**U. S. Food and Drug Administration**
**Center for Food Safety and Applied Nutrition**
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# Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

# Overarching Principles: Kinetics and Pathogens of Concern for All Technologies

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## 1.KINETIC PARAMETERS FOR INACTIVATON OF MICROBIAL POPULATIONS

### 1.1. Models and Parameters

Kinetic parameters and models are used for the development of food preservation processes to ensure safety. They also provide the tools to compare the impact of different process technologies on reduction of microbial populations. The parameters used to analyze and report the reduction of a microbial population as a function of process parameters include empirical coefficients experimentally determined from microbial reduction kinetics, as well as constants from expressions based on chemical reaction kinetics. The purpose of this section is to present the models and kinetic parameters used to present and compare microbial inactivation data from thermal, pressure and electromagnetic processes.

#### 1.1.1. Rate constants

The traditional approach to describing changes in microbial populations as a function of time has used the survivor curve equation:

$$\log [N / N_0] = -t / D \quad (1)$$

where:

N = microbial population at any time, t

$N_0$ = initial microbial population

D = decimal reduction time, or time required for a 1-log cycle reduction in the microbial population.

The corresponding model from chemical reaction kinetics is the first-order kinetic model:

$$dN / dt = -kN \quad (2)$$

where:

k = reaction rate constant (first-order), or the slope of the natural logarithm of survivors in contrast to time for the microbial population.

Equation (2) can be integrated to obtain a more familiar expression for the reduction of microbial populations:

$$\ln [N / N_0] = - k t \quad (3)$$

By comparing Eq. (1) and (3), the relationship between the decimal reduction time and the first-order reaction rate constant is:

$$k = 2.303 / D \quad (4)$$

The primary parameters (D-value or k) would describe the microbial population reduction at a constant and defined temperature, pressure and/or electric field. The inherent assumption in the use of these models (and the corresponding parameters) is that the reduction in microbial population is described by the first-order reaction model. Alternative models are being developed to explain microbial inactivation kinetics when the linearity of the data is questionable (Peleg and Cole 1998; Anderson 1996). If there is evidence of a different reaction model, different parameters need to be identified and used for process development and prediction purposes.

Only a limited amount of the published data on microbial inactivation has been analyzed using the reaction rate model to quantify first-order rate constants (k). On the other hand, most published data on changes of food quality attributes have been presented as reaction rate constants (k). As indicated by the relationship between D-value and k, published data can be easily transformed.

### 1.1.2. Temperature coefficients

Traditionally, the influence of temperature on microbial population inactivation rates has been expressed in terms of the thermal resistance constant (z-value) using the following model:

$$\log [D / D_R] = -(T - T_R) / z \quad (5)$$

The thermal resistance constant z(T) is the temperature increase needed to accomplish a 1-log cycle reduction in the D□value. The reference decimal reduction time ($D_R$) is the magnitude at a reference temperature ($T_R$) within the range of temperatures used to generate experimental data. Microbial populations with higher resistance to temperature change are described by larger z(T). The most evident examples are the larger z(T) for spores compared to the ones for vegetative cells.

An alternative model for describing the influence of temperature on microbial population reduction rates is the Arrhenius equation. The model illustrates the influence of temperature on the reaction rate constant (k), as follows:

$$k = k_0 \exp [-E / R T_A] \quad (6)$$

where:

$k_0$ = Arrhenius Constant

E = Activation Energy Constant

$T_A$ = Absolute Temperature

R = Universal Gas Constant

Based on the Arrhenius model (Eq. 6), the slope of ln (k) in contrast to $1/T_A$ plot determines the temperature coefficient E (activation energy constant) . The activation energy constant describes the influence of temperature on the magnitude of the first-order reaction rate constant (k).

When the thermal resistance model and the Arrhenius model are applied to microbial population reduction rate data over the same temperature range, a relationship between the 2 coefficients [z(T) and E] is evident. By comparison of Eq. (5) and (6), the following relationship can be obtained:

$$E = 2.303 R T_A^2 / z \quad (7)$$

The temperature used in Eq. (7) should be selected as a mid-point in the range of temperatures used to generate the original experimental data. Equation (7) does suggest that the relationship between the 2 temperature coefficients [E and z(T)] depends on temperature. The magnitudes of the 2 coefficients, however, are significantly different, and any influence of temperature is negligible as long as the temperature reference is within the range used for data collection. The use of the coefficients [z(T) or E] should be limited to the range of temperatures used to obtain experimental D-values. The z(T) should only be used with a defined reference temperature as emphasized by Datta (1992).

The use of the first-order models and the corresponding models for temperature influence must be applied within the limits of data used to generate the parameters within the

expressions. The estimation of the kinetic parameters from the appropriate model requires careful attention to statistical limits created by the experimental data. Several authors, including Arabshahi and Lund (1985) and van Boekel (1996), have demonstrated the influence of statistical parameters on the use of the prediction models.

### 1.1.3. Pressure coefficients

There are only limited references to parameters used to describe the influence of pressure on the rate of microbial population reduction. Zook and others (1999) have used a parameter similar to the thermal resistance constant z(T), based on the following model:

$$\log[D / D_R] = -(P - P_R) / z \quad (8)$$

where:

$D_R$ = decimal reduction time at a reference pressure ($P_R$).

In this report, the pressure coefficient will be defined as:

z(P) = the pressure increase required to accomplish a 1-log cycle reduction in the decimal reduction time (D-value).

In order for pressure resistance constant z(P) to be meaningful, it is important to include a minimum of 3 D-values in the analysis of data. All D-values must be obtained at the same temperature and above the threshold pressure needed for the target microbial inactivation. The threshold pressure (or critical pressure) is the pressure below which microbial inactivation does not occur.

An alternate model to describe the influence of pressure on microbial inactivation rates is based on the Eyring equation, as proposed by Weemaes and others (1999). The model describes the reaction rate constants (k) as follows:

$$\ln (k) = \ln(k_R) - [V( P - P_R)/RT_A] \quad (9)$$

where:

$k_R$ = reaction rate constant at reference pressure ($P_R$)

V = activation volume constant

P = pressure

$T_A$ = absolute temperature

The activation volume constant (V) is the pressure coefficient obtained from the slope of the ln (k) in contrast to ($P - P_R$) plot. The magnitude of V increases as the slope of the

plot increases. When the rate of microbial inactivation increases significantly with small changes in pressure, the magnitude of the V will be larger. Alternatively, smaller values of V describe microbial populations with inactivation rates that would change less when pressure changes. As suggested when describing z(P) values, it is important for all reaction rate constants (k) used in the analysis to be measured at the same temperature. For the activation volume constant (V) to be useful and meaningful, the k constant should be measured at pressures above the threshold pressure needed to inactivate the target microbial population.

### 1.1.4. Electric field coefficients

As in the case of pressure processes, when microbial populations are exposed to pulsed electric fields (PEF), the electric field intensity applied should be above the threshold electric field intensity, the critical electric field intensity for the target microorganism. A model similar to those for temperature and pressure can be used to describe the influence of electric field intensity on the rate of microbial population reduction. The proposed model would be:

$$\log [D / D_R] = -(E - E_R) / z \quad (10)$$

where:

$D_R$ = decimal reduction time at a reference electric field intensity ($E_R$).

The electric field coefficient in this model is defined as:

z($E$) = the increase in electric field intensity ($E$) required to reduce the decimal reduction time (D) by 1-log cycle at a specific temperature and pressure.

All D-values used in this type of analysis should be acquired at the same temperature and pressure. A minimum of 3 D-values should be obtained for the data analysis.

An alternative model for describing the influence of electric field intensity on the survival of a microbial population was proposed by Peleg (1995). The model is based on the Fermi equation and can be expressed as:

$$N / N_0 = 1/\{1 + \exp[( E - E_d ) / K]\} \quad (11)$$

where:

$E_d$ = the electric field intensity when microbial population has been reduced by 50%.

$K$ = a coefficient with magnitude based on the slope of the survivor curve obtained at several levels of electric field intensity.

This model has been applied to survivor data for several different microbial populations to generate typical magnitudes of the coefficient (*K*) (Peleg 1995). Larger magnitudes of the coefficient would suggest a higher resistance to changes in electric field intensity.

A similar model has been proposed and used by Hulsheger and others (1981) and applied by Jeyamkondan and others (1999). The model describes the survivor number as a function of electric-field strength and treatment time:

$$N / N_o = \{ t / t_c \}^{[-(E - Ec) / K]} \qquad (12)$$

where:

t = treatment time

$t_c$ = critical treatment time or treatment time below which no inactivation of microorganisms occurs

$E_c$ = critical electric field strength or electric field strength below which no inactivation of target microorganism occurs

*K* = specific rate constant

The model proposed by Hulsheger and others (1981) is similar to Eq. (11), but accounts for exposure time at a given electric field intensity. The coefficient (*K*) has a similar relation to electric field intensity as in Eq. (11) and the relative magnitudes should be interpreted in the same manner.

### 1.2. Kinetic Parameters for Inactivation of Microbial Pathogens

The purpose of this section is to provide an overview and discussion on the kinetic data of microbial population inactivation. This section addresses the use of kinetic parameters for development of processes and the comparison of parameters obtained for various microorganisms, including a discussion on the limitations of the parameters. Finally, the research needs will be addressed, with specific attention to recommendations on experimental approaches to be considered in the future.

Kinetic parameters describing the inactivation of microbial pathogens are presented in Tables 1A, B and C and are a summary of parameters presented in other sections of this report. The intent of the summary is to provide an overview and a comparison of the kinetic parameter magnitudes for the various microorganisms for each process technology. The parameters defined in Section 1.1. (D-value and z(T), z(P), z(*E*), E, k, K and V) have been calculated from data previously reported and using the models in Section 1.1 for thermal, pressure and PEF technologies. The parameters for thermal treatment also apply to microwave energy and electrical resistance (ohmic) processes, as well as any other technology where temperature is the primary factor in reduction of the microbial population. Likewise, the parameters for pressure or PEF treatments should

apply to any process where pressure or electricity is the primary critical factor in reducing microbial populations. It must be noted that, given the scarcity of data, these are estimated parameters and there is an imminent need for more research in this area. Although this report contains references to several other technologies, the quantity of data describing the influence of the treatment on reduction of microbial populations is insufficient at this time.

Like in most of the published literature, in this report data have been analyzed assuming that the reduction in microbial populations follows a linear first-order model, with the exception of the PEF parameters that will be discussed in Section 1.2.2.4. The potential of non-linear inactivation data or the use of alternative models cannot be ignored. Because there is currently insufficient information on alternative models to allow the type of comparisons being considered in this portion of the report, these issues will be discussed when describing the specific technologies.

The use of consistent parameters for all preservation technologies should improve the efficiency of future investigations and encourage uniformity in the methodologies for establishment of minimum process requirements.

### 1.2.1. Process development

The parameters presented in Tables 1A, 1B and 1C parallel the traditional parameters used for development of thermal preservation processes. The basic model for process development is based on the survivor curve Eq. (1) or (2):

$$F = - D \log [ N_0 / N ] = D \log [ N / N_0 ] \quad (13)$$

or:

$$F = - \ln [ N_0 / N ] / k = \ln [ N / N_0 ] / k \quad (14)$$

where F is the total time required to reduce the microbial population by a specified magnitude needed to ensure product safety, under the conditions defined by D-value or k. The basic model assumes a linear first-order relationship between microbial population and time. Currently, there is a lack of historical evidence to support alternative models; however, there is considerable discussion about the appropriateness of using a first-order model to describe the reduction in microbial population for all preservation technologies. For example, models for PEF technology as presented in Eq. (11) and (12) should continue to be evaluated, but at this time, input parameters for these models are limited.

### 1.2.2. Inactivation data and parameters

#### 1.2.2.1. Limitations of the calculated parameters

A few limitations need to be considered when interpreting the parameters presented in Tables 1A, B and C. Care should be taken when they are intended to be used as tools to

develop processes, to compare the resistance of different microbial populations, or to identify appropriate surrogate microorganisms.

As illustrated in this report, the kinetic parameters for microbial populations exposed to thermal treatments have been assembled over a significant period of time. Over time, the published literature has included kinetic parameters needed to respond to most process, product and microbial situations. The parameters provide a sound basis to develop processes for the microwave energy and electrical resistance (ohmic) technologies. In addition, the available parameters provide a sound basis to compare different microbial populations and the influence of different product environments on the parameter magnitudes. The key issue for these electrothermal treatments is the lack of conclusive evidence on the existence of non-thermal effects influencing the reduction in microbial populations. It is believed, however, that those effects would add an extra factor of safety to the preservation process (see Microwave and Ohmic and Inductive Heating chapters).

In general, the data used to determine the D-values (and k-values) for pressure processes appear to be adequate. The limitations to these data are primarily associated with temperature control during pressure treatments. In addition, when temperature changes have been reported, the influence on the kinetic parameters has not been analyzed. The evidence suggesting a synergistic impact of pressure and temperature is too limited for use in process evaluation.

The most serious deficiency in pressure process kinetics is that most of the parameters (D and k) have been measured at a single pressure. Only 4 studies (Rovere and others 1996; Kalchayanand and others 1998; Zook and others 1999; Reddy and others 1999) have used 3 to 5 pressure levels, while controlling all other factors influencing the parameters. The results from these studies are adequate to evaluate the pressure coefficient [z(P)] and/or activation volume [V]. With exception of the 4 publications cited above, the estimated parameters are limited by the number of pressure magnitudes used, the lack of temperature control and the lack of multiple data for the same microorganism and/or product/substrate. By overcoming these limitations, parameters from future investigations will meet the needs of process development and product/microorganism comparisons.

The data available on the influence of PEF on microbial populations have many limitations. As will be indicated during the discussion of parameters in Table 1C, the kinetic parameters (D-value or k) are based on 2 points on the survivor curve, the initial population and the final population. It should be recognized that the values of parameters in Table 1C were not based on linear regression analysis. In addition, temperature controls and collection of multiple data points at the same temperature level are lacking.

At this time, no single report has measured the inactivation of microbial populations at several levels of electric field strength, leading to the quantification of the PEF coefficient z(*E)* . Although 3 such coefficients are presented in Table 1C, these coefficients have been estimated based on kinetic parameters reported in separate investigations and must be used with these limitations in mind. There are no published reports that evaluate the potential for a synergistic influence of electric field strength and

temperature. There are only 2 reports with kinetic parameters based on Eq. (11) and (12) and these reports provide limited parameters on microorganisms of food safety concern. They do not include any of the microorganisms of food safety concern.

### 1.2.2.2. Thermal processes

The literature provides an impressive array of kinetic parameters to be used in the development of thermal processes. In addition to data and parameters on inactivation of microbial populations, Table 1A includes additional information on the medium used and specific experimental conditions (that is, temperature) when available. The time parameters are the decimal reduction time (D-value) and the corresponding rate constant (k). The temperature coefficients include the thermal resistance constant [z(T)] and the activation energy constant (E).

The kinetic parameters calculated for the thermal inactivation of microbial pathogens in Tables 1A, B and C should be considered when using any process technology where temperature is the primary mode of microbial inactivation. The most promising alternative thermal processes to reduce pathogenic microbial populations are microwave energy and electrical resistance (ohmic), which are included in this report. As suggested in the chapters on microwave and ohmic and inductive heating, it is assumed that the direct influence of microwave energy or electrical resistance on microorganisms is negligible. Therefore, thermal kinetic parameters should be considered for the above-mentioned electrothermal processes (ohmic, inductive and microwave heating).

Kinetic parameters for vegetative cells of *Salmonella* serovars*,* pathogenic *Escherichia coli, Yersinia enterocolitica,* pathogenic *Vibrio* spp*., Aeromonas hydrophila, Campylobacter jejuni, Listeria monocytogenes* and *Staphylococcus aureus* are presented in Table 1A. In general, the D-values are relatively small and the k-values are relatively large for the vegetative microorganisms normally targeted in pasteurization or other mild thermal processes. Other than the abnormally high D-values (low k-values) for *Salmonella* pathogens in milk chocolate, *Salmonella* Typhimurium and *L. monocyctogenes* are the most thermally resistant vegetative microorganisms. The largest D-value for Salmonella Typhimurium is 18.3 min (k= 0.126/min) at 55C. For *L. monocyctogenes*, the largest D-value is 16.7 min (k = 0.14/min) at 60C. The largest D-values for *E. coli* are 6.6 min (55 °C) for O111:B4 and 6.4 min (57 °C) for O157:H7. Based on limited data for O157:H7 in ground beef, a z(T) of 5.3 °C has been estimated. Other significant magnitudes for D-values include 6.6 min for *A. hydrophila* at 48 °C and 16.7 min for *L. monocytogenes* in cured ground beef at 60 °C. A z(T) of 5.56 °C for *L. monocytogenes* in milk has been estimated, based on published data.

In general, the thermal resistance constants z(T) for the vegetative microorganisms fall in the range between 4 and 7.7 °C. This range includes a z(T) of 5.3 °C for *E. coli* O157:H7 in ground beef and of 5.56 °C for *L. monocytogenes* in milk, both estimated from limited data presented in the references cited. The larger z(T) presented include 12.4 to 25 °C for *Vibrio* species (in fish products) and 17.7 to 18.9 °C for *Salmonella* serovars (in milk

chocolate). These abnormally high z(T) for vegetative microorganisms should be noted for these products and may be specifically associated with them.

*S. aureus,* a vegetative microorganism that produces a heat-stable toxin, has D-values similar to other vegetative populations. The z(T) of 9.5 °C is relatively high and must be considered when developing processes for situations where *S. aureus* could present a health hazard.

The largest D-value (smallest k-value) reported at 110 °C for toxin-producing, sporeforming microorganisms is 12.42 min (0.185/min) for *Clostridium botulinum* proteolytic Type B spores in pureed peas. Most other D-values are in the more typical range of 1 to 3 min for spore-forming microorganisms. Other values to be noted are the D-value of 36.2 min (k = 0.064/min) for *Bacillus cereus* spores at 95 °C and 100 min (k = 0.023/min) for *C. botulinum* non-proteolytic Type E spores at 70 °C. When expressed at 110 °C, these D-values become 1.18 min for the *B. cereus* spores and less than 1 sec for the Type E spores.

Data for *Bacillus subtilis* spores have been included in Table 1A to illustrate the influence of ohmic heating on inactivation kinetics. These data were reported by Cho and others (1999) and indicate that the reduction in D-value (higher k-value) and the increase in z(T) (lower E) when using ohmic heating are statistically significant. These results suggest an independent and additional inactivation mechanism due to the electric current during the ohmic heating. The overall influence of these non-thermal effects, however, is not sufficient to consider the use of alternate kinetic parameters for development of ohmic heating processes. These authors have demonstrated that a 2-stage process involving ohmic heating, interrupted by a 20-min incubation, resulted in enhanced inactivation of *B. subtilis* spores. This increase in inactivation has been attributed to the positive influence of electric treatment on spore germination.

Separate data for microwave heating are not included in this section. The non-thermal effects of microwave processes on microbial inactivation have not been confirmed and appear to be of insufficient magnitude to be considered during development of processes.

### 1.2.2.3. Pressure processes

For processes involving the use of pressure for reduction of microbial populations, the F-value is the time the product needs to be exposed to the specified pressure and other conditions (that is, temperature) to accomplish the recommended amount of inactivation. Since the application of most of the pressure technologies involves instantaneous adjustment to the process pressure, the use of the basic model is straightforward. The pressure coefficients [z(P) or V] provide users with the flexibility to select the most appropriate pressure for the specific application. For pulsed-pressure technologies, the model would need to incorporate the influence of time and incremental pressure. In this case, the estimation of kinetic parameters will require the measurement of other variables.

The kinetic parameters for inactivation of microbial populations due to pressure are presented in Table 1B. The time parameters, decimal reduction time (D-value) and first-order rate constant (k), were calculated based on the reduction in microbial population at a constant pressure. The pressure coefficients are z(P) and the activation volume constant (V), as defined in Section 1.1.3. and indicate influence of pressure on the rate of inactivation. In most references cited, there are insufficient data to estimate these coefficients. Special consideration needs to be given to the combined use of pressure and temperature. Based on the current available information, the z(P) and z(T) parameters should be adequate for process development. The combined influence of pressure and temperature on inactivation kinetics for microbial populations has been investigated, although not extensively. Published reports suggest a synergistic impact of pressure and temperature on inactivation rates, but additional investigations are needed. The independent influence of pressure on rates, as indicated by the z(P) or V parameters, needs to be clearly established. The influence of temperature can be quantified in several ways, but the optimum approach would be based on the dependence of z(P) or V on temperature. Although minimum pressure thresholds for microbial inactivation are not presented in this section, these parameters are discussed in the section on high pressure processing.

Several investigations on Salmonella indicate that decimal reduction times (D-value) range from 1.48 to 6 min (k = 0.348 to 1.556/min), with pressure having an obvious influence on the rate. Most of the studies have been conducted at ambient temperatures (20 to 25 °C). The D-values for *E. coli* are as high as 15 min (k = 0.154/min) at 300 MPa and 6 min (k = 0.384/min) at 600 MPa for O157:H7. There are insufficient data to establish the influence of pressure or temperature and therefore z(P) or z(T) were not estimated.

Pressure appears to have a significant influence on inactivation rates for populations of *S. aureus,* apparently one of the most pressure-resistant vegetative bacteria, as suggested by D-values of 7.14 min (k = 0.323/min) at 600 MPa compared to 150 min (k = 0.015) at 400 MPa. D-values reported for 500 MPa are lower than the ones for 400 MPa, but were measured in a different medium and may be influenced by temperature. However, in comparable experiments, inactivation rates of selected strains of various *Listeria* spp. with, for example, D-values ranging from 1.48 min (k = 1.556/min) at 350 MPa to 15 min (k = 0.154/min) at 400 MPa were lower than the ones for *S. aureus*. These data were measured at ambient temperatures (20 to 25 °C). Recently, D-values of over 5 min were also reported for *L. monocytogenes* at 345 MPa and 25 °C (Alpas and others 1999).

Comprehensive data on inactivation rates of *Clostridium sporogenes* spores were reported by Rovere and others (1996). These data indicate that D-values are 0.695 min (k = 3.314/min) at 800 MPa at 108 °C compared to 16.772 min (k = 0.136/min) at 600 MPa at 90 °C. The magnitudes of these D-values are similar to the D-value of 12 min at 680 MPa reported in a separate investigation (Crawford and others 1996), even though the latter was measured at ambient temperatures. From the Rovere and others (1996) data, the influence of pressure on inactivation rate, z(P), were estimated to be 725 MPa at 93 °C, 962 MPa at 100 °C and 752 MPa at 108 °C. The inconsistent influence of

temperature on z(P) may be associated with the limited range of temperatures and pressures used in the experimental investigation, as well as adequacy of temperature control during data collection.

Recent inactivation data for *C. botulinum* Type E Alaska and Type E beluga (Reddy and others 1999) indicate that their D-values were in the same range as for *C. sporogenes.* The D-values for *C. botulinum* Type E Alaska were lower in crab meat than in a buffer. The D-values for *C. botulium* Type A 62-A are generally higher than the values for *C. sporogenes,* even when considering the influence of temperature and pressure. The pressure coefficient z(P) for the Type A 62-A data was 1524 MPa. Surprisingly, this value was much higher than the z(P) values reported for *C. sporogenes,* even though data from *C. sporogenes* were recorded at lower temperatures.

An in-depth investigation of pressure inactivation of *Saccharomyces cerevisiae* in orange and apple juice has been reported by Zook and others (1999). The calculated D-values were 10.81 min (k = 0.21/min) at 300 MPa, where temperatures have been maintained at levels between 34 and 43.4 °C. These D-values are slightly higher than the ones reported earlier by Parish and others (1998). For apple juice and orange juice z(P) were 115 MPa and 117 MPa, respectively. These values are much lower than those reported for *C. sporogenes* and *C. botulinum.* Since data for 5 different pressures have been reported by Zook and others (1999), the activation volumes (V) could be estimated to be $1.24 \times 10^{-4}$ for orange juice and $1.37 \times 10^{-4}$ $m^3$/mole for apple juice.

In summary, the most pressure-resistant pathogenic vegetative cell populations appear to be those of *E. coli* O157:H8 with a D-value of 6 min (k= 0.384/min) at 600 MPa, and *S. aureus* with a D-value of 7.14 min (k = 0.323/min) at 600 MPa. The most pressure-resistant spores appear to be *C. sporogenes* with a D-value of 16.772 min (k = 0.138/min) at 600MPa (T = 90 °C) and *C. botulinum* Type A 62-A with a D-value of 6.7 min (k = 0.344/min) at 827 MPa (T = 75 °C). The pressure coefficient z(P) of 1524 MPa at 75 °C for *C. botulinum* Type A 62-A constitutes an additional indication of the pressure resistance of the spore populations. A recent report shows little if any inactivation after 30 min of *C. botulinum* 17B and Cap 9B exposure to 827 MPa at 75 °C (Larkin and Reddy 1999).

### 1.2.2.4. Pulsed electric field processes

Currently, the majority of the kinetic parameters for the PEF technologies are in a form that fits the basic model [Eq. (13) or (14)]. Even with the limitations mentioned above, the use of the parameters and model to establish process time (F) would seem appropriate in the short term. Models, such as Eq. (11) or (12), provide desirable alternatives, but a great effort would be needed to evaluate them. The use of z(*E*) values provides the users with flexibility to select the optimum electric field strength for a given product and to evaluate the influence of other factors such as synergistic effects of electric field strength and temperature. Adequate inactivation data for estimating the kinetic parameters for microbial populations exposed to PEF are scarce. The information presented in Table 1C compares decimal reduction times (D-value) and first-order rate constants (k), for

experiments where electrical field strength (*E*) and initial temperature were mostly available. Three different PEF coefficients have been presented: the z(*E),* the specific rate constant (K) from the Hulsheger model (Hulsheger and others 1981) and a similar constant (K) based on the analysis by Peleg (1995).

It should be noted that the D-values (k-values) have been determined from measurements of microbial population reduction after 1 exposure time to a given electrical field strength. The parameters obtained should be considered with this limitation. Furthermore, there is no evidence that the survivor curve during exposure to a pulsed electric field is described by a first-order model. The parameters are presented in this report to allow for more direct comparisons of the effectiveness of PEF in reducing different microbial populations, as well as to note the influence of the media on microbial inactivation. In addition, the D-values (k) provide a more direct approach to evaluating the influence of electric field strength on the rate of microbial population reduction. As will be emphasized later in this section of the report, there is a great need to better understand survivor curve shapes for microbial populations exposed to pulsed electric fields.

The results in Table 1C clearly indicate that the D-values are several orders of magnitude smaller than the same parameters for thermal or pressure processes. Assuming first-order kinetics through 6-12Ds, this suggests a significant advantage for PEF, when compared to the other technologies. This assumption may not be valid because inactivation of 99.9% of a cell population is frequently difficult to achieve.

Several investigations have reported data on reduction of *E. coli* populations exposed to PEF. The highest D-values are 4500 µs (k = 0.051 X 10-2 /µs) at 16 kV/cm and 17.8 µs (k = 12.94 X 10-2 /µs) at 70 kV/cm. Using a limited number of D-values, a z(*E*) of 41 kV/cm has been estimated. Note that this magnitude is based on less than ideal data, collected at temperatures ranging from 15 to 37 °C. The D-value of 4500 µs, at 16 kV/cm and 37 °C for *E. coli* would suggest that this microorganism is one of the more PEF resistant vegetative cell populations.

The investigations on the influence of PEF on *Salmonella* Dublin, *S. aureus* and *Zygosaccharomyces bailii* provide only limited amounts of data. The D-values for *S. aureus* are very similar to the magnitudes for *E. coli*, with values of 4000 to 6000 µs at relatively low electric field strength (16 kV/cm) and temperatures of 30-37 °C.

The data for the *Listeria* spp. indicate that D-values are as low as 18.8 µs at 50 kV/cm for *Listeria innocua* and as high as 540 µs at 20 kV/cm for *L. monocyctogenes.* Since these data were measured at relatively low temperatures (10 to 50 °C), the parameters would indicate that *Listeria* is one the more resistant vegetative cell populations to a PEF treatment.

The D-values of 50-60 µs (k = 3.84 to 4.61 X 10-2 /µs) at 50 kV/cm for *B. cereus* spores are higher than for other microbial populations at the same field strength and temperature. Two D-values (17.5 to 26.3 µs) for *B. subtilis* spores at the same pressure and from 2 different investigations were considerably lower than the D-values for *B. cereus* spores.

Using the D-values for *B. subtilis* spores at 3 different electrical field strengths and within an ambient temperature range, a z(*E*) of 15.5 kV/cm has been estimated. Unexpectedly, this magnitude is much lower than the one estimated for vegetative cell populations (that is, *E. coli* with a z[E] of 41 kV/cm). These observations need more comprehensive investigation before any conclusions are reached.

Several investigations have reported data on inactivation of *S. cerevisiae* when exposed to PEF. Overall, the D-values vary significantly depending on the electric field strength and temperature. In general, the magnitudes are larger than *E. coli,* lower than *L. monocytogenes* and much less than *B. subtilis* spores. An z(*E*) of 17 kV/cm has been estimated from data reported for PEF treatments of *S. cerevisiae* in apple juice, much lower than the value estimated for *E. coli* (41 kV/cm) and similar to the one of *B. subtilis* spores (15.5 kV/cm).

The influence of electrical field strength (*E*) on the rate of microbial population inactivation may also be estimated from the coefficient (K). These parameters have been reported for a limited number of microbial populations. Among them, the populations with greater resistance to PEF would include *Escherichia* spp*., Listeria* spp*., Pseudomonas* spp. and *Klebsiella* spp. The coefficient z(*E*) was highest for *Escherichia* spp., which was higher than the one for *B. subtilis* spores. Data are insufficient to make valid comparisons of the relative resistance for vegetative and spore populations to PEF.

In summary, the survivor data for microbial populations exposed to PEF are too limited to be used in reaching definite conclusions about the magnitude of the kinetic parameters. In addition, data are not adequate to calculate parameters to compare the relative resistance of various microbial populations to PEF. For instance, data based on the same field strength and temperature are lacking. In addition, only a few of the published reports provide information on the threshold field strengths needed to initiate inactivation.

### 1.3. Future Research Needs

This section focuses on the research needs associated with kinetic parameters to be used for development of food preservation processes to ensure safety. For several technologies discussed in this report, the data necessary to estimate kinetic parameters are lacking. If these technologies are to evolve to industrial applications, kinetic data must be collected in the future.

The following is a list of research areas that need further investigation:

- Evaluation of the adequacy of a linear first-order survivor curve. Although there is evidence of various types of deviation from the historical model, a universally accepted alternative has not evolved. Future research on an appropriate model would be beneficial to all preservation technologies.
- Investigation on the influence of pressure on reduction of microbial populations using the proper experimental design (statistically valid, collection of data at different pressures and control of temperature and product), so that z(P) and/or

activation volumes (V) are quantified. These investigations should also evaluate synergistic effects between pressure and temperature.

- Research on developing an experimental protocol for obtaining statistically reliable kinetic parameters to describe survivor curves for microbial populations exposed to PEF. These studies should incorporate multiple levels of electric field intensity, as well as the potential for synergy with temperature.
- Further research on the PEF microbial inactivation models presented as Eq. (11) or (12). The investigations need to provide reliable kinetic parameters for these models and for the microbial population of interest in food safety.

**Table 1a. Kinetic parameters for inactivation of microbial population for thermal processes**

| Process Technology | Microorganism | Substrate | Time Parameter | | Temperature Coefficient | | Temperature | Other | Referen |
|---|---|---|---|---|---|---|---|---|---|
| | | | (D) (min) | (k) (1/min) | Z(T) (C) | (E) (kJ/mole) | (C) | | |
| **Thermal** | **Vegetative Cells** | | | | | | | | |
| | *Salmonella serovars* | Milk | 0.018-0.56 | 4.113-127.9 | 4.4-5.6 | 392-499 | 65.6 | | ICMSF(1 |
| | *S.Senftenberg* | various foods | 0.56-1.11 | 2.075-4.113 | 4.4-5.6 | 392-499 | 65.5 | | ICMSF(1 |
| | *S.Typhimurium* | TBS + 10-42%MS | 4.7 - 18.3 | 0.126-0.49 | 4.5-4.6 | 448-458 | 55 | | ICMSF(1 |
| | *S. Senftenberg* | Milk chocolate | 276 - 480 | 0.005-0.008 | 18.9 | 120 | 70-71 | | ICMSF(1 |
| | *S.Typhimurium* | Milk chocolate | 396 - 1050 | 0.002-0.006 | 17.7 | 128 | 70-71 | | ICMSF(1 |
| | *S.Typhimurium* | Ground beef | 2.13 - 2.67 | 0.86-1.08 | | | 57 | | ICMSF(1 |
| | *S.Eastbourne* | Milk chocolate | 270 | 0.0085 | | | 71 | | ICMSF(1 |
| | *Escherichia coli ATCC* | Dairy products | 1.3-5.1 | 0.45-1.77 | | | 57.2 | | ICMSF(1 |
| | *E. coli O111:B4* | Skim/Whole milk | 5.5-6.6 | 0.35-0.42 | | | 55 | | ICMSF(1 |
| | *E. coli O157:H7* | Ground beef | 4.1-6.4 | 0.36-0.56 | | | 57.2 | | Line ar others (1 |
| | *E. coli O157:H8* | Ground beef | 0.26-0.47 | 4.9-8.86 | 5.3 | 401 | 62.8 | | Line ar others (1 |
| | *Yersinia enterocolitica* | Milk | 0.067-0.51 | 4.52-34.4 | 4-5.78 | 367-530 | 60 | | ICMSF(1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Vibrio parahaemolyticus* | Fish homogenate | 10 - 16 | 0.144-1.05 | 5.6-12.4 | 159-352 | 48 | | ICMSF(1 |
| *V. parahaemolyticus* | clam/crab | 0.02-2.5 | 0.92-115 | 5.6-12.4 | 166-368 | 55 | | ICMSF(1 |
| *V. cholerae* | crab/oyst | 0.35-2.65 | 0.87-6.58 | 17-21 | 101-125 | 60 | | ICMSF(1 |
| *Aeromonas hydrophila* | Milk | 2.2-6.6 | 0.35-1.05 | 5.2-7.7 | 256-379 | 48 | | ICMSF(1 |
| *Campylobacter jejuni* | Skim milk | 0.74 - 1.0 | 2.3 - 3.11 | | | 55 | | ICMSF(1 |
| *C. jejuni* | Beef/Lamb/Chicken | 0.62 - 2.25 | 1.0 - 3.72 | | | 55-56 | | ICMSF(1 |
| *Listeria monocytogenes* | Milk | 0.22 - 0.58 | 3.97 - 10.47 | 5.5 | 386 | 63.3 | | ICMSF(1 |
| *L. monocytogenes* | Meat products | 1.6 - 16.7 | 0.14 - 1.44 | | | 60 | | ICMSF(1 |
| *Staphylococcus aureus* | Milk | 0.9 | 2.56 | 9.5 | 224 | 60 | | ICMSF(1 |
| *S. aureus* | Meat macerate | 6 | 0.384 | | | 60 | +500 ppm nitrite | ICMSF(1 |
| *S. aureus* | Pasta | 3 | 0.768 | | | 60 | aw = 0.92 | ICMSF(1 |
| *S. aureus* | Pasta | 40 | 0.0576 | | | | T=60C, aw = 0.8 | ICMSF(1 |
| *S. aureus* | Phosphate buffer | 2.5 | 0.921 | | | 60 | pH = 6.5 | ICMSF(1 |
| **Spores** | | | | | | | | |
| *Bacillus cereus* | various | 1.5 - 36.2 | 0.064 - 1.535 | 6.7 - 10.1 | | 95 | | ICMSF(1 |
| *Clostridium perfringens* | Phosphate buffer | 0.015 - 8.7 | 0.265 - 15.35 | | | 90 | pH = 7.0 | ICMSF(1 |
| *C. perfringens* | Phosphate buffer | 3.15 | 0.731 | | | 104.4 | pH = 7.0 | ICMSF(1 |
| *C. perfringens* | Beef gravy | 6.6 | 0.349 | | | 104.4 | pH = 7.0 | ICMSF(1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Clostridium botulinum 62A* | Vegetable products | 0.61 - 2.48 | 0.929 - 3.775 | 7.5 11.6 | | 110 | | ICMSF(1 |
| *C. botulinum 62A* | Phosphate buffer | 0.88 - 1.9 | 1.212 - 2.617 | 7.6 - 10 | | 110 | pH = 7.0 | ICMSF(1 |
| *C. botulinum 62A* | Distilled water | 1.79 | 1.287 | 8.5 | | 110 | | ICMSF(1 |
| *C. botulinum B* | Phosphate buffer | 1.19 - 2.0 | 1.152 - 1.935 | 7.7 - 11.3 | | 110 | pH = 7.0 | ICMSF(1 |
| *C. botulinum B* | Vegetable products | 0.49 - 12.42 | 0.185 - 4.7 | 7.4 - 10.8 | | 110 | | ICMSF(1 |
| *C. botulinum E* | Seafood | 6.8 - 13 | 0.177 - 0.339 | 9.78 | | 74 | | ICMSF(1 |
| *C. botulinum E* | Oyster homogenate | 72 - 100 | 0.023 - 0.32 | 6.8 - 7.5 | | 70 | | ICMSF(1 |
| *Bacillus subtilis* | 0.1% NaCl | 32.8 | 0.0702 | 8.74 | 293 | 88 | Conventional | Cho ar others (1 |
| *B. subtilis* | 0.1% NaCl | 30.2 | 0.0763 | 9.16 | 282 | 88 | Ohmic | Cho and others (1999) |

**Table 1b. Kinetic parameters for inactivation of microbial population for pressure processes**

| Process Technology | Microorganism | Substrate | Time Parameter | | Pressure Coefficient | | Pressure | Pressure Threshold | Other | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | (D) (min) | (k) (1/min) | [z(P)] (MPa) | (V) (m3 /mole) | (MPa) | | | |
| **Pressure** | **Vegetative Cells** | | | | | | | | | |
| | *Campylobacter* | | < 2.5 | >0.92 | | | 300 | | | Smelt and Hellemons (1998) |
| | *Salmonella serovars* | | | | | | | | | |
| | *Salmonella Senftenberg* | Buffer | 6 | 0.384 | | | 345 | | T=230C | Metrick and others (1989) |
| | *S. Senftenberg* | | 5 | 0.461 | | | 300 | | | Smelt and Hellemons (1998) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Salmonella Enteritidis* | Meat | 3 | 0.768 | 450 | | Patterson and others (1995) |
| *Salmonella Typhimurium* | Milk | 3 | 0.768 | 350 | | Patterson and others (1995) |
| *S. Typhimurium* | Meat | 1.48 | 1.556 | 414 | T=25C | Ananth and others (1998) |
| *S. Typhimurium* | | 0.6 | 3.838 | 345 | T=50C | Kalchayanand and others (1998) |
| *Yersinia enterocolitica* | Milk | 3 | 0.768 | 275 | | Patterson and others (1995) |
| *Escherichia coli* | | 7.5 - 15 | 0.154 - 0.307 | 300 | | Smelt and Hellemons (1998) |
| *E. coli* | Milk | 1 | 2.303 | 400 | T=50C | Gervilla and others (1997b) |
| *E. coli* | Meat | 2.5 | 0.92 | 400 | | Patterson and Kilpatrick (1998) |
| *E. coli* | Milk | 1 | 2.303 | 450 | T=25C | Gervilla and others (1997a) |
| *E. coli* | Buffer | 3 | 0.768 | 700 | | Patterson and others (1995) |
| *E. coli O157:H7* | Milk | 3 | 0.768 | 400 | T=50C | Patterson and Kilpatrick (1998) |
| *E. coli O157:H8* | | 6 | 0.384 | 600 | | Smelt and Hellemons (1998) |
| *E. coli O157:H7* | | 0.7 | 3.29 | 345 | T=50C | Kalchayanand and others (1998) |
| *Staphylococcus aureus* | | 150 | 0.015 | 400 | | Smelt and Hellemons (1998) |
| *S. aureus* | Milk | 2.5 | 0.92 | 500 | T=50C | Patterson and Kilpatrick (1998) |
| *S. aureus* | Meat | 3 | 0.768 | 500 | T=50C | Patterson and Kilpatrick (1998) |
| *S. aureus* | | 7.9 | 0.292 | 500 | | Smelt and Hellemons (1998) |
| *S. aureus* | | 7.14 | 0.323 | 600 | | Smelt and Hellemons (1998) |
| *S. aureus* | Buffer | 3 | 0.768 | 700 | | Patterson and others (1995) |
| *S. aureus 582* | | 0.6 | 3.838 | 345 | T-50C | Kalchayanand |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | and others (1998) |
| *Listeria monocytogenes* | | 1.48 - 13.3 | 0.173 - 1.556 | | 350 | 101 strains | Smelt and Hellemons (1998) |
| *L. monocytogenes* | Milk | 3 | 0.768 | | 375 | | Patterson and others (1995) |
| *L. monocytogenes* | Meat | 2.17 | 1.061 | | 414 | T=25C | Ananth and others (1998) |
| *L. monocytogenes ScottA* | Meat | 3.5 | 0.658 | | 400 | T=Amb. | Mussa and others (1999) |
| *L. monocytogenes* | | 5.0 - 15 | 0.154 - 0.461 | | 400 | | Smelt and Hellemons(1998) |
| *L. monocytogenes* | | < 2.5 | > 0.92 | | 500 | | Smelt and Hellemons(1998) |
| *Listeria innocua* | Eggs | 3 | 0.768 | | 450 | T=20C | Ponce and others(1998) |
| *L. monocytogenes* | Ground Pork | 1.89 - 4.17 | 0.552 - 1.219 | | 414 | T=25C | Murano and others (1999) |
| *L. monocytogenes* | Ground Pork | 0.37 - 0.63 | 3.656 - 6.224 | | 414 | T=50C | Murano and others (1999) |
| *L. monocytogenes ScottA* | | 4 | 0.576 | | 345 | T=50C | Kalchayanand and others (1998) |
| *Pseudomonas fluorescens* | Milk | 1 | 2.303 | | 300 | T=50C | Gervilla and others (1997b) |
| *P. fluorescens* | Milk | 1 | 2.303 | | 400 | T=10C | Gervilla and others (1997b) |
| *P. fluorescens* | | 0.6 | 3.838 | | 345 | T=50 | Kalchayanand and others (1998) |
| **Spores** | | | | | | | |
| *Clostridium sporogenes* | | 12 | 0.192 | | 680 | | Crawford and others (1996) |
| *C. sporogenes* | | 16.772 | 0.138 | | 600 | T=90C | Rovere and others (1996b) |
| *C. sporogenes* | | 6.756 | 0.341 | 725 (90C) | 700 | T=93C | |
| *C. sporogenes* | | 5.306 | 0.434 | | 800 | T=93C | |
| *C. sporogenes* | | 3.502 | 0.658 | | 600 | T=100C | Rovere and others (1996b) |
| *C. sporogenes* | | 3.186 | 0.723 | 962 (100C) | 700 | T=100C | |
| *C. sporogenes* | | 2.857 | 0.806 | | 800 | T=98C | |
| *C. sporogenes* | | 1.282 | 1.796 | | 600 | T=108C | Rovere and |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | others (1996b) |
| *C. sporogenes* | | 0.901 | 2.556 | 752 (108C) | | 700 | T=108C | |
| *C. sporogenes* | | 0.695 | 3.314 | | | 800 | T=108C | |
| *Clostridium botulinumTypeE Alaska* | Buffer | 8.77 | 0.263 | | | 758 | T=35C | Reddy and others (1999) |
| *C. botulinumTypeE Alaska* | Buffer | 2.64 | 0.872 | | | 827 | T=35C | |
| *C.botulinumTypeE Beluga* | Crab meat | 3.38 | 0.681 | | | 758 | T=35C | Reddy and others (1999) |
| *C.botulinumTypeE Beluga* | Crab meat | 1.64 | 1.404 | | | 827 | T=35C | |
| *C. botulinumTypeE Alaska* | Crab meat | 2 | 1.152 | | | 758 | T=35C | Reddy and others (1999) |
| *C. botulinumTypeE Alaska* | Crab meat | 1.76 | 1.309 | | | 827 | T=35C | |
| *C.botulinumTypeA 62-A* | Buffer | 13.21 | 0.174 | | | 414 | T=75C | |
| *C.botulinumTypeA 62-A* | Buffer | 12.6 | 0.183 | | | 551 | T=75C | |
| *C.botulinumTypeA 62-A* | Buffer | 10.59 | 0.218 | 1524 | 4.39x10-6 | 689 | T=75C | Reddy and others (1999) |
| *C.botulinumTypeA 62-A* | Buffer | 9.19 | 0.251 | | | 758 | T=75C | |
| *C.botulinumTypeA 62-A* | Buffer | 6.7 | 0.344 | | | 827 | T=75C | |
| **Yeast** | | | | | | | | |
| *Saccharomyces cerevisiae* | orange juice | 10.81 | 0.21 | | | 300 | T=34C | Zook and others (1999) |
| | | 2.8 | 0.82 | | | 350 | T=36.8C | |
| | | 0.97 | 2.37 | 117 | 1.241X10-4 | 400 | T=37.2C | |
| | | 0.5 | 4.61 | | | 450 | T=39.7C | |
| | | 0.18 | 12.79 | | | 500 | T=43.4C | |
| *S. cerevisiae* | apple juice | 9.97 | 0.231 | | | 300 | T=34C | Zook and others (1999) |
| | | 0.88 | 2.617 | 115 | 1.371X10-4 | 400 | T=37.2C | |
| | | 0.28 | 4.798 | | | 450 | T=39.7C | |
| | | 0.15 | 15.35 | | | 500 | T=43.4C | |
| *S. cerevisiae* | | 1.27 | 1.813 | | | 350 | pH=3.7 | Parsih and others |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | (1998) |
| | *S. cerevisiae* | | 0.067 | 34.373 | | 500 | | pH=3.7 | |

## Table 1c Kinetic parameters for inactivation of microbial population for PEF processes

| Process Technology | Microorganism | Substrate | Time Parameter | | PEF Coefficient | | Field Strength | Other | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | (D) | (k) | [Z(E)] | (K) | | | |
| **Pulsed Electric Fields** | **Vegetative Cells** | | (microsec) | (x 10-2/microsec) | (kV/cm) | (kV/cm) | (kV/cm) | | |
| | *Escherichia coli* | Skim milk | 38.4 - 44.8 | 5.14 - 6.0 | | | 20 -45 | T=15 C | Martin-Belloso and others (1997b) |
| | *E. coli* | SMUF | 17.8 | 12.94 | | | 70 | T=20 C | Zhang and others (1995a) |
| | *E. coli* | Milk | 333 | 0.692 | | | 22 | | Grahl and others (1992) |
| | *E. coli* | SMUF | 4000 - 4500 | 0.051 - 0.058 | | | 16 | T=37 C | Pothakamury and others (1995) |
| | *E. coli* | Buffer | 75 - 100 | 2.3 - 3.07 | | | 20 | T<30 C | Hulsherger and Nieman (1980) |
| | *E. coli* | Phosphate buffer | 270 | 0.853 | ~41 | 6.3 - 8.1 | 20 | T=20 C | Hulsheger and others (1983) |
| | *E. coli* | 0.1% NaCl | 100 | 2.3 | | | 19.5 | T=20 C | Sale and Hamilton (1967) |
| | *E. coli* | Phosphate buffer | 2 | 115.15 | | | 40 | | Matsumoto and others (1991) |
| | *E. coli* | Potato dextrose | 16 - 32 | 14.39 | | | 40 | T=15 C | Zhang and others (1994b) |
| | *E. coli* | Skim milk | 64 - 96 | 2.4 - 3.6 | | | 40 | T=15 C | Zhang and others (1994b) |
| | *E. coli* | Skim milk | 27.4 - 49.6 | 4.64 - 8.41 | | | 50 | T<30 C | Qin and others(1995c) |
| | *E. coli* | SMUF | 26.7 | 8.63 | | | 50 | T<30 | Qin and |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | C | others(1995c) |
| *Salmonella Dublin* | Skim milk | 4 - 42.4 | 0.054 - 0.52 | | 15 - 40 | T=15-40C | Sensoy and others (1997) |
| *S. Dublin* | Milk | 360 | 0.64 | | 36.7 | T=63 C | Dunn and Pearlman (1987) |
| *Salmonella Typhimurium* | NaCl | 4 | 57.58 | | 83 | | Gupta and Murray (1989) |
| *Listeria monocytogenes (Scott A)* | Milk | 150 - 200 | 0.012 - 0.015 | | 30 | T=10-50C | Reina and others (1998) |
| *L. monocytogenes* | Phosphate buffer | 540 | 0.426 | 6.4 - 6.5 (2 - 2.8) | 20 | | Hulsheger and others (1983) |
| *Listeria innocua* | Skim milk | 76.9 | 2.995 | | 50 | T=15-28 C | Fernandez and others (1999) |
| *L. innocua* | Skim milk | 26.7 | 8.625 | | 50 | T=22-34 C | Calderon-Miranda (1998) |
| *L. innocua* | Liquid Whole Egg | 18.8 | 12.25 | | 50 | T=26-36 C | Calderon-Miranda (1998) |
| *Staphylococcus aureus* | SMUF | 4000 - 6000 | 0.038 - 0.058 | | 16 | T=37 C | Hulsherger and others (1983) |
| *S. aureus* | SMUF | 4000-4500 | 0.052 - 0.058 | | 16 | T<30 C | Pothakamury and others (1995) |
| *S. aureus* | Phosphate buffer | 360 | 0.64 | 2.6 (2.0) | 20 | | Hulsherger and others (1983) |
| *Lactobacillus delbrueckii* | SMUF | 2000-2400 | 0.096 - 0.115 | | 16 | T<30 C | Polhakamury (1995) |
| *Lactobacillus delbrueckii* | Buffer | 1022 | 0.225 | (1.6) | 25 | T=60 C | Jayaram and others (1992) |
| *Pseudomonas fluorescens* | Skim milk | 22.2 | 10.374 | | 50 | T=15-28 C | Fernandez and others |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (1999) |
| *Pseudomonas auriginosa* | Phosphate buffer | 308.6 | 0.746 | | 6.3 (1.8 - 2.6) | 20 | | Hulsheger and others (1983) |
| *P. fluorescens* | Water | 3.3 | 69.79 | | | 10 | T = 20C | Ho and others (1995) |
| *Klebsiella pneumoniae* | Phosphate buffer | 360 | 0.64 | | 6.6 | 20 | | Hulsheger and others (1983) |
| **Spores** | | | | | | | | |
| Bacillus cereus | 0.15% NaCl | 50 - 60 | 3.84 - 4.61 | | | 50 | T=25 C | Marquez and others (1997) |
| *Bacillus subtilis* | 0.15% NaCl | 17.5 - 26.3 | 8.76 - 13.16 | | | 50 | T=25 C | Marquez and others (1997) |
| *B. subtilis* | SMUF | 2500 - 3000 | 0.077 - 0.092 | | | 16 | T<30 C | Pothakamury (1995) |
| *B. subtilis* | Pea soup | 11.3 | 20.38 | ~15.5 | | 33 | T<5.5 C | Vega-Mercado and others (1996a) |
| *B. subtilis* | SMUF | 425 - 520 | 0.44 - 0.54 | | | 16 | | Qin and others (1994) |
| **Yeast** | | | | | | | | |
| *Saccharomyces cerevisiae* | NaCl | 61.5 | 3.745 | | | 35 | | Jacob and others (1981) |
| *S. cerevisiae* | Phosphate buffer | 270 | 0.853 | | (2.3) | 20 | | Hulsheger and others (1983) |
| *S. cerevisiae* | Water | 4666 | 0.049 | | | 20 | | Mizuno and Hori (1988) |
| *S. cerevisiae* | Potato dextrose | 8.7 | 26.47 | | | 40 | T=15 C | Zhang and others (1994b) |
| *S. cerevisiae* | Apple juice | 102.9 - 135 | 1.706 - 2.238 | ~17 | | 12 | T=4-10 C | Zhang and others (1994a) |
| *S. cerevisiae* | Apple juice | 42.9 - 428.6 | 0.537 - 5.368 | | | 25 | T<30 C | Qin and others (1995a) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | Apple juice | 0.83 | 277.47 | | 50 | T=22-29 C | Qin and others (1995a) |
| *Candida albicans* | Phosphate buffer | 240 | 0.96 | 2.2 (1.2 - 3.1) | 20 | | Hulsheger and others (1983) |
| *Zygosaccharomyces bailli* | Juices | 0.4 - 0.7 | 3.29 - 5.76 | | 32 - 36.5 | T=20 C | Raso and others (1998) |

## 2. MICROBIOLOGICAL CRITICAL FACTORS

The efficacy of a preservation technology is influenced by a number of microorganism-related factors that are generally independent of the technology itself. These include the type and form of target microorganism; the genus, species and strain of microorganism; growth stage; environmental stress selection mechanisms; and sublethal injury. Each of these factors influences the resistance of a microorganism to a preservation process, independently of the apparent inactivation capacity of that particular process.

Among the food microbial hazards, bacteria are generally the most resistant microorganisms of concern and therefore should be the primary targets in most preservation processes. In most cases, microorganisms other than bacteria will be destroyed before or concurrently with pathogenic and spoilage bacteria; however, in designing processes to inactivate all pathogens, it is also advisable to consider the resistance properties in foods of other microorganisms such as yeasts, molds, parasites and protozoa, that may persist in or grow in foods.

A few genera of foodborne bacteria (for example, *Clostridium* spp. and *Bacillus* spp.) are capable of existing in 2 forms: active vegetative cells and dormant spores. These 2 forms often differ in their resistance properties to heat, chemicals, irradiation and other environmental stresses. In that same manner, studies have shown that spores are typically more resistant than vegetative cells to the alternative processing technologies evaluated in this report. For pasteurization purposes, one is mostly concerned with the inactivation of vegetative cells of disease-producing microorganisms. In order to have a commercially sterile product, however, one must devise a process that inactivates all microbial spores (usually targeting spores of *C. botulinum*) capable of germinating and growing in the food under normal storage conditions.

Differences in resistance of microorganisms may be found not only between genera and species but also between strains of the same species. For instance, some bacterial strains with unique resistance to thermal inactivation, irradiation and high pressure processing have been identified. It is possible that, in the future, a pathogenic "super bug" would emerge. If this occurs, this pathogen should be considered a possible food safety hazard and the process would have to be redesigned to specifically inactivate it. Alternatively, if the "super bug" is not a pathogen or spoilage microorganism, it may be very useful as a possible surrogate during development and validation of a process. Another factor that

can affect resistance of bacteria to preservation processes is stage of growth. It appears that cells in exponential or log phase of growth are generally less resistant than cells in stationary phase. The development of stress resistance proteins in stationary phase may be the cause of this phenomenon.

One of the basic principles of microbial genetics is that extreme environments that would kill most bacterial cells result in the selection of mutants resistant to severe conditions. These environmental conditions encountered by a population of cells may induce a stress "defense mechanism" in some of them. This selection process has been scientifically supported by studies suggesting that bacterial stress may induce hypermutability. Hypermutability would in turn lead to a microbial population of greater resistance (Buchanan 1997). Therefore, the exposure of cells to some form of stress may induce and allow the survival of microorganisms with unusually higher durability to a given inactivation process. Mazotta (1999) found that the heat resistance of acid- or salt-adapted, heat-shocked, or starved *E. coli* O157:H7 cells was higher than that of cells grown to exponential or stationary phase under optimum conditions. He suggested that it would be appropriate to use stress-inducing culture conditions when studying the thermal resistance of vegetative pathogens in specific products in order to add an extra factor of safety to the process. Lou and Yousef (1997) determined that sublethal stresses to ethanol, acid, hydrogen peroxide, heat, or salt had variable effects on subsequent exposure of *L. monocytogenes* to lethal levels of the same stressors. For example, heat shocking increased the resistance of the microorganism to ethanol, hydrogen peroxide and salt, but not to acid. Davidson (1999) reviewed the impact of stress induction on resistance to food antimicrobials. He stated that resistance could be acquired through previous exposure or adaptation due to cross-protection from environmental or processing factors including stresses such as heat or acid. A number of studies like the ones described have demonstrated the occurrence of stress-induced enhanced resistance to inactivation. The questions relative to process design and verification are: (1) Are the microorganisms and food environments likely to be of the type involved in stress induction? (2) Would stress induced resistance possibly occur at any point in the food processing operation? and (3) If it did, would it significantly impact the inactivation process leading to possible underprocessing? Considering that in most food processing systems the design is to have microorganisms exposed only once to a stress-inducing factor (for example, heat, acid, antimicrobials and so on), the development of a resistant population is not likely to occur. One possible exception might be the case of previously processed material that is reprocessed into the streamline. In those cases, in-depth studies of the impact of stress induction during the processing are needed.

Another microbial-related factor that influences the effectiveness of a process is the susceptibility of the microorganism to cellular injury. The effectiveness of an inactivation process is often measured by enumerating any surviving organisms (using biological indicators or surrogates) in a selective medium. Under the best circumstances, a processed microorganism would be either viable or dead; however, inactivation often results in a continuum of effects with some degree of injury. Injured cells can be easily underestimated, resulting in misleading conclusions about the efficiency of the method. The detection and enumeration of injured microorganisms require special procedures.

Often, injury is identified when surrogate organisms are enumerated using a selective culturing medium (generally a medium containing a chemical inhibitor that allows growth only of the particular microorganism being enumerated) in contast to a non-selective medium. It is often desirable to use selective media in the field to ensure growth of only the surrogate microorganism, and not of background microflora. The choice of "best" method to enumerate the test organism will largely depend on the experimental variables and the researcher's experience with field studies.

## 3. PATHOGENS OF PUBLIC HEALTH CONCERN

In the United States, foodborne diseases caused by microorganisms can be attributed primarily to pathogenic bacteria, enteric viruses and protozoa (Anonymous 1999; Carsberg 1999; Jackson and others 1997; Katsuyama 1993; Varnam and Evans 1991). The following bacteria are known to be responsible for causing foodborne disease: *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens*, pathogenic *Escherichia coli*, *Listeria monocytogenes, Salmonella*, *Shigella*, *Staphylococcus aureus*, pathogenic *Vibrio* spp. and *Yersinia enterocolitica.* The viruses of concern in foods are Hepatitis A, Norwalk, Norwalk-like and Rotavirus (CDC 2000; Mead and others 1999). *Cryptosporidium parvum*, *Cyclospora cayetanensi*s, *Giardia lamblia* and *Toxoplasma gondii* are all parasites of concern, in part because they produce resistant cysts. When exploring the new preservation technologies, their preservation level should be compared to that of classical pasteurization or commercial sterilization technologies. Therefore, in an attempt to determine the pathogens of greatest public health concern for new technologies, the resistance of pathogens to heat will be examined.

### 3.1. Vegetative Bacteria Inactivated by Cooking and Pasteurization

***Salmonella.*** Bacteria of the genus *Salmonella* is one of the most well-known and frequently encountered pathogens in foods. Approximately 2,200 serovars of *Salmonella enterica* subsp. *enterica* exist and can be isolated from meats, poultry, eggs, raw milk, water, fish, shellfish, feeds, fruits and vegetables. Because *Salmonella* serovars are natural contaminants of intestinal tracts of animals, birds and reptiles, they may contaminate food and equipment through secondary contamination. Therefore, inactivation of this pathogen through processing and avoidance of post-processing contamination is very important. The infectious dose of this microorganism can be very low: It has been demonstrated that it may take only one cell to cause a person to become ill. This makes effective pasteurization critical to produce safe food.

Inadequate heating of foods of animal origin or cross contamination are the primary vehicles for salmonellosis outbreaks. Meat and poultry (that is, beef, turkey, chicken and pork) and homemade ice cream (generally due to the use of raw eggs), fruits and vegetables and salads have been the most frequently reported items (CDC 2000). Raw or improperly pasteurized milk and eggs, as well as other foods have also been associated with salmonellosis. Inadequate cooking or processing, improper cooling, ingestion of raw

products and cross contamination of foods after cooking seem to be the major sources of *Salmonella* serovars.

The maximum growth temperature for *Salmonella* serovars is 49.5 °C. The microorganism is considered to be sensitive to heat and is killed easily by pasteurization of milk equivalent to 71.7 °C for 15 s. The heat resistance of *Salmonella* serovars depends on factors such as serovar type, water activity, pH and heating medium. The heat resistance of serovars in various foods is shown in Table 2. *Salmonella* serovar Senftenberg is generally considered to be the most resistant strain (Tables 2 and 3). Therefore, any heat-resistant studies using *Salmonella* serovars should include serovar Senftenberg strain 775W, unless it is not relevant for the application. Then it would serve only as a point of reference for heat resistance.

***Shigella.*** Bacteria of the genus *Shigella,* the causative microorganism for shigellosis, is a member of the *Enterobacteriaceae*. It is a gram-negative, non-sporeforming non-motile rod. The organism has a growth range of 10 to 47.2 °C with an optimum of 37 °C. The 2 primary foodborne pathogens are *S. flexneri* and *S. sonnei*. The microorganism is carried by humans and primates and is spread to food by carriers and contaminated water. *Shigella* strains are not particularly heat resistant. Approximately 5 min at 63 °C inactivates most strains of *S. flexneri* and *S. sonnei*. The main foods implicated in outbreaks of *Shigella* spp. are salads and seafoods that become contaminated during handling by infected workers or by unclean and unsanitized food contact surfaces. Control of *Shigella* is best accomplished by hygiene, health education, water disinfection and sanitation along with mild heat treatment where necessary.

**Pathogenic *Escherichia coli***. *Escherichia coli* is a gram-negative, motile, facultative anaerobe non-sporeforming rod. The source of the microorganism is generally the gastrointestinal tract of warm-blooded animals but it can also be found in water. Five to 6 types of diarrheagenic *E. coli* are known today, including enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, enteroadherent and enteroaggregative. These strains may cause neonatal, infantile, traveler's, or bloody diarrhea. Some produce toxins while others are invasive. Enterohemorrhagic *E. coli* (EHEC) causes a sequela called hemolytic uremic syndrome. Foods implicated in outbreaks of EHEC include ground beef, roast beef, alfalfa sprouts, raw milk, apple cider, meat sandwiches, mayonnaise, lettuce and dry salami. Food process inactivation of this bacterium is best accomplished by: 1) adequate cooking of all meat products to a center point temperature of 165-180$^{o}$F and 2) a professional sanitation program in place to inactivate the bacteria and prevent post-processing contamination or cross-contamination of processed food with raw product. The heat resistance of *E. coli* is equivalent to or slightly lower than *Salmonella* serovars (Table 4). Therefore, a heat process sufficient to inactivate *Salmonella* serovars will also likely inactivate *E. coli*.

***Yersinia enterocolitica.*** *Yersinia enterocolitica* is a pathogen that causes a foodborne infection with an onset time of 3-7 d. The symptoms of yersiniosis include severe abdominal cramps which mimic appendicitis, watery diarrhea, vomiting and fever. Pork and pork products, milk and foods washed in contaminated water (for example, tofu)

have all been implicated in outbreaks. Cross contamination can also cause a problem in ready-to-eat foods. *Yersinia enterocolitica,* as a psychrotroph can grow at refrigeration temperatures. In fact, cold storage can be selective for the microorganism. *Yersinia enterocolitica* has very low heat resistance in milk (Table 5).

***Vibrio.*** Three species of *Vibrio* are potential pathogens in food: *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. All are found in the marine environments and contaminate foods via contaminated water. Foods associated with *Vibrio* spp. foodborne infections include seafoods, raw vegetables, milk and inadequately sanitized water. Keeping raw product at low temperatures prior to processing helps to slow growth, and heating above 60 °C should easily inactivate this heat-sensitive organism (Table 6).

***Aeromonas hydrophila***. *Aeromonas hydrophila* has many pathogenic properties resembling *V. vulnificus*, that is, gastroenteritis in healthy individuals or septicemia in individuals with underlying chronic disease (for example, leukemia, carcinoma and cirrhosis) and those treated with immunosuppressive drugs or who are undergoing cancer chemotherapy or with impaired immune systems. Infections among healthy people are generally self-limiting whereas children are at the greatest risk. Species of *A. hydrophila* are ubiquitous in freshwater environments. Although *A. hydrophila* can be isolated from a wide range of foods at the retail level, outbreaks are generally small. The organism is a well-established component of raw meat spoilage and is found on beef, pork, lamb, poultry, fish and shellfish. It is also a common component of raw milk and raw vegetables. *Aerominas hydrophila* is eliminated by mild heat treatments (Table 7).

***Campylobacter jejuni***. *Campylobacter jejuni* is the leading cause of foodborne illness in humans in the United States (CDC 1999). The organism causes a diarrheal infection but can also have a more severe sequela known as Guillain-Barre Syndrome (0.2-2 cases/1000 cases of *C. jejuni*; paralysis, demyelination of nerves). The microorganism is generally unable to grow in foods; however, it often finds entry to food via human carriers or contamination. In heat-treated or dehydrated foods, contamination may not be a problem, but raw refrigerated foods of animal origin can be a source. The organism can be isolated from all common food animals and birds. Foods commonly associated with infection include raw milk, poultry, red meat and contaminated water sources. The microorganism is extremely sensitive to heat and would be inactivated at temperatures as low as 55 °C (Table 8).

***Listeria monocytogenes***. *Listeria monocytogenes* is the cause of a foodborne illness known as listeriosis. There are 13 serovars of pathogenic *L. monocytogenes,* but according to Raccourt and Cossart (1997), 95% of human isolates are serotypes 1/2a, 1/2b and 4b. The microorganism often attacks persons with suppressed immune systems, including pregnant women, neonates, the elderly and persons immunosuppressed by medications. Manifestations of listeriosis include abortion, perinatal septicemia and meningitis. The mortality rate associated with listeriosis is ca. 30% (Rocourt and Cossart 1997; V.J. Scott, personal communication). Although rarely described, *L. monocytogenes* can grow to high populations in temperature-abused food resulting in severe gastroenteritis after consumption. *Listeria monocytogenes* is truly ubiquitous and can

survive for long periods of time under extreme and adverse conditions. *Listeria monocytogenes* can multiply in foods stored at refrigeration temperatures so risk may increase during storage. It has been found in raw milk, raw milk cheese, soft-ripened cheeses, raw meats and seafood. There have been cases of illness from coleslaw and other raw vegetables that have been fertilized with animal manure or wastewater, and then not rinsed and cleaned prior to preparing and eating. Recently, an outbreak of listeriosis caused by *L. monocytogenes* serotype 4b resulted in 50 cases and 8 deaths linked to consumption of hot dogs and/or deli meats (CDC 1998). *Listeria monocytogenes* may be controlled in food processing plants with a good sanitation program and prevention of cross-contamination between raw and finished product. In addition, heating equivalent to milk pasteurization (71.7 °C for 15 s) or heating to 62.8 °C for 30 min or above should inactivate the microorganism in milk or other foods (Tables 9 and 10).

### 3.2. Vegetative Bacteria Inactivated by Pasteurization but Able to Produce a Heat-stable Toxin

***Staphylococcus aureus***. *Staphylococcus aureus* can produce a toxin in improperly stored food that, if ingested, will produce mild to severe symptoms of nausea, cramps, vomiting, diarrhea and prostration in 2-7 h, lasting 1 to 2 d. The enterotoxins produced by *S. aureus* are resistant to heating (up to a $D_{110C}$ = 10 min as measured by bioassays), including low-acid canned food processing. Many healthy people harbor *S. aureus.* It can be found in the nose, throat, hands, fingertips, hair and skin. Any food that is contaminated with the organism and supports growth can potentially develop this bacterial toxin. Proteinaceous foods (for example, chicken, turkey, meat, fish, dairy products, salad vegetables, ham), potatoes, cream-filled products and cream sauces are commonly involved in outbreaks. As the microorganism does not compete well in mixed populations, it is generally not a problem in unheated foods; however, when other naturally occurring bacteria are killed by cooking or inhibited from growth and *S. aureus* is later introduced by humans, it survives and grows. Consequently, it may be found in prepared foods such as salads, custards and cream-filled products. *Staphylococcus aureus* is also resistant to low water activities and survives curing solutions that contain salt or sugar. The cells of the microorganism are not heat resistant (Table 11) and should easily be killed by any mild heat treatment. *Staphylococcus aureus* is best controlled by preventing contamination.

### 3.3. Sporeforming Bacterial Organisms That Survive Cooking and Pasteurization and Produce Toxin

***Bacillus cereus***. *Bacillus cereus* is a sporeforming organism that produces 2 types of illness: the diarrheal syndrome, which develops within 20 h following ingestion, or the emetic (vomiting) response, which occurs 1 to 5 h after ingestion. The illnesses are the result of toxins associated with growth of the microorganism in foods (emetic) or the gastrointestinal tract (diarrheal). The diarrheal (enterotoxin) is produced during exponential growth in the gastrointestinal tract, while the emetic toxin is produced by cells growing in the food product (Granum 1997). Dairy products, cereals, meats and fried rice are commonly the foods involved in foodborne illness. Since the microorganism does produce spores, it is considered resistant to at least pasteurization

conditions. In fact, the spores of *B. cereus* have $D_{95C}$ values ranging from 1.5 to 36.2 min (Table 12). One thing that is apparent concerning the heat resistance of *B. cereus* spores is how variable their heat resistance is in the same food product. It is generally thought that *B. cereus* do not survive the low-acid canned food process. For instance, using the highest z(T) (10.1) and D-value (36.2) in Table 12, the $D_{121C}$ is 5.8 s, which confirms that this heat susceptibility is the case (ICMSF 1996).

***Clostridium perfringens.*** *Clostridium perfringens* food poisoning is the result of an enterotoxin produced in the gastrointestinal tract by *C. perfringens.* After ingestion, the microorganisms multiply and sporulate in the small intestine, releasing the enterotoxin and causing symptoms including abdominal cramps and diarrhea (McLane 1997). Meat and poultry are the most common foods associated with *C. perfringens* food poisoning. Foodborne illness caused by this microorganism is generally the result of poor refrigeration and inadequate reheating of cooked foods. Spores are often found on raw meats and may survive cooking of beef or poultry. If foods are inadequately cooled, the spores may then germinate and outgrow. If the food is then inadequately reheated, cells may continue to grow and reach large numbers. Ingestion of large numbers of organisms is necessary both for the microorganism to survive passage through the stomach and to initiate growth and toxin production in the intestines. As can be seen in Table 13, the D-value of *C. perfringens* spores at 98.9 °C may be as high as 31.4 min in beef gravy. Therefore, a low number of spores could potentially survive cooking of a meat in sauce; however, the preferred method of control of this microorganism is not necessarily initial heating but rather adequate cooling and adequate reheating following cooling to inactivate any cells produced during cooling.

***Clostridium botulinum***. *Clostridium botulinum* is a common soil bacterium that produces heat-resistant spores. This organism produces a potent neurotoxin that may be toxic to both humans and animals. The toxin is considered heat-labile and can be inactivated by heating to 80 °C for 10 min. When ingested, the toxin is absorbed and irreversibly binds to peripheral motor nerves causing paralysis and possible death without antitoxin treatment. Vegetables can carry heat-resistant Type A, B and F *Clostridium botulinum* spores that are a major concern in low-acid canned foods. Type E spores also can be found in fish and seafood products. The heat resistance as measured by D-values of *C. botulinum* Types A and proteolytic B spores generally ranges from 0.6 to 3 min at 110 °C (Tables 14 and 15). A comparison of Type A and B spores in similar products is shown in Table 16. *Clostridium botulinum* Type E spores are much less resistant than Type A or B and can be inactivated at or below 100 °C (Table 17).

### 3.4. Cyst-producing Protozoa That Can Remain Infectious in Unpasteurized Foods

***Cryptosporidium* and *Cyclospora*.** The protozoa, *Cryptosporidium parvum* and *Cyclospora cayetanensis*, are not able to replicate in foods, but they do produce cysts that can remain infectious in foods for extended periods of time. Since these organisms appear to have a low infective dose, their presence can contribute to infection, causing diarrhea in the general population. These protozoa may contaminate water systems since the cysts have a high tolerance for disinfectants, such as chlorine. Washing food with contaminated

water can infect foods with cysts. Thermal resistance values for *Cryptosporidium* oocysts of $D_{60\,°C}$ of 20 s in distilled water and $D_{71.7\,°C}$ of <1 s have been reported in milk. According to Rose and Slifko (1999), the heat resistance of *Cyclospora* may be similar to that of *Cryptosporidium* (Table 18).

### 3.4. Enteric Viruses That Can Cause Foodborne Infection from Unpasteurized Foods

**Hepatitis A virus, Rotavirus and Norwalk virus.** Several outbreaks of foodborne illness have been attributed to the viral contamination of shellfish and of unprocessed fruits. Hepatitis A virus and other enteric viruses may be found in shellfish taken from waters polluted by sewage. Fruits grown in fields where human waste or sludge is used as fertilizer have the potential for contamination by enteric viruses. Foods most vulnerable to viral contamination would be those not receiving a heat pasteurization step.

## 4. SURROGATE ORGANISMS TO VALIDATE PROCESSING PARAMETERS

### 4.1. Thermal, electrothermal and non-thermal food processing

The establishment of traditional thermal processes for foods has been based on 2 main factors (Anonymous 1989): 1) knowledge of the thermal inactivation kinetics of the most heat-resistant pathogen of concern for each specific food product and 2) determination of the nature of heat transfer properties of the food system, generally defined by a heat transfer rate. These 2 factors are used to calculate the scheduled process, thereby ensuring inactivation of pathogen(s) in that product. The validity of the established process is often confirmed using an inoculated test pack study. An inoculated pack study would be tested under actual plant conditions (this includes processing and control equipment, product and packaging) to reproduce the process in every detail. Since it is unwise to introduce viable pathogens into the production area, surrogate organisms are often utilized in the inoculated pack study, and their inactivation is measured to validate the process. Surrogates play an important role as biological indicators that can mimic the thermal inactivation properties of a pathogen and can help to detect peculiarities or deviations in the processing procedure.

One of the challenges in using new processing technologies for food preservation and pathogen inactivation is to determine if traditional methodologies can be used to establish and validate the new process. For practical purposes, the mechanism of microbial inactivation under electrothermal processes is basically the same as under conventional thermal processes: that is, heat inactivation. Thus, the 2 factors described above, which are well established for thermal processes (Anonymous 1996), should be used as a basis for establishing and validating scheduled electrothermal processes. It is also appropriate to use surrogate organisms to assist in determining and validating the process effectiveness. Regarding other preservation processes not based on heat inactivation (that is, high pressure, pulsed electric field, pulsed light), nonpathogenic surrogates still need to be identified and their significance evaluated. To accomplish this, more research needs

to be done in the area of inactivation kinetics of pathogens by new technologies as well as in the identification of non-pathogenic candidates useful as surrogate organisms.

### 4.2. Importance of Surrogates

Surrogate organisms are invaluable in confirming the efficacy of thermal-based processes. Their use, as opposed to using actual pathogens, derives from the need to prevent the introduction of harmful organisms into the production facility area. The consequences of mishandling a pathogen in the presence of workers, product and equipment (from safety to legal liability issues) could be devastating. Therefore, the use of surrogates by processing companies is of great importance to ensure microbiological safety of the process. For instance, surrogates have been used for many years in the low-acid canning industry to establish and validate the destruction of *C. botulinium* spores. The use of nonpathogenic spores of the putrefactive anaerobe *C. sporogenes,* or spores of the flat-sour thermophilic organism *Bacillus stearothermophilus* as surrogates for *C. botulinium,* have helped the industry develop processes that ensure products are safe and commercially sterile.

### 4.3. Criteria for Surrogates

The ideal surrogate would be the pathogen (or target organism) itself that had been transformed into a nonpathogenic form using genetic engineering techniques. Such an approach to surrogate selection is generally not followed due to possible reversion to pathogenicity or possible detection of false positives during routine testing. Generally, surrogates are selected from the population of well-known organisms that have well-defined characteristics and a long history of being nonpathogenic. In selecting surrogates, the following microbial characteristics are desirable:

- Nonpathogenic.
- Thermal or other inactivation characteristics that can be used to predict those of the target organism.
- Durability to food and processing parameters similar to target organism (for example, pH stability, refrigeration stability, oxygen tolerance and so on.).
- Stable thermal and growth characteristics that are similar from batch to batch.
- Easily prepared into high-density population forms.
- Once prepared, population is constant until utilized.
- Easily enumerated using rapid and inexpensive detection systems.

- Easily differentiated from other natural flora.
- Follows inactivation kinetics in a manner similar to pathogens receiving the same inactivation treatment.
- Genetically stable so results can be reproduced independently of laboratory or time of experiment.
- Will not establish itself as a "spoilage" organism on equipment or in the production area.
- Not susceptible to injury or reversible inactivation properties.

Ideal surrogates, with all of the features described above, are scarce. Generally, surrogates will have many of the criteria, as is the case with the traditional surrogates used in low-acid canned foods processing validation.

### 4.4. Surrogates for Pasteurized Products

Rather than using biological indicators as a basis for process validation, pasteurization processes have traditionally been evaluated and monitored using enzymatic destruction. Milk pasteurization has relied on the inactivation of the naturally occurring phosphatase enzyme as confirmation that product has received the proper heat treatment. This approach is not quantitative and is specific to pasteurization of milk. To obtain quantitative information to support the development and validation of thermal processes, the use of bacterial surrogates is preferred to the use of naturally occurring enzymes. Research, however, is progressing on the identification and use of proteins and enzymes with inactivation kinetics comparable to microorganisms of concern, and there is a great potential in using them as chemical indicators.

Selection of surrogate organisms for pasteurization of products is a relatively new task for food scientists. The literature basically lacks information on recommendations for useful surrogate organisms. Therefore, a good deal of research and development may be required for progress to be made. Efforts are needed to identify and establish surrogates that meet many of the criteria listed above and can be utilized in process development for pasteurized foods.

Pathogens of public health significance in foods are vegetative cells of both gram-positive and gram-negative organisms as well as protozoan cysts. These organisms are inactivated fairly rapidly at pasteurization temperatures and are not good candidates as surrogates. Thermoduric lactic acid bacteria are also present in many foods. These organisms may survive the process and should be evaluated for their possible value as surrogates. They include the lactic streptococci (*Streptococcus thermophilus*), the lactobacilli (*Lactobacillus delbrueckii* spp., *Lactobacillus bulgaricus*, *Lactobacillus lactis*) and *Pedioccocus* spp. Other related lactic acid bacteria to consider as possible surrogates would include strains of *Lactococcus* and *Leuconostoc*. In cases where *Listeria*

*monocytogenes* is the pathogen of interest, strains of *Listeria innocua* have served as non-pathogenic surrogates. In addition, nonpathogenic strains of *E. coli* have served as surrogates for *E. coli* O157:H7. In cases such as these, where surrogates are utilized, it should be proven that the surrogates are suitable for use based upon the above criteria.

### 4.5. Surrogates for Low-acid Canned Food Products

Methodologies for validating low-acid-canned food (LACF) processes have been in existence for quite some time. The regulatory agencies require that scheduled processes for LACFs be established by a person or organization having expert knowledge of thermal processing requirements for foods packaged in hermetically sealed containers. The biological validation of an electrothermal process such as ohmic heating or inductive heating may be designed and performed following conventional heating biological validation procedures; however, the mode of heat generation, heat distribution and location of coldest point(s) need special considerations when validating an electrothermal process and cannot be extrapolated from conventional thermal processes, as will be described in the subsequent sections of this report. These issues need to be investigated by experts in electrothermal processes.

Where novel non-thermal processes are being investigated, the application of traditional and classical approaches, such as the use of the formula described by C.O. Ball in the 1920's, may not be applicable. The approaches to process development for novel processes may initially be mostly empirical and strongly supported by biological validation until the nature of the kinetics of inactivation is more fully understood and hence predictable. It remains to be seen if the use of organisms like *C. sporogenes* or *B. stearothermophilus* will be acceptable surrogates for *C. botulinium* in many of these novel non-thermal processing applications. As will be described in the following sections of this report, future research needs to address the resistance of pathogens of concern and the identification of appropriate surrogates for the specific non-thermal processes.

### 4.6. Other Considerations

The use of surrogate organisms to determine and validate processes for electrothermal processes will be challenging, especially for pasteurization processes. The following are some further points to consider while undertaking this endeavor:

- Keep the approach as easy, accurate and simple as possible.
- Design the process so that the surrogate exhibits a predictable time-temperature profile that correlates to that of the target pathogen.
- Be attentive to the introduction of system modifications or variables, which could alter the heat-transfer properties of the food leading to inaccurate results (for example, thermocouple probes changing heating rates, nutrients added to the product for surrogate growth altering viscosity and so on).

- Validate the susceptibility or tolerance of a surrogate, if it is known.
- When first developing a process, working with a mixture of potential surrogate strains may be useful in narrowing the parameters toward establishing an effective process.

## THERMAL RESISTANCE TABLES

**Table 2.** Heat resistance of serovars of *Salmonella* in various foods (ICMSF 1996).

| Serovar | Food | Temp (°C) | D-value (min) | Approx. z-value (°C) |
|---|---|---|---|---|
| Senftenberg | beef boullion | 65.5 | 0.66 | |
| | pea soup | 65.5 | 1.11 | 5.6 |
| | skim milk | 65.5 | 1.11 | |
| | Milk | 65.6 | 0.56 | 4.4 |
| | Milk chocolate | 70 | 480 | 18.9 |
| | Milk chocolate | 71 | 276 | |
| Typhimurium | Milk chocolate | 70 | 1050 | 17.7 |
| | Milk chocolate | 71 | 396 | |
| | TSB[1] + 10% MS[2] | 55.2 | 4.7 | 4.5 |
| | TSB + 30% MS | 55 | 11 | 4.6 |
| | TSB + 42% MS | 55.1 | 18.3 | |
| | ground beef | 57 | 2.13-2.67 | |
| Eastbourne | Milk chocolate | 71 | 270 | |
| none specified | Ground beef | 57.2 | 4.2 | |

[1]Trypticase soy broth

[2]Milk solids

**Table 3.** Heat resistance as measured by the D-value at 65.6 °C (150 0F) and approximate z-values of *Salmonella* serovars in milk (ICMSF 1996).

| **Serovar** | **$D_{65.6C}$ (s)** | **Approx. z-value (°C)** |
|---|---|---|
| Anatum | 1.4 | 5.0 |
| Binza | 1.5 | 5.3 |
| Cubana | 1.8 | 5.6 |
| Meleagridis | 1.1 | 5.4 |
| Newbrunswick | 1.3 | 4.5 |
| Senftenberg | 34.0 | 4.4 |
| Tennessee | 1.4 | 4.9 |

**Table 4.** Effect of heating on pathogenic and non-pathogenic strains of *Escherichia coli* (ICMSF 1996).

| **Product** | **Strain** | **Temp (°C)** | **D-value (min)** |
|---|---|---|---|
| Raw milk | ATCC 9637 (NP)[1] | 57.2 | 1.3 |
| Chocolate milk | ATCC 9637 (NP) | 57.2 | 2.6 |
| 40% Cream | ATCC 9637 (NP) | 57.2 | 3.5 |
| Ice cream mix | ATCC 9637 (NP) | 57.2 | 5.1 |
| Skim milk | O111:B4 | 55 | 5.5 |
| Whole milk | O111:B4 | 55 | 6.6 |
| Ground beef [2] | O157:H7 | 57.2 | 4.5 |

| | | | |
|---|---|---|---|
| Ground beef [3] | O157:H7 | 57.2 | 4.1-6.4 |
| Ground beef [2] | O157:H7 | 62.8 | 0.4 |
| Ground beef [3] | O157:H7 | 62.8 | 0.26-0.47 |

[1]NP, non-pathogenic

[2]Doyle and others 1984

[3]Line and others 1991

**Table 5.** Effect of heating on *Yersinia enterocolitica* in milk; where measured, z-value ranged from 4.0-5.78 °C (ICMSF 1996).

| **Temperature (°C)** | **D-value** |
|---|---|
| 51.7 | 23.4-29.9 |
| 55 | 1.8-2.2 |
| 57.2 | 4.6-14.7 |
| 58 | 1.4-1.8 |
| 60 | 0.067-0.51 |
| 62 | 0.15-0.19 |
| 62.8 | 0.012-0.96 |
| 65 | 0.028 |
| 68.3 | 0.09 |

**Table 6.** Heat resistance of *Vibrio* species (ICMSF 1996).

| **Species** | **Product** | **Temp (°C)** | **D-value (min)** | **z-value (°C)** |
|---|---|---|---|---|
| *parahaemolyticus* | | | | 5.6-12.4 |
| | fish homogenate | 48 | 10-16 | |

| | | | | |
|---|---|---|---|---|
| | clam homogenate | 55 | 0.02-0.29 | |
| | crab homogenate | 55 | 2.5 | |
| *cholerae* | | | | |
| | 1% peptone | 60 | 0.63 | 17 |
| | crab homogenate | 60 | 2.65 | 21 |
| | oyster homogenate | 60 | 0.35 | |
| *vulnificus* | SIMILAR TO *V. parahaemolyticus* | | | |

**Table 7.** *Aeromonas hydrophila* heat resistance (ICMSF 1996).

| **Product** | **Temp (°C)** | **D-value (min)** | **z-value (°0C)** |
|---|---|---|---|
| Raw milk | 48 | 3.3-6.2 | 5.2-7.7 |
| Saline | 48 | 2.2-6.6 | 5.2-7.7 |

**Table 8.** *Campylobacter jejuni* heat resistance (ICMSF 1996).

| **Product** | **Temp (°C)** | **D-value (min)** |
|---|---|---|
| Skim milk | 50 | 1.3-5.4 |
| | 55 | 0.74-1.0 |
| Ground beef | 50 | 5.9-6.3 |
| | 56 | 0.62-0.96 |
| Lamb | 50 | 5.9-13.3 |
| | 55 | 0.96-1.26 |
| Cooked chicken | 55 | 2.12-2.25 |

**Table 9.** Resistance of *Listeria monocytogenes* to heat in milk products (ICMSF 1996).

| **Products** | **Temp (°C)** | **D-value (min)** |
|---|---|---|
| Raw milk, raw skim milk, raw whole milk, cream | 52.2 | 24.08-52.8 |
| | 57.8 | 3.97-8.17 |
| | 63.3 | 0.22-0.58 |
| | 66.1 | 0.10-0.29 |

**Table 10.** Heat resistance of *Listeria monocytogenes* in various products at 60 °C (ICMSF 1996).

| **Product** | **D60 °C - value (min)** |
|---|---|
| Ground meat | 3.12 |
| Ground meat, cured | 16.7 |
| Fermented sausage | 9.2-11 |
| Roast beef | 3.5-4.5 |
| Beef | 3.8 |
| Beef homogenate | 6.27-8.32 |
| Naturally contaminated beef | 1.6 |
| Weiner batter | 2.3 |
| Chicken leg | 5.6 |
| Chicken breast | 8.7 |
| Chicken homogenate | 5.02-5.29 |
| Carrot homogenate | 5.02-7.76 |

| | |
|---|---|
| ***RANGE*** | ***1.6-16.7*** |

**Table 11.** *Staphylococcus aureus* vegetative cell heat resistance (ICMSF 1996).

| **Product** | **Temp (°C)** | **D-value (min)** | **Notes** |
|---|---|---|---|
| Milk | 50 | 10 | z = 9.5 °C |
| | 55 | 3 | |
| | 60 | 0.9 | |
| | 65 | 0.2 | |
| | 70 | 0.1 | |
| | 75 | 0.02 | |
| Meat macerate | 60 | 6.0 | + 500 ppm nitrite |
| Pasta, semolina-egg dough | 60 | 3 | $a_w$ = 0. 92 |
| | | 5 | $a_w$ = 0. 87 |
| | | 8 | $a_w$ = 0. 85 |
| | | 12 | $a_w$ = 0. 83 |
| | | >40 | $a_w$ = 0. 80 |
| | | 32 | $a_w$ = 0. 76 |
| | | 22 | $a_w$ = 0. 61 |
| Phosphate buffer | 60 | 2.5 | pH = 6.5 |

**Table 12**. *Bacillus cereus* spores heat resistance in various media at 95 °C (ICMSF 1996).

| **Heating medium** | **D95 °C (min)** | **z-value (°C)** |
|---|---|---|

| | | |
|---|---|---|
| 0.06 M phosphate, pH 7.0 | 2.6-21.7 | 10.0-10.1 |
| 0.05 M phosphate, pH 7.0[1] | 2.1-3.4 | |
| Phosphate, pH 7.0 | 13 | |
| Distilled water | 1.5-36.2 | |
| Water | 36 | 6.7-8.3 |
| Infant formula, pH 6.3 | 2.7-15.3 | 8.1-8.7 |
| Milk | 1.8-19.1 | 9.4 |
| Bread | 2.9-36.2 | |
| **RANGE** | **1.5-36.2** | **6.7-10.1** |

[1]Contains sorbitol, glycerol or NaCl

**Table 13.** Heat resistance of *Clostridium perfringens* spores (ICMSF 1996).

| **Product/Heating Medium** | **Temperature (°C)** | **D-value (min)** |
|---|---|---|
| Phosphate buffer, pH 7.0 | 90 | 0.015-8.7 |
| Phosphate buffer, pH 7.0 | 98.9 | 18.6 |
| | 104.4 | 3.15 |
| | 110 | 1.29 |
| Beef gravy, pH 7.0 | 98.9 | 31.4 |
| | 104.4 | 6.6 |
| | 110 | 0.5 |

**Table 14.**Heat resistance of *Clostridium botulinum* strain 62A (Type A) spores at 110 °C. (ICMSF 1996).

| Product | D-value (min) | z-value (°C) |
|---|---|---|
| Asparagus, canned, pH 5.04 | 1.22 | 8.8 |
| Asparagus, canned, pH 5.42 | 0.61 | 7.9 |
| Corn, canned | 1.89 | 11.6 |
| Macaroni creole, pH 7.0 | 2.48 | 8.8 |
| Peas, puree | 1.98 | 8.3 |
| Peas canned, pH 5.24 | 0.61 | 7.6 |
| Peas, canned, pH 6.0 | 1.22 | 7.5 |
| Spanish rice, pH 7.0 | 2.37 | 8.6 |
| Spinach, canned, pH 5.37 | 0.61 | 8.4 |
| Spinach, canned, pH 5.39 | 1.74 | 10.0 |
| Squash | 2.01 | 8.2 |
| Tomato juice, pH 4.2 | 1.50-1.59[1] | 9.43 |
| Tomato juice, pH 4.2 | 0.92-0.98 | - |
| Phosphate buffer, M/15, pH 7.0 | 0.88 | 7.6 |
| | 1.74 | 10.0 |
| | 1.34 | 9.8 |
| | 1.6-1.9 | 8.1-9.2 |
| | 1.01 | 9.1 |
| Distilled water | 1.79 | 8.5 |

[1]Strain A16037

**Table 15.** Heat resistance of *Clostridium botulinum* proteolytic Type B spores at 110 °C (ICMSF 1996).

| **Product** | **D-value (min)** | **z-value (°C)** | **Strain** |
|---|---|---|---|
| Asparagus, canned, pH 5.04 | 1.09 | 9.7 | 213B |
| Asparagus, canned, pH 5.42 | 1.06 | 7.9 | 213B |
| Beans, snap | 0.86 | 9.7 | 213B |
| Beets | 1.17 | 10.8 | 213B |
| Carrots, fresh | 0.94 | 9.4 | 213B |
| Corn | 1.03 | 10.0 | 213B |
| Corn, puree | 2.88 | 10.6 | 213B |
| Corn, canned | 2.15 | 9.6 | 213B |
| Milk solids, whole, 20%, pH 6.34 | 0.93 | 7.9 | 213B |
| Mushrooms, puree | 0.49-0.99 | - | 7 strains |
| Peas, puree | 2.14-12.42 | 8.3 | 213B |
| Peas, canned, pH 5.6 | 3.07 | 10.1 | 213B |
| Peas, canned, pH 5.94 | 1.52 | 7.4 | 213B |
| Rock lobster, liquor | 2.97-3.33 | 10.6 | A35 |
| Spinach, fresh | 1.75 | 10.3 | 213B |
| Spinach, canned, pH 5.39 | 1.54 | 8.6 | 213B |
| Spinach, canned, pH 5.37 | 1.19 | - | 213B |
| Phosphate buffer, M/15, pH 7.0 | 1.85 | 7.7-11.3 | 213B |
|  | 1.4 | 8.5 | 213B |
|  | 1.6 | 8.3 | 213B |

| | | | |
|---|---|---|---|
| | 1.19 | 9.1 | 213B |
| | 2.0 | 9.1 | 213B |
| | 1.5 | 10.1 | Amanna |
| | 2.0 | 9.0 | 169B |

**Table 16.** Comparison of heat resistances as measured by D-value in minutes of *Clostridium botulinum* Type A and B spores in similar products at 110 °C (ICMSF 1996).

| **Product** | **Type A** | **Type B (Proteolytic)** |
|---|---|---|
| Asparagus, canned | 0.61-1.22 | 1.06-1.09 |
| Corn, canned | 1.89 | 2.15 |
| Peas, puree | 1.98 | 2.14-12.42 |
| Peas canned | 0.61-1.22 | 1.52-3.07 |
| Spinach, canned | 0.61-1.74 | 1.19-1.54 |
| Phosphate buffer, M/15, pH 7.0 | 0.88-1.9 | 1.19-2.0 |

**Table 17.** Heat resistance of *Clostridium botulinum* non-proteolytic Type E spores in seafood products (ICMSF 1996).

| **Product** | **Temp (°C)** | **D-value (min)** | **z-value (°C)** |
|---|---|---|---|
| Blue crabmeat | 74 | 6.8-13.0 | - |
| Oyster homogenate | 70 | 72 | 7.5 |
| Oyster homogenate + 1% NaCl | 70 | 100 | 6.8 |
| Oyster homogenate + 0.13% K sorbate | 70 | 72 | 7.4 |
| Oyster homogenate + NaCl + K sorbate | 70 | 79 | 7.3 |

| | | | |
|---|---|---|---|
| Menhaden surimi | 73.9 | 8.66 | 9.78 |

**Table 18.** Heating resistance of protozoa in water systems and in foods

| Organism | Heating Med | Survival | Time/Temp. | Reference |
|---|---|---|---|---|
| *Anisakis pseudoterranova* | fish/medium | maximum survival time | 1 min, 60 °C | 1 |
| *Anisakis anisakis* | fish/medium | maximum survival time | 10 s, 55 °C | 1 |
| *Cryptosporidium* oocysts | distilled water | 3-log reduction | 1 min, 60 °C | 2 |
| Cryptosporidium *oocysts* | Milk | 6-log reduction | 5 s, 71.7 °C | 2 |
| *Taenia* cysticerci | | inactivated | 60 °C | 1 |

[1]ICMSF 1996

[2]Rose and Slifko 1999

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