# Studies on the Heat Resistance of Wild Yeasts and Bacteria in Beer[1]

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**ABSTRACT**

Detailed kinetic studies on wild yeast and heat-resistant brewing bacteria showed that the Z value used in current lethal-rate calculations in brewing is not suitable. An alternative method, based on the D values of the most heat-resistant brewing microbe, is proposed. A complete reinvestigation of pasteurization in brewing is suggested.

Key words: *Bacteria, Decimal reduction time (D value), Pasteurization units, Wild yeasts, Z value*

Compared to other food industries, breweries have a rather straightforward and limited spectrum of microorganisms to deal with (1,2,7,8,11,16). Moreover, because none of the microbes are pathogenic, microbial spoilage and profit loss are considered the most important problems. Pasteurization has been the primary approach to securing biological stability of the product and prolonging its shelf life. The commercial rule of thumb has been to use a time-temperature relationship of 15 min at 60°C (140°F) (4,5,25), ie, 15 pasteurization units (PU), where 1 PU is defined as exposure to 60°C for 1 min. Nevertheless, even though the cycle of pasteurization (heating up, holding, and cooling down) is fully understood and fully considered in the use of lethality curves and pasteurizer recorders (10) to assess killing before and after attainment of the maximal temperature, a wide variance occurs in the degrees of heat and numbers of pasteurization units used in North American breweries. Although laboratory tests indicate that values from 1–5 PU are effective in achieving commercial sterility (6,28), 8–30 PU are generally used (3,6,22,28), perhaps to have a built-in safety factor in case of possible resistant contaminants.

The purpose of these experiments was to reassess the death rates of wild yeast in beer (using moist heat destruction), by comparing their decimal reduction times at a given temperature ($D_{temp}$) and phantom thermal death time (TDT) curves to those obtained from some very heat-resistant brewing bacteria.

Lactic acid bacteria have been shown to possess considerably less heat resistance than some yeasts (21,27), although Splittstoesser et al (27) described a fructose-requiring wine *Lactobacillus* with a $D_{60}$ value of 1.7 min in wine. In addition, Put et al (23) showed that sporogenous yeasts had higher heat resistance than asporogenous yeasts. In fact, *Saccharomyces cerevisiae* had the highest heat resistance of all yeasts isolated from soft drinks. The brewing literature, however, is very inconsistent. For example, Epstein and Dee Snell (5) reported that *Lactobacillus* and *S. pastorianus* require 58°C for 10 min and 60°C for 15 min, respectively, for death, whereas Laufer and Brenner (17) reported them to need less than 50°C/10-min treatments. Lund (19) found a *L. lindneri* strain to be very resistant and Del Vecchio et al (4) reported an abnormal yeast and used it to provide a Z value of 12.5°F (6.94°C), which is now used for lethal rate calculations by the industry.

We believe that only by careful and exhaustive examination of a large number of yeast and brewing bacteria will this controversy be solved so that the brewing industry can safely minimize its energy expenditure for pasteurization of beer. Only very accurate enumeration of cell viability, using techniques such as membrane filtration (12), the attemperated dilution blank method (instant heat-up times allowing for examination of up to six or seven logs of cell death) (20), and the multiple point method, will provide precise enough measurement of kinetic variables of cell death to allow these decisions to be made.

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## EXPERIMENTAL

### Microorganisms

The microorganisms used in this study (Table I) were selected from the National Collection of Industrial Bacteria (Torry Research Station, Abbey Road, Aberdeen, Scotland) and the National Collection of Yeast Cultures (Brewing Industry Research Foundation, Nutfield, Surrey) catalogs as beer spoilage organisms. *S. carlsbergensis* L-5 was obtained from Molson Breweries of Canada Ltd., Montreal. Bacteria were of the most heat-resistant spoilage bacteria described in prior literature.

All organisms were checked for culture homogeneity by streaking for isolated colonies on tomato juice agar plates.

For experimental purposes, yeast cultures were cultivated in wort broth with 10% beer and bacterial cultures in de Man-Rogasa-Sharpe broth (MRS) (Oxoid) with 10% beer. Media was dispensed into 250-ml side-arm Erlenmeyer flasks and autoclaved. A Klett-Summerson colorimeter (No. 66 red filter, Klett Mfg. Co. Ltd., New York, NY) was used to monitor growth. Cells were harvested only from the early stationary phase of the growth curves because heat sensitivity varies with physiological age, stationary phase cells being more heat resistant (9). The relationship between Klett units and cell number for each microbe was established so that cell number in individual heat-resistance experiments could be balanced.

### Heat Stress Experiments

Yeasts and bacteria were subjected to moist heat destruction over the range of 37–53°C, using the attemperated dilution blank method (20). A 250-ml microbiological dilution blank filled with 99 ml of beer was placed into a 30-L water bath equipped with a Lauda B1 thermostatically controlled heat circulator set at the stipulated temperature. A second dilution blank of the identical beer contained a thermocouple connected to a model 850Z (Cole Parmer Ltd., Chicago, IL) digital display. When the bottle was stabilized at the desired temperature, 1.0 ml of the yeast or bacterial slurry at $10^8$–$10^9$/ml was added, homogenized, and agitated by

**TABLE I**
**Microbes Used in Studies on Heat Resistance**

| Microbe | Source and Description |
|---|---|
| Yeasts | |
| *Saccharomyces carlsbergensis*[a] | MBCL[b]-L5 |
| *Hansenula anomala*[a] | NCYC[c] 682 |
| *Pichia membranaefaciens*[a] | NCYC 326 |
| *Sacch. willianus*[a] | NCYC 114 |
| *Candida mycoderma* | NCYC 327 |
| *Torulopsis colliculosa* | NCYC 608 |
| *Kloeckera apiculata* | NCYC 328 |
| Heat-resistant brewing bacteria | |
| *Pediococcus acidilactici* | NCIB[d] 6990 |
| *Lactobacillus frigidus* | NCIB 8518 |
| *L. delbrueckii* | NCIB 8130 |

[a]Sporogenous yeasts.
[b]Molson Breweries of Canada, Ltd.
[c]National Collection of Yeast Cultures.
[d]National Collection of Industrial Bacteria.

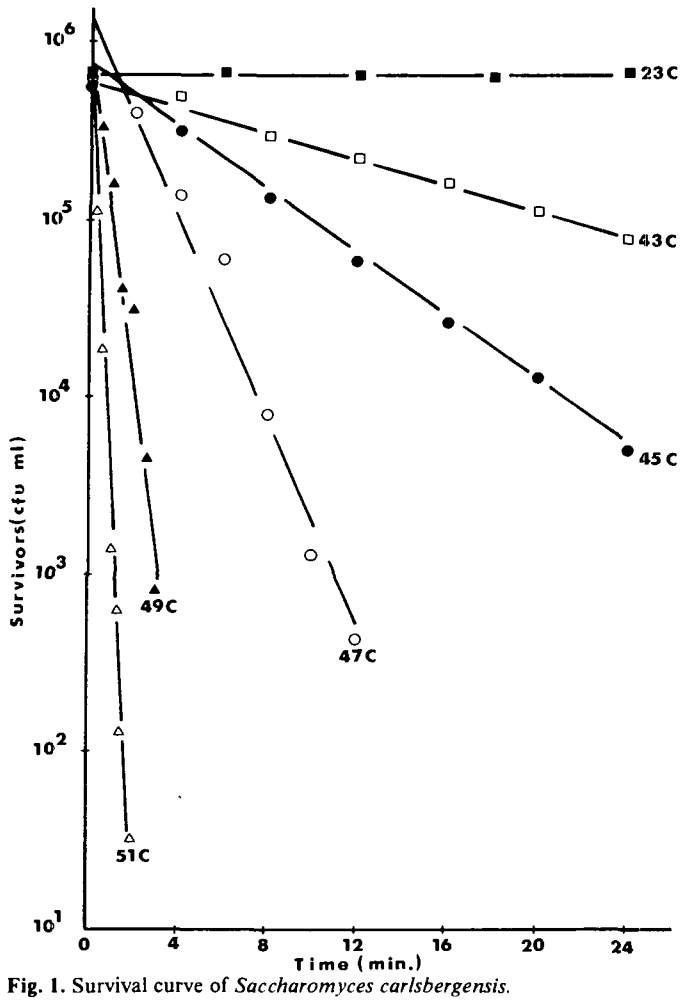


Fig. 1. Survival curve of *Saccharomyces carlsbergensis*.

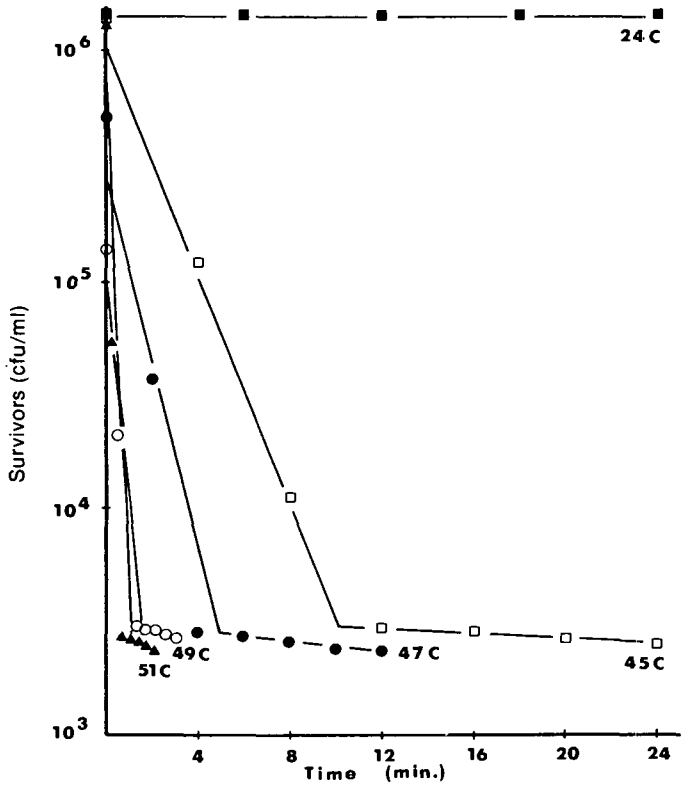


Fig. 2. Survival curve of *Saccharomyces willianus*.

hand to prevent flocculation. Aliquots of 1.0 or 11.0 ml were sampled at fixed intervals. These aliquots were cooled before enumeration by adding them directly to a 99-ml 0.1% peptone dilution blank held at 20° C (or to a cooled test tube when undiluted samples were plated). Controls were conducted to ensure that death did not occur in unheated beer during the experiment.

**Heating Menstrum**

A commercial lager beer (Molson Canadian), used as the heating menstrum for all studies, was formulated using 11.5° P corn grit adjunct wort. Its pH was 4.0 and it contained 5.0% v/v alcohol. Beer (apparent extract 2.1) was degassed before use by vigorous stirring at 20° C so that pipetting would not be inaccurate. The pH was not affected by the degassing step. The choice of menstrum influences resistance of organisms. Increases in alcohol content and decreases in pH or carbohydrate levels of beer promote more rapid death at a given temperature (9,20). Organisms are more resistant as total solids and pH increase (to near neutrality) and as alcohol is lowered.

**Viable Cell Counts**

The membrane filtration technique for viable counting as previously described (12) was used throughout the experiment.

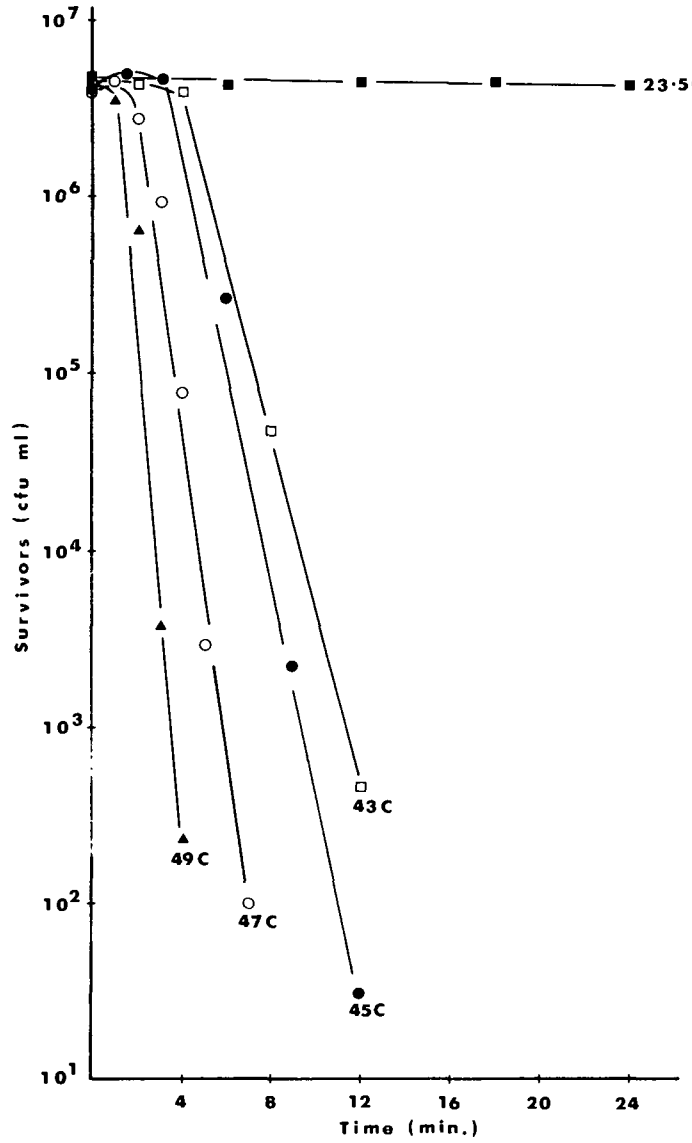


Fig. 3. Survival curve of *Lactobacillus delbrueckii*.

Ten-milliliter aliquots (in triplicate) of the appropriate dilutions of the heat-treated organisms were filtered through sterile 0.45-$\mu$m (47-mm diameter) cellulose ester (Millipore) membranes. Sterile 0.1% peptone water was used to rinse the holders and to make all dilutions. Membranes were then incubated on agars manufactured by adding Difco agar to pH 6.8 wort broth (yeast), to pH 6.8 MRS (Oxoid) broth (*Pediococcus*), or to pH 6.8 MRS (Oxoid) broth with 0.5% maltose as recommended by Lawrence and Leedham (18) for lactobacilli. Media was neutralized because of the effect that pH may play in recovery of heat-shocked microbes (20). Membrane filter holders were autoclaved or were sterilized dry using an ultraviolet sterilizer (Millipore Corp., Mississauga, Ont.).

Plates were incubated aerobically for two to three days at 27°C. *Pediococcus* and *Lactobacillus* were incubated at 27°C anaerobically in a $CO_2$ incubator that had been evacuated and filled twice with beverage grade $CO_2$. Plates were counted after four to five days.

**Treatment of Data**

Survival curves were obtained by plotting on 5–7-cycle semilog paper the mean of triplicate viable counts vs the time interval of sampling. The goodness of fit of these curves was determined by the linear regression method. D values (time required for a decrease in viability to 10% of original level) at each temperature were calculated from the slopes. TDT curves were constructed using linear regression. $D_{60}$ values were obtained by extrapolation.

The quadratic regression method was used to determine the equations of the curvilinear response of *L. frigidus*, and calculus was applied to determine the maximum slope of the survival curves.

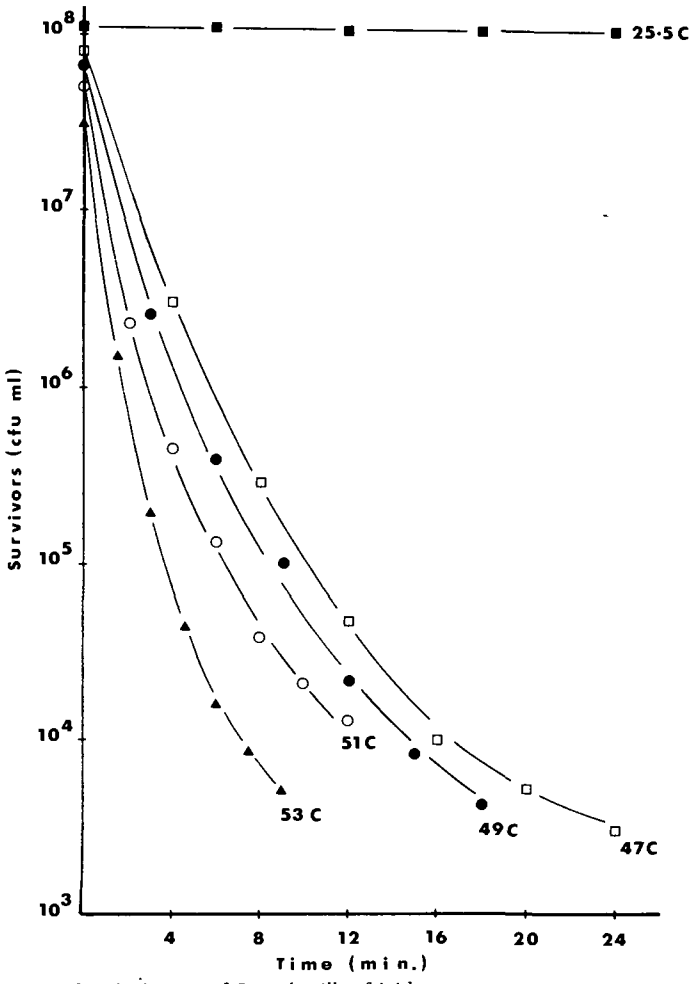


**Fig. 4.** Survival curve of *Lactobacillus frigidus*.

Statistical analysis was applied to calculate the coefficient of variation (c.v.) of various phantom TDT curves. Confidence limits of $D_{60}$ values were determined. All calculations were done in an Apple II Plus computer with the Visicalc software package (Apple Computer Inc., Cupertino, CA.)

**Simulated Pasteurization**

Presterilized beer bottles were filled with 340 ml of degassed beer to which $4.0 \times 10^7$ *Pediococcus acidilactici* per milliliter had been added. Bottles were sealed with specially designed C-10 locking connectors and C-18 beer bottle receptacles with attached 13-cm needlelike thermocouples (No. CNL, O.F. Ecklund Inc., Cape Coral, FL). Thermocouples are designed to reach the zone of the bottle that heats most slowly. Thermocouples were attached to a Process Multipoint Recorder, (model III, Honeywell, Fort Washington, PA) with 24-channel capacity. The temperature of each bottle was recorded every 15 sec.

At initiation of the experiment, bottles were plunged into racks in a shaker waterbath (Warner Chilcott Laboratories), which was adjusted to 75 rpm and kept at 62.0°C by two Lauda circulators (Brinkmann Instruments, Rexdale, Ont.). The agitation produced a pasteurization curve not unlike that obtained industrially. Cooling was by removal of bottles at the appropriate time into 15°C water. Survivors were enumerated by the membrane filtration technique (12). PU valves and total decimal reduction times were determined for each minute of heating.

## RESULTS AND DISCUSSION

Figures 1–4 show the survival curves for *S. carlsbergensis, S. willianus, L. delbrueckii,* and *L. frigidus*. Control experiments ensured that resuspension of the organisms into beer did not result in significant death unless temperatures were elevated. The sets or families of lines illustrated represent the four situations encountered when heat was applied in this study to the seven yeasts and three bacteria. All other yeasts and *P. acidilactici* were patterned exactly after Fig. 1 (all showing normal death kinetics with different slopes).

Semilog plots of survivors vs time gave straight lines with statistically significant negative correlation coefficients (99% confidence level) when plotted using the linear regression method. Rates of death, as expected, increased with increasing temperature, as can be seen when D values were plotted against temperature

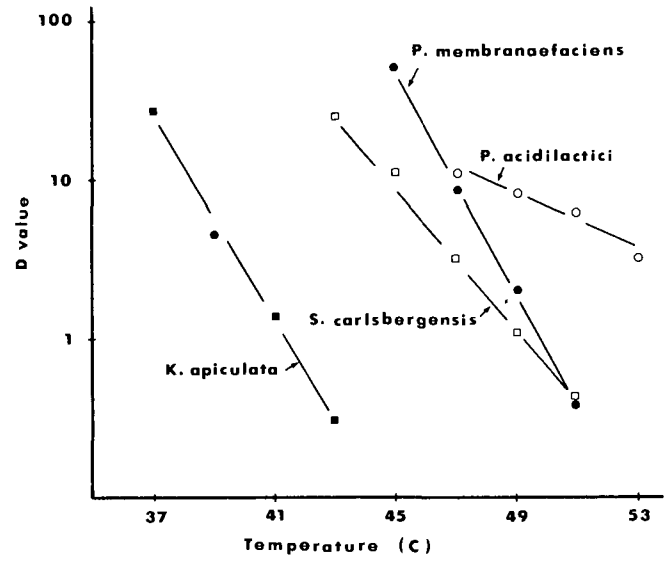


**Fig. 5.** Phantom thermal death time curves for some of the organisms with normal survival curves.

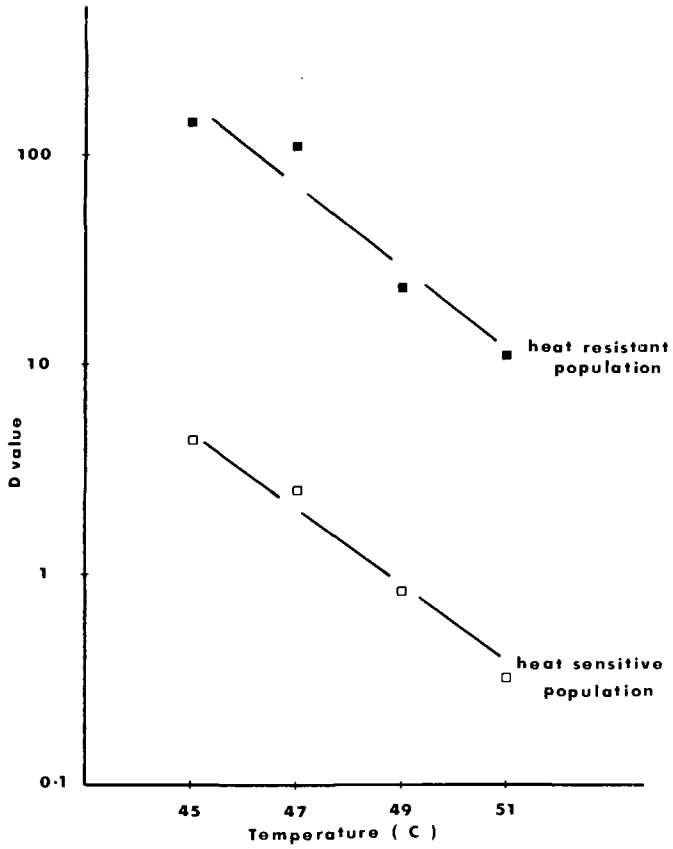


**Fig. 6.** Phantom thermal death time curves for *Saccharomyces willianus.*

(Fig. 5). For all seven microbes with survivor curves similar to Fig. 1, this plot, the phantom TDT curve, resulted in straight lines when either Log D or D on a semilog plot was plotted against temperature.

In Fig. 2, showing *S. willianus*, biphasic survival curves were obtained. In this case, D values could be obtained for both the heat-sensitive and the more resistant portions of the population. The explanation for biphasic kinetics appeared simple, but it had to be proved. The presence of a contaminant yeast or bacteria in the culture was ruled out. Then, an aliquot of the surviving population (after the inflection point of the line) was taken, regrown, and subjected to a similar heat treatment. Virtually identical kinetics were obtained, indicating that the surviving population grew up in new medium into the same heterogeneous biphasic population. Although we believed this proved the presence of a low but significant spore population with higher resistance, a further experiment was conducted to stimulate higher sporulation using acetate medium (13). Although the increase in spore percent was not large (from 0.03 to 0.25%), the population still yielded biphasic kinetics, this time with a higher level of more-resistant cells. The phantom TDT curves for both population segments are drawn in Fig. 6.

Figure 3 shows the survival curve of *L. delbrueckii.* This organism does not begin to die immediately on treatment but produces a plateau or shoulder in the curve (9). We have assumed that these organisms are mostly paired, as seen in shaken culture suspensions, and that the subsequent straight-line kinetics best represents death of derived single cells as measured by colony-forming units. This data treatment has been recommended earlier (24). The phantom TDT curve is found in Fig. 7.

Finally, Fig. 4 shows the survival curve of *L. frigidus.* This organism is more difficult to handle because of its tendency to form longer chains that cannot be broken up by shaking. The change in slope of the survival curve with time is believed to be caused by the fact that single cells require one target "hit" for death to occur,

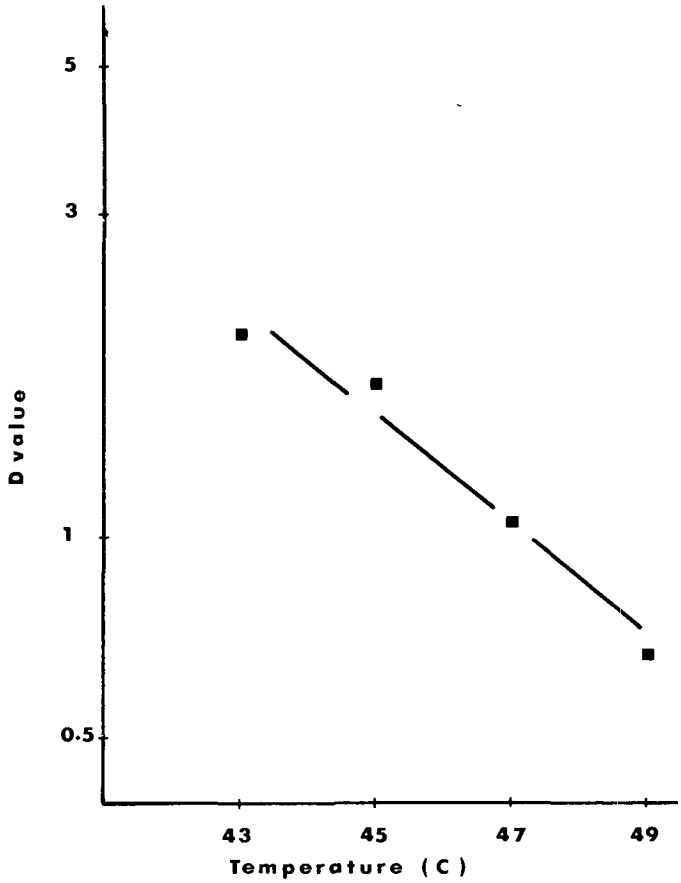


**Fig. 7.** Phantom thermal death time curve for *Lactobacillus delbrueckii.*

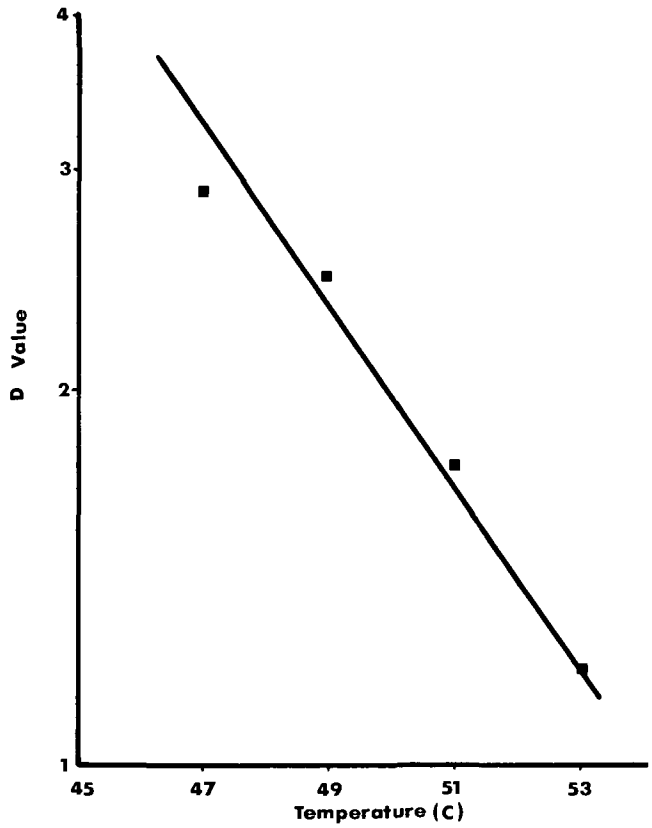


**Fig. 8.** Phantom thermal death time curve for *Lactobacillus frigidus.*

whereas doubles, triples, quadruples, etc. require correspondingly more heat before a loss of one colony-forming unit is seen by membrane filtration. This results in increasing cell resistance to heat by some members of the population (9), probably as chain length increases. As a result, the lines curve, and meaningful data could only be obtained by using quadratic regression (polynomial) to determine the equations of the curvilinear survival curves. Calculus was applied to determine the maximal slope. The D values so obtained were used to draw the phantom TDT curve (Fig. 8) for this organism.

Summaries of the kinetics of death and the statistical analysis on survival curves of all organisms are found in Table II. At temperatures of significance to brewers (ie, 60°C) *none* of the yeasts or yeast spores were particularly heat resistant. In fact even 1 min at 60°C would be sufficient to kill hundreds of logs of these brewing contaminants. *Kloeckera apiculata* is particularly sensitive to heat,

**TABLE II**
**Kinetic Parameters of Brewing Microbes Subjected to Moist Heat Destruction**

| Organism | Temperature (°C) | D Value (min) | Correlation Coefficient[a] | Z Value[b] for Organism (°C) |
|---|---|---|---|---|
| *Candida mycoderma* | 45 | 31.250 | −0.998 | 3.721 |
| | 47 | 4.237 | −0.990 | |
| | 49 | 1.992 | −0.999 | |
| | 51 | 0.649 | −0.970 | |
| *Kloeckera apiculata* | 37 | 27.027 | −0.992 | 3.174 |
| | 39 | 4.608 | −0.996 | |
| | 41 | 1.412 | −0.974 | |
| | 43 | 0.318 | −0.966 | |
| *Hansenula anomala* | 45 | 6.800 | −0.997 | 4.622 |
| | 47 | 2.618 | −0.998 | |
| | 49 | 0.873 | −0.989 | |
| | 51 | 0.354 | −0.986 | |
| *Pichia membranaefaciens* | 45 | 62.500 | −0.988 | 2.806 |
| | 47 | 8.929 | −0.977 | |
| | 49 | 2.040 | −0.999 | |
| | 51 | 0.430 | −0.964 | |
| *Saccharomyces carlsbergensis* | 43 | 26.316 | −0.997 | 4.415 |
| | 45 | 11.236 | −0.990 | |
| | 47 | 3.559 | −0.983 | |
| | 49 | 1.068 | −0.980 | |
| | 51 | 0.464 | −0.994 | |
| *Sacch. willianus* | | | | |
| Heat sensitive | 43 | 12.195 | −0.989 | 4.122 |
| | 45 | 3.665 | −0.970 | |
| | 47 | 1.626 | −0.983 | |
| | 49 | 0.386 | −0.987 | |
| Heat resistant | 47 | 125.000 | −0.952 | 2.742 |
| | 49 | 14.706 | −0.994 | |
| | 51 | 3.745 | −0.982 | |
| | 53 | 0.730 | −0.995 | |
| *Torulopsis colliculosa* | 43 | 8.771 | −0.992 | 5.005 |
| | 45 | 2.444 | −0.979 | |
| | 47 | 0.885 | −0.980 | |
| | 49 | 0.573 | −0.976 | |
| *Lactobacillus frigidus* | 47 | 2.857 | −0.995 | 15.395 |
| | 49 | 2.494 | −0.987 | |
| | 51 | 1.742 | −0.984 | |
| | 53 | 1.188 | −0.994 | |
| *Pediococcus acidilactici* | 47 | 11.765 | −0.994 | 11.170 |
| | 49 | 8.772 | −0.997 | |
| | 51 | 6.250 | −0.996 | |
| | 53 | 3.333 | −0.998 | |
| *L. delbrueckii* | 43 | 2.028 | −1.000 | 12.257 |
| | 45 | 1.701 | −0.995 | |
| | 47 | 1.063 | −0.991 | |
| | 49 | 0.678 | −0.983 | |

[a] Of survival curves.
[b] Slope of phantom thermal death time curve.

so much so that normal plate counts poured with 45–50°C molten agar would kill most if not all of a culture of this microbe. The importance of tempering molten agar used in the pour plate method of yeast enumeration is stressed to food microbiologists for all yeasts studied here. At temperatures near 50°C, all yeasts died very quickly. This was another reason why membrane filtration was used in this study to enumerate cells.

Table II also shows results for three heat-resistant bacteria. Although most brewery bacteria are comparatively more heat sensitive, two of the three bacteria chosen display a good deal of resistance to heat. Because yeasts of the type usually found in beer can be disregarded in determining conditions for pasteurization, all decisions about time-temperature relationships should be based on $D_{60}$ values for organisms such as *P. acidilactici*, which in this case displays a $D_{60}$ of 0.867.

Table III shows a detailed statistical analysis of the phantom TDT parameters. The c.v. gives a relative indication of the reliability of the phantom TDT curves. In general, yeasts exhibited smaller c.v. values than did bacteria. The 95% confidence interval of log $D_{60}$ shows the limits of variation of the predicted values. The calculation of the standard error of the predicted sample mean was based on the number of items (K items as described by Sokal and Rohlf [26]) used in the experimental survival curves. Thus, if the experiment could be conducted at 60°C, seven samples would be taken to determine the death rate of the organisms. The confidence intervals were not symmetrical when transformed from log to linear scale.

Consideration of the tables shows that considerable error may result if one considers only the lethal rate ($L_t$) equation:

$$L_t\,(°C) = \frac{1}{\text{Log}^{-1}\left(\frac{60-t}{Z}\right)}$$

where Z, the negative reciprocal of the slope of the phantom TDT curve according to Del Vecchio et al (4), was 6.94°C.

Although Hansen and Riemann (9) quoted Von Schelhorn as saying "a high resistance of an organism may be correlated with a high Z value" and although Z is significant in describing an organism's heat resistivity (15), we contend, as do others (14), that Z is *not* a measure of heat resistance. It is the only variable pertaining to the organism in the above formula and therefore cannot be used to calculate lethality. The following three examples should illustrate this point.

Figure 9 illustrates a hypothetical example (similar to that seen with *Kloekera* and *Pichia* wild yeasts or with *P. acidilactii* and *L. delbrueckii* [Table II]), comparing two phantom thermal death curves with similar slope (Z). The two curves, however, occur over vastly separated temperature gradients. In this case, a similar Z value certainly does not describe heat resistance nor would it

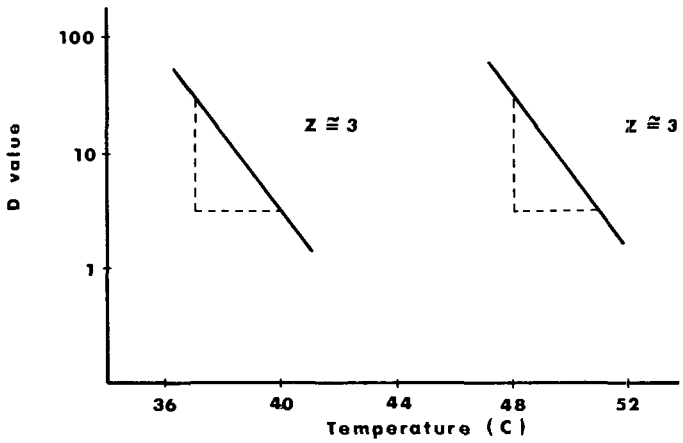


**Fig. 9.** Hypothetical phantom thermal death time curves of similar slope (Z value) obtained over vastly separated temperature gradiants.

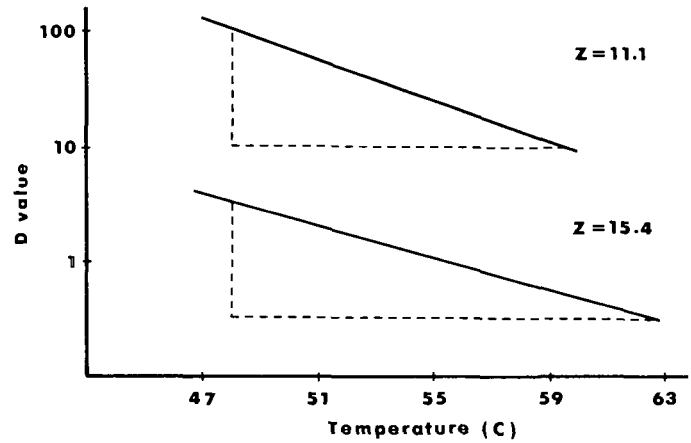


**Fig. 10.** Hypothetical phantom death time curves of similar slope (Z value) obtained over the same temperature gradiant.

**TABLE III**
**Statistical Analysis of Extrapolated $D_{60}$ Values**

| Organism | c.v.[a] | Log $D_{60}$ ($D_{60}$)[b] | S. E. of Pred. Mean[c] | Log 95% C.I.[d] | Antilog 95% C.I.[e] |
|---|---|---|---|---|---|
| *Candida mycoderma* | 14.492 | −2.667 (0.002) | 0.480 | (−4.732, −0.601) | ($1.851 \times 10^{-5}$, 0.250) |
| *Kloeckera apiculata* | 5.309 | −5.865 (0.0000014) | 0.338 | (−7.319, −4.411) | ($4.802 \times 10^{-8}$, $3.880 \times 10^{-5}$) |
| *Hansenula anomala* | 2.545 | −2.411 (0.00388) | 0.068 | (−2.703, −2.119) | ($1.993 \times 10^{-3}$, $7.596 \times 10^{-3}$) |
| *Pichia membranaefaciens* | 4.433 | −3.605 (0.00025) | 0.195 | (−4.442, −2.767) | ($3.610 \times 10^{-5}$, $1.711 \times 10^{-3}$) |
| *Saccharomyces carlsbergensis* | 3.721 | −2.401 (0.004) | 0.114 | (−2.763, −2.038) | ($1.725 \times 10^{-3}$, $9.158 \times 10^{-3}$) |
| *S. willianus* | | | | | |
| Heat sensitive | 7.239 | −3.034 (0.00092) | 0.251 | (−4.113, −1.955) | ($7.707 \times 10^{-5}$, $1.108 \times 10^{-2}$) |
| Heat resistant | 6.461 | −2.722 (0.0019) | 0.245 | (−3.775, −1.669) | ($1.678 \times 10^{-4}$, $2.143 \times 10^{-2}$) |
| *Torulopsis colliculosa* | 14.695 | −2.538 (0.0029) | 0.419 | (−4.342, −7.343) | ($4.552 \times 10^{-5}$, $1.844 \times 10^{-1}$) |
| *Lactobacillus frigidus* | 13.872 | −0.357 (0.440) | 0.094 | (−0.760, 0.045) | (0.174, 1.110) |
| *Pediococcus acidilactici* | 13.519 | −0.062 (0.867) | 0.126 | (−0.603, 0.479) | (0.249, 3.011) |
| *L. delbrueckii* | 12.958 | −1.043 (0.091) | 0.152 | (−1.696, −0.390) | (0.020, 0.407) |

[a] Coefficient of variation of phantom thermal death time curve = $(S_b/b) \times 100\%$, where $S_b$ = standard deviation of the slope and b = absolute value of the slope.

[b] Extrapolated value from the phantom thermal death time curve.

[c] Standard error of the predicted sample mean of log $D_{60}$ based on number of samples; thus, K = 7 except *L. delbrueckii*, where K = 4 (26).

[d] 95% confidence interval based on (n−2) degrees of freedom for log $D_{60}$; n = 4 in all cases except *S. carlsbergensis*, where n = 5.

[e] Actual 95% confidence interval.

predict similar lethality of the two yeasts or the two bacteria by the formula!

Figure 10 describes two organisms (similar to *L. frigidus* and *P. acidilactici*) with similar Z values and kinetics obtained over the same temperature range. One organism, however, is markedly more resistant (has higher D values) than the other, and again the similar Z value inserted into the formula above would not result in lethality descriptions appropriate for both microbes!

Figure 11 shows hypothetical phantom thermal death curves for two microbes (similar to the vegetative cells and spores of *S. willianus*) that have different Z values over different temperature ranges. Here, although the spores have a smaller Z value, they are more resistant than vegetative cells. This is contrary to the statement by Von Schelhorn (cited in 9) and to what is predicted by the use of Z values alone in Del Vecchio's formula for lethal rate, in which his abnormally heat-resistant yeast became the example for all to use in subsequent brewing pasteurization.

Our suggestion, following careful examination, is that after compiling extensive data on heat resistance of many brewery contaminants (using D values at 60°C, for example), the inactivation factor (IF) concept (14) should be applied, using the equation[2]:

$$IF = 10^{(\text{treatment dose/D value})}$$

For example if the $D_{60}$ value of the most resistant microbe in brewing was 1 min and the total treatment dose selected was 9 min (considering fractional D values at each temperature of heating and cooling as well as a holding time), then the IF would be $10^9$. If the average number of organisms per bottle of beer was 1,000, this

[2]This concept is valid as long as an exponential phantom TDT curve is obtained. If the curve has a shoulder, IF is overestimated; if the curve has a tail, IF is underestimated.

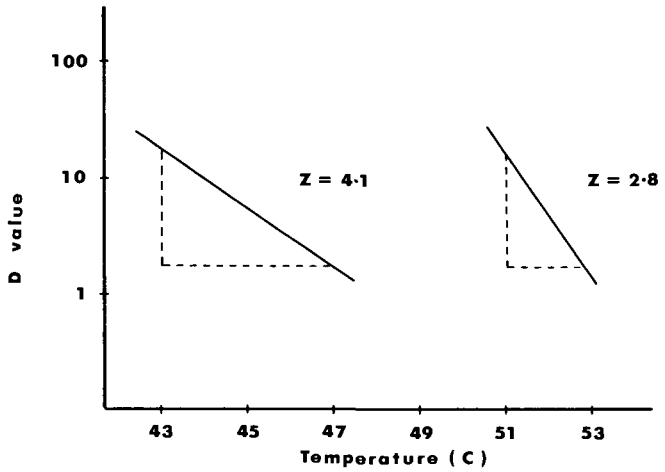


**Fig. 11.** Hypothetical phantom thermal death time curves with different slopes (Z values) obtained over different temperature gradiants.

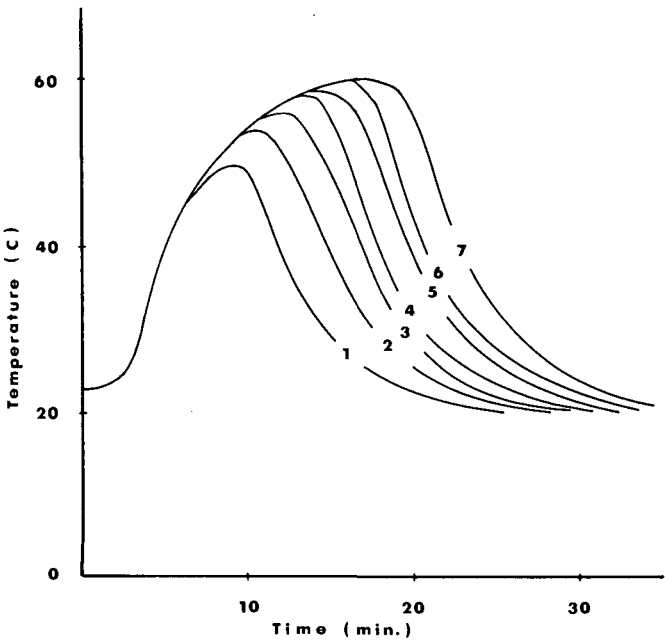


**Fig. 12.** Heating curves for simulated industrial pasteurization.

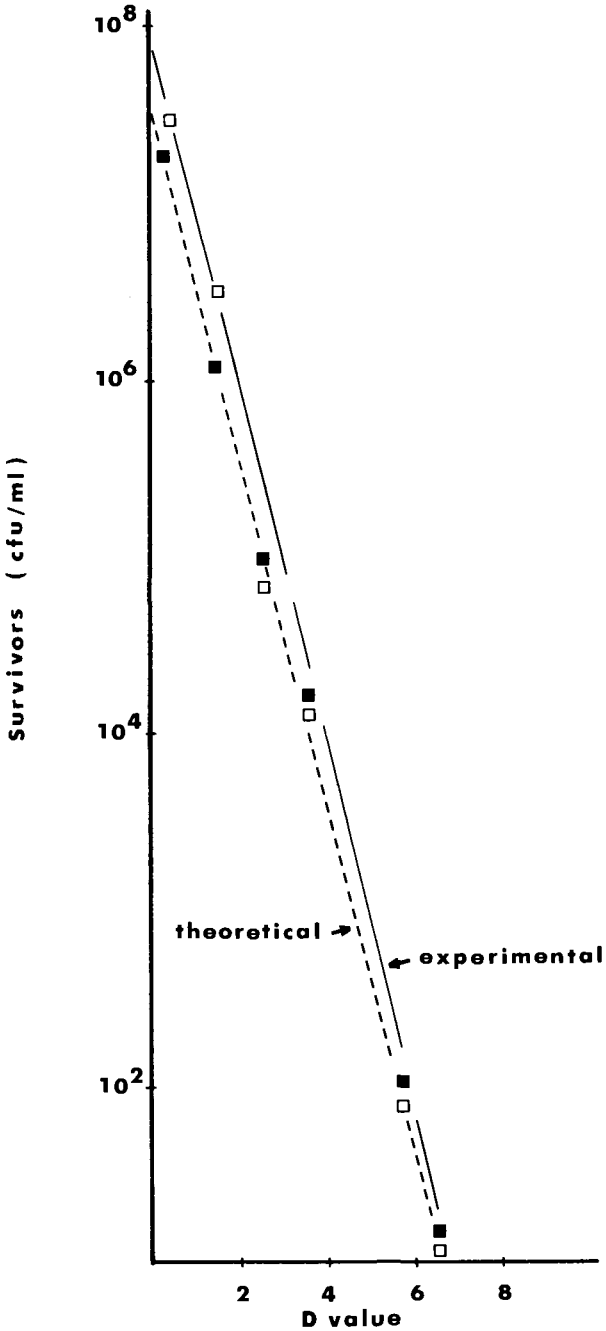


**Fig. 13.** Predictive cell death (by D values) vs actual experimentally measured cell death, using *Pediococcus acidilactici*.

treatment should yield the expectation of one contaminated bottle in $10^6$ bottles of pasteurized beer, ie,

**TABLE IV**
**Theoretical D Values and Obtained Experimental Data for Simulated Pasteurization of Contaminated Beer**

| Curve[a] | PU[b] | $D_{total}$[c] | Survivors per Milliliter[d] |
|---|---|---|---|
| 1 | 0.06 | 0.337 | $2.8 \times 10^7$ |
| 2 | 0.55 | 1.54 | $3.2 \times 10^6$ |
| 3 | 1.16 | 2.62 | $6.9 \times 10^4$ |
| 4 | 1.68 | 3.45 | $1.3 \times 10^4$ |
| 5 | 3.28 | 5.59 | $8.0 \times 10^1$ |
| 6 | 4.01 | 6.44 | $1.2 \times 10^1$ |
| 7 | 6.10 | 8.81 | 0 |

[a]Curves 1–7 are shown in Fig. 12.
[b]Pasteurization units, 1 PU = 60°C for 1 min.
[c]$D_{4^\circ-60^\circ C}$ based on *Pediococcus acidilactici.*
[d]Initial population $4.0 \times 10^7$/ml.

$$\text{Degree of sterility} = \frac{\text{IF}}{\text{Average no. of organisms per bottle}}$$

The importance of low numbers of microbes in unpasteurized beer can be easily shown to plant personnel using these formulas. The canning industry, for example, uses a 12 D approach (IF = $10^{12}$), basing the heat treatment on the D value of pathogenic, heat-resistant *Clostridium botulinum* spores and the risk of economic loss from the death of a consumer.

Figure 12 shows the heat processing curves for the samples taken and the corresponding table (Table IV) shows the theoretical D values and obtained experimental data for the simulated pasteurization of contaminated beer. The predicted death of *P. acidilactici* was calculated from D values at various temperatures obtained from the phantom TDT curve.

The closeness of the actual data to predicted values (Fig. 13) proves the value of laboratory experiments that are less practically designed. These values can be used for prediction of industrially oriented pasteurization. The fact that D values are proportional to kill and that pasteurization units do not necessarily correspond with cell death should be noted. Even so, pasteurization of beer infected with $4 \times 10^7$ pediococci per milliliter was effected by only 6.1 PU. The level of contamination (and resistance of the only organism added) far exceeds that usually found in unpasteurized beer destined to be bottled.

In summary, we believe that the information reported here should rekindle interest in rigorously testing a large number of other isolates of *Lactobacillus, Pediococci*, other bacteria, and wild yeast in order to determine the most heat-resistant microbe on which to base time-temperature levels to maximize energy utilization but safely accomplish pasteurization.

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## LITERATURE CITED

1. Ault, R. G. *J. Inst. Brew.* 71:376, 1975.
2. Brenner, M. W., and Hsu, W. P. *Tech. Q. Master Brew. Assoc. Am.* 8:45, 1971.
3. Coleman, M. *Brew. Guard.* 105(10):51, 1976.
4. Del Vecchio, H. W., Dayharsh, C. A., and Baselt, F. C. *Am. Soc. Brew. Chem., Proc. 1951*, p. 45.
5. Epstein, S. S., and Dee Snell, F. *J. Inst. Brew.* 46:175, 1940.
6. Findlay, W. P. K. Modern Brewery Technology. The Macmillan Press: London, 1971, p. 214.
7. Gilliland, R. B. *Brew. Guard.* 96:37, 1967.
8. Gilliland, R. B. *Inst. Brew. (Aust. N.Z. Sect.) Proc. 16th Cont., Sidney, N.S.W., 1980,* p. 135.
9. Hansen, N. H., and Riemann, H. *J. Appl. Bact.* 26(3):314, 1963.
10. Hough, J. S., Briggs, D. E., and Stevens, R. Malting and Brewing Science. Chapman and Hall Ltd.: London, 1971, p. 555.
11. Ingledew, W. M. *J. Am. Soc. Brew. Chem.* 37:145, 1979.
12. Ingledew, W. M., Burton, J. D., Hysert, D. W., and Van Gheluwe, G. *J. Am. Soc. Brew. Chem.* 38:125, 1980.
13. Ingledew, W. M., and Casey, G. P. 1982. *Brew Dig.* In press.
14. Ingram, M. Sporeformers as food spoilage organisms. Page 549 in: Gould, G. W., and Hurst, A., eds. The Bacterial Spore, Academic Press: New York, 1969.
15. King, L. M., Egan, L., Schisler, D., and Hahn, C. W. *J. Am. Soc. Brew. Chem.* 36(3):144, 1978.
16. Kleyn, J., and Hough, J. *Ann. Rev. Microbiol.* 25:583, 1971.
17. Laufer, S., and Brenner, M. W. *Am. Soc. Brew. Chem., Proc. 1940*, p. 30.
18. Lawrence, D. R., and Leedham, P. A. *J. Inst. Brew.* 85:119, 1979.
19. Lund, A. *J. Inst. Brew.* 52:207, 1946.
20. Menegazzi, G. S., and Ingledew, W. M. *J. Food Sci.* 45(2):182, 196, 1980.
21. Murdock, D. I., Troy, V. S., and Folinazzo, J. F. *Food Res.* 18:85, 1972.
22. Portno, A. D. *J. Inst. Brew.* 74:291, 1968.
23. Put, H. M. C., De Jong, J., Sand, F. E. M. J., and Van Grinsven, A. M. *J. Appl. Bact.* 40:135, 1976.
24. Russell, A. D. The destruction of bacterial spores. Page 521 in: Hugo, W. D., ed. Inhibition and Destruction of the Microbial Cell. Academic Press: New York, 1971, p. 512.
25. Scruggs, C. E., and Baselt, F. C. *Wallerstein Lab. Commun.* 18:159, 1955.
26. Sokal, R. R., and Rohlf, F. J. Biometry, the Principles and Practice of Statistics in Biological Resesarch. W. H. Freeman and Co.: San Francisco, 1969.
27. Splittstoesser, D. F., Lenk, L. L., Wilkinson, M., and Stamer, J. R. *Appl. Microbiol.* 30:369, 1975.
28. Wilcox, I. C. *J. Inst. Brew.* 72:236, 1966.

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