reduction procedure was used in these studies; however, instead of the spores being deposited directly in the product they were contained in plastic rods, as described by Pflug (1976). Since the spores do not interact with the product, the calibration of the BIUs is not product specific.

The objective of these experiments was to gather data that could be used to: (1) evaluate our ability to correct F-value data gathered using a biological measuring system with a z-value of, for example, 7.8°C to an F-value for a z-value of 10°C; (2) determine if biological measuring units should be calibrated at a temperature within one or two degrees of the test temperature; and (3) compare F-values obtained using physical and biological measuring systems. (For definitions of symbols, refer to the Appendix.)

MATERIALS & METHOD

Experimental plan

Two series of experiments were carried out at the Green Giant

Company pilot plant facility in Le Sueur, Minn., on two separate dates: Aug. 11, 1977, and Jan. 16, 1978. Each experiment consisted of six individual heating tests. Three heating medium temperatures were used: 115.6, 121.1, and 127.8°C. Duplicate tests were carried out at each temperature. In each heating test, four containers were fitted with thermocouples and ten containers were fitted with BIUs.

In addition to the field tests, spore destruction tests were carried out in the laboratory at 115.0, 121.1, and 127.0°C to obtain calibration data for use in determining F(BIO)-values.

Spores

A suspension of *Bacillus stearothermophilus* spores was used to fill the plastic rod units. The spores were grown in March, 1977, from *Bacillus stearothermophilus*, ATCC 7953, using nutrient agar supplemented with 5.0 ppm $MnSO_4$. The spores were cleaned using insonation to free the spores from vegetative debris and repeated washings with water for injection (USP). After cleaning, the spores were suspended in 50X Butterfield's phosphate buffer (0.015M) made up with water for injection (USP). They were stored at 4°C.

Biological indicator units

The plastic rods used in this study were similar to those described by Pflug (1976). The BIUs were prepared in May, 1977, using the previously described *Bacillus stearothermophilus* spores suspended in 50X Butterfield's buffer. Each rod was filled with about 0.28 ml of spore suspension (7×10^6 spores); the prepared BIUs were coded Lot 7.

Calibration of BIUs

The calibration of the Lot 7 BIUs was carried out in a miniature retort at temperatures of 115.0, 121.1, and 127.0°C. Two tests at each temperature were carried out, one close to the Aug. 11, 1977 field studies and the other close to the Jan. 16, 1978 field studies.

Five randomly-selected rods were heated at each time interval. To determine the initial number of spores per rod, five randomly-selected rods were heated for 15 min in a boiling water bath. After heating, all BIUs were cooled in an ice water bath and held in ice water until recovery procedures were started. Summary information including D(T) values, length of the 95% confidence interval (CI) expressed as a percent of the D(T), intercept ratio (IR) and heating times for the calibration tests is shown in Table 1. The number of surviving spores per BIU was determined using plate count procedures. The recovery medium for all calibration and field tests was Difco Tryptic Soy Agar, Lot 636483. Incubation was at 55°C for 48 hr. A calibration graph and table of the number of surviving spores per BIU as a function of the sterilizing value at the calibration temperature were prepared at each calibration temperature.

Installing BIUs in metal containers

To install a BIU in a can, a hole was punched in the end of the 303 × 406 cans. An Ecklund receptacle was then installed in the end of the container. Immediately before filling the cans, the plastic rod BIU was screwed into place, as shown in Figure 1.

The BIUs were 75 mm long and inserted along the central axis of the container with the calibrated spores located along the center line as the containers rolled, and therefore, were at the slowest heating zone in the container.

Field test procedures

The plastic rod BIUs were carried to the plant in ice water in insulated containers. They remained in the ice water until they were placed in the cans.

An FMC Steritort was used in all experiments. The operating temperatures used were 127.8, 121.1, and 115.6°C. Two replicate tests were carried out at each temperature for both Series I and Series II. In all tests the reel speed was 10.6 rpm.

Empty 303 × 406 cans were fitted with either Lot 7 BIUs or Ecklund needle-type thermocouples. The cans were then filled with whole kernel corn, brine was added, the cans were sealed, and then loaded into the Steritort and heated. Fresh corn was used for Series I and thawed frozen corn for Series II. In each experiment, there were four cans containing thermocouples and ten cans containing BIUs.

A summary of the test temperature and heating time conditions for Series I and II experiments is shown in Table 2. The heating times at each heating medium temperature were selected so that in Series I experiments the number of survivors per BIU would be near the end of the calibration curve and in Series II near the beginning of the calibration curve.

Spore recovery procedures

After the heating and cooling process was completed, the cans containing the BIUs were recovered, opened, and the rods removed. Using a Vortex mixer, the rods were agitated for 15 sec, opened, and the spore suspension was removed using 0.25 ml glass tuberculin syringes. In Series I, a single, undiluted 0.05 ml aliquot of the spore suspension was placed in a 100 mm diameter plastic petri plate using the glass syringe. Again using the 0.25 ml syringe, 0.1 ml of the spore suspension was deposited in a 25 × 150 mm test tube containing 9.9 ml of a phosphate buffer. The dilution blank was shaken, and duplicate 0.2 ml and 0.01 ml aliquots were plated in 100 mm diameter petri plates. In Series IIA and IIB, 0.1 ml of the sample was placed in a dilution bottle containing 100 ml phosphate buffer. The dilution blank was shaken and duplicate 0.1 and 1.0 ml samples were plated. In Series II, C through F, the same dilution was made as in A and B; however, duplicate 0.01 and 0.1 ml samples were plated. About 30 ml of Tryptic Soy agar was added to each plate. The plates were incubated at 55°C for 48 hr and the colony-forming units counted.

Analysis of data

The thermocouple data were recorded by a Honeywell strip chart temperature recording potentiometer. The data were manually taken off the strip chart and tabulated. The F_o-values were determined by the General Method (Bigelow et al., 1920); the several F_o-values for each test were averaged and the mean reported as the F_o (PHY)-value.

The F(BIO)-values were calculated from the plate count data. The number of colony-forming units per plate was multiplied by the dilution factor to obtain the number of surviving spores per BIU. Since duplicate aliquots were plated for each BIU, there were two estimates of the number of surviving spores per BIU. The geometric mean of the numbers of survivors of the BIUs of each test was determined, and using this mean value, the appropriate F(BIO)-value

Table 1—Temperatures, D- and IR values, and heating times at each test condition for the calibration experiments

Temp °C	Date of test	D(T) min	Length of 95% CI as a % of the D(T)	IR[a]	Heating times
115.0	Sept.	40	31	1.10	20, 50, 80, 110, 140
	Dec.	38	28	1.09	10, 20, 50, 80, 110, 140, 170
121.1	July	5.4	21	1.14	4, 8, 12, 16, 20, 24, 28
	Dec.	6.1	26	1.07	2, 4, 12, 20, 28, 30
127.0	Sept.	0.72	8	1.23	2, 2.5, 3, 3.5, 4
	Dec.	0.64	7	1.26	2, 2.5, 3, 3.5, 4

[a] $IR = \log Y_o / \log N_o$

Table 2—Temperatures and heating times for the two series of experiments

Test no.	Test temp (°C)	Heating time[a] (min)
Series I Experiments		
A	127.8	8.0
B	127.8	8.0
C	121.1	26.5
D	121.1	26.5
E	115.6	112.0
F	115.6	112.0
Series II Experiments		
A	127.8	6.0
B	127.8	6.0
C	121.1	17.0
D	121.1	17.0
E	115.6	74.0
F	115.6	74.0

[a] Time from steam-on to steam-off

was determined from the appropriate calibration chart. For each test, the standard deviation and coefficient of variation were calculated based on the logarithm of the numbers of survivors for each of the BIUs.

Calibration charts at 115.0 and 121.1°C were used with the data from tests carried out at 115.6°C, and calibration charts at 121.1 and 127.0°C were used with the data from tests carried out at 127.8°C. Calibration charts at 115.0, 121.1, and 127.0°C were used with the data from the tests at 121.1°C.

To obtain F_0(BIO)-values it was necessary to correct the F(BIO)-values for the difference in the z-value of the spores of 7.8°C (14°F) and a z-value of 10°C (18°F). For tests carried out at 115.6 and 127.8°C, the three-step conversion described by Pflug and Christensen (1980) was used to obtain the F_0(BIO)-value. When calibration data at 121.1°C was used to evaluate data at 121.1°C, only the Y_2 correction was made.

The three-step conversion, A to B, B to C, and C to D, is shown below:

$$\overset{(A)}{F(T_c, z)} \times Y_1 = \overset{(B)}{F(T_1, z)}$$

$$\overset{(B)}{F(T_1, z)} \times Y_2 = \overset{(C)}{F(T_1, 10°C)}$$

$$\overset{(C)}{F(T_1, 10°C)} \times Y_3 = \overset{(D)}{F(T_{ref}, 10°C)}$$

The factor, Y_1, to convert from A to B, was calculated using the equation:

$$Y_1 = 10^{(T_c - T_1)/z}.$$

The factor, Y_3, to convert from C to D, was calculated using the equation:

$$Y_3 = 10^{(T_1 - T_{ref})/10°C}.$$

The factor, Y_2, to convert from B to C, is the ratio of the sterilization value calculated using the second z-value (in this analysis, 10°C, 18°F) to the sterilizing value calculated using the first z-value (in this analysis, 7.8°C, 14°F) for the specific f_h- and j_c-values of the canned food product. It is calculated at the heating medium temperature (T_1).

$$Y_2 = \frac{F(T_1, z_2)}{F(T_1, z_1)} = \frac{F(T_1, 10°C)}{F(T_1, 7.8°C)} = \frac{F(T_1, 18°F)}{F(T_1, 14°F)}$$

Data were tabulated by Pflug and Christensen (1980) for a range of f_h-values for a j_c of 1.40.

If z_2 is larger than z_1, then $F(T_1, z_2)$ will be larger than $F(T_1, z_1)$; the ΔF [$\Delta F = F(T_1, z_2) - F(T_1, z_1)$] is a function of $F(T_1, z_1)$ and while ΔF increases as the $F(T_1, z_1)$ increases, the rate of increase approaches zero when $F(T_1, z_1)$ is very large.

In the correction calculation sequence $A \times Y_1 = B$, $B \times Y_2 = C$, and $C \times Y_3 = D$, we will use the following alternative method for the second calculation: $B + \Delta F = C$. Brine-packed whole kernel corn in 303 × 406 cans heated in a Steritort has an f_h-value of the order of 5.0 min; this value will be used in making the Y_2 correction. The correction values were taken from the table (in Pflug and Christensen, 1980) for the appropriate change in z from 14°F to 18°F.

RESULTS

THE RESULTS from the BIU tests in 303 × 406 cans of whole kernel corn in brine heated in a Steritort are summarized in Table 3. The raw data from the BIUs in each test were combined; the geometric mean, the standard deviation, and coefficient of variation were determined and are shown in Table 3. The result of this analysis was used with the BIU calibration data to determine the F(BIO)-value; these data are also shown in Table 3. The mean F_0(PHY)-values calculated from heat penetration data gathered during each experiment are also shown in Table 3. The conversions of the F(BIO)-values to F_0(BIO)-values are shown in Table 4. The data are presented in Table 4 in a form that makes it possible to compare the F_0(BIO)-values obtained using different calibration charts and to observe the effect of converting the data from the z-value of the spores in the BIUs to a z-value of 10°C.

The data in Table 4 make possible a comparison of the biologically determined F_0-values and the physically determined F_0-values at the three different test temperatures. The average deviations are also shown in Table 4, both as F_0-value differences and as a ratio of the F_0-value difference to the F_0(PHY)-value.

DISCUSSION

IN THIS STUDY, data were generated to provide information on three aspects of the use of biological monitoring devices: (1) the need to calculate sterilizing values on a common basis, that of a z-value of 10°C (18°F), and to

Table 3—Geometric mean, standard deviation, and coefficient of variation of the raw test data and the F(BIO)-value determined from the BIU calibration curve and the F_0(PHY)-value for each test

	Geometric mean of the no. of organisms surviving per BIU	Std dev	Coefficient of variation	$F(T_c, 7.8°C)$ T_c close to T_1, (min)	F(121.1°C, 7.8°C)	F_0(PHY)
Tests carried out at 115.6°C						
t_h = 112 min						
IE	3.5811	1.06	0.297	144	24.4	32.8
IF	3.3406	0.53	0.159	148	25.2	33.7
t_h = 74 min						
IIE	5.8142	0.20	0.034	87	13.6	19.2
IIF	5.8389	0.19	0.033	87	13.4	19.1
Tests carried out at 121.1°C						
t_h = 26.5 min						
IC	1.5102	0.60	0.394	31[a]	31[a]	28.9
ID	1.3972	0.43	0.307	31[a]	31[a]	28.2
t_h = 17 min						
IIC	5.4326	0.13	0.023	16.0	16.0	13.1
IID	5.4065	0.19	0.036	16.2	16.2	14.4
Tests carried out at 127.8°C						
t_h = 8 min						
IA	.8087	0.35	0.429	5.1[a]	32[a]	18.6
IB	.9484	0.53	0.560	4.9[a]	32[a]	23.9
t_h = 6 min						
IIA	6.0409	0.42	0.070	1.2	11.8	6.4
IIB	6.1308	0.26	0.042	1.1	11.0	6.1

[a] Values estimated from extrapolated survivor curve data.

evaluate the ability to convert the results obtained using a biological indicator with a z-value of, for example, 7.8°C, to a sterilizing value based on a z-value of 10°C; (2) obtain data to determine if biological indicator units should be calibrated at a temperature that is within one or two degrees of the test temperature or if the BIUs can be calibrated at a convenient reference temperature; and (3) obtain data to determine if the F_o(BIO)- and F_o(PHY)-values are comparable.

In this study we have used a calibration chart to relate numbers of surviving spores with sterilization values. We believe that this gives more accurate results than using the equation F(BIO) = D(log N_o − log N) because the semilogarithmic survivor curve is not always a straight line. In this study, the spores in the BIUs yielded nearly a straight line semilogarithmic survivor curve at 127.0°C but produced curves at 115.0 and 121.1°C. In Table 1 are shown the D- and IR values for the spores in the BIUs; at the same test temperature, the survivor curves were similar in shape and there was good agreement in the D-values. The effect of the curvature of the semilogarithmic survivor curve appears as an increase in the confidence interval (CI) of the D-value. In Table 1, the CI is expressed as a percentage of the D-value. Because we are using linear regression (log N vs U) to determine the slope and then the D-value (D = −cotan θ), the CI increases as the data deviate from a straight line. Therefore, the large CI values in Table 1 are not indicative of poor survivor curve data but rather are large because of the deviation of the data from linearity. When there is deviation of the data (log N vs U) from linearity, the use of the curve formed by the data to calculate the F(BIO)-value will give a more accurate result than using the D-value calcu-

Table 4—F(BIO)-value data, conversion of the F(BIO) to F_o(BIO)-values, F_o(PHY)-values, and differences in the F_o(BIO) and F_o(PHY)-values

Test conditions	F(BIO) (min)	Y_1	F(T_1, 7.8°C) (min)	ΔF	F(T_1, 10°C) (min)	Y_3	F_o(BIO) (min)	F_o(PHY) (min)	F_o(BIO) minus F_o(PHY)	$\frac{F_o(BIO) - F_o(PHY)}{F_o(PHY)}$
Tests carried out at 115.6°C										
t_h = 74 min										
Calibration graph at 115.0°C										
IIE	87	0.838	72.9	0.7	73.6	0.282	21	19.2	1.8	0.094
IIF	87	0.838	72.9	0.7	73.6	0.282	21	19.1	1.9	0.099
Calibration graph at 121.1°C										
IIE	13.6	5.07	69.0	0.7	69.7	0.282	19.7	19.2	0.5	0.026
IIF	13.4	5.07	67.9	0.7	68.6	0.282	19.4	19.1	0.3	0.016
t_h = 112 min										
Calibration graph at 115.0°C										
IE	144	0.838	121	1.00	122	0.282	34.4	32.8	1.6	0.049
IF	148	0.838	124	1.00	125	0.282	35.2	33.7	1.5	0.044
Calibration graph at 121.1°C										
IE	24.4	5.07	124	1.00	125	0.282	35.2	32.8	2.4	0.073
IF	25.2	5.07	128	1.00	129	0.282	36.4	33.7	2.7	0.080
Tests carried out at 121°C										
t_h = 17 min										
Calibration graph at 115.0°C										
IIC	98	0.165	16.2	0.7	16.9	1.00	16.9	13.1	3.8	0.290
IID	99	0.165	16.3	0.7	17.0	1.00	17.0	14.4	2.6	0.180
Calibration graph at 121.1°C										
IIC	16.0	1.00	16.0	0.7	16.7	1.00	16.7	13.1	3.6	0.275
IID	16.2	1.00	16.2	0.7	16.9	1.00	16.9	14.4	2.5	0.174
Calibration graph at 127.0°C										
IIC	2.9	5.71	16.6	0.7	17.3	1.00	17.3	13.1	4.2	0.321
IID	2.9	5.71	16.6	0.7	17.3	1.00	17.3	14.4	2.9	0.201
t_h = 26.5 min										
Calibration graph at 115.0°C										
IC	186	0.165	30.7	0.7	31.4	1.00	31.4	28.9	2.5	0.086
ID	188	0.165	31.0	0.7	31.7	1.00	31.7	28.2	3.5	0.124
Calibration graph at 121.1°C										
IC	31[a]	1.00	31	0.7	31.7	1.00	31.7	28.9	2.8	0.097
ID	31[a]	1.00	31	0.7	31.7	1.00	31.7	28.2	3.5	0.124
Calibration graph at 127.0°C										
IC	4.7[a]	5.71	26.8	0.7	27.5	1.00	27.5	28.9	−1.4	−0.048
ID	4.8[a]	5.71	27.4	0.7	28.1	1.00	28.1	28.2	−0.1	−0.004
Tests carried out at 127.8°C										
t_h = 6 min										
Calibration graph at 121.1°C										
IIA	11.8	0.138	1.63	0.39	2.02	4.68	9.45	6.4	3.05	0.476
IIB	11.0	0.138	1.52	0.38	1.90	4.68	8.89	6.1	2.79	0.457
Calibration graph at 127.0°C										
IIA	1.7	0.790	1.34	0.37	1.71	4.68	8.00	6.4	1.60	0.250
IIB	1.6	0.790	1.26	0.37	1.63	4.68	7.63	6.1	1.53	0.251
t_h = 8 min										
Calibration graph at 121.1°C										
IA	32[a]	0.138	4.43	0.45	4.88	4.68	22.8	18.6	4.2	0.226
IB	32[a]	0.138	4.43	0.45	4.88	4.68	22.8	23.9	−1.1	−0.046
Calibration graph at 127.0°C										
IA	5.1[a]	0.790	4.03	0.44	4.47	4.68	20.9	18.6	2.3	0.124
IB	4.9[a]	0.790	3.87	0.44	4.31	4.68	20.2	23.9	−3.7	−0.155

[a] Estimated from survivor curve extended beyond data points.

lated from the straight line regression analysis of log N vs U.

In previous studies, Pflug and Smith (1977) found that the z-value of *Bacillus stearothermophilus* spores decreases from about 10°C in the temperature range 100–110°C to about 7.8°C in the range 115–125°C. We conclude from these data that if the z-value of the biological indicator organism is substantially different from 10°C, then the F(BIO)-values must be converted from the z-value of the biological indicator organism to a z-value of 10°C if the biological and physical F-values are to be comparable.

The reported F_o(BIO)-values have not been corrected for heating and cooling lags of the BIUs. Studies have been carried out to evaluate the heating and cooling lag correction factors that are associated with the use of plastic rod BIUs. When the f_h- and f_c-values of the product in the container are small, of the order of 2 to 10 min, the correction to be applied to the sterilization time at heating medium temperature (U) is small, between 0 and –0.5 min. The explanation for the observation that spores in the BIU receive a greater U-value than spores in the surrounding product is that during heating, the temperature of the spores in the BIU cavity tend to follow closely the temperature of the product on the outside of the BIU. The temperature difference across the BIU wall decreases with increasing f_h-value. At the start of cooling, the temperature of the spore suspension inside the rod cavity lags behind the temperature of the product surrounding the BIU. The effect appears to be a difference in the transition temperature change characteristics of the product in the container and the spore suspension in the plastic rod cavity. Measurements of this lag in the start of cooling have yielded U-values that are as much as 0.5 min larger inside the BIU cavity than in the product immediately outside the BIU.

In Table 4 are shown F_o(BIO) data that can be used to compare the effect of using calibration graphs at different temperatures on the resulting F_o(BIO)-value. The comparison of the F_o(BIO)-values for the two replicate tests at each temperature are, in general, extremely good. The variation, when calibration graphs of different temperatures are used expressed as a percentage of the mean for any one test condition, was less than 15% for all comparisons, except IIA and IIB where differences between the F_o(BIO) for a 121.1° calibration chart were about 20% greater than when a 127.0°C chart was used. For both of the tests carried out at 127.8°C, the 127.0°C calibration chart gave lower F_o (BIO)-values. In all other comparisons, there did not appear to be any consistent trend.

The good agreement between F_o(BIO)-values calculated using calibration graphs at different temperatures indicates that the calibration graphs need not be at the test temperature as long as the z-value of the spores of the BIU units is known, and the data are corrected if the z-value is not 10°C (18°F).

Comparing the F_o(PHY)-values with the F(BIO)-values for data at a reference temperature of 121.1°C and the BIU z-value of 7.8°C shown in Table 3, we observe that the noncorrected F(BIO)-values differ both in magnitude and direction from the F_o(PHY)-values. When the data are corrected (data shown in Table 4), there is general agreement (very good at 115.6°C, good at 121.1°C, and fair at 127.8°C) between the F_o(PHY) and F_o(BIO) data; where there are differences, the F(BIO)-value consistently exceeds the F_o(PHY)-values. For the very short heating times at 127.8°C, the negative lag correction factor (U-value correction of 0 to –0.5 min) of the plastic rod units is an explanation for some of this difference.

The maximum lag correction value that we have observed is –0.5 min. This error is, in general, inconsequential at low temperatures where heating times are long. However, at high temperatures the error can be quite large. For example, a sterilization value of 5.0 min measured by a BIU at 127.8°C with the correction will be reduced to 4.5 min. When the sterilization value at test temperature decreases, we anticipate that the correction will also decrease and that when the sterilization value at test temperature is near 1 min, as it was in Test IIA and B, the correction will no longer be –0.5 min but will be much smaller.

The difference between the F_o(BIO)- and F_o(PHY)-values increases as heating medium temperature increases and the heating times become shorter. The potentiometer used in these experiments was calibrated by a commercial instrument company; Ecklund needle-type thermocouples were used. Differences between the F_o(BIO)- and F_o(PHY)-values expressed as a percentage of the F_o(PHY) were less than 10% at 115.6°C, averaged about 16% for tests carried out at 121.1°C, and were somewhat variable at 127.8°C. At 127.8°C, the average deviation was less than 15%, excluding the results of IIA and IIB, which average 36%.

There can be temperature measurement errors as well as biological system errors. In Tests IA and B, there is variation in the F(PHY)-values between the two replicates, A and B, but close agreement in the F_o(BIO)-values. This result suggests that the F_o(PHY) in either IA or B may have an error. The potentiometer used to gather the time-temperature data for the F(PHY)-values could have a calibration error. Also, there could be a thermocouple error. An error of ± 0.3°C will produce about a ± 6% variation in the U-value. If the heating medium temperature is 127.8°C, the error in F_o will be about ± 30%.

We believe that the results of this study indicate that BIUs can be used effectively to measure the F_o-value delivered to containers of food heated in continuous agitating sterilization machines at an accuracy level that is essentially on a par with the calculation of sterilization values from time-temperature data gathered in the field using thermocouples. The accuracy of any system is a function of the design of the system, the calibration of the system, and the reproducibility and expertise of those utilizing the system. The use of BIUs requires quantitative bacteriological procedures. Using quantitative microbial procedures and properly calibrated BIUs, we believe that F_o-values can be routinely measured to within ± 15% of the true F_o-value.

APPENDIX

D(T)

The D-value is an index of microbial destruction. It is the time required at a temperature, T, to reduce a specific microbial population by 90%. It is the negative reciprocal of the slope of the line fitted to the graph of the logarithm of the number of survivors versus time.

F(T, z), F_o, F_o(BIO), F_o(PHY)

The equivalent time at a specific temperature, T, delivered to a container or unit of product for the purpose of sterilization, calculated using a specific value of z. F_o indicates that the temperature was 250°F and the z-value was 18°F. F_o(BIO) indicates that the F_o-value was measured biologically; F_o(PHY) that it was determined from data measured physically.

f, f_h

The temperature response parameter, f, is the time required for the straight line fitted to the log-linear portion of a heating or cooling curve to traverse one log cycle; it is the time required for the temperature difference between product and heating or cooling medium to decrease by 90%; f_h identifies the heating parameter.

IR

The intercept ratio, IR, is a parameter of a semilogarithmic survivor curve when the regression line fitted to the

data does not pass through the N_o point. It is equal to the log of the zero time intercept of the regression line (Y_o) divided by the log of N_o ($IR = \log Y_o / \log N_o$).

j

Lag factor of the semilogarithmic heating curve for a specific location in a product in a container.

$$j = \frac{\text{(heating or cooling medium temp)} - \text{(Y-intercept temp)}}{\text{(heating or cooling medium temp)} - \text{(initial product temp)}}.$$

j_c

The cooling lag factor $[(j_c = (T_2 - T_{Ba}) / T_2 - T_B)]$.

$T, T_o, T_1, T_2, T_{Ba}, T_{ref}, T_c$

T is the variable product temperature. T_o is the initial product temperature. T_1 is the heating medium temperature. T_2 is the cooling medium temperature. T_B is the product temperature at steam off. T_{Ba} is the temperature intercept value at steam off of the line fitted to the log-linear portion of the cooling curve. T_{ref} is the reference temperature used in calculating sterilizing values. T_c is the BIU calibration temperature.

U

The equivalent sterilizing value using the heating medium temperature, T_1, as the reference temperature and with a specified z-value; $U = F(T_1, z)$.

z

Measure of the direction of the thermal death time curve, the number of degrees of temperature change necessary to cause the F-value to change by a factor of ten.

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