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Inactivation kinetics of food-borne pathogens subjected to thermal treatments: a review

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ABSTRACT

Thermal processing technologies are safe and easy to control methods without leaving residues, and could be used to inactivate food-borne pathogens, ensure food quality and provide the food with sufficient stability during storage. Establishing inactivation kinetics of food-borne pathogens is essential in developing effective pasteurisation protocols without damaging food quality. This study presents a comprehensive review of recent progresses in inactivation kinetics of food-borne pathogens. It covers theoretical bases and experimental methods for developing thermal inactivation kinetics of food-borne pathogens. Finally, it proposes possible recommendations on the future research directions of establishing inactivation kinetic models for food-borne pathogens in thermal processing.

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Introduction

Foods contaminated with food-borne pathogens are of great food safety concerns to consumers, manufacturers and requlatory agencies. Table 1 shows food-borne outbreaks associated with foods, such as meat, tomato and low-moisture food contaminated by Salmonella spp, Escherichia coli, Listeria monocytogenes and E. sakazakii [1–9]. Thermal processing technology has been used and approved by regulatory and international agencies to inactivate food-borne pathogens and ensure food stability. It includes conventional and novel thermal processing technologies. Conventional thermal technologies, such as hot air, hot water, hot oil and steam, can be efficiently used to eliminate Salmonella and Escherichia coli O157:H7 in food [10-14]. Novel thermal technologies, such as infrared (IR), radio frequency (RF), microwave (MW) and ohmic heating, can be effectively used for pasteurising food-borne pathogens. For example, IR has been used to inactivate Salmonella in almonds [15]. RF heating has ability for pasteurising Salmonella in almonds [16] and Salmonella and Escherichia coli O157: H7 on black and red pepper spices [17]. MW is used for inactivating Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes in salsa [18]. Ohmic heating has been used to inactivate Escherichia coli O157:H7, Salmonella Typhimurium and Listeria monocytogenes in orange and tomato juice [19]. Populations of foodborne pathogens during heat treatment change with heating temperature, water activity (a_w) , heating rate, pH, heat shock, recovery medium and composition/physical characteristics of the foods [20-27]. For example, when the ground beef inoculated with Escherichia coli O157:H7 was cooked in a water bath for 1 h at temperature of 55-62.5 °C, the D-value (the time required at a certain temperature to reduce a specific microbial population by 90% or by a factor of 10) was significantly lower in ground beef adjusted with pH 4.5 than pH 5.5 [22]. The heat resistance as explained by D-value of Escherichia coli O157:H7 in ground beef decreased with the addition of arvacrol and cinnamaldehyde [21]. Water activity, a_{wr} is an effective measure of the availability of water in a food system. It is defined as the ratio of the vapour pressure in food and the saturated vapour pressure of water at the same temperature. The range of a_w is normally from 0.0 to 1.0. If the water activity of food is less than 0.6, almost all microbial activities are inhibited. As the a_w value increases from 0.0 to 1.0, the heat resistance (D-value in min) of foodborne pathogens decreases. For example, the heat resistance of Salmonella PT 30 in almond kernels with water activity of 0.601 is higher than that of 0.946 [20]. However, only limited numbers of temperature-time combinations for food-borne pathogens can be used for experiments due to labour costs and time limitations.

Models are effective tools in the development of thermal treatments for controlling pathogens in a more systematic and efficient manner compared with traditional methods. The kinetic models allow for prediction of the inactivation rate of pathogens with treatment times not only under isothermal but also non-isothermal conditions through other incorporated models. Therefore, thermal inactivation kinetic models developed from carefully planned experiments are used to estimate times and temperatures required to achieve specific log-reductions of food-borne pathogens.

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Table 1. Food-borne outbreaks associated with various foods contaminated with pathogens.

| Pathogens | Food commodities | Country and infestation, no. | Year | Reference |
|------------------------|--------------------------------------|------------------------------|------|-----------|
| Salmonella | Peanut butter | United States, 42 | 2013 | [1] |
| | Pine nuts | United States, 42 | 2013 | [2] |
| | Almond | United States/Canada, 29 | 2004 | [3] |
| Escherichia coli | Hazelnut | United States, 8 | 2012 | [4] |
| | Cookies | United States, 76 | 2012 | [5] |
| Salmonella | Chilli powder | Germany, – | 2013 | [6] |
| E. sakazakii | Infant formula | Japan, – | 2010 | [7] |
| Listeria monocytogenes | Ready-to-eat meats | United States, 2500/per year | 1999 | [8] |
| Salmonella | Tomato, serrano and jalapeño peppers | United States, 1400 | 2010 | [9] |

In recent years, some research papers about thermal inactivation kinetics of pathogens in food and agricultural products have been published. For example, inactivation kinetics of Salmonella spp. under conventional and novel thermal technologies are reviewed but only the primary model (the food-borne pathogen evolution as a function of heating time) is concerned [28]. However, since the inactivation kinetics are actually influenced by several factors, such as different bacterial strains, age of the culture, food composition (fat, NaCl, pH and a_w), processing parameters, and physiological state of the organisms, some researchers have established secondary models for predicting survival curves under different conditions [20,24,29-32]. Also omnibus models incorporating the primary and the secondary models are further considered for predicting survival curves of pathogens [23,33,34]. So far, there is little systematic review on complex inactivation kinetics of food-borne pathogens subjected to thermal treatments.

Objectives of this review are to present an overview of the recent research progress in thermal inactivation kinetics of food-borne pathogens. It involves theory foundations and experimental methods, and makes the comparison among the common thermal death kinetic models. Finally, this review proposes possible recommendations on developing trends and research directions for achieving food-borne pathogen inactivation in thermal processing.

Definitions of kinetic models

Mathematical models are becoming important tools for describing and predicting the growth, survival and inactivation responses of pathogens under specific environmental conditions. Typically, a predictive kinetic model comprises two parts, namely, a primary model that describes the foodborne pathogen evolution as a function of heating time. Secondary model was developed by using second-order response surface regression or step-wise regression to predict D-value or some other parameters got from primary model according to any other independent variables, such as pH, water activity and heating rate, which are obtained by experiments. Omnibus models incorporating primary and secondary models are used for predicting survival curves with any specified values of independent variables, such as temperature, water activity, heating rate and so on. The model accuracy (goodness of fit of the experimental data to the models) is assessed by using the coefficient of determination R^2 , root mean square error (RMSE), accuracy factor parameter A_f and bias factor B_f , which are showed as following:

$$R^{2} = \frac{SS_{R}}{SS_{T}} = \frac{\sum_{i} (\text{predicted value} - \text{average})^{2}}{\sum_{i} (\text{measured value} - \text{average})^{2}}$$
(1)

$$RMSE = \sqrt{\frac{\sum (\text{measured value} - \text{predicted value})^2}{n-1}}$$
 (2)

$$A_{\rm f} = 10 \frac{\sum \left| \log(\text{predicted value}/\text{measured value}) \right|}{n} \tag{3}$$

$$B_{\rm f} = 10 \frac{\sum \log(\text{predicted value/measured value})}{n}$$
 (4)

n means the number of measured data.

 R^2 measures how well further outcomes are predicted by a linear or nonlinear model. If R^2 is close to one, it means high accuracy of the model [35]. RMSE is used to measure the average deviation between the observed and predicted data sets. The lower RMSE value indicates the better fit of the data to the model [36]. A_f and B_f are used to estimate the percentage discrepancy between observed and estimated values [37,38].

Experimental methods to obtain thermal inactivation kinetic data

Pathogenic suspension directly subjected to heating

Pathogenic suspension directly subjected to heating is a common technique for studying thermal inactivation kinetics of pathogens [39,40]. Submerged coil heating apparatus is commonly used for determining the thermal inactivation of Listeria monocytogenes [41] and Salmonella Serovars [42]. The temperature range of this apparatus for determining thermal inactivation of pathogens is from 20°C to 90°C. Also, glass tube and test tube methods submerged in water baths are commonly used for studying the thermal inactivation of Entero-coccusfaecalis, E. coli and mould [43-45]. For example, Boutibonnes et al. [43] pipetted bacterial culture into glass tubes and placed into a 37 °C (control) water bath for characterising the heat shock response in Entero-coccusfaecalis. Lee and Kang [44] placed inoculated tubes with E. coli suspension immersed in a water bath (55 °C). Fujikawa et al. [45] compared the thermal inactivation kinetics of mould spores by using the glass capillary tube (1 mm in inner diameter) and test tube methods (10 mm in inner diameter) at the temperature of 60°C for 14 min, and found that there were no significant differences in values of the inactivation rate and the

delay of fast declines in survival curves between the two methods.

In addition to final temperature and holding time, heat resistance of a pathogen is mainly affected by sample compositions, pH values of the heating medium, and growth temperatures. Thermal kinetic models obtained by pathogen suspension may not be suitable for typical commercial preparation procedures in real food. For example, the *D*-value of *E. coli O157:H7* at 55 °C is 15.4 min in brain heart infusion (BHI) broth [46], 26.5 min in beef [22] and 45.4 min in apple juice [47].

Subjecting inoculated food to heating

Improved methods for obtaining information on kinetic response of food-borne pathogens should simulate the real environment for the pathogens in food samples. The experimental methods are developed based on different food states.

Studying thermal inactivation kinetics in liquid food

The liquid foods can be easily put in and taken out because of the fluidity, thus the tube methods in water or oil baths are commonly used for studying the thermal inactivation kinetics. Gabriel and Nakano [47] placed inoculated apple juice in glass tubes and then heated in a water bath, and their results showed that the D_{55} value was the highest when the food-borne pathogens grew in 1% glucose before inoculated into apple juice. Huemer et al. [48] used glass tubes in a silicone oil bath to study the thermal inactivation kinetics of spores of Bacillus sporothermodurans in milk. Odlaug and Pflug [49] put tomato juice in an aluminium thermal-deathtime tube (a tube made by aluminium is used for evaluating the population changes of food-borne pathogens with heating time at a given temperature) to study thermal resistance of clostridum botulinum spores. The heat resistance of the same pathogens under different test conditions showed differences in thermal inactivation kinetics [50,51]. For example, D-values obtained in 9-mm test tubes were about 8-29 times larger than those obtained using 3-mm capillary tubes for Yersinia enterocolitica strains in physiological saline at 60°C due to non-isothermal test conditions in 9-mm test tubes. Test methods have been developed eliminate to

non-isothermal conditions by thermal-death-time capillary tubes and aluminium test cells due to shorter come-up time (the time needed for the sample core temperature to reach the set-point temperature) [50–54].

Studying the thermal inactivation kinetics in solid food

For solid food samples, such as a variety of meat, nuts and powder, the requirements for experiment methods are not only obtaining minimum come-up time, but also facilitating easy loading and unloading samples. Sterile bags are commonly used for pasteurisation of pathogens in meat samples since they can endure heating temperature. For example, sterile stomach bags (Whirl-Pak filter bags) are used for uniform thickness of less than 1 mm of around beef [30], around chicken [33], catfish and tilapia [11], chicken shawirma [55] and peanut butter [56] for achieving short come-up time. Sterile nylon/polyethylene vacuum bags are applied for ground pork and turkey [57] and almonds [58]. Whirl-Pak filter bags are made of polyethylene and the same as sterile nylon/polyethylene vacuum bags. These bags can be used for pasteurisation of pathogens in solid food when the sample temperature is lower than 80 °C [11,30,33,55-58]. There have other methods to obtain thermal inactivation kinetics for pathogens in solid food, such as glass beaker/polypropylene jar in RF systems [17,59], polypropylene container in MW systems [18], glass vials when the heating temperature above 100 °C [60], aluminium thermal death tubes or cells for viscous materials, including solid foods with wide temperature ranges [20,61].

Studying inactivation kinetic models for liquid, semi-solid and solid foods

Aluminium test cells (TDT disk) designed by Chung *et al.* [52] are widely used for determining the heat resistance of pathogens due to high thermal conductivity, good corrosion resistance, machinability, wide temperature range beyond 100° C and ease of use [20,51–53]. It is made of aluminium alloy and consists of two parts: a base and a screwed-on cap to allow easy loading and unloading of the sample. The schematic diagram of aluminium cells is shown in Figure 1. Gurtler *et al.* [62] evaluated the glass capillary tube and TDT disk methods for determining thermal inactivation kinetics of



Figure 1. Schematic diagram of aluminium cells (\emptyset : 18 × H: 4 mm) [52].

Salmonella in liquid whole egg, and results showed that the *D*-value in the aluminium tube method was nearly the same as that in glass capillary tube method. Basaran-Akgul [63] compared the glass capillary tube and aluminium tube methods for *clostridium sporogenes PA 3679* inactivation in carrot juice, and reached the same conclusion. However, the capillary tube is not suitable for solid foods due to the difficulty of fitting samples into it. Aluminium test cells can be used for both liquid and solid samples.

Heating rates show a significant effect on thermo-tolerance of pathogens, and slower heating rates often result in enhanced heat resistance of pathogens with large D-values at the same target temperatures [51,53,64]. Stephens et al. [64] guantified the effect of heating rates on the thermal inactivation of Listeria monocytogenes with a programmable heating block, and the maximum thermo-tolerance was enhanced at rates of heating $\leq 0.7 \,^{\circ}$ C/min. Foster *et al.* [65] developed a new apparatus to control the heating rate for pasteurisation of pathogens in food by setting a starting temperature, an end temperature, heating time, hold time and cooling time by user-friendly software (Figure 2). However, this apparatus only provides a fast heat treatment to the surface of single food sample, and could be improved by using the sample core temperature to cover the pathogen's resistance over the whole volume and multiple samples to accelerate the experimental process [66,67].



Figure 2. Vertical section (a) and plan view (b) of the apparatus [65].

A unique heating block system (HBS) has been designed to heat liquid, semi-solid and solid foods over a wide range of controlled heating rates for determining the inactivation kinetics of bacterial spores [68]. The HBS consists of a heating unit, which is the same as aluminium test cell, a data acquisition/control unit, and a computer (Figure 3). Heating rate, set-point temperature and holding time are controlled by the customised Visual Basic software and two PID controllers via a solid-state relay. This system can help in precisely characterising the heat resistance of pathogens in foods.

Thermal death kinetic models for food-borne pathogens

Models to describe the thermal inactivation kinetics for foodborne pathogens ranged from primary models (Table 2) with the influence of temperature to omnibus models (Table 3) with all influence factors, such as temperature, time, PH, water activity, heating rate, salinity, etc. This section introduces some of the most often-used models.

Primary modelling

First-order kinetic model

Many published studies on the thermal inactivation of pathogens in food products have shown the first-order thermal inactivation kinetic model [20,69–71] due to the uniform temperature assumption in the product during heat processing [72]. The general form of the first-order kinetic model can be expressed as follows [73]

$$\frac{dc}{dt} = -kc \tag{5}$$

where *c* represents microbial population, *t* is the heating time under isothermal conditions (min) and *k* is the rate constant (\min^{-1}) . This equation can be integrated into a form below:

$$\lg c = \lg c_1 - \frac{kt}{2.303}$$
 (6)

$$t = (\lg c_1 - \lg c) \times \frac{2.303}{k} \tag{7}$$

$$t = (\lg c_1 - \lg c) \times D \tag{8}$$

where c_1 represents the initial microbial population and c is the final microbial population after heat treatment. The susceptibility of pathogens to heat at a specific temperature is characterised by the value of D, which is defined as the time (min) for one log reduction at a constant temperature. Plotting log D-values against temperature often reveals a linear relationship, commonly referred to the thermal death time curve. A *z*-value is obtained as the temperature increase (°C) needed to result in 1-log reduction of D-value from the thermal death time curve:

$$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}}$$
(9)

where D_T is the value of D measured at temperature T, T_1 and T_2 are two different temperatures. The *z*-value is also



Figure 3. Schematic diagram of the TDT HBS [68].

Table 2. Primary model of pathogens under thermal treatments.

| Pathogens | Media | Treatment | Method | Kinetic models | Reference |
|--------------------------------------|------------------------------------|--------------------------------|-----------------------------------|----------------------------|-----------|
| Salmonella | Milk chocolate | Hot water (50–70 °C) | Sterile bags | Biphasic | [10] |
| Escherichia coli O157:H7, Salmonella | Catfish and tilapia | Hot water (55–65 °C) | Sterile plastic bag | Weibull | [11] |
| Enteritidis PT 30 or Salmonella 775W | Almonds | Hot oil (116–127 °C) | Stomacher bag | Weibull (Upward concavity) | [14] |
| Salmonella PT 30 | Almonds | Hot water (56–80 °C) | TDT cell | First order, Weibull | [20] |
| Listeria monocytogenes | Broth culture | Hot water (55–65 $^{\circ}$ C) | TDT tubes | Sigmoidal | [39] |
| Salmonella PT4 | Broth | Hot water (49–60 $^{\circ}$ C) | Flask | Biphasic | [40] |
| Escherichia coli K-12 | Mashed potato | Hot water (57–63 $^{\circ}$ C) | Capillary tube | First order | [50] |
| PA 3679 spores | Phosphate buffer/ mashed potato | Hot air (121 °C) | TDT celĺ | First order | [52] |
| Salmonella spp | Turkey and pork | Hot water (50–66 °C) | Nylon/polyethylene vacuum bags | Weibull | [57] |

obtained by the -1/slope of the regression equation of the log *D*-value against temperature.

For eukaryotic cells, first-order rate kinetics (Arrhenius) is usually used to describe eukaryotic inactivation, but there is a breakpoint around 42.5 °C due to the onset of thermotolerance below this threshold. The Arrehenius model has shown to hold up to 57 °C [74]. For the pathogens, if these show the log-linear behaviour, first-order rate kinetics would be used to describe inactivation characteristics. However, most survival curves do not show log-linear behaviour, because of heterogeneity within the cell population, clumping of a small number of cells, poor heat transfer through the heating menstruum (non-uniform treatments), heat adaptation of pathogens during a heat treatment, protection by fat, protein content and dead cells, and inadequate enumeration methods. Deviations from the first-order kinetics have been frequently observed. This deviation must be taken into account to avoid under- or over-processing of food. Thermal inactivation kinetic models, such as Weibull's survival curves, Biphasic, and Empirical sigmoid models, are commonly used to characterise the non-linear survival curves.

Weibull's survival models

Microbial inactivation using conventional and novel thermal technologies often does not follow the first order kinetics. Sometimes it shows upward and downward concavities. Models derived from the Weibull distribution have the ability to describe these survival trends. The primary model [73] of Weibull's survival curves can be expressed as:

$$\ln \frac{P}{P_0} = -b(T)t^{n(T)} \tag{10}$$

where P(t) is the momentary microbial count, P_0 is the initial microbial count, *b* and *n* are temperature (*T*) dependent coefficients, b(T) is a rate parameter and n(T) is a measure of the

semi-logarithmic survival curve's concavity. When n(T) < 1, the curve presents an convex, suggesting that some microorganisms are more resistant than others or protected by various factors, which make them survive under testing conditions or the remaining population becomes progressing sturdier. When n(T) > 1, the curve is concave, indicating that accumulated damage makes the surviving cells more susceptible to lethal treatment. When n(T) = 1, it means a linear semi-logarithmic survival curve. Examples of published survival curves of Salmonella fitted with Equations (5) and (10) as primary model are shown in Figure 4. The curve shown in Figure 4 presents a first order kinetic model and the Weibull distribution with convex, and the parameters in Equations (5) and (10) are used to generate the curves. Weibull's survival curves can also be used to estimate the inactivation of Salmonella spp. under isothermal and non-isothermal conditions. Equation (10) is used to estimate the inactivation of Salmonella spp. under isothermal conditions. Equation (11) incorporating changed temperature over time is used to estimate the inactivation of Salmonella spp. for the nonisothermal process, and the momentary slope of nonisothermal is the same as isothermal at the momentary temperature [57].

$$\frac{\mathrm{dln}\frac{P}{P_0}}{\mathrm{d}t} = -b[T(t)]n[T(t)] \left\{ \frac{-\mathrm{ln}\frac{P}{P_0}}{b[T(t)]} \right\}^{\frac{n(t(t))-1}{n[T(t)]}}$$
(11)

where T(t) is the time corresponds to the momentary logarithmic survival ratio $\ln \frac{P}{P_0}$. When pathogens are primarily exposed to low heating rates, in which the cells are exposed to sub-lethal temperatures for long time and then to lethal temperature, the cell may yield heat adaptation, which can be estimated by using the Weibull inactivation model [75]. The Weibull model could be used to estimate log-reduction of target pathogens when the sample temperature and exposure time are given during the heating process.

Biphasic model

Biphasic survival curves proposed by Kamau *et al.* [76] can be used to express broken curves [40], which represent a mix of two fractions of different heat resistances. The equation is shown as following:

$$P(t) = e^{-k_1 t_1} + e^{-k_2 (t-t_1)}$$
(12)

where t_1 is the critical time at which the rate changes, k_1 and k_2 , are the temperature-dependent inactivation rates at the first and the second phases. Examples of published survival curves of *Salmonella* fitted with Equation (12) as a primary model are shown in Figure 5. It describes the heat resistance of *Salmonella* PT4 with high-density stationary-phase populations heated at 60 °C, and the curves show biphasic curves.

Several reactions leading to the population changes of pathogens can take place simultaneously. Those reactions can be parallel or sequential and their contributions to the overall heat resistances vary. This biphasic model represents a mix of two species or strains, which have different heat resistances, pathogen in broth with high-density stationary-phase populations or pathogen inoculated into agricultural products with lower water activity ($a_w < 0.6$), such as flour, peanut butter and chocolate. For example, a mix of two species or strains having different heat resistances shows biphasic curves [77]. The heat resistance of Salmonella PT4 with high-density stationary-phase populations heated at 60 °C shows biphasic curves due to the leakage from early heat casualties [40]. When Salmonella weltevreden was inoculated into flour with an initial water activity range from 0.2 to 0.6 before heating, a biphasic curve was observed, which shows an initial rapid decline during the first 10 min, then followed by a linear survival curve [78]. The thermal inactivation curves of Salmonella during milk chocolate conching at 70°C show a rapid death in the first 180 min and then a lower inactivation of Salmonella [10]. When peanut butter inoculated into Salmonella was heated in a circulating water bath at temperatures from 70 °C to 90 °C, the inactivation curves show rapid death in the first 10-20 min and follow by lower death rates thereafter [79,80]. These results demonstrate that, for any temperature, as the initial water activity of agricultural product was lower than 0.6 prior to heating, the thermal inactivation kinetics shows biphasic survival curves. That is because the food-borne pathogens may aggregate into particles within or near the aqueous phase, while others are located in more hydrophobic and fatty environment, and the solid particles are not fully overlaid by the fatty phase at the beginning of heating, which leads to less resistance.

Empirical sigmoid model

Empirical sigmoid model can be used to express asymptotic convex behaviour of a variety of pathogen survivor curves. It is justified by the existence of a distribution of heat resistance within the bacterial population [81]. The equation is shown as following:

$$\log N(t) = \log N_0 - \log (1 + \exp (a + b \ln (t)))$$
(13)

where *a* and *b* (>0) are location and dispersal parameters, which are estimated from experimental data, log N_0 is the initial microbial count, log N_t is the number of cells at time *t*,

and t approaches infinity when the derivative of the right side of Equation (1) approaches 0. This model represents non-isothermal inactivation when "flat shoulder" and "lag period" are shown in Figure 6. For example, survival curves of Listeria monocytogenes in broth are described by the sigmoid model under heat treatment at temperatures of 55, 60 and 65 °C after heat-shocking at 42 °C for 1 h with non-selective enumeration agar [39]. When Salmonella serotypes were inoculated into ground chicken at three temperatures of 60, 65 and 71.1 °C and four concentrations of two antimicrobials (cinnamaldehyde and carvacrol), most inactivation curves display both upward concavity and tail, thus the sigmoid model is useful for predicting the heat resistance of Salmonella serotypes in ground chicken [33]. Tailing phenomena should be considered when heat-damaged cells were repaired in foods to evaluate the efficiency of the food cooking process.

Secondary model

If the inactivation kinetics are approximately described by a primary model and the parameter dependences on all the pertinent factors are adequately elucidated for specific technologies and products, secondary model may be able to predict inactivation in similar matrices (similar independent variables) and under different operating conditions. Secondary model is developed by using response surface analysis or step-wise regression to predict D-value, b, n or 7.0 log relative reduction (Ln $t_{7,0}$) based on the primary model, such as first-order, the Weibull or sigmoid model [20,30,33,82-84]. For example, secondary model developed from response surface analysis was used for predicting D-values of Salmonella PT30 in almonds for any combinations of temperature, a_w and their interactions shown in Equation (14) [20], and D-values of L. monocytogenes for any combinations of temperature, sodium lactate and sodium diacetate in beef [83]. A step-wise regression was used to determine the independent parameters to predict the Ln t_{7.0} values of Salmonella in ground chicken based on the sigmoid model [33].

$$log D = -296.569 + (607.617a_w) + (6.444T) - (270.392a_w^2) - (0.030T^2) - (11.580a_wT)$$
(14)
+ (3.894a_w^2T) + (0.039a_wT^2)

Global approach: omnibus models

Omnibus model is a model type that fits the primary and the secondary models at the same time by using all the data from the experimental curves [24,33,34]. Based on the empirical sigmoidal model, the omnibus mixed-effect model is shown as following [24]:

1 + 1

$$\log N_{ijk} = \log N_{0j} \exp \left(\frac{t}{\chi_j}\right)^{\beta_j} + \varepsilon_{ijk}$$

$$\ln \chi_j = a_1 + a_2 SPP + a_3 pH + a_4 NaCl^2 + a_5 SPP^2 + a_6 pH^2$$

$$+ a_7 NaCl \times pH + a_8 NaCl \times T + a_9 pH \times T + u_j$$

$$\ln \beta_j = b_1 + b_2 pH + b_3 NaCl + b_4 NaCl^2 + b_5 NaCl \times pH + v_j$$

$$\log N_{0j} = \log N_{0mean} + w_j$$
(15)

Table 3. Secondary and omnibus model of pathogens under thermal treatments.

| Pathogens | Media | Treatment | Method | Kinetic models | Reference |
|------------------------------|----------------|---|---------------|--|-----------|
| Salmonella Enteritidis PT 30 | Almonds | Hot water (70–80 °C), water activity | TDT cell | Secondary polynomial models | [20] |
| Escherichia coli O157:H7 | Ground beef | Hot water (55–62.5 °C), pH, NaCl, SPP | Stomacher bag | Omnibus model (Weibull) | [24] |
| Listeria monocytogenes | Ground turkey | Hot water (55–65 °C), sodium chloride and green tea | Stomacher bag | Second-order response surface regression | [29] |
| Listeria monocytogenes | Ground beef | Hot water (60–73.9 °C), salt, sodium pyrophosphate and sodium lactate | Stomacher bag | Secondary response surface regression (Weibull) | [30] |
| Salmonella serovars | Ground chicken | Hot water (60–71.1 °C), cinna- maldehyde and carvacrol | Stomacher bag | Omnibus model (Sigmoid model) | [33] |



Figure 4. Heat inactivation kinetics of *Salmonella Enteritidis PT 30* inoculated in almond kernel flour with water activity (a_w) of 0.888 at different temperatures ($c_0, 68 \,^\circ C_i \, (c_0, 68 \,^\circ$

The parameters of χ and β from the Weibull model are expressed as a function of the environmental variables: temperature (*T*), pH, salt percentage concentration (NaCl) and sodium pyrophosphate percentage concentration (*SPP*). *u* and *v* are added to the mean of the intercepts a_1 and b_1 due to some fraction of the variability in the scale and shape parameters. *w* means initial microbial concentration. log N_0 is a variable from condition to condition. ε means residual error. log N_{ijk} means concentration taken at the time *k* in the food sample *i* exposed at the environmental condition *j*.

Omnibus models are used to predict survival curves for any specified values of temperature, water activity, pH, NaCl and other parameters. Juneja *et al.* [23] observed concave upward survival curves and developed an omnibus model for predicting times needed to obtain 6.5-log lethality between 55 °C and 71.1 °C in ground beef supplemented with salt (0–4.5%), sodium pyrophosphate (0–0.5%) and sodium lactate (0–4.5%). Juneja *et al.* [33] also compared the accuracy of omnibus sigmoid model to that of omnibus log-linear model with tail for predicting the 7.0-log reduction times in ground chicken under three temperatures and four concentrations of two natural antimicrobials, as shown in Figure 7. Juneja *et al.* [24] developed a non-linear mixed effect omnibus model and indicated that the model can be used to assist meat processors in determining the processing times and temperatures required to achieve specific log reduction of *E. coli O157:H7* in ground beef.

Omnibus model provides guidance on how changes in food formulation parameters affect the heat resistance of pathogens. It assists food processors to design thermal processes for estimating lethal treatment, i.e. the processing times and temperatures required to achieving specific log reductions of the pathogen, thus developing safe cooking processes. The model prediction accuracy would depend on the online measurement of operational conditions and food property changes during the heat process.



Figure 5. Biphasic thermal inactivation curves in Salmonella Enteritidis PT4. Mean numbers of survivors and standard deviations at $60 \degree C$ (a), $59 \degree C$ (b), $58 \degree C$ (c), $57 \degree C$ (d), $56 \degree C$ (e) and $55 \degree C$ (f), showing the best-fit lines for initial and tailing populations. The arrows indicate the limits of detection [40].



Figure 6. Empirical sigmoid curves of the (a) untreated, (b) heat-shocked and (c) selected cultures (subcultures of cells survived from a heat treatment of 60° C for 20 min) of *Listeria monocytogenes* at (1) 55 °C and (2) 60 °C. Plotted points are the means of the observed values. The lines are the predicted survivor curves. Vertical bars indicate one standard deviation [39].



Figure 7. Survival curves of *Salmonella* in ground chicken for the different combinations of cinnamaldehyde and carvacrol concentrations at the temperature of 60°C, as modelled by individual regressions and mix-effects omnibus regressions for both the log-linear with tail and the empirical sigmoidal inactivation models. Mean predicted value and 90% confidence intervals are shown for the omnibus regressions [33].

Model comparisons and applications

Selection of the appropriate primary and omnibus models depends on principle of parsimony and the goodness of fit: pooled variance, pooled Bayesian Information Criterion (BIC) and Akaike's Information Criterion (AIC). Pooled variance is calculated as an average of variance, BIC is calculated as an average of the individually fitted curves weighed by the number of observations for each survival curve [24,33], and AIC is a measure of the relative quality of statistical models for a given set of data [85]. The models with the lowest pooled variances, BIC and AIC, mean a good fit with a manageable number of parameters, and therefore are chosen for further analyses. For example, Juneja et al. [33] compared pooled variances and BIC for each of the nine models in their research, and then concluded that the empirical sigmoid and log-linear models were the most parsimonious and chosen for further analyses. Then secondary model was obtained separately for parameters (such as a and b in the empirical sigmoid) from the primary model in terms of the independent variables. Lastly, models incorporating the primary and the secondary models were built for predicting survival curves with any specified values. For example, Juneja et al. [33] reviewed two models for Salmonella serotypes in ground chicken, and recommended the omnibus sigmoid model to predict the 7.0-log lethality more accurately than the omnibus log-linear model with tail due to lower pooled variance and BIC.

Traditionally, D- and z-values are used for describing the efficacy of static pathogen inactivation treatment and F-values (the time required to achieve a specific reduction in microbial numbers at a given temperature) are used for evaluating that of a dynamic process. For the non-linear microbial inactivation kinetics during thermal treatment, traditional values cannot be employed, therefore, Weibull's parameters, such as b and n, are used to calculate the time of the first decimal reduction [86] or the time to reduce the number of microorganisms by a factor of 10 [87]. Magnetic hyperthermia (MH) as an alternative chemical-free method was used for disinfecting food spoilage bacteria in planktonic cells and biofilms since they have a greater and faster bactericidal effect and can overcome the limitation of biocides by acting on microbial cells regardless of their metabolic states. Thus, it was a potential disinfection method in food-related environments [88,89]. The heat was produced under an applied oscillating magnetic field by spin relaxation processes. The mild heat value (MH-value) defined as the time needed to achieve a predefined microbial reduction at a reference temperature and a known thermal resistant constant is used to assess the efficacy of a mild thermal treatment, in which the inactivation kinetics are not log-linear [90].

Conclusion and suggestions for future research

Several models have been used to study the thermal inactivation kinetics of food-borne pathogens. For example, firstorder kinetic model expresses log-linear behaviours obtained from uniform heating treatment. Weibull's models are used for estimating inactivation of pathogens under isothermal and non-isothermal conditions. Biphasic model expresses broken curves obtained from mixed species, which have different heat resistances, pathogen in broth with high-density stationary-phase populations or pathogen inoculated into agricultural products with lower water activity ($a_w < 0.6$). The empirical sigmoidal model represents non-isothermal inactivation characteristics when "flat shoulder" and "lag period" are present. Secondary model was developed by using second-order response surface regression or step-wise regression to predict D-value or some other parameters got from primary model according to any other independent variables, such as pH, water activity and heating rate, which associated with experiment. Omnibus models incorporating primary and secondary models are used for predicting survival curves with any specified values of independent variables. The thermal inactivation kinetics of food-borne pathogens in meat and low moisture foods are studied by using ground meat, powder or other model foods, which have the same physicochemical characteristics with real food. That's because in real food, there has complexity of various physical and chemical reactions in the process of heat treatment. Future research on developing thermal inactivation kinetic models associated with pasteurisation should focus on the following areas:

Applications of thermal inactivation kinetics of pathogens in real food

Due to some food quality characteristics, experimental conditions and some other restrictions, model food having the same physicochemical characteristics with real food has been widely used instead of an actual food matrix (real food with various independent variables, which are associated with experiment) to establish the thermal inactivation kinetics. The models made from model food are able to predict the pathogen behaviour in the most of the foods, but it is impractical to predict the process time required to achieve a 3-log reduction in the products with high sugar content and lower water activity. Since food is a complex system composed of a variety of ingredients, the interior of food may have physical or chemical reactions under heat treatment, and be accompanied by synergistic or antagonistic effects between reactions. Therefore, there have many unknown factors when using thermal inactivation kinetic model developed from the model food for guiding the heat treatment of real food. It is needed to conduct further research to validate the reliability and effectiveness of thermal inactivation kinetic model obtained from the model food.

Omnibus model needs to be developed and validated

Heating by conventional and novel thermal processing is influenced by the temperature, composition of materials, water activity, preheating, heating rate, heat shock protein and the type of heating equipment. However, the primary model only takes temperature and time into account, the secondary model assesses *D*-value or some other parameters got from primary model based on the pertinent factors, such as pH, water activity and heating rate, which associated with experiment. Omnibus models incorporating primary and secondary models are used for predicting survival curves with any specified values of independent variables. These models can be used for predicting survival curves under any specified environmental conditions, which associated with experiment. Therefore, omnibus models need to be properly established or validated first, and then used for predicting the destruction rate of the pathogens under commercial thermal processing conditions in the near future.

Non-isothermal conditions need to be considered

Many of the researchers rely on isothermal treatment conditions during heating to develop fundamental kinetic models. However, non-isothermal conditions in commodities during thermal treatment are commonly observed in real food, therefore heat transfer theory via simulation along with thermal inactivation kinetic models of pathogens should be considered together for pasteurisation of foods.

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