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Review

Modeling pathogen growth in meat products: future challenges

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Meat products are perishable foods and unless stored under proper conditions spoil quickly. In addition, if pathogens are present, meat products may become hazardous for consumers. Pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. can grow and cause illness by the ingestion of the bacterial cells, therefore, assurance of meat safety and quality is of utmost importance. The emergence of low infectious dose pathogens, i.e. those that may cause disease at 1–10 organisms ingested, presents a significant challenge to predictive microbiology. In order to become a better tool for the meat industry and consumers, the mathematical models that form the basis for predicting microbial growth should (1) be validated in the actual food rather than in lab media, (2) take into account the cumulative effect of any temperature fluctuation that regularly occurs in distribution, and (3) keep in mind that pathogen initial count is usually unknown, and may be below the detection limit. This review presents some background on how to address these challenges.
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Meat products are perishable foods and unless stored under proper conditions, the growth of both spoilage bacteria and pathogens can occur. Pathogens such *as Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* spp. can cause illness by the ingestion of the bacterial cells at low doses. Incidents, such as the Bil Mar and the Rochester Meat food poisoning outbreaks, occurred despite their approved HACCP plans. These outbreaks stress the need for a precise and reliable model and data for predicting pathogen growth in meat and its products.

From a legal standpoint, in the US, Sec 402(a)(1) of the Federal Food Drug and Cosmetics Act (FFDCA) states that a food is adulterated if it contains any poisonous or deleterious substance that "may render" the food injurious to health. Essentially this means that a food is legally subject to seizure or recall if it contains any detectable level of pathogen. Although the FFDCA specifies no numbers, in Sec 7106.18 of the Compliance Policy Guides Manual (CPGM), the Food and Drug Administration (FDA) established the principle of adulteration at the level of detection for dairy foods which has been become the standard practice for all other foods. This principle has been upheld by the courts, for example is US vs. Seabrook International Foods (501 F.Supp 1086; 1980) where the "may render" clause was interpreted to mean any detectable level of pathogen, *Salmonellae* in this case.

Meats are controlled under the Federal Meat Inspection Act (FMIA) and are regulated differently. Although the FMIA has a similar language to the FFDCA, the Food Safety Inspection Service (FSIS) has more specific rules for pathogens in meat. Under the new required HACCP plan for both raw and processed meat, USDA requires mandatory testing by the manufacturer for generic *E. coli* which may indicate the presence of pathogens (9 CFR Part 304). USDA also will randomly test meat products for specific pathogens. Thus in 64FR 2803-2805 (19 January 1999), the USDA states that the detection of *E. coli* O157:H7 at any level makes the meat (beef in this case) adulterated, i.e. the level of detection is the end of shelf life based on safety. The same policy was established in 64FR 28351-28353 (26 May 1999) for *Listeria* in ready to eat meats, i.e. any finding of *Listeria monocytogenes* makes the product unsafe. While not established directly, USDA also considers detection of *Salmonella* to be adulteration, but

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has only taken action after several consecutive positive findings based on statistical sampling approach. This performance standard (63FR 1800–1803; 12 January 1998) deems ground beef to be adulterated if five of 53 samples (>7.5%) are positive for *Salmonella*. In 1999, this standard was applied to Supreme Beef in Texas, and although the Federal District Court did not uphold this standard and ruled in favor of Supreme Beef, its decision was reversed upon appeal. Supreme Beef had argued that Salmonellae are a problem at slaughter and thus they get bad product that was inspected and passed by the USDA so it was not their problem.

Despite the tremendous progress in bioanalytical techniques, for most pathogens the limit of detectability generally is one microbe in 25 g of food. Hypothetically, if the food contains less than one detectable pathogen in 25 g, it is considered 'safe' although it can become unsafe by the time it is placed on the retail shelf if the organism is held under conditions where it can grow. If a product was contaminated with pathogens at a level below the detection limit, we can define the shelf life of a product based on safety as the time at which the pathogen reaches a detectable count. This of course will depend on the initial count, N_0, which could be variable, and the temperature history during transport, retail and home holding. Thus food products may become microbiologically unsafe before or very close to the end of their sensory quality shelf life if temperature abused as has been presumed to be the case in the Bil Mar food incident. The current procedures of microbiological challenge testing (MCT) are valid only for the specific conditions of the experiment and cannot be used when processing, product composition, environmental conditions, etc., are changed [1].

A number of internal and external factors affect microbial growth in meat, with temperature being the primary extrinsic controlling factor. Despite the recent improvements in meat production and distribution, cases of food poisoning continue to rise in most countries [2]. Predictive microbiology is a promising area of food microbiology, providing databases and software packages that can be an effective tool for risk assessment. However to date, these tools have been mainly used for research purposes because of practical limitations. The emergence of low-infectious dose pathogens, particularly with ground beef products presents a significant challenge to predictive microbiology [3]. In most models, competition between microorganisms in foods is not considered. Growth of pathogens in meat products will depend on the initial population density and that of competing organisms [4]. In order to become a better tool for the food industry and consumers, models should (1) be validated in the actual food rather than in lab media, (2) take into account the cumulative effect of temperature fluctuation, and (3) be able to predict the time to reach infectious levels even for unknown initial levels that may be below detection.

Modeling microbial growth

For many homogeneous microbial populations, the growth in either nutrient media or foods can be described by the curve in Fig. 1. The stationary and death phases are not applicable to shelf life based on either quality or food safety, as by the time the population reaches those levels, the food would be unacceptable from a sensory quality standpoint as well as essentially unsafe, once detectable levels of pathogens are reached. Environmental conditions, food composition and growth status of the microbes (lag, log or stationary phase) can affect the growth rate. Since food becomes microbiologically unsafe before the stationary phase is reached, one should try to optimize analysis of the lag time and initial exponential growth rate in the log phase. Determining these kinetic parameters and modeling the quantitative effect of various hurdles on them is critical for risk assessment. The most important parameter in the prediction of pathogen growth is the time at which the organism is detectable at 1 CFU/25 grams (i.e. $\sim 4\times10^{-2}$ CFU/gram). This time corresponds to t_d in Fig. 1, which shows a different lag time, t_1. The t_d could be shorter or longer than t_1. This time may be different than the lag time t_L, which is based on counts of 10^1–10^2 CFU/g with a variability of 1 log cycle. Generally t_L is considered to be the intersection of the first lag line with the steep log phase line. Importantly, most studies used to validate mathematical models for growth use a N_0 of 10^2 CFU/g or larger, but the actual N_0 for undetected pathogens would be less

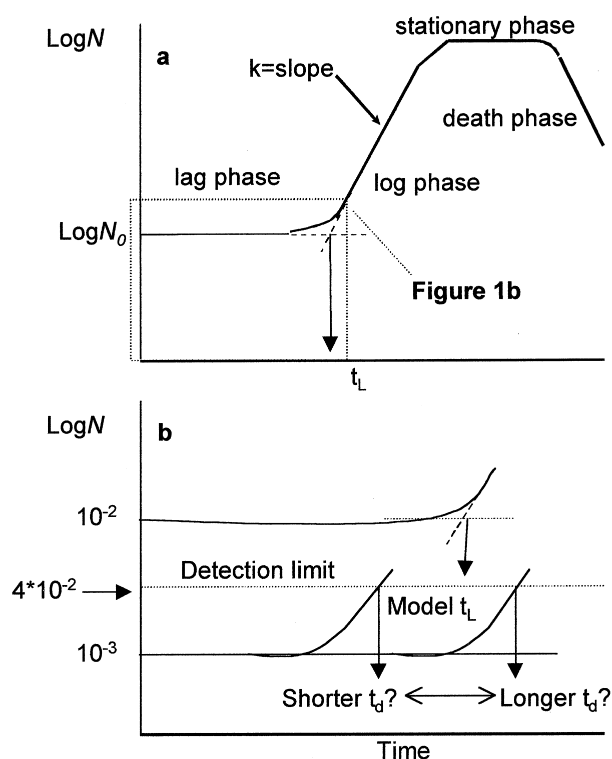

Fig. 1. A typical microbial growth curve.

than $\sim 4\times10^{-2}$ CFU/gram. However, the current mathematical models have not addressed this parameter.

For the exponential phase, Monod [5] stated that the rate at which the population increases is proportional to the number of members in the population. Therefore, the specific growth rate or generation time should become constant for constant environmental conditions. This assumption is correct for a limited period of time, in which there is no substrate limitation or any other environmental change, such as change in pH. The integrated form of the Monod model is:

$$N = N_0 \exp[k(t - t_L)] \quad (1)$$

where N is the number of organisms at time t, N_0 is the initial number and at the end of the lag time, k is the specific growth rate, and t_L is the lag time.

The specific growth rate can be derived from the slope of the plot of ln N vs. t (for $t > t_L$). The lag time, t_L can be determined graphically as the intersection of the lag line and the linear regression line of the exponential growth phase. This model is simple and fairly accurate and has been used extensively, including for mixed flora [6,7].

Another approach to describe the microbial growth curve in Fig. 1 is by a non-linear model first introduced by Gibson *et al.* [8], and called the Gompertz function. The basis for this equation is that changes in the specific growth rate of microbes are induced by nutrient limitations and production of toxic metabolites. This also assumes an even lag period for different N_0s. Typically the specific growth rate will increase to a maximum, and then will decrease. The Gompertz function has the form:

$$\mathrm{Log}N = a + c\exp\{-\exp[-b(t - m)]\} \quad (2)$$

where N CFU unit^{-1} at time t, a = asymptotic log count of bacteria at infinitely short times, c = asymptotic amount of growth that occurs as t increases indefinitely (number of log cycles of growth), m = time at which the absolute growth rate is maximal, and b = relative growth rate at m. Using these four parameters and a non-linear regression of log N vs. time, one can fit the data and obtain values of the maximum growth rate and the time to reach that rate [8,9]. Zwietering *et al.* [10] suggested that the parameters of their modified Gompertz model for *Lactobacillus plantarum* can be made temperature dependent by either logarithmic transformation of the lag time or square root transformation of the specific growth.

Numerous other models have been developed based on either the Monod model or Gompertz function. These different kinetic models have been thoroughly reviewed and classified [11–14]. All models use the N_0 value as a given initial data point, which is generally an inoculum of 10^2–10^3 CFU/g not the real world of pathogens. The problem with modeling pathogens growth is that the initial count is legally expected to be below the level of detection. However, even if we assume that some pathogens are present, we still cannot answer the question of whether they will grow and what will be the relationship of t_d to t_L? In addition, we do not know the effect of the inoculum size and initial state of the organism (i.e. from lag, log or stationary phase) on the t_L. It is the aim of this review to address these questions as these are key in doing a risk assessment of any possibly contaminated food including meat.

The effect of temperature

Numerous studies have evaluated the effect of environmental factors such as temperature [15], oxygen [16], and level of mixed microbial population in the food [17–19] on microbial growth. However, the most important factor for controlling microbial growth in the production and distribution chain of chilled foods is temperature. Although microbial growth occurs from about −8 to +90°C, the growth rate of most microbes of significance to food poisoning decreases above 35–40°C. Within the practical distribution range for refrigerated products (0–35°C), temperature affects the duration of the lag phase, the rate of growth, and the final cell numbers [15]. Food-poisoning bacteria can multiply within a temperature range from about 0–50°C [20], however, refrigerated storage will favor gram-negative bacteria and psychrotrophic pathogens, whereas higher storage temperature may favor mesophilic food-born spoilage microorganisms [15]. It is presumed that the temperature will also affect the multiplication rate below the detection limit, but how this is affected is unknown.

Arrhenius model

Based on thermodynamic considerations, the Arrhenius model has had success in describing the temperature dependence of many chemical reactions related to shelf life of food [21]. Since the replication of the gene during cell division is a chemical process, it seems logical that the growth rate would follow the Arrhenius law for a certain temperature range. The form of the Arrhenius model is:

$$k = k_0 \exp(-E_A/RT) \quad (3)$$

where k is the microbial growth rate constant, k_0 is the 'collision' or 'frequency' factor, T is the absolute temperature (K), R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and E_A (J mol^{-1}) is the activation energy. The activation energy is a measure of the temperature sensitivity of the reaction(s) responsible for microbial growth. This sensitivity can be calculated from the slope of a plot of ln k vs. $1/T$. The Arrhenius model has been

applied to microbial growth in many foods including packaged refrigerated beef [22]. The Arrhenius model could also be used to model the temperature dependence in both of the lag phases, the lower one of which would be the most critical phase for prediction of safe shelf life under variable temperature conditions, where the initial microbial load is below detectability as would be expected for pathogens. To make the Arrhenius plot, the inverse of the lag time is used, however, the fit is usually not as good as for the growth rate data from the exponential phase [79,80].

Ratkowsky's equation

Ratkowsky *et al.* [25] proposed an empirical equation for the temperature dependence of microbial growth up to the optimum temperature (T_{opt}) as:

$$\sqrt{k} = b(T - T_{min}) \quad (4)$$

where k is the specific growth rate, b is the slope of the regression line of $\sqrt{k}$ vs. the temperature, T is the test temperature, and T_{min} is the notational microbial growth temperature where the regression line cuts the temperature axis at $\sqrt{k}=0$. They applied the equation to more than 50 sets of growth data with excellent fit, and in another study for an additional 30 organisms [26], again in both cases using data beyond the lag time.

The basic equation was extended to cover the whole temperature range for growth and to account for the drop in growth rate above the T_{opt} [26]. The non-linear regression model is:

$$\sqrt{k} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \quad (5)$$

where c is an additional parameter to enable the model to fit the data for temperatures above T_{opt}, and T_{max} is the upper temperature where the regression line cuts the temperature axis at $\sqrt{k}=0$. This equation was modified by Zwietering *et al.* [27] to make it applicable for $T > T_{max}$. Their modified function was selected as the most suitable model for specific growth rate as a function of temperatures, and also in comparison to other models. In this equation, T_{min} is a notational temperature and does not represent the actual zero growth temperature, in some cases, T_{min} for growth may be several degrees below the freezing point, where the water activity and other environmental factors are altered [25,26]. Despite this criticism, the Ratkowsky equation predictions of the measured T_{min} for the lag phase was used in modeling the effect of temperature on coliform growth in meat [28]. McMeekin *et al.* [29] suggested that the non-applicability of the Arrhenius model for modeling the temperature-dependence of microbial growth is a result of the change in E_a value with temperature. They related the activation energy to the Ratkowsky equation by:

$$E_A = 2RT^2/(T - T_{min}) \quad (6)$$

Thus, the change of E_A for a given organism is greater for a low value of $T - T_{min}$ ($5 < T < 30°C$). However, this correction may not be fully correct since the pre-exponential factor (k_0) in the Arrhenius equation may also be changing with temperature. It should be noted that Fu *et al.* [24] demonstrated with *Pseudomonas fragi* growth in a simulated milk system, that if enough data points are collected over time (i.e. 15 or more), and at least five temperatures are used, there is a good fit for both the Arrhenius model and the Ratkowsky equation. This study demonstrates the benefit of using many data points to fit simple equations, rather than using over manipulated empirical equations.

Log shelf-life model

For a temperature difference from 20 to 30°C, a simple plot of the log time to reach some value N (e.g. shelf life) vs. temperature is also a fairly good straight line [15]. The equation takes the form of:

$$t_s = t_0 \exp(-bT) \quad (7)$$

where t_s is the shelf life at temperature T (°C), t_0 is the shelf life at 0°C, and b is the slope of ln t_s vs. T. From this plot, the increase in rate for a 10°C increase in temperature (Q_{10}) can be estimated from:

$$Q_{10} = e^{10b} \quad (8)$$

It is therefore possible to use the value of Q_{10} as a measure of a temperature dependence of growth. This, plot and the derived Q_{10} has been used to explain the effect of temperature on loss of quality, nutrient loss as well as microbial growth and shelf life [15]. The Q_{10} value is usually assumed constant over a small temperature range as noted. The reported values of Q_{10} for microbial growth under refrigeration conditions range from 2 to 10 [15]. Such a relationship was demonstrated for the shelf life of pasteurized milk [30].

This model is also supported by the data provided by Rosso *et al.* (1996) for the growth of *L. monocytogenes* in minced beef. This model thus becomes useful even if we cannot measure the growth rate below the detection limit. If we take a meat product and inoculate it with the pathogen to below the detection limit, then all we need to do is store at four or five constant temperatures and measure a time to detection. If the plot of log t_d vs. the temperature is a straight line as with eqn (7), then the behavior of the organism is predictable. This plot would then allow us to predict the time to detect at any temperature, or for a time–temperature sequence. This

approach has been successfully applied to the data of Lindroth and Genigeorgis [16] for time to detect of botulinum toxin in fish. Thus there should be no reason it would not apply to the detection time for vegetative cells. It should also be noted that this bot toxin data has a good fit using the Arrhenius relation, and over the narrow temperature range shows an excellent fit for ln t_d vs. T (°C). Thus it can be asumed to apply to detection limits for other pathogens.

Pathogen growth under fluctuating environmental conditions

Besides initial contamination, temperature abuse may be the next most important factor leading to a food borne illness outbreaks but the extent of the risk-increase due to temperature fluctuation is unknown. Data in the literature for the effect of temperature-fluctuation conditions on pathogen growth are minimal. For CAP/MAP foods, a major question is whether spoilage bacteria will result in end of sensory based shelf life before the pathogen detection limit is reached, when the product is exposed to temperature abuse.

Mathematical modeling for prediction of bacterial growth for a given time–temperature history may not be effective if there is a time–temperature history effect. A history effect is one in which the actual growth rate that is measured after a temperature shift is significantly different from that predicted by a database model done at the same temperature as noted in Fig. 2. Several studies at fluctuating temperatures suggested a history effect [24,32–34], yet others did not find it [35,36]. It should be noted that there have been no studies to determine the effect of fluctuations of water activity, oxygen level and carbon dioxide level on growth, and other important factors if temperature is controlled.

As shown in Fig. 2, if an organism is grown to level A at 20°C and then transferred to 4°C, the Arrhenius or the shelf life models will predict that it will then grow at the same rate as found when it is tested only at 4°C.

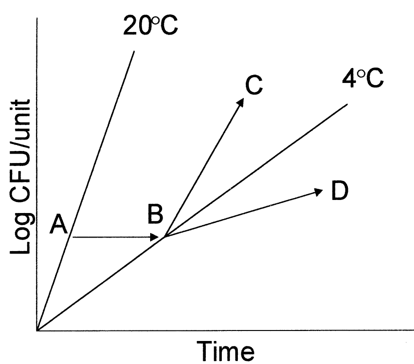

Fig. 2. Example of history effect when product is shifted after exposure at one temperature (A) to a second temperature (B). (C) represents a positive history effect, while (D) represents a negative effect.

However, some metabolic effects may cause the growth to be faster (B to C) or slower (B to D) than predicted. If a history effect exists, than any temperature dependent model will result in errors unless the effect is small. This history effect may also independently affect the lag phase further complicating any predictive model.

Predicting the growth of microbial pathogens in a food system is problematic as in sensory shelf life prediction for chemical reactions, due to the different temperature sensitivities of different deterioration modes [21]. Each organism of the natural flora is affected differently by temperature change, a_w and gas composition. However, given that everything but temperature is maintained constant, the effect of time–temperature history can be integrated simply by the following equation:

$$f(A) = \sum k_{[f(T_i)]} \mathrm{d}t \tag{9}$$

where $f(A)=$ quality function = ln N/N_0 for first order microbial growth, $k_{[f(T_i)]}$ = time–temperature dependence of quality loss rate or microbial growth rate. For pathogens this would be $1/t_d$. Taoukis and Labuza [37,38] solved this equation using the Arrhenius relation for temperature dependence. Dividing the time into short intervals of constant temperature, and then summing up the right hand side of eqn (9) simplifies the solution as shown in eqn (10). The value of k for each temperature in the $t-T$ sequence can be calculated using any temperature growth model. This approach is essentially similar to the original temperature dependent model used to predict the required time needed to reach a 12-log reduction during a heat sterilization process.

$$\ln(N/N_0) = \sum_{i=1}^{i=n} k_i \Delta t_i \tag{10}$$

A number of researchers have tried to attack the fluctuating temperature problem by designing new models [39], using the Monod equation [40], or fitting the models for a specific process [23,41–43]. Brocklehurst *et al.* [44] tested the effect of temperature fluctuations on the growth of *Salmonella typhimurium* in broth and gelatin gel. They found that for systems stressed by pH or NaCl, when temperature fluctuated between 4 and 22°C or 12 and 22°C, the experimental data did not fit the prediction. It should be noted that the generation time was shorter than the predicted value when the fluctuation cycle period was longer, as would be expected in real life. This observation may be due to a different behavior of the bacteria in fluctuating temperatures than predicted by models developed for constant temperature.

Several studies of microbial growth have shown history effects [24,32–45], however, only the study of Fu *et al.* [24] examined the statistical significance of the difference between predictive models and the history effect. The growth rate of *P. fragi* in a skim milk model system under non-isothermal conditions, was affected positively by the history (i.e. higher than predicted from isothermal data), while the history effect on the lag phase was negative (i.e. shorter than under the isothermal conditions). These effects were more profound when the temperature changes were stepwise vs. occurring in a sine wave. Buchanan and Klawitter [46] showed that prior incubation temperature affects the growth of *Listeria monocytogenes* Scott A at 5°C. The duration of the lag phase at 5°C decreased when either aerobic cultures were previously grown at 28°C or when anaerobic cultures were grown at 13°C or less. This is the same effect as was found by Fu *et al.* [24], however, Buchanan did not find an effect on the growth rate in the log phase.

Based on the above results, one concern with pathogens below the detection limit is that the actual time to detect may be shorter than would be predicted if the product goes through temperature fluctuations. No data exists to confirm this disparity, but it is a very critical point for proper risk assessment.

Modeling the growth of pathogens in ground or emulsified meat products

Food-born pathogens are adventitiously introduced into food, and thus are very likely to occur in very low concentration especially if the product is ground after contamination. Since food is considered safe until pathogens are detected as was illustrated earlier, the growth that one needs to model occurs during the time where we cannot detect the bacteria. Importantly, growth of the pathogen accelerated by temperature fluctuations observed in normal distribution can turn an apparently safe product into a health risk. Thus modeling of pathogen growth should be conceptually different than modeling the growth of spoilage organisms since we have no estimate of N_0.

The Monod model was used to describe the growth of *Listeria innocua* in sausages, however, with an initial inoculum of 10^4 bacteria per gram [47]. The Gompertz function was used to describe the growth of *E. coli* O157:H7 in raw ground beef [48], of *Shigella flexneri* in canned beef/chicken/vegetable broth [49], of *Bacillus cereus* as a function of temperature, pH and water activity [50], and of *Yersinia enterolytica* at low temperature [51]. A modified Gompertz function was also used to model the growth of *Aeromonas hydrophila* as a function of temperature, pH and water activity [52,53], and *L. monocytogenes* [54]. All these models used data collected from experiments that had initial counts of $N_0 = 10^3$–10^4 CFU/ml, and none of these studies challenged the question of whether the model is valid for a very low inoculum below the detection limit, and how it would apply to fluctuating temperatures. Interestingly, Gay *et al.* [55] found a different lag time (i.e. time to reach exponential growth) when *L. monocytogenes* was inoculated in liquid media at 10^1 (~7.7 days) or 10^3 (<1 day) cells per ml.

As previously noted, a critical question in predicting growth of pathogens is the ability of the models to predict growth at different temperatures. Several studies found large discrepancies between the predictive models and the actual measurements. Both the USDA Pathogen Modeling Program (PMP), and the UK Food Micromodel (FMM), showed that the measured lag phases were shorter than the predicted value for *L. monocytogenes* in sterile homogenized foods, held at 12–35°C [56]. Very recently, te-Giffel and Zwietering [57] performed an extensive validation of various models for the growth of *L. monocytogenes*, and found that all models had a very poor fit to the results obtained in meat. On the other hand, the results of Rosso *et al.* [31] for the growth of *L. monocytogenes* in refrigerated and minced beef suggest that the ln of generation time correlated very well with temperature, i.e. a plot based on eqn (7).

The best way to keep meat products safe is to prevent contamination in the first place, but if they are unavoidably contaminated, an applicable kill step should be used to reduce or eliminate the contaminants and the treated product should be held under conditions that minimize the growth of any surviving pathogens. The first may be addressed by the application of HACCP plans, but if the meat has been contaminated by an undetectable amount of pathogens, one needs a best estimate of the length of the lag phase in terms of time to detection. Use of kill steps such as heating, high pressure or irradiation have been addressed by others. As for holding, one can therefore define the lower lag period as the shelf life of the meat product based on safety concerns, which presumably is longer than based

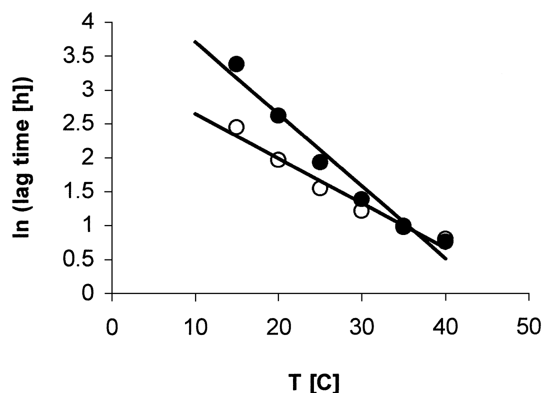

Fig. 3. Log shelf life plot for the lag time (h) of *Salmonella typhimurium* in lean (○) and fat (●) beef [58].

Table 1. Temperature dependence of the lag time period vs. temperature for pathogens

Pathogen	Product	N_0	Temperature range (°C)	r^2	n	Q10	Ref.
L. monocytogenes	Baby food (chicken)	10^3–10^4 [a]	12- 35	0.987	4	2.09	[56]
E. coli O157:H7	Ground beef	10^3–10^4 [a]	12- 35	0.767	3	2.78	[48]
E. coli O157:H7	Model media	NA [c]	12- 37	0.878	4	2.79	[61]
E. coli O157:H7	Model media	NA	12- 37	0.943	4	2.48	[61]
S. typhimurium	Lean beef	10^7 [b]	15- 40	0.968	6	1.94	[58]
S. typhimurium	Fat beef	10^7 [b]	15- 40	0.966	6	2.90	[58]
S. typhimurium	Chicken breast	10^3 [a]	10-37	0.833	4	3.28	[60]

[a] CFU/g.
[b] CFU/cm^2.
[c] NA, not reported.

on quality concerns. This means that the spoilage organism goes through the lag phase and into log phase of growth much before we reach t_d. The lag time for the growth of *S. typhimurium* inoculated at 10^7 CFU/cm^2 on the surface of sterile beef was modeled by Dickson *et al*. [58] using the Gompertz function. They used an exponential-decay function (11) to model the relationship between lag and generation times to the temperature.

$$Y = D + \{E[e^{-F(T)}]\} \tag{11}$$

where Y is the lag or generation time, T is the temperature, and D, E, and F are derived parameters. These models were used successfully for time-temperature integration, and prediction of *S. typhimurium* populations on inoculated beef tissue cooled at 6 or 9°C/h. As shown in Fig. 3, a plot of the ln of lag time in terms of t_L in Fig. 2 for both lean and fat samples vs. the temperature gave straight lines. In these cases, the initial inoculum on the surface of the beef was 10^7 CFU/cm^2 (which is about $10^{3.5}$ CFU/g). Using the shelf-life plot approach of Taoukis and Labuza [59], the temperature sensitivity (Q_{10}) of the growth of Salmonella was 1.94 for lean meat and 2.90 for fatty meat.

The lag time for a number of pathogens as a function of temperature was reported in several studies. Walls and Scott [56] used the Gompertz function to model the growth of *L. monocytogenes* inoculated at 10^3–10^4 CFU/g in baby food containing chicken and vegetables by the Gompertz function. Oscar [60] modeled the growth of *S. typhimurium* inoculated at 10^3 CFU/g on the surface of cooked chicken breast using a two-phase linear growth model. Walls and Scott [56] measured growth of *E. coli* O157:H7 inoculated at 10^3–10^4 CFU/g in raw ground beef and modeled the lag time by using the Gompertz function. Buchanan and Bagi [61] reported the lag time for *E. coli* O157:H7 inoculated in medium as a function of temperature, initial pH, sodium chloride content, and sodium nitrite. As shown in Table 1, most studies have high linear correlation of ln (lag time) vs. temperature, when using the shelf-life plot method. The low correlation for the ground beef sample is very likely a result of too few temperatures ($n = 3$) over a wide range (12–35°C) of temperatures, thus emphasizing the need for more temperatures. Modeling such data may assist in predicting the time to detect pathogens at different temperatures; however, will not take into account any temperature history effect.

Conclusions and future work

Predictive microbiology is a very powerful tool in predicting cell-death-kinetics and the determination of the conditions for the 'commercial sterilization' of canned foods or pasteurization of refrigerated foods. In recent years, there has been a great interest in predictive microbiology and in the use of modeling to predict bacterial growth in meat and other foods. However, despite the extensive work in this field, it seems that most models do not address the following basic questions.

1. Can we use models that were developed for initial counts of 100 cells per gram or more for predicting growth in systems that contain less than four cells per 100 g?
2. Will the time to detect pathogens follow the same temperature dependence as found for the lag phase when using higher inocula levels?
3. What will be the effect of inoculum physiological state (lag/log or stationary phase) on the growth parameters below the detection level.
4. What is the effect of temperature fluctuations on pathogen growth in meat and its products in the lower lag phase, i.e. is there a temperature history effect, and what direction does it take.

Answering these questions is a critical step towards the implementation of predictive microbiology models to the determination of the 'safe shelf life' of fresh meat and meat products.

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