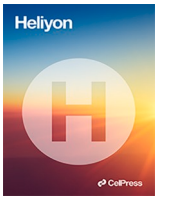
Heliyon 10 (2024) e36977

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Kinetic study of the thermal inactivation of *Weizmannia coagulans* during food thermal processing

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ARTICLE INFO

Keywords:
Prediction
Bigelow model
Performance
Accuracy

ABSTRACT

Weizmannia coagulans has attracted attention due to its remarkable health benefits for human, but the dynamic changes of its viable bacteria during thermal processing have been less reported. In this study, a predictive model for the survival of *Weissmanella coagulans* during thermal processing of food was developed and validated during the processing of coffee, tea, instant noodles, calcium milk biscuits, muffin cake and steamed buns. The kinetics of heat inactivation activities of *Weizmannia coagulans* VHProbi C08 and *Weizmannia coagulans* GBI-30, 6086 at 85, 95, 105, 110 and 115 °C were investigated, and their coefficients of determination were greater than 0.91 and 0.87, and the root-mean-square errors were less than 0.64 and 0.43, respectively. The z-values of VHProbi C08 and GBI-30, 6086 were obtained by Bigelow model fitting as 36.1 °C and 36.9 °C, respectively. The developed prediction model was applied to the thermal processing of six food products and the measured values were all within ±0.5 Log_{10} (CFU/mL) of the predicted values, indicating high prediction accuracy. The model predicts the survival of *Weissmanella coagulans* simply by obtaining the initial number of viable bacteria and the change in temperature. These suggested that the model can be used as an effective tool to evaluate the stability of *Weizmannia coagulans* in food thermal processing.

1. Introduction

Probiotics are living microorganisms, which can regulate intestinal flora and improve human health [1]. In the past few years, probiotics have received more and more attention. The global market size of probiotics may be as high as $111.2 billion in 2030, according to the forecast of Grand View Research, Inc. At present, many probiotic strains have been isolated and studied; such as *Bifidobacterium longum* BB536, *Bifidobacterium lactis* Bi-07, *Lacticaseibacillus rhamnosus* GG, and *Lacticaseibacillus plantarum* WCFS1 and other strains have been proved to be beneficial to human health and have been applied to products [2–5]. Studies have shown that foods or medicines contain 10^6–10^7 CFU/g of probiotics that can have beneficial effects on human health [6]. In addition, probiotics can be used as a means of degrading harmful ingredients in foods, such as *Clostridium tyrobutyricum* Z816 degrading β-LG to reduce milk allergy [7]. However, the fact is that the survival of most probiotics in non-refrigerated environments decreases rapidly, and it is difficult to survive for more than 10 min at temperature above 55 °C. This severely restricts the application of probiotics and increases

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https://doi.org/10.1016/j.heliyon.2024.e36977
Received 18 May 2023; Received in revised form 25 August 2024; Accepted 26 August 2024
Available online 28 August 2024

the storage and transportation costs of probiotics.

Weizmannia coagulans (synonym: *Bacillus coagulans*)is a lactic acid-producing and spore-forming bacterium with probiotic characteristics and high-temperature resistance [8]. It was identified by the Food and Drug Administration of the general recognition as safe (GRAS) status in 2012 [9], and included in the list of strains that can be used for food by the National Health Commission of the People's Republic of China in 2016 [10]. The research on the health function of *W. coagulans* mainly focused on the treatment of gastrointestinal (GIT) diseases; such as *W. coagulans* Unique IS2 alleviated irritable bowel syndrome in children aged 4–12 [11]; *W. coagulans* LBSC ameliorated acute diarrhoea [12]; *W. coagulans* MTCC 5856 shortened the duration of diarrhoea in children [13]. Moreover, the health functions of *W. coagulans* in anticancer, antibacterial and alleviating severe depression have also been reported [14]. Currently, *W. coagulans* have been widely used as ingredients for various functional foods; e.g., bakery products, beverages, jellies and confections, pasta, etc.; due to their ability to form spores that remain active under unfavorable environmental conditions [15].

W. coagulans is used in heat-processed foods because of its good heat resistance. Previous studies showed that the survival rates of *W. coagulans* MTCC 5856 in coffee and tea after brewing were 94.94 % and 99.76 %, respectively [16]. Another study reported that the survival rate of *W. coagulans* MTCC 5856 was 88.94 % and 94.56 % after cooking in pancakes and wheat noodles; furthermore, it maintains 73 % survival after cooking at 260 °C for 5 min [17]. Despite *W. coagulans* is heat resistance, its survival rate varies at different heating temperatures and times. In addition, it is time-consuming and laborious work to detect the viable count of each product by traditional methods such as plate, especially in exploring the best heat treatment process. Therefore, it is important to establish a dynamic prediction model of the number of viable bacteria changing with heating temperature and time, which is suitable for various foods. This can not only predict the viable count of food after thermal processing, but also select the appropriate thermal processing conditions according to the ideal viable count.

In previous studies, the Arrhenius, Bigelow, and Weibull model has often been used for the kinetic study of thermal inactivation of microorganisms. The Arrhenius model describes a linear relationship between the logarithm of the rate of inactivation and the reciprocal of temperature [18]. Bigelow model describes a linear relationship between temperature increase and the logarithm of heat treatment time, which Ball modelled to quantify thermal sensitivity through the parameter *z*, i.e. an increase in temperature reduces heating time by a factor of 10 [19]. Moreover, the parameter D (decimal reduction time) is used to quantify heat resistance [20]. The Arrhenius approach is widely used by chemists in chemical engineering, whereas the Bigelow approach is more commonly used by microbiologists in food process engineering. Although the input variable for the Arrhenius model is temperature and the input variable for the Bigelow model is the temperature inverse, the estimates of *D* values are very close [21]. In addition, there was no significant difference in the quality of fit between the two models in the temperature range below 100 °C.

In the above context, the aim of this study was to develop a model for predicting the survival of *W. coagulans* VHProbi C08 and *W. coagulans* GBI-30, 6086 in foods under different temperature during heat treatment and to validate the model in several common heat-processed products. It provides a rapid and simple technical method for evaluating the stability of *W. coagulans* in functional food processing.

2. Materials and methods

2.1. Strains and reagents

W. coagulans VHProbi C08 was isolated from sour cabbage, and it was preserved in China Center for Type Culture Collection (CCTCC, Wuhan, China), with preservation number M2019738. *W. coagulans* GBI-30, 6086 was provided by Ganeden, Inc. (Mayfield Heights, OH, USA). Yeast powder, peptone, beef extract and agar powder supplied by HuanKai Biology (Guangzhou, China). $MnSO_4$, NaCl, K_2HPO_4, $MgSO_4$, $7H_2O{\cdot}FeSO_4$, $7H_2O{\cdot}ZnSO_4$, $5H_2O{\cdot}CuSO_4$, $7H_2O{\cdot}CoSO_4$, glycerol, glucose purchased from Macklin (Shanghai, China).

2.2. Establishment of dynamic model

2.2.1. Preparation of W. coagulans spores

W. coagulans with high spore number was prepared using previously described media formulations [22]. The medium consisted of 3 g/L yeast powder, 5 g/L peptone, 2 g/L beef extract, 0.005 g/L $MnSO_4$, 2 g/L NaCl, 3 g/L K_2HPO_4, 0.02 g/L $MgSO_4$. Its pH was adjusted to 6.3 ± 0.2 and autoclaved at 115 °C for 30 min. *W. coagulans* VHProbi C08 and *W. coagulans* GBI-30, 6086 were inoculated 5 % (v/v) into a 500 mL flask containing 100 mL medium. The flasks were shaken at 40 °C for 48 h at 210 r/min. The cultured bacterial liquid was centrifuged at 4 °C and 5000×*g* using a centrifuge (5804/R, Eppendorf, Germany), the precipitate was mixed with 40 % glycerol suspension and placed in a water bath at 80 °C for 12 min to remove the vegetative cells. After cooling immediately, the spore solution was stored at −40 °C.

2.2.2. Thermal inactivation at different temperatures

The temperature tolerance of *W. coagulans* at 85, 95, 105, 110, and 115 °C was assayed. In short, 1 mL spore solution of *W. coagulans* VHProbi C08 or *W. coagulans* GBI-30, 6086 was added to a 10 mL screw glass tube, and then 1 mL ultrapure water was added and mixed on a vortex oscillator (MIX-2500, yooning, Cnina) for inactivation experiments at 85 and 95 °C. In addition, 1 mL spore suspension and 1 mL rapeseed oil were mixed for heat inactivation at 105, 110 and 115 °C. The tubes were placed in silicone oil bath and heated to between at 85 and 115 °C for different time intervals. The time for the sample to reach the target temperature was about

2 min. Subsequently, the tube was cooled in a cold water bath, and the number of viable spore was counted. The spore suspension in the glass capillary tube was diluted 10 times in sterile saline, and the viable spores were counted on glucose yeast extract agar medium (GYEAM) by the pour plate method [23]. The GYEAM consisted of 5.0 g/L yeast powder, 5.0 g/L peptone, 5.0 g/L glucose, 0.5 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.3 g/L $MgSO_4$, 1.0 mL trace element solution (10 g/L NaCl, 18 g $7H_2O{\cdot}FeSO_4$, 16 g/L $MnSO_4$, 1.6 g/L $7H_2O{\cdot}ZnSO_4$, 1.6 g/L $5H_2O{\cdot}CuSO_4$, 1.6 g/L $7H_2O{\cdot}CoSO_4$), 15 g/L agar powder. The pH of medium was adjusted to 6.3 ± 0.2, and heated at 121 °C for 15 min. The plate was anaerobically cultured at 40 ± 2 °C for 48 h. The average number of viable spores was expressed as Log_{10} CFU.

2.3. Model validation in heat-treated food

Predictive modelling of thermal inactivation of *W. coagulans* based on Bigelow model was validated under six different dynamic temperature profiles. The temperature black box (L91-1H, HangZhou Loggertech, China) was used to monitor the temperature change in real time, and temperature data were obtained with a time interval of 2 s. For each selected sample, one biologically independent experiment with three replicates (n = 3) was performed.

2.3.1. Model validation in the brewing process of hot drinks and convenience foods

Changes in the viable counts of *W. coagulans* during the brewing process of tea, instant coffee and instant noodles made with hot water were verified. Instant coffee, instant tea and instant noodles were added with sterile water at 90 °C, and the addition amounts were 150, 250 and 500 mL, respectively. At the same time, two kinds of *W. coagulans* were added separately. The temperature probe of the temperature black box was completely immersed below the liquid level to monitor the temperature change in real time. The samples were taken out at intervals to detect the viable cell count using the method described in Section 2.2.2.

2.3.2. Model validation during baking of baked goods

In order to verify the accuracy of the model predictions of the present invention, the number of surviving *W. coagulans* VHProbi C08 and *W. coagulans* GBI-30, 6086 were determined during the processing of bakery products (milk calcium biscuits and muffin cakes). The formula of milk calcium biscuits was shown in Supplementary Table 1. The method of preparation was to melt the butter by heating, then add eggs, *W. coagulans* solution, milk, high calcium milk powder, sugar, baking soda and low gluten powder stirred evenly; the obtained dough was extruded with a biscuit mold (15 g), placed in an oven (K30FK601, Yuanmai, China) at 170 °C for 6 min, and cooled to below 40 °C to remove the cake. The recipes for muffin cake preparation were summarized in Supplementary Table 2. The preparation procedure was that muffin cake powder, eggs, and water mixed with *W. coagulans* were added to a bowl, stirred until smooth without particles; then the vegetable oil was added and stirred uniformly. The stirred sample was put into a muffin paper cup (40 g) and placed in the oven (upper fire temperature 160 °C, bottom fire temperature 180 °C) baking 15 min, cooled to below 40 °C and removed. The temperature probe of the temperature black box was inserted into the center of biscuits and cakes to monitor the temperature change in real time. The number of viable bacteria in the sample was detected at the beginning and end of baking.

2.3.3. Model validation in the process of cooking pasta

The changes of viable counts of *W. coagulans* VHProbi C08 and *W. coagulans* GBI-30, 6086 during Chinese steamed bun bread cooking were detected. The Chinese steamed buns were prepared according to the method previously described [24], with some modifications to the recipe (Supplementary Table 3). Briefly, highly active dry yeast (Angel Yeast, China) and wheat flour (100 g) were dissolved in water, activated at 30 °C for 15 min. The mixture was mixed with *W. coagulans*, wheat flour, baking soda, and sucrose in a flour mixer (M5, Hauswirt, China) for 10 min. Moreover, keep the dough at 30 °C for 1.5 h, then take it out and mix well to drain the gas and cut into small even doughs (100 g). Finally, the dough was cooked on the steamer (ZGS302002, Midea, China) for 20 min, turned off the fire and stand for 5 min, took out the steamed bread and cool naturally. Samples were taken every 15 min to detect the residual number of viable bacteria during steamed bread cooking.

2.4. Data analysis

The development of the thermal inactivation prediction model of *W. coagulans* referred to the Bigelow Model [25], with slight modifications. In short, the change in the number of the viable cells at different temperatures measured in Section 2.2.2 over time was fitted with equation (1).

$$\mathrm{Log}(N_t) = \mathrm{Log}(N_0) - \frac{t}{D_T} \tag{1}$$

where N_0 is the original the viable cells count of *W. coagulans*; N_t is the number of viable cell after heat treatment time t; D_T is the inactivation rate of *W. coagulans* at a temperature (T), i.e., the time required for *W. coagulans* to decrease by an order of magnitude at T.

The minimum residual sum of squares (RSS) of the measured value and the fitted value of the number of viable bacteria was calculated. The programming solver function in the Excel 2016 loading term was used to minimize the RSS. The inactivation curve of *W. coagulans* at different heat inactivation temperatures was fitted, and the inactivation rate D_T value at different heat inactivation temperatures was obtained. The D_T value can also be related to T by equation (2). The accuracy of the developed model was evaluated

by graphical comparison between observed and predicted values, the coefficient of determination (R^2) and the root mean square error (RMSE) [26].

$$D_T = D_{ref} \times 10^{\frac{T-T_{ref}}{z}} \tag{2}$$

where T_{ref} is a reference temperature, D_{ref} is the inactivation rate of *W. coagulans* at T_{ref}, z is the temperature increase required to reduce the D_T value by 10 times, which is the reciprocal of the slope of the logarithmic D_T value versus the temperature.

In order to understand the number of viable bacterial residues of *W. coagulans* at dynamic temperatures, equation (3) (the differential form of equation (1)) was used to describe the variation of ΔN_t in a certain Δt period.

$$\frac{dN_t}{dt} = \frac{\Delta N_t}{\Delta t} = \frac{LogN_i - LogN_{i-1}}{t_i - t_{i-1}} = -\frac{1}{D_{T-int}} \tag{3}$$

where N_{i-1} is the number of viable bacteria at time t_{i-1}, and $D_{T\text{-}int}$ is the inactivation rate within time $\Delta t = 1/30$ min.

At any temperature corresponding to any time point, the $D_{T\text{-}int}$ at that time point is represented by the D_T average in time Δt. Equation (3) was used to calculate the ΔN_t value in time Δt, N_i is the sum of $N_{t\text{-}1}$ and ΔN_t, and and then the corresponding N_i value under time i was calculated. For the evaluation of model prediction results under fluctuating temperature, the evaluation criteria for

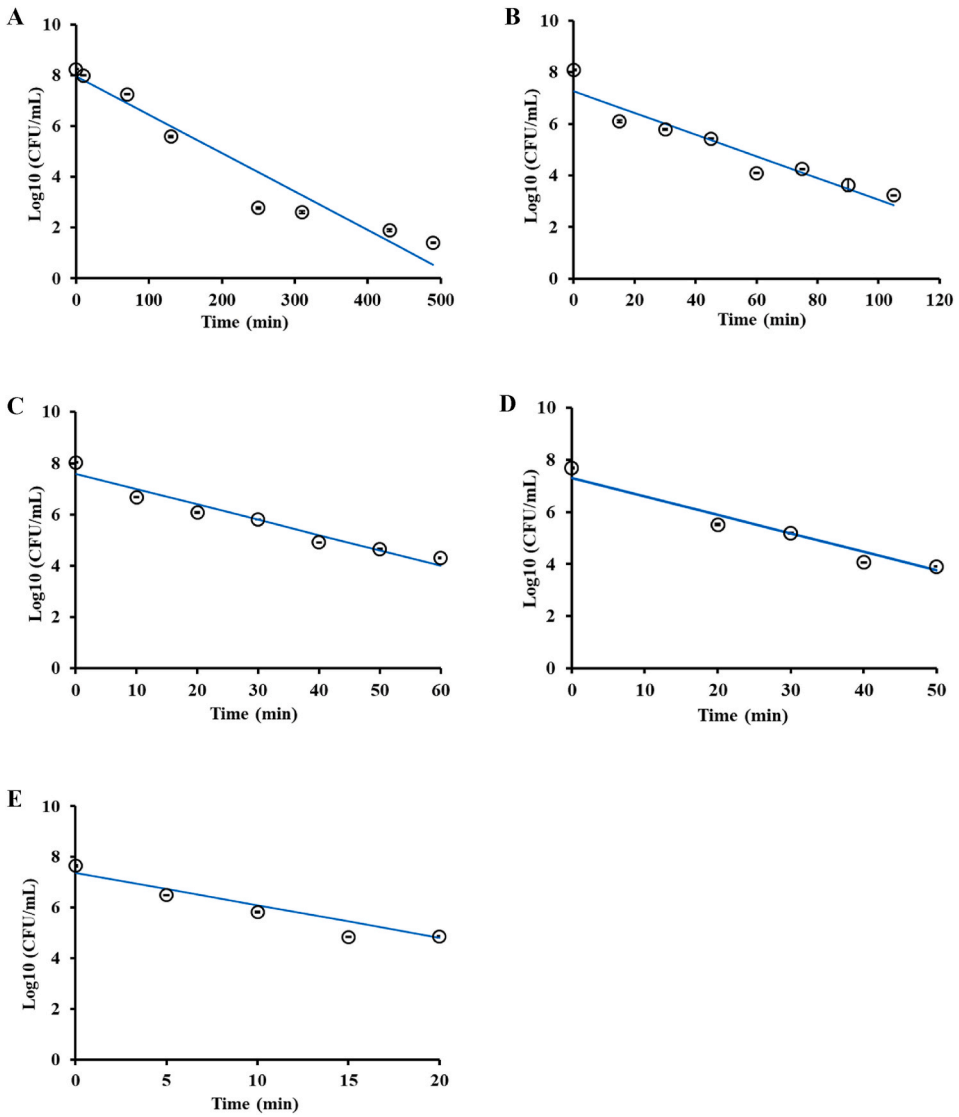

Fig. 1. Inactivation kinetics of *W. coagulans* VHProbi C08 at different temperatures. A, 85 °C; B, 95 °C; C, 105 °C; D, 110 °C; E, 105 °C. The points (○) are observed values and the blue solid lines (—) indicate the fitting of the Bigelow model to the experimental data. Mean values ± standard deviation of one independent experiment with three replicates are shown.

the acceptable simulation zone (ASZ) commonly used in the international mainstream academic circles was adopted. The predictive performance of the developed model in predicting *W. coagulans* VHProbi C08 and GBI-30, 6086 in different food products under dynamic temperature conditions was assessed by ASZ method [27]. The range of ±0.5 log-units of the predicted values was defined as the ASZ region and the model was considered reliable when at least 70 % of the actual observations were located in this region [28].

3. Results and discussion

3.1. Development of the kinetic model

The effect of temperature (85, 95, 105, 110 and 115 °C) on *W. coagulans* VHProbi C08 and GBI-30, 6086 activity results are shown in Fig. 1(A–E) and Fig. 2(A–E), respectively. With the increase of temperature and heating time, the number of live spores gradually decreased. After heating at 115 °C for 20 min, the number of viable spores of VHProbi C08 decreased from 7.64 to 4.85 Log_{10} (CFU/mL) ($p < 0.05$) (Fig. 1E), and GBI-30, 6086 decreased from 7.13 to 4.23 Log_{10} (CFU/mL) ($p < 0.05$) (Fig. 2E), respectively. Viable spore counts of both VHProbi C08 and GBI-30, 6086 were reduced at temperatures ranging from 85 to 115 °C, and the higher the heat inactivation temperature, the faster the number of living spores decreased. Notably, after heating at 115 °C for 20 min, *W. coagulans*

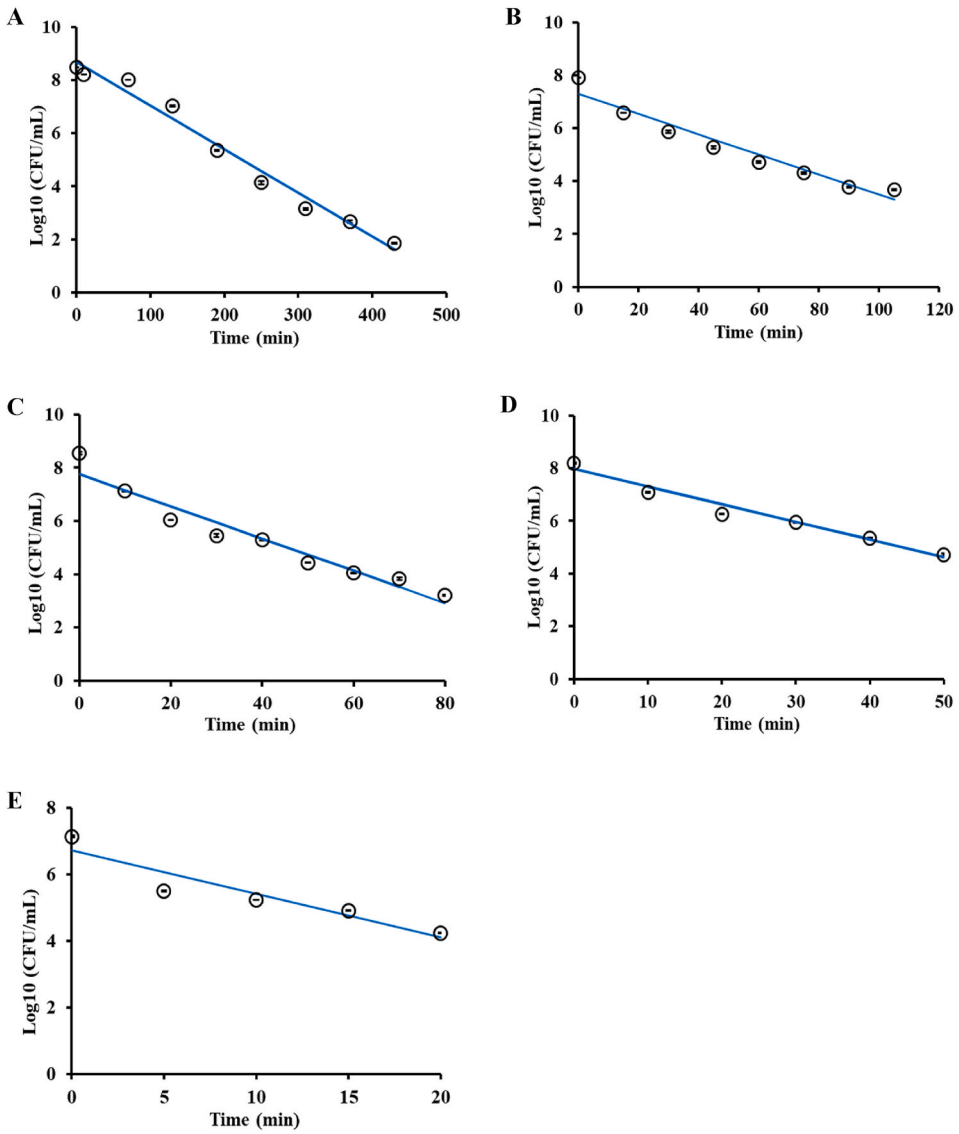

Fig. 2. Inactivation kinetics of *W. coagulans* GBI-30, 6086 at different temperatures. A, 85 °C; B, 95 °C; C, 105 °C; D, 110 °C; E, 105 °C. The points (○) are observed values and the blue solid lines (▬) indicate the fitting of the Bigelow model to the experimental data. Mean values ± standard deviation of one independent experiment with three replicates are shown.

VHProbi C08 and GBI-30, 6086 still survived many spores, and their heat resistance was far superior to the common probiotics such as *L. casei*, *L. brevis* and *L. plantarum* [29]. As such, these two *W. coagulans* have broad prospects for use in thermally processed foods.

The D_T values of VHProbi C08 and GBI-30,6086 at different temperatures were calculated by Bigelow model fitting, the results were shown in Fig. 3A and B. It decreases with the increase of temperature. When the heat inactivation temperature was 110 °C, the D_T values of VHProbi C08 and GBI-30, 6086 were 14.06 and 14.97 min, respectively. However, previously reported D_T values of *B. coagulans* MTCC 5856 and *B. coagulans* ATCC 31284 were less than 5 min [17].

The T_{ref} of both VHProbi C08 and GBI-30, 6086 is 85 °C, the D_{ref} is 66.04 min and 60.89 min respectively, and the *z*-values calculated from equation (2) are 36.1 °C and 36.9 °C, respectively (Table 1), which were higher than the previously reported for *W. coagulans* [30]. One reason for this difference in thermal stability is due to strain specificity, which has been reflected in previous studies [31]. In general, spores have heat-resistant properties that are responsible for the survival of *W. coagulans* in thermal conditions [32]. We found 48 genes related to spore formation in VHProbi C08 (GenBank accession: CP080329.1), and 43 genes in GBI-30, 6086 (GenBank accession: GCA_000756285.1), which were higher than *W. coagulans* ATCC 7050 (16 genes, GenBank accession number: CP009709.1) with *z*-values of 16.5 °C [33]. Furthermore, In the whole genome of VHProbi C08 and GBI-30,6086, there are more abundant spore proteins and small acid-soluble spore proteins related genes. Water content of the spore nucleus, mineral ions, spore proteins and small acid-soluble spore proteins saturate spore DNA thereby protecting it from heat damage [34]. Therefore, the different characteristics of spores formed between different strains of *W. coagulans* lead to the difference of heat resistance. On the other hand, the difference between the heating medium of the strain in this study and the previous report is also the reason for the different *z*-values. For example, *W. coagulans* NTCC4522 had a *z*-value of 12.8 °C in tomato compared to 11.29 °C in asparagus [21]; and the *z*-value of *W. coagulans* 185A was 10.2 °C at atmospheric pressure [31], while it was 33 °C at a pressure of 600 MPa [35].

The R^2 values and RMSE values of the model are displayed in Table 2. For the Bigelow and secondary model, the R^2 values of VHProbi C08 and GBI-30,6086 were in the range of 0.91–0.96 and 0.88–0.98, respectively, indicating that the model performed well. In addition, the RMSE values were 0.09–0.64 and 0.08–0.42, respectively, which were lower than those previously reported for similar models [36]. It was generally believed that the smaller the RMSE value, the better the adequacy of the model to describe the data [37]. Therefore, the model developed in this study has an acceptable analytical ability to analyse the dynamics of viable spore numbers during thermal inactivation.

The Bigelow model was originally developed to quantify microbial inactivation in the canning industry, which is generally considered to follow first-order kinetics. A feature of the model is the focus on the effect of temperature on microorganisms. This feature is particularly useful for the food industry because food is a complex model and lacks very detailed knowledge and necessary tools [38]. Despite many reports suggest that Weibull models are superior to Bigelow model in some aspects, Bigelow model is simpler and can effectively describe the experimental data of microbial heat extinguishing in food [21,39]. In addition, our results also proved that Bigelow model has good performance.

3.2. Model validation

In the model validation experiment, the reference temperature was 85 °C, the D_T values of each temperature point can be calculated according to equation (2). After the initial spore number was known, equation (3) was solved by Euler's method to obtain the predicted value of viable spores at each temperature point.

3.2.1. Model validation in the brewing process of hot drinks and convenience foods

The established prediction model of viable cell survival was verified in the brewing process of coffee, tea and instant noodles. The temperature dependence of the measured values and model predictions were shown in Fig. 4A, B, C, D, E and F. After adding sterile water at 90 °C, the temperature of coffee, tea and noodles increased immediately and then decreased gradually. During this process, the measured and predicted values of the number of viable spores of strains VHProbi C08 and GBI-30, 6086 decreased slightly in the early stage. With the decrease of temperature, there was no significant change in the measured and predicted values of viable spore

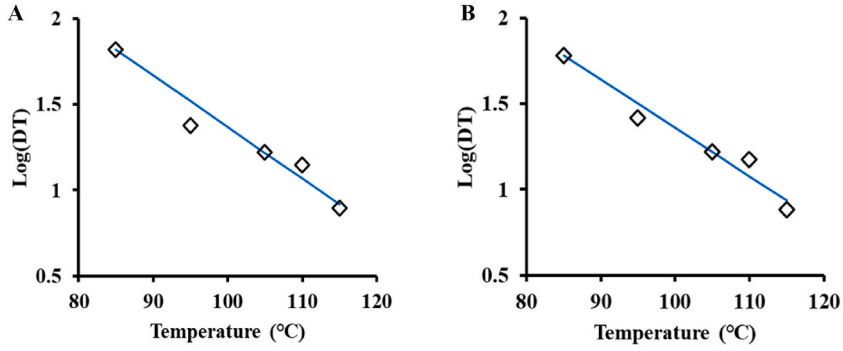

Fig. 3. D-values of *W. coagulans* VHProbi C08 (A) and *W. coagulans* GBI-30, 6086 (B) exposed to different temperatures. The quadrilaterals (◇) are actual detection value and the blue solid lines (—) indicate the fitting of the secondary model to actual detection value.

Table 1
The D_T and z values of *W. coagulans*.

W. coagulans	Temperature (°C)/D_T (min)					z (°C)	Heating media
VHProbi C08	85/66.04	95/33.02	105/16.51	110/11.68	115/8.26	36.1	Water/oil
GBI-30, 6086	85/60.89	95/26.21	105/16.53	110/14.97	115/7.68	36.9	Water/oil
ATCC 7050	80/33.3	90/14.79	100/4.75	105/1.43		16.5	Tomato sauce
ATCC 185A	100/1.41	105/0.53	110/0.14			10.2	Tomato juice
ATCC 185A	75/2.1	85/1.6	95/0.91	100/0.73	105/0.19	33	Tomato juice 600 Mpa
ATCC 8038	95/7.05	100/2.56	105/1.18	110/0.2		10	Tomato juice
NTCC4522	105/0.79	111/0.26	120/0.045	123/0.031	126/0.018	12.8	Tomato
NTCC4522	102/1.6	111/0.28	117/0.074	123/0.026	129/0.06362	11.29	Asparagus

Table 2
The goodness of fit statistics of various models used in this study.

model	Temperature (°C)	VHProbi C08		GBI-30, 6086	
		R^2	RMSE	R^2	RMSE
Bigelow model	85	0.94	0.64	0.98	0.38
	95	0.91	0.48	0.95	0.35
	105	0.94	0.32	0.94	0.42
	110	0.96	0.32	0.97	0.23
	115	0.92	0.32	0.88	0.43
Secondary model	–	0.95	0.09	0.96	0.08

number.

During the brewing of coffee, tea and instant noodles, even if the live spores of VHProbi C08 and GBI-30, 6086 were exposed to 90 °C, their activity remained unchanged, which proved that both strains showed high resistance to brewing conditions of coffee, tea and instant noodles. Common probiotics, such as *L. rhamnosus*, *L. planturum* and *L. casei*, do not have high-temperature tolerance [40]. These proved that VHProbi C08 and GBI-30, 6086 were more suitable for brewing drinks. Similarly, *W. coagulans* MTCC 5856 has high survival in tea and coffee brewing process [16]. These facts also highlight the great application potential of *W. coagulans* in thermally processed foods.

3.2.2. Model validation during baking of baked goods

The results showed that the observed and predicted values of viable spore numbers of strains VHProbi C08 and GBI-30, 6086 at the beginning and end of baking of calcium milk biscuits are basically consistent (Fig. 5A and B). Moreover, there was no significant change in the number of viable spores of strains VHProbi C08 and GBI-30, 6086 in 0–3 min (temperatures below 107 °C) based on the prediction curve. When the temperature was higher than 107 °C, the number of viable spores of VHProbi C08 decreased from 7.91 to 6.69 Log_{10} (CFU/g) ($p < 0.05$). When the temperature was higher than 107 °C, the number of viable spores of GBI-30, 6086 decreased from 8.44 to 7.08 Log_{10} (CFU/g) ($p < 0.05$). Similar results were observed in the baking process of the muffin cake, and the observed and predicted values of the number of live spores were basically the identical. These indicated that the model we established is suitable for predicting the number of viable spores of *W. coagulans* during the processing of baked foods.

Notably, during the thermal processing of calcium milk biscuits and muffin cakes, the temperature tolerance of *W. coagulans* was different. Among them, the temperature tolerance in the calcium milk biscuit was up to 107 °C, while in the muffin cake was 94 °C. This showed that the ingredients of calcium milk biscuits can significantly improve the temperature tolerance of the strain, which can be used as a potential material for probiotic embedding carriers, probably due to the greater amount of milk contained in the composition of calcium milk biscuits. Previous studies have shown that proteins in milk bind to ions and small molecules, and their gel properties, excellent surface and self-assembly properties help maintain microbial stability [41]. In addition, even though the number of viable spores in calcium milk biscuits and muffin cakes decreased significantly after thermal processing, the number of live spores was still greater than 6 Log_{10} (CFU/g), which was sufficient for exerting the probiotic function of probiotics.

It is of interest to describe the response of *W. coagulans* to external conditions through mathematical modelling and thus predict changes in viable spore counts during food processing. Previously, a predictive model for the growth of *W. coagulans* in food based on the effects of temperature, pH and water activity has been developed by Misiou et al. [26]. Although the thermal inactivation kinetics of *W. coagulans* (ATCC 8038, ATCC7050, and 185A) in tomato product has been studied [31,33], to the best of our knowledge, this study is the first to report a predictive model for the change in the number of residual viable spores of *W. coagulans* during food thermal processing.

3.2.3. Model validation in the process of cooking pasta

The model's prediction of the number of viable spores of *W. coagulans* during steamed bread cooking is shown in Fig. 6A and B. The number of viable spores of VHProbi C08 and GBI-30, 6086 at the beginning and the end of steamed bread cooking were in general agreement with the predicted values. Moreover, there was no significant change in the number of viable spores of VHProbi C08 and GBI-30, 6086 at temperatures below 88 °C. When the temperature was higher than 88 °C, the predicted number of viable spores of

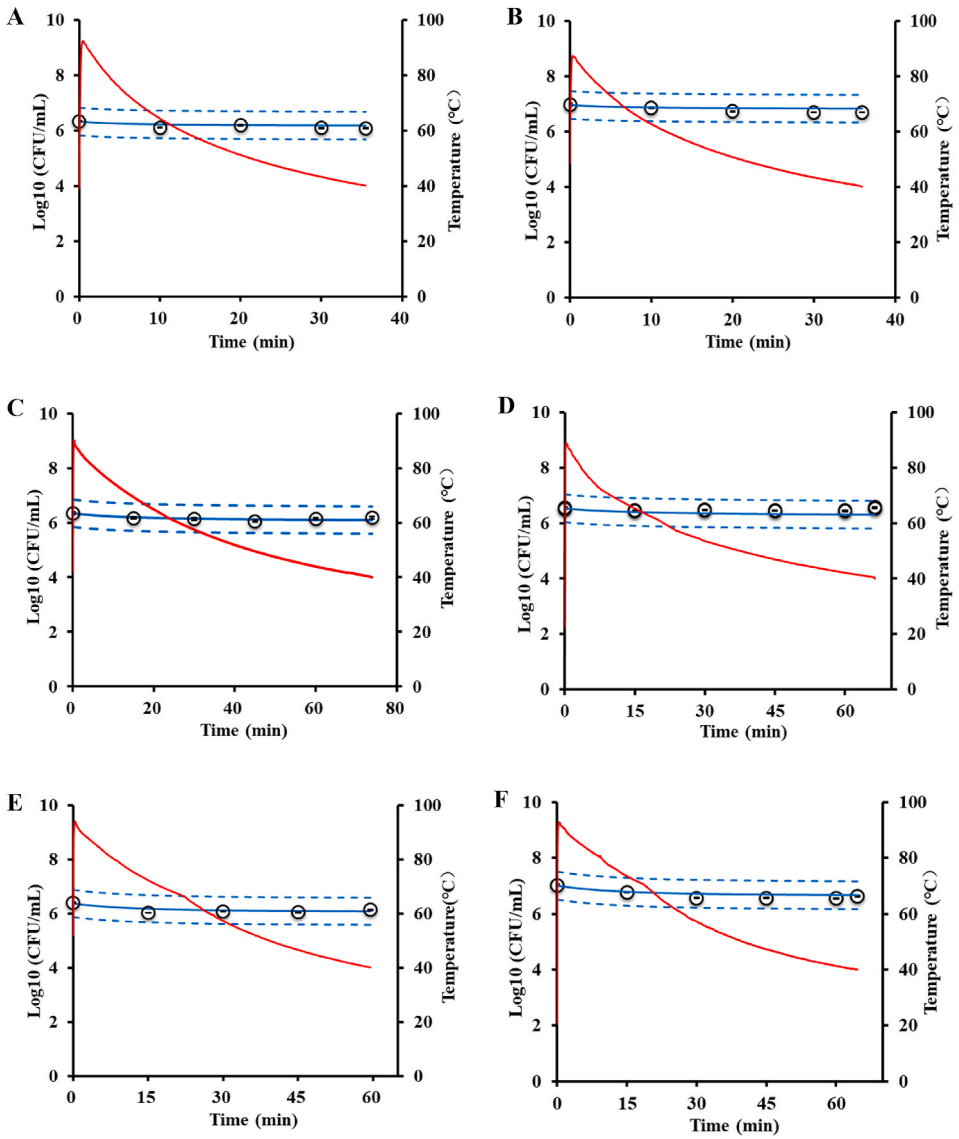

Fig. 4. Comparison between observed (○) and predicted data (▬) of two strains during the brewing process of instant coffee (A, VHProbi C08; B, GBI-30, 6086), tea (C, VHProbi C08; D, GBI-30, 6086), and instant noodles (E, VHProbi C08; F, GBI-30, 6086). Blue dashed lines (▬ ▬ ▬) show the acceptable simulation zone. Red solid lines (▬) indicate temperature changes. Mean values ± standard deviation of one independent experiment with three replicates are shown.

VHProbi C08 decreased from 8.17 to 7.42 Log_{10} (CFU/g) ($p < 0.05$), the GBI-30, 6086 viable spores decreased from 8.13 to 7.37 Log_{10} (CFU/g) ($p < 0.05$).

The prediction model we established mainly explored the effect of temperature on *W. coagulans* inactivation. Previous studies have reported that the pH and moisture content of the sample also affect the heat inactivation of microorganisms [42]. However, this study found that the prediction model established by only considering the temperature factor also had good accuracy. This may be due to the different species of microorganisms. Measurements from the six validation experiments ranged between ±0.5 log units of the predicted values, and the measured values of the viable spores of *W. coagulans* were in general agreement with the predicted values. In addition, the observation minus the predicted value is the prediction error (PE) of each observation, which is an important index for the analysis of the ASZ. The PE value of 0 is considered to be a perfect prediction, and the prediction error value between −1.0 and 0.5 is considered to be acceptable. The analysis method has also been applied to the evaluation of many model predictions [42,43]. The PE value of this study was also between −1.0 and 0.5. In summary, the prediction results of this study are acceptable.

Prediction model is of great significance in estimating the number of microorganisms in the food chain and has great application in the risk assessment of public health safety caused by controlling microbial pathogens. Such as, heat inactivation of *Salmonella* on leafy greens and *E. faecium* in wheat flour were modelled based on the Bigelow model [44,45]. All of these studies concluded that the

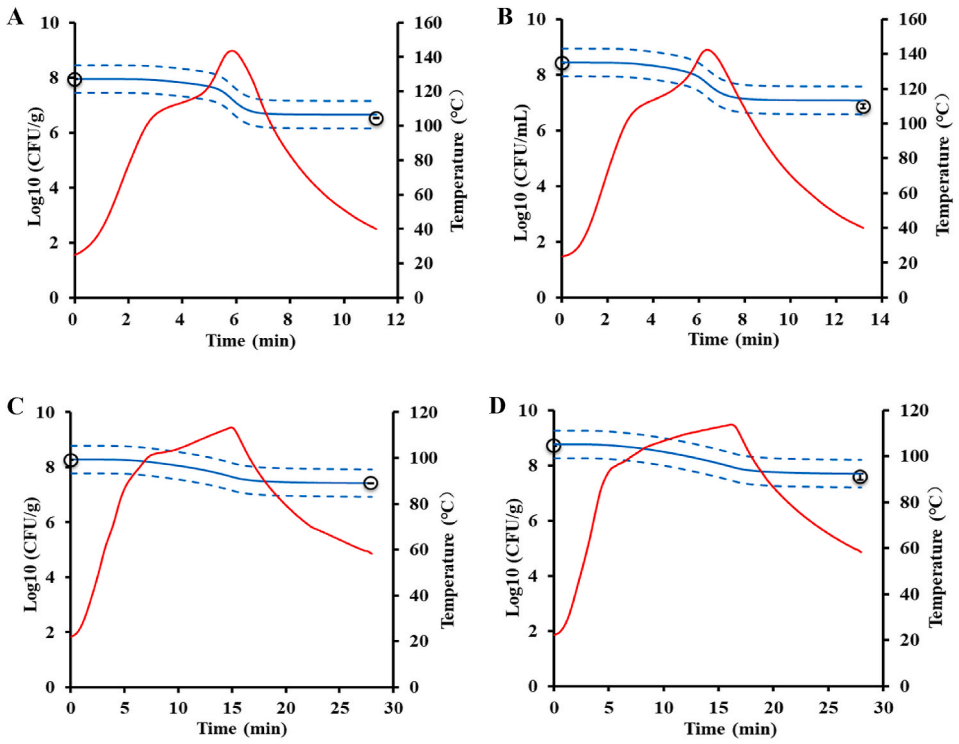

Fig. 5. Comparison between observed (○) and predicted data (▬) of two strains in the baking process of calcium milk biscuits (A, VHProbi C08; B, GBI-30, 6086) and muffin cakes(C, VHProbi C08; D, GBI-30, 6086). Blue dashed lines (▬ ▬ ▬) show the acceptable simulation zone. Red solid lines (▬) indicate temperature changes. Mean values ± standard deviation of one independent experiment with three replicates are shown.

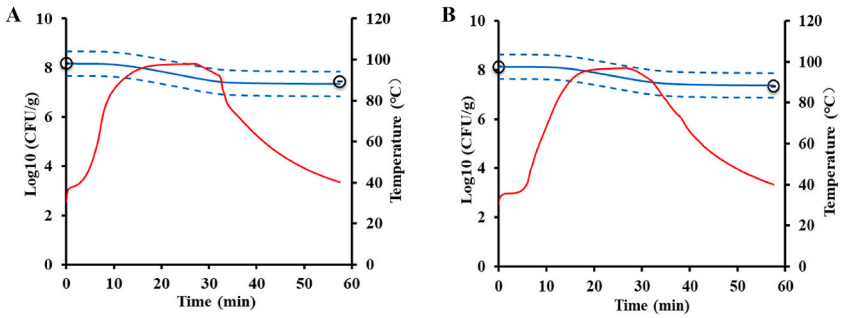

Fig. 6. Comparison between observed (○) and predicted data (▬) of two strains in the cooking process of steamed bread (A, VHProbi C08; B, GBI-30, 6086). Blue dashed lines (▬ ▬ ▬) show the acceptable simulation zone. Red solid lines (▬) indicate temperature changes. Mean values ± standard deviation of one independent experiment with three replicates are shown.

Bigelow model fits the inactivation data better [46]. Analogously, the predictive model for heat inactivation of *W. coagulans* based on Bigelow model performed well in this study. The model we developed was validated with good accuracy in six thermally processed products. The model only needs to know the initial live microbial biomass and the temperature change during processing to predict the live microbial residue. This will help to select the appropriate temperature and time for food thermal processing to ensure that *W. coagulans* is present in food at an adequate level of vitality, thereby expanding the application range of probiotics and improving the value of products. In the future, the combination of our modelling with advanced sensing techniques, such as the Electrochemiluminescence assay [47], will allow for more precise monitoring of changes in *W. coagulans* during thermal processing of foodstuffs.

4. Conclusion

In this study, a prediction model for the survival rate of *W. coagulans* during food thermal processing was developed and validated in the processing of coffee, tea, instant noodles, calcium milk biscuits, muffins and steamed buns. The thermal inactivation kinetics of

W. coagulans VHProbi C08 and *W. coagulans* GBI-30, 6086 were investigated at 85, 95, 105, 110 and 115 °C, and z-values of 36.1 °C and 36.9 °C were obtained by Bigelow model fitting, respectively. The measured values of the developed model for thermal processing of the six foods were within ±0.5 Log10 (CFU/mL) of the predicted values, indicating a high degree of prediction accuracy. The prediction model established in this paper will further promote the application of *W. coagulans* in functional foods or functional food products.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Shudong Peng: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Chaoqun Guo:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Xiaoyuan Zhang:** Writing – original draft, Formal analysis. **Xinping Bu:** Formal analysis. **Xinping Li:** Methodology. **Hongchang Cui:** Formal analysis. **Zhi Duan:** Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This research was financially supported by the Mountain Tai New Strategy Industry Leader Program (Grant No. tscy20180317).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36977.

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