Quantification of microbial quality and safety in minimally processed foods

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Abstract

To find a good equilibrium between quality and margin of safety of minimally processed foods, often various hurdles are used. Quantification of the kinetics should be used to approach an optimum processing and to select the main aspects. Due to many factors of which the exact quantitative effect is unknown (uncertainty) and the often large (biological) variability, this is not a straightforward and easy procedure. For describing the kinetics one can start by describing the development in time (primary models), followed by models describing the parameters of the primary models as function of process variables (secondary models). Due to the complexity and large variability and uncertainty, it is best to start process analyses globally, using simple kinetic models.

Various types of primary and secondary models will be commented upon and some criteria for model choice will be given. Especially, if dimensionless numbers can be defined, then these can be used for a structured analysis, clearly quantifying main aspects.

Important points for the development of models and interpretation of modelling results will be presented. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Quality; Safety; Modelling; Dimensionless numbers

1. Introduction

Often the optimisation of quality and margin of safety of food products gives a conflict, by increasing the safety (for example, more rigorous heat treatment, the higher concentration of preservatives), the quality decreases, and vice versa. In order to determine a compromise between quality and margin of safety, quantification should be performed. However, in order to quantify total food production and distribution processes, much information must be gathered about the kinetics of the spoilage organisms and pathogens, the quality attributes of the product and in many cases the kinetics of physical processes like heat and mass diffusion. For quality, one can concentrate on various quality attributes originating from physical (e.g. drying), (bio)chemical (e.g. colour change), enzymatic (e.g. action of lipases) or microbial processes (e.g. acidification).

In minimally processed foods, one aims at reducing the negative influence of processing (mainly the heat treatment) by combining various hurdles, in order to be able to guarantee an acceptable safety level, with an acceptable shelf life (Leistner & Gorris, 1995). It is practically impossible to have perfect knowledge of all factors of a food product, micro-organism, and production and storage conditions influencing, either directly or indirectly, safety and/or quality. Therefore, there is always a certain level of uncertainty. Since safety of food products is a prerequisite, one often wants to take “no risk” and use many “safety factors” if there is uncertainty (Smith, Imfeld, Dayan, & Roberfroid, 1999). This means that if one has more knowledge, one can better locate the risks, probably allowing less over-processing.

In addition to this uncertainty, there is also often a large variability. There is a biological variability (for example of raw material or contaminating organisms), process variability (for example daily variation), and variability after processing (for example storage time, storage temperature), at the distribution and consumer level. This variability increases the need for safety factors. In the same manner as with uncertainty, one can also see this as a challenge. If one can reduce the
variability by better control, one can decrease the safety factors, and thus reduce over-processing.

This is well represented by Cole’s cliff (Cole, 1994), Fig. 1. If one can better locate where one is (and where the cliff is!), then one can reduce the safety factors (from 1 to 2), and thus better target the process (reduce uncertainty). If one can reduce the variability, then one can also move closer to the cliff with the same (very small) risk to fall off (from 2 to 3).

2. Kinetics and probability

One of the differences in approach between quality and safety is that for quality one is often interested only in the kinetics of the process, while for safety, apart from the kinetics, the probability is also often relevant (probability of contamination, probability of survival) (Cassin, Lammerding, Todd, Ross, & McColl, 1998). It is best to clearly separate the probability and the kinetics. If, for example, the probability is determined that a certain level is reached in a certain time (one-point determination), then it depends on both the probability of a viable organism present, its initial level and the kinetics, so probability and kinetics cannot be separated.

Thus, one should make a distinction between probability and number. If numbers are always larger than one per product unit, then one can see the process as continuous (probability not relevant). If numbers are smaller than one per product unit, then this means that there is a probability that an organism is present and it becomes discrete, with some products with one organism, others with no organisms (Zwietering & van Gerwen, 2000). In that case, both probability and number are of relevance. For example, in Table 1, the number after heating is calculated as 4E-6 cfu (concentration of 4Ee-8 cfu/g with a 100 g product), quite largely below 1. This means that one in 2.5E5 products contains at least one organism, and the others will have no organisms Table 2.

In order to obtain transparency and optimal control of safety, it is best to give attention to the whole chain from farm to fork. Often one reasons only from raw material to exit of the product from the factory, but also primary production and consumer handling are in many cases relevant. Although more difficult to control, the main critical control points (CCPs) and main stages can be situated before or after the factory.

Since analysing the whole chain with all its aspects (for both quantitative HACCP analysis and a quantitative risk assessment) is a rather heavy task, it is better to start globally in order to detect the important determinants. This analysis does not have to be accurate, but should reveal the orders of magnitude of the determinants. One can then better invest afterwards in getting more accurate estimates of the main stages, than to spread all efforts evenly over the whole chain.

Together with expertise, quantifying to estimate the order of magnitude is better than basing oneself on expertise only.

One can separate a process in stages and determine for each stage the contamination (probability and

<table>
<thead>
<tr>
<th>Stagea</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Assemblage</td>
<td>Cooking</td>
<td>Cool 1</td>
<td>Cool 2</td>
<td>Cool 3</td>
<td>Storage 1</td>
<td>Transport</td>
<td>Storage 2</td>
</tr>
<tr>
<td>t (h)</td>
<td>0.1</td>
<td>0.0833</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>100</td>
<td>8</td>
<td>48</td>
</tr>
<tr>
<td>T (°C)</td>
<td>25</td>
<td>65</td>
<td>37</td>
<td>20</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>k (h⁻¹)</td>
<td>0.951</td>
<td>−218.4</td>
<td>1.65</td>
<td>0.5722</td>
<td>0.0661</td>
<td>0.0019</td>
<td>0.0228</td>
<td>0.0661</td>
</tr>
<tr>
<td>r_c (cfu/g)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SC</td>
<td>0.0413</td>
<td>−7.906E</td>
<td>0.1791</td>
<td>0.0621</td>
<td>0.0072</td>
<td>0.0819</td>
<td>0.0793</td>
<td>1.3787</td>
</tr>
<tr>
<td>CC</td>
<td>0.30103</td>
<td>0.16275</td>
<td>7.401</td>
<td>0.22063</td>
<td>0.12879</td>
<td>0.09775</td>
<td>0.06704</td>
<td>0.04890</td>
</tr>
<tr>
<td>C (cfu/g)</td>
<td>2.2</td>
<td>3.98E-08</td>
<td>1.511</td>
<td>2.897</td>
<td>3.962</td>
<td>5.991</td>
<td>8.391</td>
<td>224.38</td>
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<tr>
<td>log(C)</td>
<td>0.34</td>
<td>−7.40</td>
<td>0.18</td>
<td>0.46</td>
<td>0.60</td>
<td>0.60</td>
<td>0.78</td>
<td>0.82</td>
</tr>
<tr>
<td>N (cfu)</td>
<td>219.96</td>
<td>3.98E-06</td>
<td>151.06</td>
<td>289.67</td>
<td>396.17</td>
<td>599.13</td>
<td>839.11</td>
<td>2248.17</td>
</tr>
<tr>
<td>log(N)</td>
<td>2.34</td>
<td>−5.40</td>
<td>2.18</td>
<td>2.46</td>
<td>2.60</td>
<td>2.78</td>
<td>2.92</td>
<td>4.35</td>
</tr>
</tbody>
</table>

*a k is the growth or inactivation rate, r_c is the contamination rate, SC is the stage characteristic (Eq. (2)), CC is the contamination characteristic (Eq. (3)), N is the bacterial number per product (cfu) and C is the bacterial concentration (cfu/g). Bold values indicate relevant changes. The temperature history in the cooling stage is approximated by three constant temperature stages.
amount) and the kinetics, finally ending up with an exposure, of which the effect on a human can be estimated (see, for example, Cassin et al., 1998). Just like all the uncertainties accumulating for the exposure estimate and the human response, one large uncertainty is also the acceptable level of risk. Zero risk does not exist, a risk of 1 in 1000 with millions of product units sold is clearly unacceptable. But to set an acceptable level is a tedious task. Putting very large effort in determining exposure accurately with a totally arbitrary ‘acceptable risk value’ or the opposite gives a wrong equilibration.

Quantifying, knowing that there are many uncertainties is still better than an ostrich policy.

### 3. Quantification and characteristic numbers

For every stage, one can make a balance, determining the inflow (contaminated numbers in product inflow), the extra contamination in the stage (from the external environment or in-line), and the kinetics (growth or death) (Zwietering & Hasting, 1997). Quantifying these three processes in one stage can actually provide an insight regarding the relative importance of the three phenomena in this stage. Additionally, over the whole process line, this gives a global view of the important phenomena.

This balance reads in words thus:

**Out** =

*Initial contamination + subsequent contamination*

*Inactivation/growth factor*

and as equation

$$N_{out} = (N_{in} + r_c)e^{kt}$$

(1)

with $N_{out}$ the outgoing concentration (cfu/g), $N_{in}$ the ingoing concentration, $r_c$ the contamination rate (cfu/g product) and $k$ the growth or inactivation rate (time$^{-1}$).

The results can be represented by characteristic numbers by defining SC and CC as the log increase due to growth (stage characteristic):

$$SC = \log(N_{out}) - \log(N_{in} + r_c) = \log(e^{kt}) = \frac{kt}{\ln(10)}$$

(2)

and contamination (contamination characteristic):

$$CC = \log(N_{in} + r_c) - \log(N_{in}) = \log\left(\frac{N_{in} + r_c}{N_{in}}\right)$$

(3)

And the total log increase is

$$\Delta \log N = \log\left(\frac{N_{out}}{N_{in}}\right) = \log\left(\frac{(N_{in} + r_c)e^{kt}}{N_{in}}\right) = CC + SC$$

(4)

These characteristic numbers can be used to compare orders of magnitudes and thus select the most important stages and phenomena, and enable ranking. It should be realised that contamination is not easy to quantify (especially for pathogens at low levels and incidence), but since it is in many cases critical, more effort should be devoted in quantifying it.

In Table 1, the results of the characteristic numbers for a process line are represented. In this example, the growth and inactivation of *Salmonella* in a product containing 100 g of chicken is predicted. Initially, the contamination is 100 cfu (so a concentration of 1 cfu/g). During processing, both the quantitative effect of temperature (SC) and the effect of additional contamination (CC) with 100 cfu/product (or $r_c = 1$ cfu/g) in every processing stage are determined. It is clear that the most relevant phenomena are inactivation in stage 2 (SC = −7.9), contamination in stage 3 (CC = 7.4) and growth in stage 8 (SC = 1.4). Except for the fact that the quantitative relevant stages are detected, even the results can be used for ranking, the recontamination in stage 3 being more important than the growth in stage 8. Although after stage 2 not all products are contaminated (number below 1), due to recontamination, all products are contaminated again. It should be noted that the predicted order of magnitude of the various processes is totally different. Even if the contamination in stage 3 is not 100 cfu/product but 1 cfu/product, the CC value changes from 7.4 to 5.4. So even with a factor change of 100 in contamination rate, the qualitative conclusion remains that contamination in this stage is important.

The results can be represented graphically in various ways, for example, by arrows (Fig. 2). In Fig. 2a, the growth in the second stage is the main determinant, while in Fig. 2b, although the initial contamination is quite large, it is reduced by inactivation. The post-contamination process is totally determining the final concentration. In this manner, one can determine and

### Table 2

Organism parameters used for the calculations in Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>$T_{min}$</th>
<th>$T_{opt}$</th>
<th>$T_{max}$</th>
<th>$\mu_{opt}$</th>
<th>$D_{ref}$</th>
<th>$T_{ref}$</th>
<th>$z$</th>
<th>log($C_{in}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>5.2</td>
<td>37</td>
<td>46.2</td>
<td>1.65</td>
<td>2</td>
<td>60</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

*Parameters from Zwietering and van Gerwen (2000).*
compare quantitatively the importance of the “highways”, which can help to direct the focus for more accurate estimations, and the potential main points for control.

The same procedure can be followed for quality attributes. In this case, probability and contamination are often not relevant. For quality, one has the same problems of variability and uncertainty, for instance, unknown aspects in the kinetics, inaccurate knowledge of consumer preference, etc.

4. Description of the kinetics

4.1. Primary models

Primary models describe the changes in time of the attribute of interest. For bacterial kinetics, growth and inactivation are possible. An often-mentioned third phenomenon, survival, can be seen as slow growth or slow inactivation, so it is not inherently different from growth or inactivation, only the time scales are larger.

Various model types can be used (from simple to more complex), examples for inactivation are given: 1st order

$$\ln \left( \frac{N}{N_0} \right) = -kt, \quad (5)$$

1st order+shoulder+asymptote (Zwietering, de Wit, Cuppers, & van ’t Riet, 1994; Buchanan, Whiting, & Damert, 1997):

$$y = 0, \quad t < \lambda,$$

$$y = -k(t - \lambda), \quad t \geq \lambda,$$

$$\ln \left( \frac{N}{N_0} \right) = \max(y, A). \quad (6)$$

During the shoulder phase \((t < \lambda)\), the level remains at the initial value, then an exponential decrease follows, which is limited by an asymptote value \((A)\). This final asymptote level can be disputed, and will often be the result of artefacts due to approaching the detection level, although often a phase with a smaller inactivation is seen (Cerf, 1977). A real horizontal asymptote meaning no inactivation at all is difficult to be appreciated biologically.

4.1.1. Sigmoidal

Empirical models for bacterial growth can also be used for inactivation, like the Gompertz model (see, for example, Bhaduri et al., 1991):

$$\ln \left( \frac{N}{N_0} \right) = -A \exp \left\{ -\exp \left( \frac{-t}{A} \right) \right\}. \quad (7)$$

Also, various mechanistic based models are proposed, like the models assuming two populations (for example Cerf, 1977):

$$\frac{N}{N_0} = f \exp(-k_1 t) + (1 - f) \exp(-k_2 t) \quad (8)$$

with \(f\) the fraction of cells with inactivation rate \(k_1\), and \((1 - f)\) the fraction with inactivation rate \(k_2\).

For a more extensive overview of primary models for growth and inactivation, the reader is referred to van Gerwen and Zwietering (1998) and McDonald and Sun (1999).

It should be in all cases realised that often variability is large and differences between models are often smaller than results of equal experiments carried out in two different laboratories. Therefore in many cases, the exponential model (Eq.(5)) is sufficient. A second remark is that the model is extrapolated over various log units, and for models with more sophisticated curvatures it is questionable if the curvature remains the same. The experiment for the model and parameter determination is carried out with concentrations, for example, from \(10^8\) to \(1\) cfu/g, and the range for practical application is an inactivation from \(10^2\) to \(10^{10}\) (Fig. 3). It can be questioned if the shape of the curve of the inactivation of the lab experiment (whatever curvature is found) is equal to the one in the food product, where the objective is to reach much lower concentrations.

4.1.2. Mechanistic models

It is clear that mechanistic models are often mathematically more complex and contain more parameters to be estimated. Their advantage, however, is that they can provide more insight and might be more reliable if tested for other conditions than those they were developed for.
It is often mentioned that mechanistic models are inherently better than empirical models. It should however be realised that this is only the case if the proposed mechanism is correct. In the following example, two mechanistic models are presented to describe the lag in bacterial growth. To develop and fit these models is relatively easy, but it is difficult or can even be considered impossible to prove that the mechanism is correct. The example concerns two different mechanistically based, realistic models that result in two different equations, but can describe the same effect.

Model 1: We assume that: (1) the cells are in such a physiological state that they cannot yet divide, and that component $B$ must first be made which is converted to $C$ before they can divide; (2) component $A$, from which $B$ is formed, is available in excess (zero-order in $A$); (3) the number of molecules $C$ per cell ($p$) is constant; therefore, the more $C$ is formed, the more cells ($X$) there will be formed: So the amount of $C$ is $p$ times the amount of biomass ($X$): $C = p \times X$ or $X = C/p$; and (4) if there are more cells, then the reactions also proceed proportionally much more quickly (first order in number). This results in the following equations ($A$, $B$, and $C$ are total amounts, not intracellular concentrations):

$$
A \xrightarrow{k_a} B \xrightarrow{k_b} C,
$$

$$
\frac{dB}{dt} = (k_a - k_B) \cdot X = k_aX - k_BBX,
$$

$$
\frac{dC}{dt} = p \frac{dx}{dt} = k_bBX.
$$

We can solve this model, for example, numerically. We can also try to interpret this equation. We can expect that if there is too little $B$ available, this must first be produced before the cell can really begin to grow; this is, therefore, the adaptation phase/lag phase. But after a time, a stationary situation ($dB/dt = 0$) could occur for $B$ (balanced growth). Then $k_a = k_B$, and therefore:

$$
p \frac{dx}{dt} = k_BBX = k_aX.
$$

If $B$ consequently reaches a stationary quantity ($B = k_a/k_b$), then the final specific growth rate becomes $k_a/p$. In the (numerical) solution it turns out that these statements tally. This is a good check of the solution and of our understanding of the system. These equations can then be simplified by defining $Q = B/k_a$ and $\mu = k_a/p$:

$$
k_a \frac{dQ}{dt} = (k_a - k_Bk_a)X, \quad \frac{dQ}{dt} = (1 - k_B)X,
$$

$$
\frac{dx}{dt} = \frac{k_b}{p} k_aQX = \mu k_BQX.
$$

It results in a model with two parameters $\mu$ and $k_B$, and two initial conditions $Q(0)$ and $X(0)$. This model can make a good description of the lag phase during bacterial growth (Fig. 4).

Model 2: Another approach could be that we assume that: (1) bacteria grow exponentially following the lag phase; and (2) every individual cell has a lag phase and due to the distribution of physiological states this lag phase is normally distributed in the population of cells (therefore, with a mean and a standard deviation). If we calculate the change in the total population with these assumptions, then we can use this as well to make a good description of the curvature of the change in the number of bacteria in time.

(1) bacteria grow exponentially following the lag phase:

$$
\frac{dN}{dt} = \mu N \text{ with } t > \lambda,
$$

$$
\ln \left( \frac{N}{N_0} \right) = \mu(t - \lambda) \text{ with } t > \lambda,
$$

where $N$ is the number of micro-organisms, $N_0$ the number at $t = 0$ (until $t = \lambda$), $\mu$ the specific growth rate and $\lambda$ the lag time.

(2) the individual lag phases are normally distributed:

$$
\lambda = \bar{\lambda} + \sigma u
$$

with $u$ following the normal distribution:

$$
f(u) = \frac{1}{\sqrt{2\pi}} \exp \left( -\frac{u^2}{2} \right).
$$

In its cumulative form

$$
F(x) = \int_{-\infty}^{x} f(u) \, du.
$$

The development in time can then be described as (see appendix):

$$
\ln \left( \frac{N}{N_0} \right) = \mu(t - \bar{\lambda})F\left(\frac{(t - \bar{\lambda})}{\sigma}\right) + \frac{\mu\sigma}{\sqrt{2\pi}} \exp \left[ -\frac{(t - \bar{\lambda})^2}{2\sigma^2} \right].
$$
This model has three parameters, $\mu$, $\gamma$ and $\sigma$ and one initial condition $N(0)$.

We can also attempt to interpret this equation. For larger times, the $e$ power will approach zero and the value of $F$ becomes 1 (the cumulative distribution becomes 1 with larger numbers) and therefore, the equation approaches exponential growth with mean lag time for larger times. We can also make a good description using this model of the course of the lag phase in bacterial growth. By making a suitable choice of the parameters, we can even let the two models fall almost precisely over each other (see Fig. 4, the two curves can even approach closer by appropriate parameter selection), from which we can conclude that the experimental confirmation of a model does not prove the correctness of a model, it only shows that the assumed mechanism is possible. Both models can describe observed results, although the starting points and the ultimate equations are totally different. In order to distinguish between the two models, experiments must be done to test the underlying assumptions, such as tracing component B and following this in time. The problem is to know which component to follow. Or we could place in each of 1000 test tubes a single microorganism and see if the time-to-turbidity is normally distributed, for example. But for testing that hypothesis, it is difficult to be sure whether there is exactly one organism in a tube.

Both models are based on a sound mechanism that is able to describe data. So we should not assume that mechanistic models that describe data are inherently better than empirical models. Only if mechanistic models are based on sound mechanisms, describing data and are tested thoroughly for their underlying assumptions, can they be said to have an advantage over empirical models.

4.2. Secondary models

4.2.1. Polynomials

The effect of environmental conditions on the parameters of the primary models, for example, the effect of pH and temperature on the specific growth or inactivation rate, is often described by polynomial models (see for example Gibson, Bratchel, & Roberts, 1988):

$$
\ln(\mu) = a + bT + cT^2 + dpH + epH^2 + fa_w + ga_w^2 + hTpH + iTa_w + jpHa_w.
$$

4.2.2. Arrhenius/Davey

For the effect of temperature, the Arrhenius model can be used

$$
k = Ae^{-E/RT}
$$

with $k$ = the reaction rate (h$^{-1}$), $A$ = collision factor (h$^{-1}$), $E$ = activation energy (J/mol), $R$ = gas constant (J/mol/K), and $T$ = absolute temperature (K). Davey (1989) extended the Arrhenius equation with other variables like

$$
\ln(k) = a + b/T + c/T^2 + da_w + ea_w^2
$$

4.2.3. Ratkowsky-type equations

Based on the Ratkowsky equation for the effect of temperature, the effect of other variables on the specific growth rate can be included (see for example McMeekin et al., 1987; Zwietering, Wijtzes, de Wit, & van ‘t Riet, 1992; Deulghere et al., 2000):

$$
\mu = c(a_w - a_{w,\min})(pH - p_{H,\min})(pH_{\max} - pH)(T - T_{\min})^2.
$$

This type of model has an advantage in that they contain interpretable parameters and the effect of the various variables can be separated, resulting in dimensionless terms:

$$
\gamma = \frac{\mu_{\text{opt}}}{\mu_{\text{opt}}} = \gamma(T) \gamma(pH) \gamma(a_w) \gamma(...).
$$

The separate factors can easily quantify the relative importance of each factor, providing quantitative insight. The cardinal-type models also have this characteristic (Rosso, Lobry, Bajard, & Flandrois, 1995).

A similar approach is developed for inactivation by Mafart (2000):

$$
D = D^*10^{-(T - T^*)/z_{pH}}10^{-(pH - p_{H^*})/z_{\mu}}10^{-(a_w - 1)/z_{aw}}.
$$

In this equation, all effects act separately on the inactivation rate, clearly quantifying the effect of each variable. Similar types of factors are derived for the effect of the recovery of the cells. This type of equation clearly shows the effect of every variable, which can detect main effects, and can help in ranking the importance of effects.

It should be noted that these equations assume multiplicative effects of the variables, with no real “interaction”. The effect of multiplication might seem a synergistic effect, two factors each having a factor of 10 inhibition, result together in a factor 100, but in principle, these equations assume separate, relative effects. It might be seen experimentally that the factor 100 inhibition gives “no growth” due to the experimental time frame. In general, the assumption of multiplicative effects gives reasonable results, except for special cases where there is a clear interaction like, for example, the interacting effect of pH and weak acids.

4.2.4. Interaction

In the use of polynomial equations the linear “interaction” terms are sometimes confused with real interaction, but it should be noted that biological
interaction is different from “statistical” interaction of linear terms in a polynomial.

If we take, for instance, the extended Ratkowsky equation with the effect of water activity (McMeekin et al., 1987), then this also results in a polynomial equation, with linear “interaction” terms, although the initial equation is based on an assumed multiplicative independent effect.

\[
\mu = b(a_w - a_{\min})(T - T_{\min})^2 \\
= b_{aw}T^2 - 2bT_{\min}a_w + bT_{\min}^2a_w \\
- b_{aw_{\min}}T^2 + 2b_{aw_{\min}}T_{\min}T - b_{aw_{\min}}T_{\min}^2 \\
= b_{aw}T^2 - cT_{aw} + da_w - eT^2 + fT - g. \tag{26}
\]

The initial equation contains three interpretable parameters \((a_{\min}, T_{\min}, \text{and } b)\) and \(b\) can be converted to \(\mu_{\text{opt}}\).

A quadratic polynomial would count 6 parameters, but as shown with the Ratkowsky equation, cubic terms seem to be necessary, which would need 10 parameters.

\[
\mu = b + cT + dT^2 + ea_w + fa_w^2 + gT_{aw}, \\
\mu = b + cT + dT^2 + eT^3 + fa_w + ga_w^2 \\
+ ha_{aw} + iT_{aw} + ha_{aw}T^2 + ia_{aw}^2T. \tag{27}
\]

So the Ratkowsky-type models can be seen as nothing more than a polynomial, but they contain interpretable and less parameters, based on a known curvature. Furthermore, the effects of all variables are clearly separated (quantifying relative effects). But it remains a challenge to determine if effects can be described really as multiplicative independent action or that there are really interactive effects. For practical usability and general applicability, suitable characteristics for models are (Rosso et al., 1995):

- simplicity
- low number of parameters
- as many interpretable parameters as possible (can be easily criticised, looked up in databases or the literature, as well as experimentally determined)
- general applicability.

For a more extensive overview of secondary models, the reader is referred to van Gerwen and Zwietering (1998) and McDonald and Sun (1999).

5. Important attention points for development and interpretation of quantification/experimentation/modelling

One important point is the accurate use of terms. Often the words fit/adjust versus predict/validate are confused. If the parameters of the model have been adjusted to the current data, then it should be named fitted or adjusted and not predicted. Only if new data are compared to model predictions, should the words predict and validate be used.

For experimentation, it is important that the kinetics is not the only phase to concentrate on, the prehistory and post-history are also important. For both growth and especially the lag phase and for inactivation, prehistory effects are quite relevant and often rather variable (difficult to control) (Robinson, Ocío, Kaloti, & Mackey, 1998). The question is, which history is to be used for the kinetic experiments. Should it be the most fail-safe choice or the most relevant one for the application? If it is chosen specific for the application, then it must be certain that this history is the encountered one, excluding all other possibilities. This is not easy, since often the ecology is not exactly known. For instance if a certain state is chosen, then is it possible that in the practical situation more resistant states exist? For example, stress can make resistance in some cases larger, in other cases weaker (Gaillard, Leguerinel, & Mafart, 1998).

The same applies for post-history effects (Mafart, 2000). Should the plating be under optimal conditions (viability) or under realistic conditions (survival in practice)? These questions are difficult to answer in general, since always using the most fail-safe selection might result in too large a safety factor, possibly resulting in unnecessary quality loss. However, choosing the average practical situation might be dangerous, since problems occur in exceptional situations.

Determination of bacteria is a further point to consider. Fast methods have the advantage that many data can be gathered with little effort, but one should consider the problem of their detection level. In certain cases, one investigates the growth in a range that is not relevant. For the growth of Listeria, for example, one is not interested in how it grows in the concentration range \(10^{-10}\), but only from \(0.01\) to \(10^7\). It should be realised that in these different ranges, different phenomena may play a role. At high levels, effects of substrate limitations and accumulation of inhibitory compounds may occur, thus resulting in dangerous conclusions. At certain limiting conditions, growth might be relevant (e.g. to a level of \(10^5\)), but may not be detected by the method. To conclude that this is a “no-growth” condition is very dangerous. Moreover, as mentioned before, the growth/no-growth depends on the experimental time frame. This must be chosen to be at least as long as the shelf-life of the products of interest. In order to conclude that there really is no growth, longer experimentation is needed.

In practice, often very large variabilities are found (see for example Fig. 5), and this together with the accuracy of the models result in the fact that models should be considered as an order of magnitude estimation and not an exact prediction. Unless all sources of variability are quantitatively known (practically impossible) this situation cannot be improved, and
even if all variability is known, it is not sure if one can only describe this variability, or really predict specific cases. This variability and uncertainty is a fact, but we should realise that we are not the only discipline that has to make decisions with the best information possible, but without knowing everything.

Finally, in order to translate probability results in particular to practical situations, one should realise that food manufacturers produce very large volumes of products, and thus need very low probabilities in order to limit problems.

6. Conclusion

In order to quantify microbial quality one should be aware of many exceptions, problems, inaccuracies, and unknowns. But that does not mean that quantification and quantitative support is not useful: it just makes the analysis more transparent and clear. If one can reduce uncertainty (more quantitative knowledge) or variability (better control), then one can decrease the safety factors, and thus reduce over-processing. Various model types can be used to describe the kinetics, which should preferably be general applicability, not too complex and contain a low number of parameters, which are interpretable. Furthermore, it is preferable if they describe the mechanism of the phenomenon of interest. But it should be realised that many mechanisms are still badly understood and that the experimental confirmation of a mechanistic model does not prove the correctness of it.

Using balances and defining characteristic numbers enables easy quantitative interpretation and ranking the relevant phenomena. Often, due to clarity, one can better criticise the weak points in the analysis. This should be seen as an advantage, clearly showing where to invest further. Using various sources of information available one can guide and support and help to structure, store and quantify information.

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Appendix A

Using the normally distributed lag time for a single cell results in
\[
\ln(N) = \mu(t - \tilde{\tau} - \sigma u) \quad \text{with} \quad t > \tilde{\tau} + \sigma u \tag{A.1}
\]
and for the entire population (this is true for large populations):
\[
\ln \left( \frac{N}{N_0} \right) = \int_{-\infty}^{\infty} \mu(t - \tilde{\tau} - \sigma u) f(u) \, du \quad \text{with} \quad t > \tilde{\tau} + \sigma u. \tag{A.2}
\]
The time is larger than the lag time (of the individual cell) if
\[
u < (t - \tilde{\tau})/\sigma. \tag{A.3}
\]
We can, therefore, also write the integral as
\[
\ln \left( \frac{N}{N_0} \right) = \mu \int_{-\infty}^{\infty} (t - \tilde{\tau} - \sigma u) f(u) \, du. \tag{A.4}
\]
This can be written as
\[
\ln \left( \frac{N}{N_0} \right) = \mu (t - \tilde{\tau}) \int_{-\infty}^{(t-\tilde{\tau})/\sigma} f(u) \, du - \mu \sigma \int_{-\infty}^{(t-\tilde{\tau})/\sigma} uf(u) \, du. \tag{A.5}
\]
Using the definitions of the normal distribution results in
\[
\ln \left( \frac{N}{N_0} \right) = \mu (t - \tilde{\tau}) F \left[ \frac{(t - \tilde{\tau})}{\sigma} \right] - \frac{\mu \sigma}{\sqrt{2\pi}} \int_{-\infty}^{(t-\tilde{\tau})/\sigma} u \exp \left( -\frac{u^2}{2} \right) \, du. \tag{A.6}
\]
This can be written as
\[
\ln \left( \frac{N}{N_0} \right) = \mu (t - \lambda) F \left[ \frac{(t - \lambda)}{\sigma} \right] \\
- \frac{\mu \sigma}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \left( \frac{(t - \lambda)}{\sigma} \right)^2 \exp \left( -\frac{u^2}{2} \right) \, du.
\] (A.7)
which can be solved to result in
\[
\ln \left( \frac{N}{N_0} \right) = \mu (t - \lambda) F \left[ \frac{(t - \lambda)}{\sigma} \right] \\
+ \frac{\mu \sigma}{\sqrt{2\pi}} \exp \left[ -\frac{(t - \lambda)^2}{2\sigma^2} \right].
\] (A.8)
This gives the final solution of the bacterial growth development in time.

References


