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Thermal inactivation of *Aspergillus flavus* in peanut kernels as influenced by temperature, water activity and heating rate

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ABSTRACT

Infection of *Aspergillus flavus*, which can produce aflatoxin, is a major problem for peanut safe storage. Thermal inactivation kinetics of *Aspergillus flavus* is essential to design an effective heat treatment process. In this study, thermal inactivation kinetics of *Aspergillus flavus* in peanut kernel flour at four water activity (a_w) levels (0.720, 0.783, 0.846, and 0.921) with three temperatures for each a_w was studied using a thermal-death-time heating block system and fitted with first-order kinetic and Weibull models. The influence of heating rates on thermotolerance of *Aspergillus flavus* was also investigated. The results showed that the Weibull distribution model had better coefficient of determination from 0.954 to 0.996, as compared to that (from 0.866 to 0.980) of the first-order kinetic model. An upward concavity was found with the inactivation curve, indicating a tailing effect. Model parameters (D, δ, and p) were estimated with the modified Bigelow equations to predict survival curves of *Aspergillus flavus* at any temperature and a_w. The reduced heat resistance of *Aspergillus flavus* at high heating rates above 1 °C/min suggests that developing fast thermal processes is preferred for pasteurizing peanuts in food industry. A case study was presented for applying the cumulated lethal time model to design the industrial heating process based on the thermal kinetics of *Aspergillus flavus*.

1. Introduction

Peanut (*Araachis hypogea* L.) is an economically important oilseed crop in the world due to its annual production of 43.98 Mt, mainly produced by Africa, China and India (FAOSTAT, 2016). However, potential peanut contaminations with aflatoxin are considered as the most serious food safety problem in the world (Bankole and Adebanjo, 2003). The aflatoxin contamination in pre-harvested peanuts is caused by the infection of *Aspergillus* species, mainly by *Aspergillus flavus* (Guo et al., 2011; Liu et al., 2017). This *A. flavus* may survive in low moisture foods for extended periods of time and may grow rapidly as soon as the storage environment becomes suitable. Therefore, it is of great importance to eliminate the *A. flavus* in peanuts before storage.

Several methods have been studied for inhibition of *A. flavus*, such as chemical fumigation (Boukaew et al., 2017), cold atmospheric plasma treatment (Sohbatzadeh et al., 2016), irradiation (Algabr et al., 2013), and chlorine dioxide gas (Yang et al., 2013). But each of these methods has limitations due to requirement of sophisticated equipment, low efficiency, high cost or harmful residues. Considering the disadvantages above, thermal processes are preferred by the food industry due to their simple, efficient and reliable treatments to inactivate micro-organisms and control enzymes. But excessive heat may cause quality deterioration, such as degradation in color, texture, nutrient content, and sensory value (Liaotrakoon et al., 2013; Ling et al., 2015).

Thermal inactivation kinetics of food-borne pathogens can effectively predict the thermal processing parameters (heating temperature and time) to avoid under or over-processing of food, which would result in the survival of micro-organisms or the deterioration of product quality. The log-linear model described by the first-order kinetics is widely used in the food industry. A mechanistic explanation for the death of micro-organisms based on this model could be caused by inactivation of some critical enzyme, which is commonly governed by the first-order kinetics (Van Boekel, 2002). However, in many cases, the microbial survival curves showed upward and downward concavities. So the classical first-order kinetics can't accurately evaluate the microbial thermal inactivation. A Weibull distribution model has the ability to adequately describe concave survival curves or log-linear ones (Bermúdezaguirre and Corradini, 2012; Mafart et al., 2002). The Weibull model has been successfully applied to characterize inactivation of micro-organisms affected by thermal treatment (Leguérinel et al., 2007; Manas et al., 2003; Takhar et al., 2009).

Temperature is not the only factor that affects microbial thermo-

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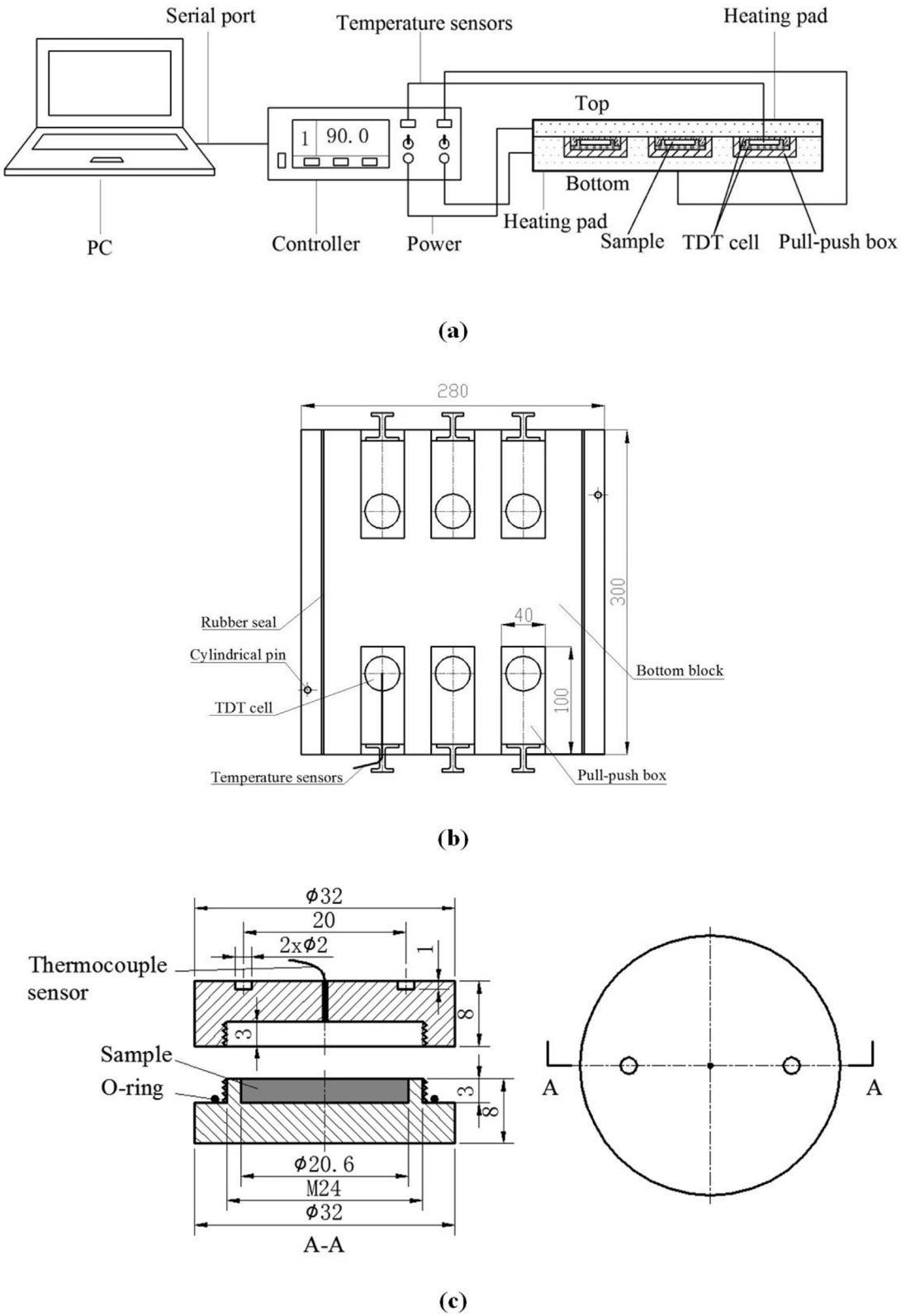

Fig. 1. Schematic diagrams of the TDT HBS (a), top view after removing the top block (b) and geometrical dimension of the TDT test cell (c) with all dimensions in mm (adapted from Kou et al., 2016).

tolerance in heat treatment. Many factors, including the composition and physicochemical properties (such as, pH, organic acids and water activity) of the heating medium, have been documented to have great effects on the microbial heat resistance (Doyle and Mazzotta, 2000; Doyle et al., 2001; Jagannath et al., 2005). Among these properties, water activity (a_w) has a pronounced influence on the heat resistance of the micro-organisms (Lang et al., 2017; Villa Rojas et al., 2013). Many studies about the effect of a_w on the thermal resistance of bacteria, such as *Salmonella* (Bermúdezaguirre and Corradini, 2012; Villa Rojas et al., 2013), *Listeria monocytogenes* (Fernández et al., 2007), and

Staphylococcus aureus (Zhang et al., 2018), have been conducted. However, the influence of a_w on the thermal inactivation of mold including *A. flavus* is not well known. Except for the temperature and water activity, the heating rates are also proved to have a significant effect on microbial heat resistance (Chung et al., 2007; Conesa et al., 2009; Kou et al., 2016).

With the thermal inactivation kinetic information for *A. flavus*, the cumulative lethal effect of a heating process can be evaluated from the measured temperature-time history through a cumulative lethal time model (Tang et al., 2000). The cumulative model could effectively predict the efficacy of a proposed thermal treatment and select suitable and safe treatment parameters for *A. flavus* inactivation in peanuts.

The objective of this study was (1) to develop two mathematical models (first-order kinetics and Weibull distribution) to estimate heat resistance of *A. flavus* in different temperature and water activity conditions, (2) use the modified Bigelow equations to evaluate the effect of temperature and water activity on the model parameters (D, δ, and p), (3) to study the thermal inactivation of *A. flavus* at 59 °C under five heating rates, and (4) apply the cumulated lethal time model to design industrial thermal treatment based on the thermal kinetics of *A. flavus*.

2. Materials and methods

2.1. Materials

Peanut kernels were purchased from a local market in Yangling, Shaanxi, China. The peanut samples were sealed into polyethylene bags and stored in a refrigerator at 4 ± 1 °C.

2.2. Sample preparation

The initial moisture content was determined by the AOAC Official Method 925.40 (AOAC, 2005). About 4–5 g peanut flour was placed in an aluminum dish and dried in a vacuum oven at 100 °C under pressure ≤0.1 kPa to a constant weight. The peanut samples were adjusted by adding a predetermined amount of distilled water to 300 g peanut kernels to obtain different moisture content or water activity (a_w) levels. Then, the adjusted samples were stored in polyethylene bags in a refrigerator at 4 °C for 5 days, and shaken 4 times a day to guarantee the uniformity of moisture distribution. After the peanut kernels achieved the specified moisture content, the kernels were milled by a grinder and passed through a no.18 mesh (16 Tyler). The water activity of peanut kernels was determined by a water activity meter (Aqua Lab 4TE, Decagon Devices, Inc., Pullman, WA, USA).

2.3. Fungal strain and spore suspension production

Aspergillus flavus (CICC 2090) was obtained from China Center of Industrial Culture Collection, Beijing, China. The strain was cultivated on Potato Dextrose Agar (PDA; Beijing Aoboxing Bio-Tech CO., LTD) and incubated at 30 °C for 7 days. Conidia were harvested from 7-day-old cultures by pouring the sterile 0.85% isotonic NaCl solution with 0.1% Tween 80 on the cultivated agar and gentle scraping the surface using a spreader (Sohbatzadeh et al., 2016). The number of conidia in the suspension was adjusted to 3×10^8 CFU/mL.

2.4. Thermal treatment

The thermal treatment was conducted in a heating block system (HBS), which was manufactured by Northwest A&F University (Kou et al., 2016). The HBS consisted of a heating unit, a data acquisition/control unit, and a computer (Fig. 1). The heating unit was composed of top and bottom blocks with eight custom-made heating pads, and 6 pull-push boxes with 6 TDT cells. Before inoculation, peanut samples and TDT cells were sterilized at 105 °C for 10 min, and 121 °C for 20 min in a vertical autoclave, respectively. After that, about 0.6 g peanut powder sample was put into the TDT cells. Then, 10 μL of *Aspergillus flavus* conidia suspension was inoculated on peanut flour, resulting in similar water activity as compared to the initial level during preliminary tests. The TDT cells were sealed and left for 24 h at ambient temperature to achieve a moisture balance.

Five TDT cells filled with 0.6 g inoculated peanut samples with four a_w levels (0.720, 0.783, 0.846, and 0.921 corresponded to sample moisture content of 6.03%, 9.77%, 13.96%, and 17.87% on wet basis) were pushed into the bottom block and heated from 25 °C to different temperatures with heating rate of 5 °C/min for different holding time. The three target temperatures with different holding times for each a_w was selected depending on the target temperature to achieve at least a 3-log reduction, e.g., 62, 68 and 74 °C for a_w of 0.720 or 59, 62 and 65 °C for a_w of 0.846. To study the influence of a_w on inactivation of *Aspergillus flavus*, a same temperature (62 °C) was included at each a_w for comparison. One TDT cell filled with un-inoculated peanut samples was used with temperature monitoring by two calibrated type-T thermocouples (TMQSS-020-6, Omega Engineering Ltd., CT, USA). The TDT cells were removed from the heating block at five different time intervals.

To further investigate the influence of heating rates on thermal inactivation of *A. flavus*, five heating rates (0.1, 0.5, 1, 5 and 10 °C/min) were chosen with a target temperature of 59 °C for a_w of 0.921. From preliminary tests, it was difficult to calculate the D-values at high temperatures due to the large population reduction after the long come up time under lower heating rates. Considering this, a lower temperature of 59 °C at a_w of 0.921 was chosen to investigate the effect of heating rates on thermal inactivation of *A. flavus* in this study. Immediately after heating, the TDT cells were pulled out and put into ice water for cooling at least 2 min. After heat treatment, peanut flours were aseptically scraped into a dilution bottle with 10 mL of sterile 0.85% isotonic NaCl solution. Then, the solution was 10-fold serially diluted in 0.9 mL of sterile NaCl solution. 100 μL of each dilution was spread on Potato Dextrose Agar and incubated at 30 °C for 7 days. The number of colony was obtained by a plate counting method. Each test was repeated in triplicate.

2.5. Modeling of thermal inactivation kinetics

The first-order kinetic and the Weibull distribution models were used to fit the thermal inactivation data. The first-order kinetic model (Peleg, 2006) was:

$$\ln N = \ln N_0 - kt \tag{1}$$

Where N is the number of microorganisms at time t (CFU/g), N_0 is the initial number of microorganisms (CFU/g), t is the time of isothermal treatment (min), and k is the first-order rate constant (min^{-1}). The equation can be changed into:

$$\log \frac{N}{N_0} = \log S(t) = -\frac{t}{D} \tag{2}$$

Where $S(t)$ is the survival ratio, and D is the decimal reduction time (min) at temperature T (°C).

The Weibull model (Peleg, 2006) can be expressed as:

$$\log S(t) = -\left(\frac{t}{\delta}\right)^p \tag{3}$$

Where δ is the scale parameter, and p is the shape parameter reflecting the curve's concavity. When $p < 1$, the semi-logarithmic curve presents an upward concavity (shoulder), indicating that the sensitive cells are inactivated first and the remaining populations are the sturdy ones, or adapting to the stress. While $p > 1$, the semi-logarithmic survival curve shows a downward concavity (tailing), which means that the remaining populations become increasingly susceptible to heat, indicating that accumulated damage makes the remaining cells more difficult to survive. A linear semi-logarithmic survival curve is a special

case of this model when $p = 1$, which means each cell is equally susceptible no matter how long the treatment lasts.

2.6. Modeling influence of temperature and water activity on thermal inactivation

To assess the influence of T and a_w on both D and δ-values, the modified Bigelow equation proposed by Gaillard et al. (1998) and Mafart et al. (2001) was used in this study as follows:

$$\log(D/D^*) = -(1/z_T)(T - T^*) - (1/z_{pH}^2)(pH - pH^*)^2 - (1/z_{aw})(a_w - 1) \quad (4)$$

Where T is temperature (°C), T^* is the reference temperature (fixed to 70 °C in this study), pH* is the pH of reference (generally, pH* = 7). D^* is the time (min) needed to achieve 1 log reduction in the population at temperature T^*, pH*, and $a_w = 1$. z_T represents the temperature increase needed to lower the D-value ten-fold. z_{pH} and z_{aw} are the distance of pH from pH* and a_w from 1, which lead to ten-fold reduction of D-value.

Since the pH was not adjusted to different levels in this study, the equation can be simplified into (Villa Rojas et al., 2013)

$$\log(D/D^*) = -(1/z_T)(T - T^*) - (1/z_{aw})(a_w - 1) \quad (5)$$

2.7. Determinations of cumulative time-temperature effects based on the first-order kinetics

The important implementation of the thermal inactivation kinetics was to optimize thermal processes. The microbial inactivation is dependent on the cumulative thermal exposure during the course of the non-isothermal treatment. The cumulative lethal effect of a given temperature-time history can be calculated using an equivalent total lethal time M_{ref} (min) at a reference temperature T_{ref} (°C) based on the first-order kinetics and the cumulated lethal time model (Hansen et al., 2004; Tang et al., 2000; Zhang et al., 2004):

$$M_{ref} = \int_0^t 10^{(T(t)-T_{ref})/z} dt \quad (6)$$

2.8. Statistical analysis

The parameters for the first-order kinetic model were obtained using linear regression in Microsoft Excel. Parameters for the Weibull model and modified Bigelow equation were obtained with SPSS Statistics 17.0.

3. Results and discussion

3.1. Thermal inactivation of A. flavus as influenced by temperature and water activity

To evaluate the influence of the temperature and a_w on the parameters of first-order kinetics and Weibull model, the D-values, the shape and the scale parameters were estimated for each survival curve obtained at different experimental conditions. Table 1 shows the D, δ, and p values obtained from the *A. flavus* heated at three different temperatures and each of four different water activity values. The determination coefficient from 0.954 to 0.996 of the Weibull distribution model was better than that from 0.866 to 0.980 of the first-order kinetic model. All the p values were smaller than 1, indicating the direct lethal effect of thermal treatment on the inactivation of *A. flavus* rather than a cumulative effect. Similar results were found in *Clostridium sporogenes* by Dong (2011), *Salmonella enteritidis* by Michalski et al. (1999), and *Listeria monocytogenes* by Chhabra et al. (1999) and Augustin et al. (1998).

The fitted first-order and Weibull models of *A. flavus* inactivation as influenced by temperature at a fixed a_w (0.783) are shown in Fig. 2. The curve slope increased with increasing temperature, indicating that lower temperature had a more obvious tailing effect. Fig. 3 shows the survival curves corresponding to *A. flavus* at 62 °C for a_w of 0.720, 0.783, 0.846 and 0.921 fitted with both Weibull model and the first-order kinetics model. The survival curve for a_w of 0.921 and 0.846 appeared to be linear, but those for 0.783 and 0.720 were slightly upwardly.

An inverse relationship between T and D-values was observed, that is, the higher temperature, the shorter time required for inactivating *A. flavus*. For example, a D-value of 43.48 min was obtained at 62 °C and a_w of 0.720, but decreased to 13.68 and 2.77 min at 68 °C and 74 °C, respectively. The δ value of the Weibull distribution tended to decrease with increasing temperature, indicating that the inactivation rate of *A. flavus* increased with increasing temperature. For example, the δ value was 6.0 at 56 °C and a_w of 0.921, but decreased to 1.7 and 1.2 at 59 °C and 62 °C, respectively. Similar trends have been found for *Salmonella Tennessee* in peanut paste by Enache et al. (2013).

Both D and δ-values decreased with increasing water activity. For example, the D and δ values were 43.48 and 41.1 at 62 °C and a_w of 0.720, respectively, but decreased to 19.96 and 10.9 at a_w of 0.783. Similar trends have been reported by Hiramatsu et al. (2005) and Archer et al. (1998). These results indicated that increasing a_w can availably reduce the thermal treatment time and achieve the desired microbial inactivation level. As an example, using the Weibull model to calculate the time for a 5-log reduction of *A. flavus*, 232.37, 123.69, 34.84 and 20.93 min were needed at 62 °C for peanut with a_w of 0.720, 0.783, 0.846 and 0.921, respectively.

3.2. Influence of temperature and water activity on D, δ and p-value

Concerning the p values showed in Table 1, the temperature and water activity had no great influence on the p-values. This observation is in agreement with those of Fernández et al. (2002) for *Bacillus cereus* spores, Couvert et al. (2005) for *Bacillus pumilus*, and Leguérinel et al. (2007) for *Salmonella typhimurium*. However, our results disagree with those obtained by Fernández et al. (2007) for *L. monocytogenes* and Hassani et al. (2005) for *Pseudomonas aeruginosa* in which the shape parameter was independent of heating temperature but dependent on the a_w or pH of the treatment medium.

It has been proved that the Weibull model shape parameter p-value should be constant if the proportion of a given microbial population is independent on the environmental conditions. Two approaches might be chosen to estimate the single P-value, one was to determine the mean value of the shape parameter estimated from each survival curve (Fernández et al., 2002). The second was to evaluate the single P-value from the whole set of survival curves, and then evaluate δ parameters for the whole set of inactivation data (Couvert et al., 2005). But the first method was not suitable because the number of data in each survival curve was different, and each data value did not have the same weight on the p-value (Couvert et al., 2005; Fernández et al., 2007). To use the Weibull model to predict the survival curves at any temperature and water activity, P-value was evaluated from the whole data and fixed at 0.66. Then, the δ-values of Weibull model were re-estimated with single P-value (0.66) for *A. flavus* (Table 2).

The values of estimated parameters D^*, δ^*, z_T and z_{aw} for the first-order kinetics and Weibull distribution models (associated p-value determined for each kinetic and single P-value evaluated from the whole set of kinetics) fitted with the modified Bigelow equation are given in Table 3. The modified Bigelow model showed a good fit for both D ($R^2 = 0.954$) and δ values ($R^2 = 0.911$ for associated p-value, and $R^2 = 0.972$ for single P-value). By combination of the parameter values in Table 3 and Eq. (3), the survival curve of *A. flavus* could be predicted whatever the temperature and a_w in peanut kernels within the experimental domain limits. Fig. 4 also shows the predictions for survival curve of *A. flavus* in peanut flour at a_w of 0.783 and 62 °C combining the

Table 1
D-values of the first-order kinetic model and δ and p values of Weibull model for *A. flavus* inactivated by thermal treatment in peanut kernels at different a_w levels and temperatures.

a_w	Temperature (°C)	First-order kinetics		Weibull model		
		D (min)	R^2	δ (CI 95%)[a]	p (CI 95%)	R^2
0.720	62	43.48	0.954	41.1 (24.9–57.4)	0.94 (0.26–1.62)	0.954
	68	13.68	0.941	6.7 (3.6–9.9)	0.57 (0.37–0.78)	0.992
	74	2.77	0.949	1.9 (0.1–3.7)	0.78 (0.26–1.30)	0.966
0.783	59	38.91	0.944	22.3 (7.1–37.5)	0.65 (0.30–1.00)	0.979
	62	19.96	0.964	10.9 (8.2–13.7)	0.66 (0.55–0.78)	0.998
	65	7.25	0.866	2.2 (−1.3–5.8)	0.49 (0.08–0.90)	0.964
0.846	59	9.49	0.940	4.2 (1.1–7.3)	0.59 (0.32–0.86)	0.987
	62	6.18	0.974	4.0 (1.9–6.1)	0.74 (0.46–1.03)	0.988
	65	3.69	0.980	3.0 (2.0–4.0)	0.81 (0.48–1.13)	0.987
0.921	56	16.86	0.908	6.0 (0.7–11.4)	0.53 (0.26–0.79)	0.986
	59	6.07	0.908	1.7 (0.5–3.0)	0.51 (0.34–0.68)	0.994
	62	3.34	0.933	1.2 (0.6–1.8)	0.56 (0.42–0.71)	0.996

[a] CI 95%: Confidence Interval.

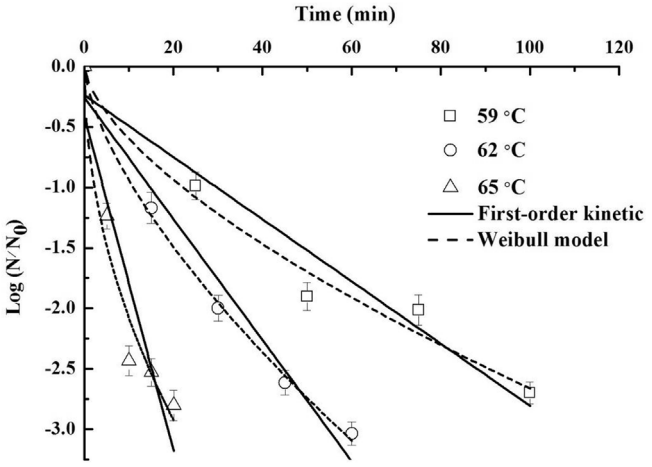

Fig. 2. Thermal inactivation (means ± standard deviation over three replicates) of *A. flavus* inoculated in peanut kernel flour with a_w of 0.783 at three different temperatures fitted with first-order kinetic and Weibull models at the heating rate of 5 °C.

Table 2
δ-values of Weibull model definite with single *P*-value (0.66) evaluated from the whole set of kinetics for *A. flavus*.

a_w	Temperature (°C)	δ (CI 95%)	R^2
0.720	62	35.2 (23.6–46.7)	0.926
	68	8.0 (7.0–9.0)	0.987
	74	1.5 (1.2–1.9)	0.959
0.783	59	22.9 (19.3–26.5)	0.979
	62	10.9 (10.4–11.5)	0.998
	65	3.7 (0.3–4.6)	0.942
0.846	59	5.0 (4.3–5.7)	0.984
	62	3.4 (3.0–3.8)	0.985
	65	2.6 (2.1–3.0)	0.977
0.921	56	8.6 (7.1–10.1)	0.974
	59	2.8 (2.4–3.3)	0.977
	62	1.6 (1.4–1.8)	0.989

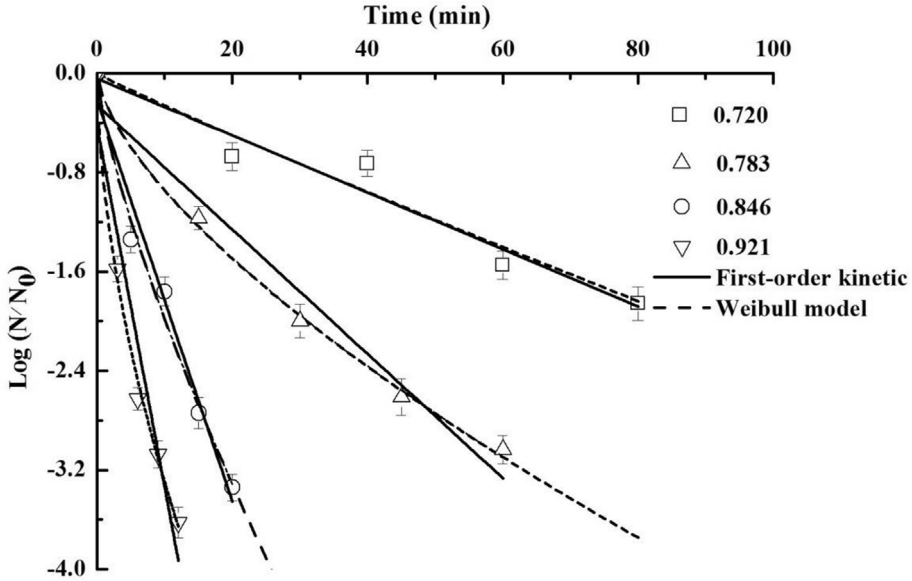

Fig. 3. Thermal inactivation (means ± standard deviation over three replicates) of *A. flavus* inoculated in peanut kernel flour at 62 °C with four different a_w levels fitted with first-order kinetic and Weibull models at the heating rate of 5 °C.

Table 3
Calculated D^*, δ^*, z_T, and z_{aw} values from the first-order kinetics and Weibull distribution models (associated *p*-value determined for each kinetic and single *P*-value evaluated from the whole set of kinetics) fitted with the modified Bigelow equation at a reference temperature of 70 °C for the thermal inactivation of *A. flavus* inoculated into peanut kernel flour at different temperatures and water activities.

Parameter	First-order kinetics	Weibull model	
		Associated *p*-value	Single *P*-value
D^*/δ^* (min) (CI 95%)	0.16 (0.03–0.28)	0.02 (−0.02–0.06)	0.03 (−0.01–0.06)
z_T (°C) (CI 95%)	9.48 (7.60–11.37)	8.51 (5.58–11.43)	9.08 (6.82–11.34)
z_{aw} (CI 95%)	0.176 (0.151–0.201)	0.120 (0.093–0.147)	0.128 (0.106–0.150)
R^2	0.954	0.911	0.972

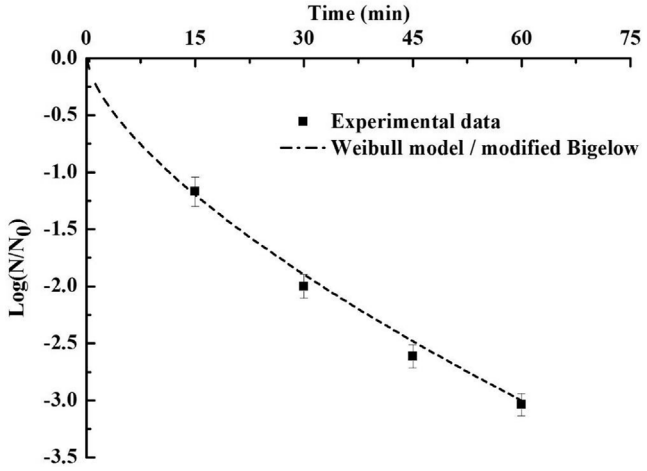

Fig. 4. Experimental data (means ± standard deviation over three replicates) and predicted survival curves for *A. flavus* inoculated in peanut kernel flour with a_w of 0.783 at 62 °C combining the Weibull model with the modified Bigelow equation.

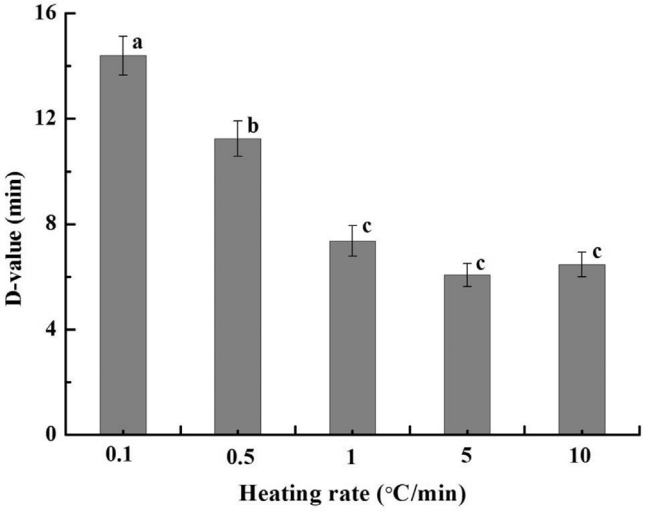

Fig. 5. *D*-values (means ± standard deviation over three replicates) for the survival curves of *A. flavus* at 59 °C with a_w of 0.921 fitted by first-order kinetic models at different heating rates (letters a, b and c indicate that means are significantly different at P = 0.05 among different heating rates).

Weibull model with the modified Bigelow equation.

3.3. Effect of heating rate on the inactivation of A. flavus

Fig. 5 shows the *D*-values of *A. flavus* as influenced by heating rate at 59 °C and a_w of 0.921. With the heating rate of 1, 5 and 10 °C/min, no significant difference (P > 0.05) was observed between the *D*-values (7.36, 6.07, and 6.46 min). But *D*-values (14.39 and 11.24 min) at 0.1 and 0.5 °C/min showed significant differences (P < 0.05) from those at high heating rates (≥1 °C/min), which was in agreement with those obtained by Kou et al. (2016) for *Escherichia coli* and by Zhang et al. (2018) for *Staphylococcus aureus*. This result indicates that smaller heating rate would enhance thermal resistance of micro-organism, which is in accordance with Chung et al. (2007) that the *D*-values obtained from the capillary tubes (higher heating rate) were 1.6–4.5 times smaller than those in the large test tubes (smaller heating rate). The enhanced thermal resistance may be attributed to heat shock conditions created by slow heating as micro-organisms experience sublethal temperatures during the long treatment time (Li et al., 2017; Wiegand et al., 2009). By considering that the slow heating rate during the non-isothermal phases of treatment may enhance heat resistance in some way, heat treatments with higher heating rates, such as dielectric heating (microwave and radio frequency energy), could be preferably applied in the food industry.

3.4. Cumulative lethal effects on A. flavus

The thermal inactivation kinetics of target pathogens was established under the isothermal condition, but most of the thermal processes were non-isothermal. Taking the radio frequency heating as an example, the temperature-time history for 2.5 kg peanuts with moisture content of 18% (a_w = 0.921) heated at electrode gap of 120 mm is shown in Fig. 6. To design the radio frequency treatment for peanut pasteurization associated with the thermal inactivation kinetics, the heating presses should be converted to isothermal process based on the cumulated lethal effect model (Eq. (6)). According to the first-order kinetics, *z* value for *A. flavus* was estimated to be 8.53 °C at a_w of 0.921 based on the data in Table 1. The equivalent lethal time M_{ref} for this heating curve (from 23 to 70 °C) at the reference temperature of 70 °C was the area of the shaded portion (0.31 min) shown in Fig. 6. The *D*-value for *A. flavus* in peanuts with a_w of 0.921 at 70 °C was 0.44 min (calculated from Eq. (5)). To obtain a 5-log reduction of *A. flavus*, 2.20 min was needed for peanuts to heat at 70 °C. For the heat treatment shown in Fig. 6, another 1.89 min holding time was required to obtain a 5-log reduction of *A. flavus*.

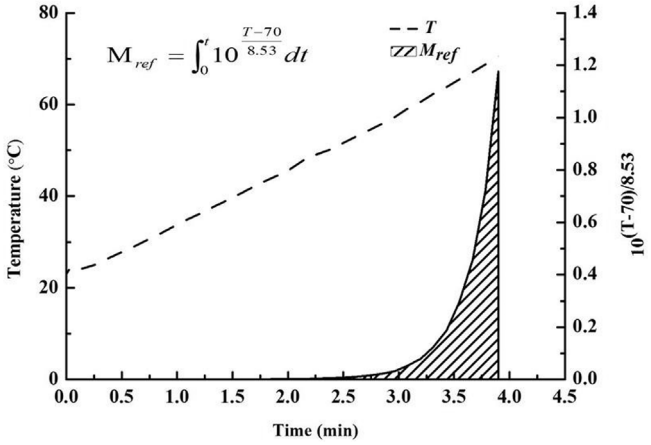

Fig. 6. Temperature-time history of radio frequency heating from 23 to 70 °C, and the equivalent lethal time M_{ref} for this heating curve at the reference temperature of 70 °C.

4. Conclusions

Thermal inactivation of *A. flavus* as influenced by temperature and water activity in peanut kernel flour was fitted with first-order kinetic and Weibull models. The Weibull model showed better coefficients of determination than the first-order kinetic one. The inactivation curves presented upward concavity characteristics, as the p values were smaller than 1, indicating a strong tailing effect for thermal inactivation of *A. flavus*. The results also showed that the heat resistance of *A. flavus* decreased with increasing a_w. The D, δ, and p values of first-order kinetic and Weibull model were analyzed. P-value was considered as a constant for its independence of temperature and a_w. The modified Bigelow equations were used to fit and predict the D and δ values at any temperature and a_w, which could be used to establish safe thermal processing protocol for pasteurizing peanuts in food industry. The influence of heating rate on heat resistance was also studied. Similar D-values were found when heating rates were above 1 °C/min, but a significant increase was observed at lower heating rates (0.1–1 °C/min). Therefore, thermal treatment technologies with higher heating rate were preferred to be used in food industry. Finally, the thermal kinetic method combined with the cumulated lethal time model was used to predict the minimum treatment time of a given temperature-time profile to achieve the required population reduction of the target microorganisms. This cumulated lethal time should allow making rapid online decision to terminate the heating process with minimum effects on product quality.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fm.2018.05.015.

References

Algabr, H.M., Zheng, T., Yu, X., 2013. Inactivation of *Aspergillus flavus* in drinking water after treatment with UV irradiation followed by chlorination. Sci. Total Environ. 463–464, 525–529.

AOAC, 2005. Official Methods of Analysis, sixteenth ed. Association of Official Analytical Chemists, Washington, DC.

Archer, J., Jervis, E.T., Bird, J., Gaze, J.E., 1998. Heat resistance of *Salmonella* weltevreden in low-moisture environments. J. Food Protect. 61, 969–973.

Augustin, J.C., Carlier, V., Rozier, J., 1998. Mathematical modelling of the heat resistance of *Listeria monocytogenes*. J. Appl. Microbiol. 84, 185–191.

Bankole, S., Adebanjo, A., 2003. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. Afr. J. Biotechnol. 2, 254–263.

Bermúdezaguirre, D., Corradini, M.G., 2012. Inactivation kinetics of *Salmonella spp.* under thermal and emerging treatments: a review. Food Res. Int. 45, 700–712.

Boukaew, S., Prasertsan, P., Sattayasamitsathit, S., 2017. Evaluation of antifungal activity of essential oils against aflatoxigenic *Aspergillus flavus* and their allelopathic activity from fumigation to protect maize seeds during storage. Ind. Crop. Prod. 97, 558–566.

Chhabra, A.T., Carter, W.H., Linton, R.H., Cousin, M.A., 1999. A predictive model to determine the effects of pH, milkfat, and temperature on thermal inactivation of *Listeria monocytogenes*. J. Food Protect. 62, 1143–1149.

Chung, H.J., Wang, S., Tang, J., 2007. Influence of heat transfer with tube methods on measured thermal inactivation parameters for Escherichia coli. J. Food Protect. 70, 851.

Conesa, R., Andreu, S., Fernández, P.S., Esnoz, A., Palop, A., 2009. Nonisothermal heat resistance determinations with the thermoresistometer Mastia. J. Appl. Microbiol. 107, 506.

Couvert, O., Gaillard, S., Savy, N., Mafart, P., Leguérinel, I., 2005. Survival curves of heated bacterial spores: effect of environmental factors on Weibull parameters. Int. J. Food Microbiol. 101, 73–81.

Dong, Q.L., 2011. Modeling the thermal resistance of *clostridium sporogenes* spores under different temperature, pH and NACl concentrations. J. Food Process. Eng. 34, 1965–1981.

Doyle, M.E., Mazzotta, A.S., 2000. Review of studies on the thermal resistance of Salmonellae. J. Food Protect. 63, 779–795.

Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W., Scott, V.N., 2001. Heat resistance of Listeria monocytogenes. J. Food Protect. 64, 410–429.

Enache, E., Ai, K., Hayman, M., Podolak, R., Black, D.G., Elliott, P.H., Whiting, R., 2013. The Heat Resistance of Salmonella Tennessee in Peanut Paste Formulations at Four Different Levels of Fat and Water Activity. Available at: https://iafp.confex.com/iafp/2013/webprogram/Paper4647.html.

FAOSTAT, 2016. Food and Agriculture Organization. Available at: http://www.fao.org/faostat/en/#home.htm.

Fernández, A., Collado, J., Cunha, L.M., Ocio, M.J., MartíNez, A., 2002. Empirical model building based on Weibull distribution to describe the joint effect of pH and temperature on the thermal resistance of *Bacillus cereus* in vegetable substrate. Int. J. Food Microbiol. 77, 147–153.

Fernández, A., López, M., Bernardo, A., Condón, S., Raso, J., 2007. Modelling thermal inactivation of *Listeria monocytogenes* in sucrose solutions of various water activities. Food Microbiol. 24, 372–379.

Gaillard, S., Leguerinel, I., Mafart, P., 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. J. Food Sci. 63, 887–889.

Guo, B., Fedorova, N.D., Chen, X., Wan, C.H., Wang, W., Nierman, W.C., Bhatnagar, D., Yu, J., 2011. Gene expression profiling and identification of resistance genes to *Aspergillus flavus* infection in peanut through EST and microarray strategies. Toxins 3, 737.

Hansen, J.D., Wang, S., Tang, J., 2004. A cumulated lethal time model to evaluate efficacy of heat treatments for codling moth Cydia pomonella (L.) (Lepidoptera: Tortricidae) in cherries. Postharvest Biol. Technol. 33, 309–317.

Hassani, M., Álvarez, I., Raso, J., Condón, S., Pagán, R., 2005. Comparing predicting models for heat inactivation of *Listeria monocytogenes* and *Pseudomonas aeruginosa* at different pH. Int. J. Food Microbiol. 100, 213–222.

Hiramatsu, R., Matsumoto, M., Sakae, K., Miyazaki, Y., 2005. Ability of Shiga Toxin-Producing *Escherichia coli* and *Salmonella spp.* to survive in a desiccation model system and in dry foods. Appl. Environ. Microbiol. 71, 6657–6663.

Jagannath, A., Tsuchido, T., Membre, J.M., 2005. Comparison of the thermal inactivation of *Bacillus subtilis* spores in foods using the modified Weibull and Bigelow equations. Food Microbiol. 22, 233–239.

Kou, X.X., Li, R., Hou, L.X., Huang, Z., Ling, B., Wang, S.J., 2016. Performance of a heating block system designed for studying the heat resistance of bacteria in foods. Sci. Rep. 6, 30758.

Lang, E., Chemlal, L., Molin, P., Guyot, S., Alvarez-Martin, P., Perrier-Cornet, J.M., Dantigny, P., Gervais, P., 2017. Modeling the heat inactivation of foodborne pathogens in milk powder: high relevance of the substrate water activity. Food Res. Int. 99, 577.

Leguérinel, I., Spegagne, I., Couvert, O., Coroller, L., Mafart, P., 2007. Quantifying the effects of heating temperature, and combined effects of heating medium pH and recovery medium pH on the heat resistance of *Salmonella typhimurium*. Int. J. Food Microbiol. 116, 88.

Li, R., Shi, Y., Ling, B., Cheng, T., Huang, Z., Wang, S., 2017. Thermo-tolerance and heat shock protein of *Escherichia coli* ATCC 25922 under thermal stress using test cell method. Emir. J. Food Agric. 29, 91–97.

Liaotrakoon, W., Clercq, N.D., Hoed, V.V., Walle, D.V.D., Lewille, B., Dewettinck, K., 2013. Erratum to: impact of thermal treatment on physicochemical, antioxidative and rheological properties of White-Flesh and Red-Flesh dragon fruit (Hylocereus spp.) purees. Food Bioprocess Technol. 6 1365–1365.

Ling, B., Tang, J., Kong, F., Mitcham, E.J., Wang, S., 2015. Kinetics of food quality changes during thermal processing: a review. Food Bioprocess Technol. 8, 343–358.

Liu, X., Guan, X., Xing, F., Lv, C., Dai, X., Liu, Y., 2017. Effect of water activity and temperature on the growth of *Aspergillus flavus*, the expression of aflatoxin biosynthetic genes and aflatoxin production in shelled peanuts. Food Contr. 82, 325–332.

Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. Int. J. Food Microbiol. 72, 107–113.

Mafart, P., Couvert, O., Leguérinel, I., 2001. Effect of pH on the heat resistance of spores comparison of two models. Int. J. Food Microbiol. 63, 51–56.

Manas, P., Pagan, R., Alvarez, I., Condon, U.S., 2003. Survival of *Salmonella senftenberg* 775 W to current liquid whole egg pasteurization treatments. Food Microbiol. 20, 593–600.

Michalski, C.B., Brackett, R.E., Hung, Y.C., Ezeike, G.O., 1999. Use of capillary tubes and plate heat exchanger to validate U.S. Department of Agriculture pasteurization protocols for elimination of *Listeria monocytogenes* in liquid egg products. J. Food Protect. 62, 112–117.

Peleg, M., 2006. Advanced Quantitative Microbiology for Foods and Biosystems: Models for Predicting Growth and Inactivation. CRC Press.

Sohbatzadeh, F., Mirzanejhad, S., Shokri, H., Nikpour, M., 2016. Inactivation of *Aspergillus flavus* spores in a sealed package by cold plasma streamers. J. Theor. Appl. Phys. 10, 99–106.

Takhar, P.S., Head, K.L., Hendrix, K.M., Smith, D.M., 2009. Predictive modeling of *Salmonella* species inactivation in ground pork and Turkey during cooking. Int. J. Food Microbiol. 5, 64–67.

Tang, J., Ikediala, J.N., Wang, S., Hansen, J.D., Cavalieri, R.P., 2000. High-temperature-short-time thermal quarantine methods. Postharvest Biol. Technol. 21, 129–145.

Van Boekel, M.A.J.S., 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. Int. J. Food Microbiol. 74, 139–159.

Villa Rojas, R., Tang, J., Wang, S., Gao, M., Kang, D.H., Mah, J.H., Gray, P., Sosamorales, M.E., Lópezmalo, A., 2013. Thermal inactivation of *Salmonella enteritidis* PT 30 in almond kernels as influenced by water activity. J. Food Protect. 76, 26–32.
Wiegand, K.M., Ingham, S.C., Ingham, B.H., 2009. Survival of *Escherichia coli* O157:H7 in ground beef after sublethal heat shock and subsequent isothermal cooking. J. Food Protect. 72, 1727–1731.
Yang, S., Bai, X.L., Jin, R.Y., Shan, F., Guo, K.X., Niu, H.G., Wu, W.D., 2013. Study on the sterilization of chlorine dioxide gas on *Aspergillus flavus*. Sci. Technol. Food Ind 34 213–212.
Zhang, L., Lyng, J.G., Brunton, N.P., 2004. Effect of radio frequency cooking on the texture, colour and sensory properties of a large diameter comminuted meat product. Meat Sci. 68, 257–268.
Zhang, L.H., Kou, X.X., Zhang, S., Cheng, T., Wang, S.J., 2018. Effect of water activity and heating rate on *Staphylococcus aureus* heat resistance in walnut shells. Int. J. Food Microbiol. 266C, 282–288.