

STATISTICAL ESTIMATION OF 12D FOR RADAPPERTIZED FOODS

INTRODUCTION

THIS REPORT is concerned with the determination of safe sterilization processes for canned food, i.e., processes which insure that the food is free of dangerous organisms. Although ionizing radiation is the method of sterilization considered here, the mathematical procedures described are equally applicable to any method of killing microorganisms in food.

We may summarize the present situation as follows. An expert committee of the United Nations Food and Agriculture, World Health Organization and the International Atomic Energy Agency (1968) has recommended a criterion of safety for radiation-sterilization which states that the probability must be no more than 1×10^{-12} that a dangerous microorganism (usually *Clostridium botulinum*) will survive the processing. The processing consists of exposing sealed cans of food to a dose of radiation under specified conditions, and the dose needed to satisfy the above criterion is called the 12D dose or minimal radiation dose (MRD). The 12D dose depends on both the microorganism and conditions (temperature, salinity, pH, etc.) in the food substrate and is a measure of the radiation resistance of the microorganisms.

The presently accepted procedure for estimating the 12D dose follows the January 1971 recommendation of the National Academy of Science-National Research Council's Advisory Committee to Natick Laboratories on Microbiology of Food. The procedure consists of a set of experiments, collectively called an inoculated pack, and a computation based on the resulting data. The experiments consist of inoculating cans of food with spores of *C. botulinum*, sealing the cans and exposing them to doses of radiation. Typically, 10^7 spores are inoculated in each can, 100 replicate cans are exposed to each dose and the doses may range from 0-5 megarads in increments of 0.5 megarads. After irradiation all cans are incubated for 6 months at 30°C. The cans are examined for swelling weekly during the first month and monthly thereafter. At the end of incubation cans are tested for toxin presence, and all cans showing neither swelling nor toxin are subcultured for surviving spores. The computation takes the resulting partial spoilage data (usually based on surviving spores) and calculates the 12D dose by using the Schmidt-Nank formula (1960).

In this paper we first present a coherent and rather simple mathematical theory that is a basis for treating the results of an inoculated pack. With the aid of this theory we then discuss the inadequacies of the accepted procedure (outlined above) and describe certain changes in both the experimental design and manner of computation that lead to an improved estimate of the 12D dose.

GENERAL THEORY

IN THIS SECTION we give a simple probabilistic theory of spore sterilization and examine the conventional experiments in the light of this theory. The theory brings one main difficulty into clear view and suggests a way of dealing with it.

We assume that each spore in a given medium, irradiated under given conditions of temperature, pH, etc. possesses a unique minimum lethal dose, X . If subjected to a dose above X , the spore will be inactivated, i.e., it will be unable to produce toxin and descendants; otherwise it

will remain dangerous. The lethal dose, X , is a random variable, and we assume that it possesses a probability distribution function, $G(x)$, and probability density function,

$$G(x) = \text{Probability that } X \leq x \quad (1)$$

$$f(x) = dG(x)/dx \quad (2)$$

The 12D dose, which we call x_c , satisfies the equation

$$G(x_c) = \text{Probability that } X \leq x_c \\ = 1 - (\text{Probability that } X > x_c) = 1 - 10^{-12} \quad (3)$$

In the experiments, n spores (typically $n = 10^7$) are put into a can and irradiated at the dose x under the test conditions. We say that a can is sterilized if all the spores in it are inactivated, and define Z_n as the minimum dose at which a can containing n spores is sterilized. Different cans will have different Z_n -values, hence Z_n is a random variable, just as X is. The distribution and density functions associated with Z_n are $\Phi_n(x)$, and $\phi_n(x)$,

$$\Phi_n(x) = \text{Probability that } Z_n \leq x \quad (4)$$

$$\phi_n(x) = d\Phi_n(x)/dx \quad (5)$$

Equation (4) means that $\Phi_n(x)$ is the theoretical fraction of cans sterilized at dose x .

There is a very important and well-known relation between $\Phi_n(x)$ and $G(x)$, which is a consequence of the fact that, if X_1, X_2, \dots, X_n are the minimum lethal doses of the n spores in the can, then Z_n is the largest of these doses. The relation, given in Gumbel (1958) and many other books on probability, is

$$\Phi_n(x) = [G(x)]^n \quad (6)$$

A somewhat different form of this relation is obtained by rewriting it as

$$\Phi_n(x) = \left\{ 1 - [1 - G(x)] \right\}^n = \left\{ 1 - n \frac{[1 - G(x)]}{n} \right\}^n \\ \approx e^{-n [1 - G(x)]} \quad (7)$$

a result which is very accurate when $n \gg 1$ and $1 - G$ is small, which is almost always the situation when we need to know $\Phi_n(x)$. Solving (7) for $G(x)$ we obtain with great accuracy

$$G(x) \approx 1 + n^{-1} \ln \Phi_n(x) \quad (8)$$

In addition we obtain from (5) and (6)

$$\phi_n(x) = n [G(x)]^{n-1} f(x) \quad (9)$$

Finally, it can be shown (Gumbel, 1958), that

$$\Phi_n(x) \approx e^{-(e^{-y})}, \phi_n(x) \approx \alpha_n e^{-(y + e^{-y})} \quad (10)$$

$$y = \alpha_n (x - U_n) \quad (11)$$

where U_n , the characteristic largest value, and α_n , the extremal intensity function, are found from

$$G(U_n) = 1 - n^{-1}, \alpha_n = nf(U_n) = n \frac{dG}{dx}(U_n) \quad (12)$$

The distribution defined by (10) to (12) is called the extreme-value distribution derived from the distribution $G(x)$. Formulae (6) and (9) are exact, and (7) and (8) are such good approximations that they too may be regarded as exact for all practical purposes. Equations (10) are approximations to the exact relations (6) and (9) and are accurate when $|x - U_n|$ is not too large. The region where (10) is most accurate is the partial spoilage range, i.e., the x -values for which $\Phi_n(x)$ is near neither zero nor one. The quantities U_n and α_n^{-1} are approximate measures, respectively, of the location and width of the partial spoilage range for cans containing n spores. As n increases, U_n (but not necessarily α_n^{-1}) increases, i.e., the partial spoilage range moves outward.

In the conventional inoculated pack N cans, each containing n spores, are exposed to a dose x , and after suitable incubation, counts are made of the number, $C(x)$, of cans that are sterilized or clean. Such a pack can be regarded as a sample of N cans, each of which has probability of sterilization $\Phi_n(x)$. It is well-known that the probability that exactly ξ cans will be sterilized is given by the binomial distribution,

Probability that ξ cans are sterilized = ξ

$$= \frac{N!}{\xi!(N-\xi)!} [\Phi_n(x)]^\xi [1 - \Phi_n(x)]^{N-\xi} \quad (13)$$

Moreover, the best estimate of $\Phi_n(x)$ that can be obtained from the data is

$$\hat{\Phi}_n(x) \equiv \text{estimate of } \Phi_n(x) = \xi/N \quad (14)$$

and, if $N \gg 1$, $\hat{\Phi}_n(x)$ is approximately normally distributed about its mean, $\Phi_n(x)$, with estimated standard deviation

$$\sigma_{\Phi} = [\hat{\Phi}_n(1 - \hat{\Phi}_n)/N]^{1/2} \quad (15)$$

To summarize, we obtain from the conventional inoculated pack an experimental fraction, (14), of cans sterilized at dose x , and this is the best obtainable estimate of $\Phi_n(x)$. If packs are run at several different doses, we obtain several points on an experimentally-determined graph of $\Phi_n(x)$. There will be some scatter or noise in this graph, much of which is caused by the sampling error (i.e., the fact that $\hat{\Phi}_n(x) \neq \Phi_n(x)$) although some may also be due to random fluctuation in spore load, n , and dose, x . Formula (15) is an estimate of the scatter at dose x due to the sampling error.

Clearly, the inoculated pack provides quite a lot of information about $\Phi_n(x)$, especially if packs are run at several different doses. However, this information is of little use unless it leads to comparable information about $G(x)$, for it is $G(x)$ that enters the calculation of the 12D dose in Equation (3). In order to apply Equation (3), we have to know both the general form and parameter values of G . We shall see later that it is relatively easy to estimate the parameter values of G from data on $\Phi_n(x)$ if the general form of G is known, but it is not easy to find the general form of G .

This seems strange at first glance, for we can find $G(x)$ from $\Phi_n(x)$ directly by means of Equation (8). The difficulty arises because the doses at which $\Phi_n(x)$ is known are far out on the right-hand tail of the distribution $G(x)$. All probability distributions look very much alike in this region, and the scatter in $G(x)$ that arises from the scatter in the estimates of $\Phi_n(x)$ will make it very difficult to see the small differences between distributions.

Of course this difficulty does not arise if the form of $G(x)$ is known. It is usually assumed (Schmidt, 1963) that $G(x)$ is of simple exponential form. There is some (perhaps inconclusive) evidence to support this assumption when the spores are in a model system (i.e., a transparent, fluid substrate), see e.g., Anellis et al. (1965). In the critique of the Schmidt-Nank calculation we shall show evidence against the assumption when spores are in a food.

Thus there is a need to determine $G(x)$ from measurements of $\Phi_n(x)$. Since the conventional inoculated pack was not designed for finding the form of $G(x)$, we should expect that other experimental designs may be superior for that purpose. Intuition suggests that differences in distributions will be most visible when we have data over a wide range in x . The simplest way of obtaining this wide range is to test at several different spore loads, i.e., values of n , because the partial spoilage range moves outward as n increases. We shall pursue this line of thought further in a later section.

A CRITIQUE OF THE SCHMIDT-NANK CALCULATION

THIS SECTION contains a sketch and critique of the Schmidt-Nank procedure for estimating the 12D dose.

The experimental procedure for a conventional inoculated pack has been described previously. The resulting data are $\Phi_n(x)$, see Equation (14), evaluated at one or more x -values. The Schmidt-Nank procedure for estimating the 12D dose is based primarily on the assumption that $G(x)$ is of simple exponential form,

$$G(x) = 1 - e^{-\lambda x}, \quad x \geq 0 \quad (16)$$

If N is the number of cans tested at dose x , n is the number of spores in each can and R is the total number of surviving spores, then some simple manipulations show that x_c can be estimated from

$$\hat{x}_c = 12\hat{D} \quad (17)$$

$$\hat{D} = \frac{x}{\log_{10} (Nn) - \log_{10} R} \quad (18)$$

Here \hat{D} is the estimated value of D , the decimating dose, i.e., dose at which the probability of spore death is

$$G(D) = 9/10$$

These formulae are not very useful as they stand because there is no practical way to measure R . In the Schmidt-Nank method this difficulty is overcome by a second assumption, namely that exactly one spore survives in every can that is spoiled (not sterilized), i.e.,

$$R = N - \xi = N(1 - \frac{\xi}{N}) \quad (19)$$

or, using (14),

$$R = N[1 - \hat{\Phi}_n(x)] \quad (20)$$

if ξ out of N cans are sterilized at dose x . This estimate of R is used in Equation (18) and permits the evaluation of \hat{D} and hence \hat{x}_c .

The Schmidt-Nank formula, (18), has been generally accepted as a simple, standard method for estimating the 12D dose. However, in recent years other procedures have been suggested as alternatives to the Schmidt-Nank formula (Anellis and Werkowski, 1968; 1971). This is evidence of growing uneasiness about the accuracy of the method, but no systematic study of its validity has appeared. One is presented in the ensuing paragraphs.

The principle criticisms that can be levelled against the Schmidt-Nank procedure are listed here and then discussed below.

- (1) The assumption of an exponential distribution may be wrong.
- (2) The assumption that one spore survives in each can that is not sterilized is questionable.
- (3) The results of using the method on experimental data are inconsistent with the assumptions.
- (4) The procedure is confusing and unclear.

First, there is much experimental evidence that something is wrong with the Schmidt-Nank formula. For, if it is applied to experimental data at several different doses, it gives an estimate of D (and hence x_c) derived from each test dose. If the theory is correct, the same D should be obtained from each test dose, aside from random fluctuations. A typical set of experimental results (Anellis and Werkowski, 1968) is reproduced in Table 1. It is clear from this and other data (Anellis et al., 1969; 1972; Grecz et al., 1965; Segner and Schmidt, 1966), that the estimate of D increases very markedly as x increases. This trend is unambiguous and far too pervasive to be attributed to any sort of randomness. It is completely at odds with the theory although it is hard to discern whether assumptions (1) or (2) or both are at fault.

Table 1—Radiation resistance of representative strains of *C. botulinum* spores in cured ham

Strain	Dose	No. of cans with viable <i>C. botulinum</i>	
		Schmidt-Nank D-value	
33A	1.0	17/20	0.148
	1.5	15/20	0.220
	2.0	8/20	0.282
	2.5	1/100	0.288
77A	1.0	16/20	0.167
	1.5	11/20	0.245
	2.0	5/20	0.309
12885A	1.0	19/20	0.149
	1.5	5/20	0.206
	2.0	3/20	0.267
	3.0	1/100	0.346
41B	.5	18/20	0.072
	1.0	13/20	0.142
	1.5	6/20	0.203
	2.5	1/100	0.282
53B	.5	19/20	0.071
	1.0	14/20	0.140
	1.5	8/20	0.203
	2.0	1/20	0.241

Second, the assumption (2) implies a relation between $\Phi_n(x)$ and $G(x)$ that is different from (6). To see this, we notice that assumption (2) implies that only two outcomes of a can sterilization experiment are possible, namely either (a) the can is sterilized or (b) exactly one spore survives in it. Hence, on this assumption

$$1 - \Phi_n(x) = \text{theoretical fraction of cans in which exactly one spore survives.}$$

Since N cans are irradiated, the total theoretical number of surviving spores is $N[1 - \Phi_n(x)]$ out of Nn spores exposed. The fraction of spores surviving is

$$\frac{N[1 - \Phi_n(x)]}{Nn} = 1 - \text{fraction killed} = 1 - G(x)$$

Therefore, we would obtain

$$\begin{aligned} \Phi_n(x) &= 1 - n[1 - G(x)] \\ \text{or } G(x) &= 1 - n^{-1}[1 - \Phi_n(x)] \end{aligned} \quad (21)$$

instead of (6), as a consequence of assumption (2). Equation (6) was derived from the reasonable assumption that the minimum sterilizing dose for a can is the minimum lethal dose for the most resistant spore in the can. Assumption (2) therefore gives results which disagree with that assumption in general, and must be logically doubtful.

Moreover, it is clear that the true total number of spores surviving radiation, R , is greater than (or equal to) the number $N - \xi$, given by Assumption (2), Equation (19). Equation (18) shows that an increase in R causes an increase in D , hence the true D -value is larger than that given by (18). However, the difference between these two D -values is usually not very great because almost always $R \ll Nn$.

Finally the Schmidt-Nank calculation is confusing because in deriving it the authors did not give any clear indication that two distinct distributions, $G(x)$ and $\Phi_n(x)$, are involved. The

formulae show it, Equations (18) and (20), but the failure to point it out explicitly has led to confusion when trying to modify the calculation.

For example, Anellis and Werkowski (1968) describe an attempt (by Weibull plotting) to ascertain the form of the partial spoilage distribution, i.e., $\Phi_n(x)$. The conclusion, that the distribution was nearly normal, is not seriously inconsistent with the form (10). However, the interpretation was marred by a number of confusing statements, evidently arising from failure to distinguish $G(x)$ from $\Phi_n(x)$.

To summarize, the most telling criticism of the Schmidt-Nank computation is that its results contradict the assumption that D is a constant. Another valid general criticism is that the derivation is confusing. The specific assumption that one spore survives in each spoiled can is illogical and should be abandoned, but it does not usually cause large errors in the estimate of D . The assumption of an exponential distribution is cast into doubt by the experimental evidence that D is not constant.

It appears, therefore, that both the experimental design of the inoculated pack and the procedure for estimating the D -value should be modified.

ALTERNATIVE DISTRIBUTION FUNCTIONS FOR SPORE DEATH

WE SHALL CONSIDER two distributions as possible replacements for the exponential distribution. They are listed below.

Weibull distribution

The distribution function is

$$\begin{aligned} G(x) &= 0, & x < 0 \\ &= 1 - \exp[-(x/\eta)^\beta], & x > 0 \end{aligned}$$

where $\eta > 0$ and $\beta > 0$. From Equations (12) we find

$$\begin{aligned} U_n &= \eta(\log_e n)^{1/\beta} \\ \alpha_n &= (\beta/U_n)\log_e n \end{aligned} \quad (22)$$

It is important to notice that the exponential distribution is a special case of this distribution, obtained by setting $\beta = 1$. $\beta > 1$ gives a higher death rate, and $\beta < 1$ a lower one, than the exponential distribution. Also, the Weibull distribution with $\beta \approx 3.26$ mimics the behavior of the Gaussian (normal) distribution in the sense that the mean, median and mode coincide when $\beta \approx 3.26$. However, the behavior of the Weibull distribution for large x is somewhat different from the normal distribution, regardless of the β -value.

Lognormal distribution

The lognormal distribution has

$$\begin{aligned} G(x) &= 0, & x < 0 \\ &= G_g[\beta \log_e(x/\eta)], & x > 0 \end{aligned}$$

where G_g is the standardized normal distribution function

$$G_g(z) = (2\pi)^{-1/2} \int_{-\infty}^z e^{-x^2/2} dx$$

If U_g and α_g are the U_n and α_n for the distribution function G_g , then Gumbel shows that

$$\begin{aligned} U_n &= \eta e^{U_g/\beta} \\ \alpha_n &= \frac{\beta \alpha_g}{\eta} e^{-U_g/\beta} \end{aligned} \quad (23)$$

and therefore

$$\alpha_n U_n = \beta \alpha_g \quad (24)$$

Other distributions, such as the normal or Gamma distributions, could also be studied, but for the sake of brevity and simplicity we shall limit ourselves to the Weibull and lognormal distribution for the present. These are natural choices, the Weibull because it is a generalization of the exponential distribution and the lognormal because it is often the governing distribution in bacteriological studies.

A NEW METHOD FOR FINDING THE DISTRIBUTION FUNCTIONS AND 12D DOSE

IN THIS SECTION we present a general method for determining the form and parameters of the distribution function $G(x)$ from measurements $\Phi_n(x)$. The basic idea is a very simple and familiar one. We hypothesize that we have a certain form of distribution, G , and we subject the data, $\Phi_n(x)$, to a transformation which would reduce the data plot to a straight line if G were of the assumed form. The straightness (absence of curvature) of the plot is a measure of how well the data support the hypothesis about the form of G , and the slope and intercept of the line provide estimates of the parameters of the distribution. In practice we usually consider several competing forms of $G(x)$, so that we subject the data to several different transformations, one appropriate for each of the competing forms. The form whose transformation produces the straightest plot is the one which fits the data best.

We illustrate the procedure by deriving the formulae appropriate to the Weibull and lognormal distributions.

Basic Formulae

When $x > 0$, the form of the Weibull distribution is

$$G(x) = 1 - \exp[-(x/\eta_w)^{\beta_w}]$$

where β_w and η_w are the parameters. We combine this with Equation (8) to obtain

$$\exp[-(x/\eta_w)^{\beta_w}] = -n^{-1} \log_e \Phi_n$$

or

$$\begin{aligned} (x/\eta_w)^{\beta_w} &= h + \log_e n \\ h &= -\log_e (-\log_e \Phi_n) \end{aligned}$$

We take logarithms again and get

$$\beta_w (\log_e x - \log_e \eta_w) = \log_e (h + \log_e n)$$

From this we see that, if we define the transformation

$$\begin{aligned} y_w &= \log_e [\log_e n - \log_e (-\log_e \Phi_n)] \\ t &= \log_e x, \end{aligned} \quad (25)$$

then we obtain the straight line relation between y_w and t ,

$$y_w = \beta_w t - \beta_w \log_e \eta_w$$

provided that G is a Weibull distribution with parameters β_w and η_w . This is the desired transformation.

For the lognormal distribution, the form when $x > 0$ is

$$G(x) = G_g [\beta_L \log_e (x/\eta_L)]$$

where β_L and η_L are the parameters. Omitting the details we find that the transformation

$$y_L = G_g^{-1} [1 + n^{-1} \log_e \Phi_n] \quad (26)$$

$$t = \log_e x$$

leads to the straight line relation

$$y_L = \beta_L t - \beta_L \log_e \eta_L$$

if G is a lognormal distribution with parameters β_L and η_L .

In practice we do not know $\Phi_n(x)$. In its place we use the quantities $\hat{\Phi}_n(x_m)$, the experimentally obtained fractions of cans sterilized [see Equation (14)], at the test doses x_m , $m = 1, 2, \dots, M$. To decide whether the true G has Weibull or lognormal form, we construct two graphs of the data points versus $\log_e x$, using Formulae (25) and (26), respectively. The transformation which produces the straighter graph of the data points corresponds to the likelier form of G . Then, if the plot is of the form

$$y = A + Bt,$$

we obtain the estimated parameters

$$\hat{\beta} = B \quad (27)$$

$$\hat{\eta} = e^{-A/B} \quad (28)$$

for whichever distribution has been chosen. The 12D dose, x_c , is estimated using

$$\hat{x}_{cW} = \hat{\eta}_w (27.63)^{1/\hat{\beta}_w} \quad (29)$$

if the Weibull distribution has been selected and

$$\hat{x}_{cL} = \hat{\eta}_L e^{(7.0345/\hat{\beta}_L)} \quad (30)$$

if the lognormal has been chosen.

Experimental design

In theory the above is easy enough, but in practice it is often difficult to tell by eye which of the two plots is straighter, especially since there is noise (random fluctuations) in the data. The discussion in the latter portion of General Theory leads us to expect that both plots will appear nearly straight if they cover only the range of x corresponding to the partial spoilage range for, say, $n = 10^7$. Figure 1 shows this very clearly. It contains the two graphs for the case where $\hat{\Phi}_n(x)$ has exactly the theoretical values, $\Phi_n(x)$, derived from a lognormal distribution with $\beta_L = 2$ and $\eta_L = 0.2$ when $n = 10^7$. The graph given by the lognormal transformation (the upper set of points in Fig. 1) is exactly straight. The graph given by the Weibull transformation (the lower set of points) is not exactly straight, but the curvature is so slight that it is hard to see which graph is straighter. If we were given only the data, we could not tell whether the distribution is lognormal with $\beta_L = 2$ and $\eta_L = 0.2$ or Weibull with $\beta_w = 0.664$ and $\eta_w = 0.0409$. Moreover, we see from Equations (27) and (28) that the 12D dose is estimated to be 6.74 if the distribution is lognormal (as it really is) and 6.06 if it is (incorrectly) thought to be of Weibull type. The large difference between these estimates of 12D attests to the importance of finding the distribution form correctly.

The discussion at the end of the General Theory section suggests that we can get around this difficulty by conducting tests at several different spore loads. This is illustrated in Figure 2, where, as in Figure 1, the data are assumed to have exactly the theoretical values derived from a lognormal distri-

bution with $\beta_L = 2$ and $\eta_L = 0.2$. However, we assume now that the partial spoilage data have been taken at three different spore loads, $n = 10^3, 10^5, 10^7$. The upper graph for the lognormal distribution is exactly straight. The curvature in the lower graph, although not overwhelming, is rather easily visible, certainly much more so than in Figure 1 which is now reproduced as the portion of Figure 2 for $n = 10^7$. We conclude that it is advantageous to test at several different spore loads.

Computation scheme

In order to choose the likelier form of G from test data, we have to decide which of the two graphs is the straighter. We have seen in Figure 2 that, even when tests are run at several spore loads, the curvature in the "incorrect" graph may not be great. Moreover, in practice the situation will be worse than shown there because of the random errors in the data. It is most desirable to have a sensitive analytical test for measuring the curvature of the graphs, rather than relying on the unaided eye.

The computational method consists of approximating the data, using the unweighted least squares procedure, by means of orthogonal polynomials

$$y = C_0 + C_1 P_1(t) + C_2 P_2(t) \tag{31}$$

the data having been first transformed by the appropriate Weibull or lognormal relation, Equation (25) or (26). Here

$$t = \log_e x, P_1(t) = t - \bar{t} \tag{32}$$

where \bar{t} is the average value of t over all the data points. $P_2(t)$ is a second degree polynomial, orthogonal to $P_1(t)$ and a constant, over the data points. Then

$$\hat{\beta} = C_1, \hat{\eta} = \exp\left[-\left(\frac{C_0 - C_1 \bar{t}}{C_1}\right)\right] \tag{33}$$

If we define

$$\hat{Q} = \sum_{j=1}^M \left\{ y_j - [C_0 + C_1 P_1(t) + C_2 P_2(t)] \right\}^2 \tag{34}$$

$$\hat{Q} = \sum_{j=1}^M \left\{ y_j - [C_0 + C_1 P_1(t)] \right\}^2$$

then a convenient measure of the curvature of the graph is

$$\rho = (M - 3)(\hat{Q} - \hat{Q})/\hat{Q} \tag{35}$$

where M is the number of partial-spoilage data points.

We determine the form of F by carrying out this computation for both the Weibull and lognormal distributions and comparing the two resulting values of ρ , ρ_w and ρ_L . If $\rho_w < \rho_L$, we conclude that spore-death is governed by a Weibull distribution; if $\rho_w > \rho_L$, then by a lognormal distribution. The quantities ρ_w and ρ_L obey the Fisher variance-ratio distribution and are the quantities that arise in the likelihood-ratio test of the hypothesis that $C_2 = 0$. Comparing the values of ρ_w or ρ_L with the tabulated values of Fisher's distribution for (1, $n-3$) degrees of freedom permits one to make confidence statements about whether $C_2 = 0$.

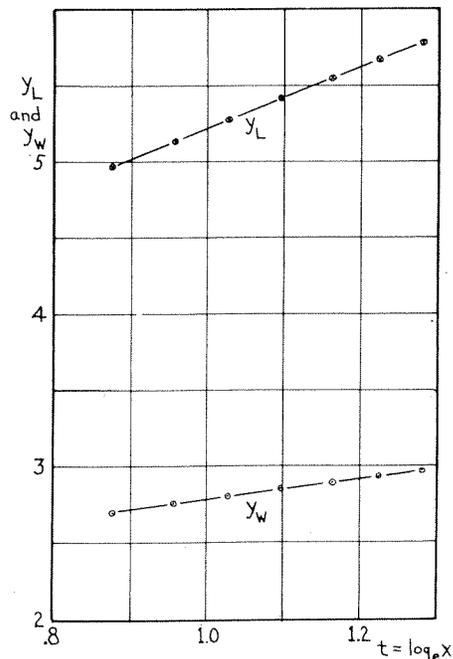


Fig. 1—Graphs of lognormal and Weibull plots of data derived from a lognormal distribution for $n = 10^7$.

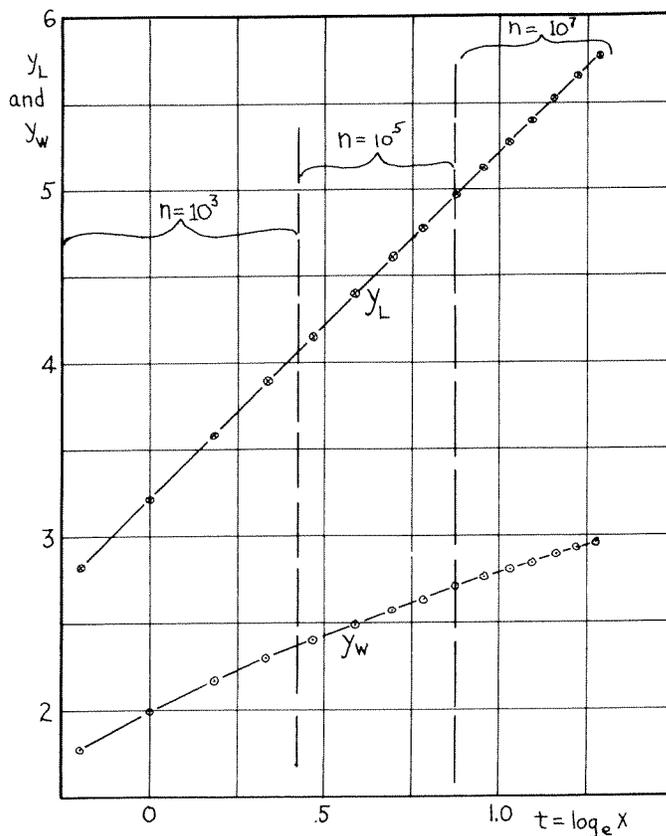


Fig. 2—Graphs of lognormal and Weibull plots of data derived from a lognormal distribution for $n = 10^3, 10^5$ and 10^7 .

Having ascertained the form of G, the constants $\hat{\beta}$ and $\hat{\eta}$ are estimated using (27) and (28), and the 12D dose is given by (29) or (30).

The experimental design described in part (b) and the computational scheme outlined above are the methods we suggest as replacements for the conventional inoculated pack and Schmidt-Nank Formula.

EXAMPLES

THIS SECTION contains two examples in which we use the preceding theory. The first example illustrates the use and accuracy of the method proposed in the previous section. Since sufficiently extensive experimental results from tests at three different spore loads were not available, it was necessary to use computer-simulated data for purposes of illustration. The second example shows how the general theory can be used with the absolute minimum amount of partial spoilage data, one point, to show that the form of G(x) is probably not exponential.

Example 1

By means of a computer program the artificial partial-spoilage data shown in Table 2 were generated as the simulated outcome of an inoculated pack. Nine partial spoilage data points were obtained at three spore loads, $n = 10^3, 10^5$ and 10^7 . The data were passed through the Weibull and lognormal transformations, Equations (25) and (26), and then subjected to the least squares process of the preceding subsections. The resulting estimates are

$$\text{Weibull: } \hat{\beta}_w = 1.582, \hat{\eta}_w = .422, \hat{x}_{cw} = 3.439, \rho_w = .069 \quad (36)$$

$$\text{Lognormal: } \hat{\beta}_L = 3.928, \hat{\eta}_L = .658, \hat{x}_{cL} = 3.941, \rho_L = 4.278 \quad (37)$$

Since $\rho_w < \rho_L$, we conclude that the true distribution is of Weibull form with parameters given in (36). Moreover, comparing the values of ρ with the 90% confidence limit of F for (1, 6) degrees of freedom, we see that ρ_L exceeds this value, 3.78, while ρ_w does not. Hence there is no reason to doubt that the distribution is Weibull, but there is a great deal of reason to doubt that it is lognormal. In this example, then, the new method would unequivocally conclude that the distribution is of Weibull form with parameters given by (36).

The data of Table 2 were generated by assuming a distribution whose true form was Weibull with

$$\beta_w = 1.700, \eta_w = .470, x_{cw} = 3.31$$

and tainting the results with various (roughly realistic) random

Table 2—Simulated outcome of an inoculated pack

Spore load n	Dose (megarads)	Fraction of cans sterilized
10 ⁷	2.4	19/40
	2.6	33/40
	2.2	33/40
10 ⁵	1.8	1/40
	2.0	18/40
	2.4	39/40
	2.4	39/40
10 ³	1.3	4/40
	1.5	17/40
	1.7	36/40

errors. Since we know what the true form is, we can verify that the method gives the correct conclusion and observe that the estimates $\hat{\beta}_w, \hat{\eta}_w$ and (especially) \hat{x}_{cw} are quite accurate despite the random errors.

The fact that the method gives decent accuracy in this example, where the correct distribution and values of $\hat{\beta}_w, \hat{\eta}_w$ and \hat{x}_{cw} are known, suggests that the method is a promising one. Many other simulated examples, not described here, have given like results. Naturally the method has to be tested on real, rather than simulated, data before a final decision is reached on its usefulness.

Example 2

An ordinary inoculated pack for FDA clearance of canned ham was run at Natick Laboratories with 10^7 spores of *C. botulinum* in each can and 100 cans at each dose. The test and analysis will be described elsewhere in detail, but the results based on can swelling may be described as follows (A. Anellis, private communication):

$x \leq 1.7$	all cans swollen
$x = 2.0$	75 out of 100 cans swollen
$x \geq 2.3$	no cans swollen

We wish to see whether these results support the assumption that the distribution is exponential.

If the distribution is exponential then we have a Weibull distribution with $\beta = 1$,

$$G(x) = 1 - e^{-x/\eta} \quad (38)$$

and because of (22)

$$U_n = \eta \log_e n$$

$$\alpha_n = 1/\eta$$

Experimentally we have obtained

$$\hat{\Phi}(2.0) = 25/100 = 0.25$$

From Equations (10) and (11) we have

$$\alpha_n(x - U_n) = y = -\log_e [-\log_e (\Phi(x))]$$

Setting $x = 2.0$ and using $\hat{\Phi}(2.0)$ as the estimate of $\Phi(2.0)$, we obtain

$$\frac{1}{\eta}(2 - \eta \log_e n) = -\log_e [-\log_e (0.25)]$$

Since $n = 10^7$ this leads to

$$\frac{2}{\eta} - 16.118 = -0.327$$

or

$$\eta = 0.1266$$

Hence, because of (38),

$$G(x) = 1 - e^{-7.899x}$$

At $x = 2.3$ we have, therefore,

$$1 - G(2.3) = 1.297 \times 10^{-8}$$

Equation (7) then implies

$$\begin{aligned} \Phi(2.3) &= \exp(-10^7 \times 1.297 \times 10^{-8}) = e^{-0.1297} \\ &= 0.8784 \end{aligned}$$

From Equation (13), with $\xi = 100$ and $N = 100$, we see that P , the probability that 100 cans are sterilized at $x = 2.3$, obeys

$$P = [\Phi(2.3)]^{100} = 0.8784^{100} \\ = 2.34 \times 10^{-6}$$

The smallness of P means that it is exceedingly unlikely that we would get 100 cans sterilized at $x = 2.3$ if the result at $x = 2.0$ is correct and if the distribution is exponential (i.e., $\beta = 1$). Since there is no reason to doubt the experimental result at $x = 2.0$, we must conclude that it is very improbable that the distribution is exponential. The conclusion would be more striking if it were based on recoverable botulinum cells, rather than visible swelling. These data are not yet available, but the smallness of P suggests that the conclusion is not sensitive to moderate changes in $\Phi(2.0)$. In any case the results tend to cast still more doubt on the assumption of exponential death for botulinum spores in canned food.

DISCUSSION & CONCLUSIONS

THE FIRST conclusion of this paper is that the accepted procedure for estimating the 12D dose of radappertized food has shortcomings that ought not to be ignored. Apparently its most serious limitation is in the assumption of exponential spore-death. If this assumption is abandoned, a procedure for finding the spore-death distribution must be provided. The experimental design and computational scheme outlined in the new method is such a procedure. If it is used, inoculated pack results can yield information about the spore-death distribution and hence well-founded estimates of the 12D dose.

Our second conclusion is that the proposed method is sufficiently promising to justify further investigation. In particular the crucial experiment consists of running tests at three or more different spore loads on an organism and substrate where the true spore-death distribution is already known with satisfactory accuracy. The doses must be closer together, and the number of replicate cans must be larger, than in previous

inoculated packs if a clear verdict is to be obtained.

Finally, we should observe that much more needs to be done before a realistic mathematical model can be obtained for such a complex process.

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