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Measuring Sterilizing Values In Containers of Food Using Thermocouples and Biological Indicator Units [1,2]

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ABSTRACT

Three series of experiments were carried out. Each experiment consisted of six or seven tests where four or five containers were fitted with thermocouples and five were fitted with biological indicator units (BIU). The sterilization value (F_0) delivered to cans of peas in brine was calculated from heat penetration data. The heat penetration data were analyzed for test-to-test reproducibility within each experimental series. Sterilization values for all tests were calculated from the BIU test results. The sterilizing values F_0 (PHY) determined from physical (PHY) heat penetration data were compared with sterilizing values F_0 (BIO) determined using the BIUs, both on the basis of accuracy and variability. The mean F_0 (PHY) - F_0 (BIO) was + 1.2 min. The mean coefficient of variation of the F_0 (PHY) was 0.03 and the F (BIO) was 0.06.

This is the report of a series of experiments carried out to evaluate the performance of thermocouples and plastic rod biological indicator units (BIU) when used to monitor the sterilization process delivered to cans of food heated in a Steritort, both in an agitating and still mode. The Steritort is a process simulator for the FMC Sterilmatic food sterilization machine.

Three series of experiments were carried out at the Green Giant pilot plant facility in Le Sueur, Minnesota. Each experiment consisted of six or seven individual heating tests where four or five containers were fitted with thermocouples and five containers were fitted with biological indicator units.

In addition to the field tests, laboratory tests were carried out to develop a calibration curve for use in the count reduction procedure. These were carried out at the University of Minnesota Environmental Sterilization Laboratory.

The objectives of these studies were to compare the F-value results obtained using thermocouples and biological indicator units when monitoring sterilization processes, and to determine if they are equally effective for agitating and still processes.

MATERIALS AND METHODS

Spores

Bacillus stearothermophilus spores were used. The spores were grown in May, 1975, from American Type Culture Strain 7953, using nutrient agar supplemented with 5 ppm of $MnSO_4$. Incubation was at 55 C for 48 h. The spores were cleaned and suspended in water for injection (USP) and stored at 4 C.

About 2 weeks before filling the rods, the spore suspension was centrifuged and resuspended in 50X standard strength Butterfield's buffer (*1*) in water for injection (USP).

Plastic rod units

The plastic rod biological indicator units (*3*) were prepared in February, 1976. Each rod contained about 0.28 ml of the spore suspension (0.7×10^7 spores). The prepared plastic rod BIUs were stored at 4 C until time of use.

Calibration experiments

The BIUs were calibrated at 121.1 C, using a miniature retort. In each calibration test three randomly-selected rods were heated for 3.8, 5.8, 7.8, 9.8, 11.8 and 13.8 equivalent minutes at 121.1 C. After heating, the rods were cooled in an ice water bath and held in ice water until recovery procedures were started. Three unheated units were analyzed to determine the initial number of spores per unit. The number of surviving spores per BIU was determined using plate count procedures. The recovery medium was soybean casein digest agar with incubation at 55 C for 48 h.

Field test procedures

The BIUs were transported to the plant in ice water in insulated containers. The BIUs were held in the ice water until they were placed in the cans.

To install a BIU or a thermocouple in a can, a hole was punched in the end of the 303 × 406 can. An Ecklund receptacle was then installed in the hole in the end of the container. Needle-type Ecklund thermocouples were installed in the thermocouple cans. Immediately before filling the cans, the plastic rod biological indicator units were screwed into place as shown in Fig. 1.

In experimental series 1 and 2, where cans were agitated during heating, two 15/16-inch-long BIUs were inserted along the center line of the container with the calibrated spores located near the geometric center, the slowest heating zone in the container. In series 3, the cans were not agitated during heating. Therefore, the spores were located near the bottom of the container, the slowest heating zone for a convection heating product. To accomplish this, 4-inch plastic rod units were used and the cans were heated with the receptacle up.

An FMC Steritort was used in all experiments; the heating medium temperature was 254 F. The reel speed was 7.2 rpm in all agitating tests. This reel speed is representative of the reel speed in FMC Sterilmatic processing machines for peas processed in commercial canning plants.

The cans were filled with 11.5 ounces of peas, brine was added until there was a 0.25-inch headspace, and the cans were sealed and then immediately heated.

Series 1. There were six experiments. In A, B, and E, the heating time was 9 min; in C, D and F, the heating time was 11 min (heating time is measured from steam on to steam off).

Series 2. There were seven experiments. In A, E and G, the heating time was 10 min. In C, D and F, the heating time was 12 min, and in B

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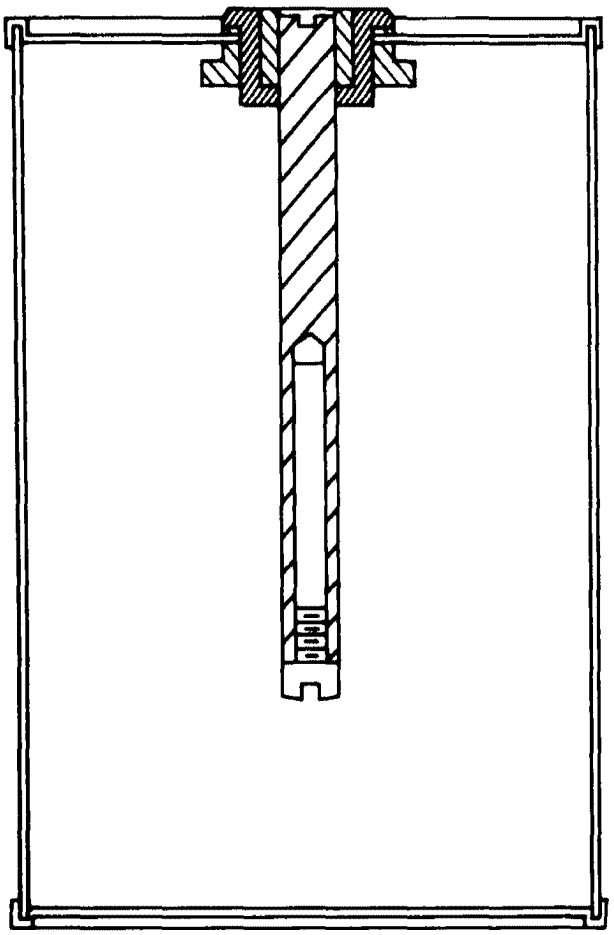

Figure 1. *Cross-section of a can containing a BIU located at the slowest heating zone.*

the heating time was 13.5 min.

Series 3. There were six experiments. The cans of peas were placed on a perforated metal shelf within the Steritort and processed under still conditions. In A, B and E, the heating time was 14 min, and in C, D and F, the heating time was 15 min.

A unique aspect of the experimental program was that within each series the location of can 2 in the Steritort, for example, the thermocouple in can 2 and the connecting harness to can 2, were the same in all six individual experiments. Thermocouple and harness placement were consistent within each series but varied between series.

Since the same thermocouple and measuring system was used, for example, for container 2 in 1A, 1B, 1C, 1D, 1E and 1F, the results can be subjected to an analysis of variance and other statistical tests to determine if the variation among containers, thermocouples and harnesses is random or whether there is bias, suggesting that particular thermocouples yield F_0-values that are greater or less than the average.

Spore recovery procedures

After the heating and cooling process was completed, the cans containing the BIUs were recovered, opened and the BIUs removed. Using a vortex mixer, the BIUs were agitated for 15 sec, opened and the spore suspension removed using a 1.00-ml glass tuberculin syringe. Duplicate 0.1-ml portions of the spore suspension were plated (using the glass syringe) in 100-mm diameter plastic petri plates. The remainder of the spore suspension was deposited as a drop in a sterile empty petri plate. Using an Eppendorf pipettor, duplicate .005-ml portions of the drop were placed in 100-mm diameter plastic petri plates. About 30 ml of soybean casein digest agar were added to each plate. The plates were incubated at 55 C for 48 h and the colonies counted.

Treatment of data

The thermocouple data were recorded on a strip chart by a temperature recording potentiometer. The data were taken off the strip chart, tabulated and then placed on a computer file. The data were analyzed using a computer program that calculated the temperature response parameter f, the lag factor j, and the length of the f-line, determined the correlation coefficient (r^2) of the fit of the f-line to the data and calculated the F_0 (PHY)-value by the General Method. F_0-values were also determined for all container heat penetration tests (CHPT) by two mathematical methods: (*a*) Ball program (*5*) and (*b*) when sufficient data were available, a program identified as HPSP that was developed in this laboratory. The F_0-values calculated by the Ball Method and by the HPSP program was compared with the F_0-values calculated by the General Method and reported as F_0-value ratios.

To prepare the calibration curve, the mean number of surviving spores per BIU as a function of the equivalent heating time at 121.1 C for each of the two calibration tests was entered into a time share computer program. The best fit second order polynominal was determined and the coefficients used to calculate the number of survivors for the range of sterilizing values over which the BIU was effective. The resulting calibration curve is shown in Figure 2; the survivor data, in the form of the mean and 95% confidence intervals for the two calibration tests, are also shown in Fig. 2.

The F(BIO)-value was calculated from the plate count data. The number of colonies per plate was multiplied by the appropriate dilution factor to obtain the number of surviving spores per BIU. Since duplicate portions were plated for each unit, there were two estimates of the number of surviving spores per BIU. F(BIO)-values were determined on the basis of the count per BIU from the calibration chart shown in Fig. 2. The F-value was obtained by averaging these two values.

The F(BIO)-value was corrected for the difference in the z-value of the spores, approximately 14 F, and the z-value of 18 F to yield an

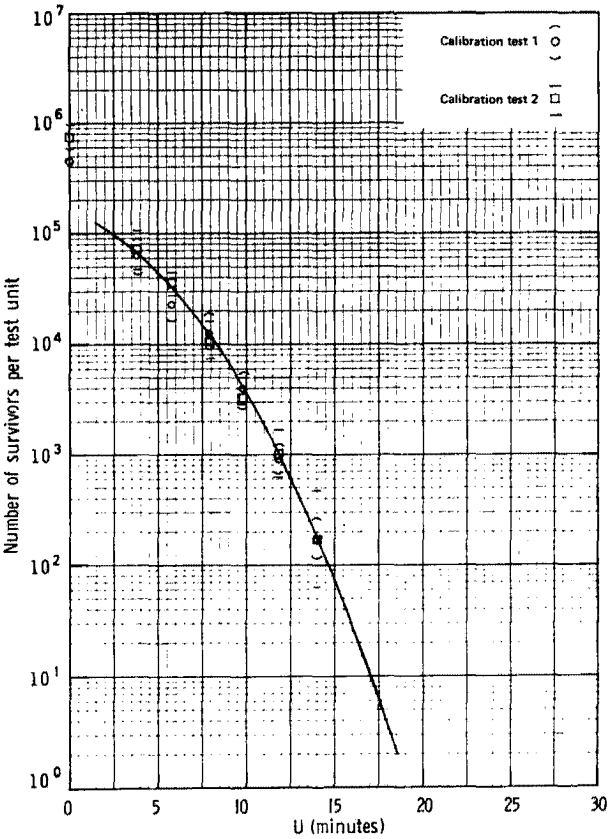

Figure 2. *Calibration curve at 121.1 C for the BIUs used in the three series of experiments; mean data values and their 95% confidence interval are shown.*

F_0(BIO)-value by the method described by Pflug (*4*). The mean F_0(BIO)-value for each experiment was obtained by averaging the F_0(BIO)-value for the five rods in each test.

RESULTS

The heat penetration test results for the three series of experiments are summarized in Table 1. Inspection of the data in Table 1 suggests that the three series of experiments were not identical. In the two series (1 and 2) where the cans were agitated, some environmental factors, probably related to the product, were present since the f_h-value for series 1 was 3.2 min with a coefficient of variation of about 0.03 in contrast to an f_h-value of 3.6 min for series 2 with a coefficient of variation of 0.04. In the non-agitated series 3, the mean f_h-value is 3.7 min and the coefficient of variation of 0.04 is almost identical with the result of the second series where the cans were agitated during heating.

The F_0-value ratios in Table 1 suggest that when the temperature response parameter and lag factor that are determined in the heat penetration data analysis are used to calculate F_0-values, the F_0-values calculated using the Ball program are about 91% of the General Method F_0-values. The HPSP program appears to give F_0-values that compare more closely (98%) to the General Method F_0-values.

In Tables 2, 3 and 4 are shown the General Method F_0-values as a function of container position. An analysis of variance test, Friedman's test (*2*), and an analysis of variance after a log transformation of the data for series 2 and for series 3 all fail to show any significant difference among container positions, thermocouple or thermocouple harness at the 90% level. This is interpreted as meaning that in these experiments there was no bias among position.

In series 1, Friedman's test of the data showed differences among positions that are significant at the 90% level but not at the 95% level. The analysis of variance tests of the data (log transformed and not transformed) showed a significant difference at the 95% level but not at the 99% level. Using the Newman-Kuels multiple comparison method, we found that only thermocouples 4 and 6 were significantly different. The results suggest that equipment performance is critical when making heat penetration tests in the Steritort and that a way should be found to validate the temperature measuring systems. In this type of equipment, where units are put on and taken off for each test and because of the agitation conditions, the thermocouple wiring system is subject to continued and deteriorating stress. The researcher must be alert to changes in the condition of either or both the thermocouple harness or the rotating thermocouple connections that can cause a

TABLE 2. *General method F_0-values calculated for experiment Series 1.*

Thermocouple No., harness and can position	Experiment Number (Heating time) 1A[a] (9 min)	1B (9 min)	1C (11 min)	1D (11 min)	1E (9 min)	1F (11 min)
2	6.2	6.2	9.5	9.8	6.2	10.0
3	6.2	5.8	9.9	9.3	5.9	10.1
4	6.3	6.0	9.2	9.2	6.0	9.8
5	6.1	6.2	9.3	9.5	6.1	10.5
6	6.6	6.2	9.5	9.6	6.9	10.9

[a]Experiment Code and heating time at 255 F.

TABLE 1. *Summary of f, j and F_0-Values for Experiments 1, 2, and 3.*

Experiment number	$\bar{f}_h$ (min)	$\bar{j}_c$	$\bar{f}_c$ (min)	$\bar{j}_c$	F_0-Values (min) Ball	HPSP	GM	F-Value ratios Ball/GM	HPSP/GM
1A	3.1	0.91	5.2	1.13	5.8	6.1	6.3	.92	.97
1B	3.3	0.89	5.4	1.12	5.7	6.2	6.1	.93	1.02
1C	3.3	0.67			8.8		9.5	.94	
1D	3.3	0.84			8.5		9.5	.89	
1E	3.3	0.85	6.3	1.12	5.5	6.1	6.2	.89	.98
1F	3.2	0.90			9.0		10.2	.90	
$\bar{x}$	3.2	0.84						.91	.99
Std. Dev.	0.083	0.089							
Coef. of Var.	0.026	0.106							
2A	3.7	0.91	6.2	1.11	6.3	6.9	6.8	.93	1.01
2B	3.4	0.92			12.9		13.9	.93	
2C	3.6	0.88			10.2		10.8	.95	
2D	3.7	0.88			9.5		10.0	.96	
2E	3.4	0.89	5.6	1.17	6.9	7.4	7.6	.91	.97
2F	3.4	0.87			10.1		10.8	.94	
2G	3.7	1.00	8.1	1.07	5.3	5.9	6.1	.90	1.00
$\bar{x}$	3.6	0.91						.93	.99
Std. Dev.	0.151	0.045							
Coef. of Var.	0.042	0.049							
3A	3.6	0.78			10.2		10.7	.94	
3B	4.0	0.80	3.6	1.36	9.2	9.0	9.5	.97	.95
3C	3.6	0.85			11.5		12.2	.94	
3D	3.7	0.85			11.0		12.1	.93	
3E	3.7	0.76	3.7	1.32	9.9	9.7	10.5	.94	.92
3F	3.8	0.74			11.1		11.9	.94	
$\bar{x}$	3.7	0.80						.95	.94
Std. Dev.	0.151	0.046							
Coef. of Var.	0.040	0.058							

systematic error in the results.

In evaluating the biological indicator results, we will first compare the mean F_0-value results from a group of replicate physical and biological tests, and secondly, we will compare variation within each group of physically and biologically determined results. The biological indicator results for the three series of experiments are summarized in Table 5. The overall performance of the BIUs, as far as measuring sterilizing values, appears to be good as indicated by the difference between the mean F_0 (PHY) and the F_0(BIO)-values of a group of replicate tests. The overall average difference F_0 (PHY) - F_0 (BIO) of the individual tests in series 1 was + 0.9 min, in series 2, + 0.2 min, and in series 3, + 2.4 min. In all three series, the F_0 (PHY)-values are higher than the F_0(PHY)-value more than 10% greater than the F_0(BIO)-values; however, only in series 3 is the mean F_0(BIO)-value. It is possible that some of the differences in the overall performance of the biological measuring system in the three experimental series is due to differences in the recovery media since there was a high degree of consistency within each experimental series. In these experiments, the TSA recovery medium was supplied by the Green Giant Company. It was not from the same lot of medium that was used in tests to develop the calibration curves. Recently, differences have been observed among lots of media. This has led to a program where medium from the same lot is used in both calibration and field tests.

A temperature calibration error of 0.5 C will produce a change in the F_0-value of about 12%. Where we are comparing F_0(PHY)- and F_0(BIO)-values there are two

TABLE 4. *General method F_0-values calculated for experiment Series 3.*

Thermocouple No., harness and can position	Experiment number (Heating time) 3A[a] (14 min)	3B (14 min)	3C (15 min)	3D (15 min)	3E (14 min)	3F (15 min)
1	10.9	9.4	12.5	12.3	9.7	12.1
2	10.6	9.3	11.6	12.0	10.8	11.4
3	11.0	9.7	12.2	12.4	10.9	12.0
4	10.9	9.4	12.4	11.5	10.6	11.6
5	10.4	9.5	12.4	12.2	10.6	12.1

[a]Experiment Code and heating time at 255 F.

TABLE 3. *General method F_0-values calculated for experiment Series 2.*

Thermocouple No., harness and can position	Experiment number (Heating time) 2A (10 min)[a]	2B (13.5 min)	2C (12 min)	2D (12 min)	2E (10 min)	2F (12 min)	2G (10 min)
2	6.3	14.8	11.0	9.7	7.4	10.6	6.0
4	6.7	13.4	11.1	9.8	7.5	10.4	6.1
5	6.7	14.2	10.7	10.3	7.8	11.0	6.1
6	7.4	13.1	10.6	9.9	7.7	11.3	6.1

[a]Experiment Code and heating time at 255 F.

TABLE 5. *Summary of The F_0 (PHY) and F_0 (BIO) results of the three series of experiments.*

Experiment number	Average sterilization value calculated from thermocouple data: F_0 (PHY) General method (min)	Std. dev. (min)	Coef. of var.	Average sterilization value determined by Biological Indicator Units: F_0(BIO)[a] (min)	Std. dev. (min)	Coef. of var.	F_0(PHY) - F_0(BIO) (min)
1A	6.3	.19	.031	5.5	.53	.093	0.8
1B	6.1	.18	.029	4.8	.36	.074	1.3
1C	9.5	.30	.031	8.2	.56	.064	1.3
1D	9.5	.23	.024	8.2	.88	.099	1.3
1E	6.2	.40	.064	6.8	2.43	(.334)[b]	-0.6
1F	10.2	.44	.043	9.1	.48	.048	1.1
			$\bar{x}$ = .0370			$\bar{x}$ = .076	$\bar{x}$ = + 0.9
2A	6.8	.21	.032	6.5	.64	.094	0.3
2B	13.9	.77	.056	13.1	.23	.016	0.8
2C	10.8	.24	.022	10.3	.47	.041	0.5
2D	10.0	.26	.026	9.5	.67	.066	0.5
2E	7.6	.18	.024	6.8	.75	.102	0.8
2F	10.8	.40	.037	11.0	.87	.072	-0.2
2G	6.1	.05	.008	6.8	.59	.080	-0.7
			$\bar{x}$ = .0293			$\bar{x}$ = .067	$\bar{x}$ = + 0.3
3A	10.7	.25	.023	8.9	.50	.052	1.8
3B	9.5	.15	.016	7.4	.36	.045	2.1
3C	12.2	.36	.030	9.5	.26	.026	2.7
3D	12.1	.36	.030	9.9	.38	.034	2.2
3E	10.5	.48	.045	7.6	.45	.055	2.9
3F	11.9	.32	.027	8.9	.53	.055	3.0
			$\bar{x}$ = .0285			$\bar{x}$ = .045	$\bar{x}$ = + 2.4
			$\bar{x}$ (19 tests) = .032			$\bar{x}$ (18 tests) = .062	

[a]Average of five BIU's.
[b]This value was eliminated in the calculation of the mean.

potential sources of error: (*a*) in calibrating the BIUs, and (*b*) in the thermocouple potentiometer system used to gather heat penetration data. Some of the differences among the three series of experiments may have been due to changes in the potentiometer calibration during this approximately 1-month period and changes in the thermocouple harness and thermocouple fittings due to normal heavy usage. It is possible that in the series 3 results, some of the differences were due to errors that might occur in the commutator system that is normally rotating but in this case was not rotating.

The accuracy of the results, as measured by the coefficient of variation, suggests that the F_0-values from time-temperature data vary less than the F_0-values measured by biological indicator units. We are limited in the conclusions we can make because of the complexity of the overall measurement problem. In this measurement situation, we have can-to-can variation that will cause the rate of heating and cooling to vary, and consequently the F_0-value received by the peas in the can will also vary among cans. Also, the thermocouples and BIUs are not in the same cans. The performance of both the thermocouple system and the biological indicator system will vary on a unit-to-unit basis. Any variation in the spore recovery manipulations will be added variation in the BIU system. In considering variation, we are using the thermocouple data as the reference base and are assuming that the difference in variation between the thermocouple-determined data and biologically-determined data are all due to aspects of the biological system. This assumption will produce an inaccuracy since it is almost certain that in both systems there is some error.

The coefficient of variation of the F_0(PHY)-values of the 19 tests ranged from 0.008 to 0.064. The mean coefficient of variation for each series is: 1, 0.037; 2, 0.029; and 3, 0.028. The coefficient of variation is smaller for series 3 (not agitated) than for series 1 or 2 where there was container agitation. The magnitudes of the mean coefficient of variation for both the F_0(PHY) and F_0(BIO) results are interesting in that the coefficients of variation are in consistent order for PHY and BIO measurements in that series 1 had the largest F_0(PHY) and F_0(BIO), and series 3 the smallest coefficient of variation values. Within experiments there does not appear to be any consistency of the coefficient of variation of F_0 (PHY) and F_0(BIO). The coefficients of variation of the F_0(BIO)-values are, in general, larger and vary more widely than for the F_0(PHY) results. The results of test 1E, in terms of its coefficient of variation, appear to be different from all other tests. Inspection of the data sheets suggested that there may have been an error in labeling the petri plates. The data for this experiment are included in Table 5, but they were not included in calculating the average coefficient of variation for the experimental series. The coefficient of variation of the remaining 18 tests ranged from 0.016 to 0.102. The mean overall value, again excluding 1E, was 0.062 min. The results of this study indicate that for peas heated in brine, the mean coefficient of variation of the F_0(PHY) is about 0.03 and for F_0(BIO) is about 0.06. On this basis, if the containers are subjected to identical heat processes and if the average F_0-value is 10 min, 67% of the F_0(PHY) should be between 9.7 and 10.3 min and 67% of the F_0(BIO) should be between 9.4 to 10.6 min.

The results of these experiments indicate that the plastic rod biological indicator units used with the count reduction procedure can be used effectively to determine the sterilizing value delivered to cans of food processed in agitating retorts. Today, we know of no other self-contained monitoring systems that generate data that are as close in agreement with F_0(PHY) as the count reduction biological monitoring system used in the tests described in this report.

In comparing F_0-values calculated from time-temperature data with F_0-values determined by biological indicator units, a greater degree of accuracy is to be expected in the F_0-values calculated from time-temperature data measured by thermocouples than from biological indicator data. The reason for expecting better accuracy from the physical system is that we are measuring temperature and time directly in a laboratory situation, whereas the biological indicator F_0-values are determined in an indirect fashion that includes: (*a*) all of the errors that might be present in the thermocouple temperature-measuring system used to calibrate the biological indicators, and (*b*) all of the additional variation due to the biological measuring system and its sensitivity to a great many uncontrolled environmental factors.

An important attribute of the biological indicator unit system is that it makes possible large numbers of F-value measurements in the same piece of equipment in the same general time period. For example, five, 10 or even 20 containers can be fitted with BIUs and allowed to proceed sequentially through filler, closing machine and retort to monitor the process delivered to a product in an agitating processing machine. The biological indicator units can therefore be used to monitor variations with time in the delivery of the sterilizing value and also may be used to determine systematic variation in the delivery of a sterilizing process as far as location in the processing equipment is concerned. Using replicate BIUs and determining the average F-values from three, five, or more units results in greater accuracy in the estimation of the F-value. The data in Table 5 suggest that if a single, properly-calibrated BIU (that is, without systematic bias) is used, then 95% of the time the calculated F-value will be within 15% of the true, delivered F-value. If three units are used, then 95% of the time the resulting average F-value will be within 9% and if five units are used, 7% of the true value.

SYMBOLS

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

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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.

F, F_0, F_0(BIO), F_0(PHY)

The F-value is the equivalent time at temperature T of a process delivered to a container or unit of product for the purpose of sterilization; it is the common measure of the level of the sterilization process and is calculated using a specific value of z. F_0 indicates that the temperature was 250 F and the z-value was 18 F. F_0(BIO) indicates that the F_0-value was measured biologically; F_0(PHY) that it was determined from data measured physically.

j

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

$$j = \frac{\text{(heating medium temperature)} \quad \text{(Y-intercept temperature)}}{\text{(heating medium temperature)} \quad \text{(initial product temperature)}}$$

r^2

Statistical correlation coefficient.

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 10.

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