Food Science of Animal Products

Research Article

Effects of licorice extract on *Paraclostridium bifermentans* spore growth and quality changes in ready-to-eat chicken breast during storage

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Abstract: This study examined the inhibitory effects of varying concentrations of licorice extract on the growth of *Paraclostridium bifermentans* spores and the quality changes in ready-to-eat (RTE) chicken breast under different storage temperatures. Results indicated significant inhibition ($P < 0.05$) of *P. bifermentans* spore growth at licorice extract concentrations ≥ 12.5 mg/mL, especially in 50 mg/mL. Compared with control group, the shelf life in treatment group (50 mg/mL licorice extract) was nearly prolonged 2-fold when storage at 15 and 20 °C. Furthermore, the Gompertz and Logistic models demonstrated a strong fit ($R^2 > 0.98$) with the growth patterns of *P. bifermentans* spores at 15, 20, and 25 °C. The Ratkowsky model provided a more detailed elucidation of the relationships among temperature, maximum growth rate, and lag phase across varying concentrations of licorice extract. The model results demonstrated that licorice extract significantly ($P < 0.05$) inhibited the *P. bifermentans* spore growth in RTE chicken breast during storage. Additionally, compared to control group, the licorice extract-treated RTE chicken breast exhibited significantly lower levels ($P < 0.05$) of pH value, thiobarbituric acid reactive substances (TBARS) value, and total volatile basic nitrogen (TVB-N) content. Correlation analysis indicated a significant positive correlation ($P < 0.01$) between the quantity of *P. bifermentans* spores and the pH value, TBARS value, and TVB-N content when storage at 15, 20, and 25 °C. This study provides a theoretical reference for extending the shelf life and maintaining the quality of RTE chicken breast, offering valuable insights for improving meat safety and quality in industrial processing.

Keywords: chicken breast; licorice extract; growth model; shelf life; quality change

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1 Introduction

Poultry products (mainly containing chicken, duck, and goose meat products) are widely accepted by consumers due to their nutritional richness (proteins and vitamins) and distinctive flavor, and their production has been continuously increasing worldwide[1–2]. Chicken meat products have many advantages, including high protein, low fat, and appropriate quality-price ratio, which are increasing flavored by consumers[3]. Therefore, various chicken products have been developed to satisfy different preferences, as ready-to-eat (RTE) chicken breast could meet the nutritional need of the people and offer advantages in convenience[4]. However, RTE chicken breast is highly susceptible to contamination by a variety of spoilage microorganisms during storage, primarily due to its high protein content and appropriate moisture nature, which create an ideal environment for microbial growth[5]. Improper storage and preservation conditions can significantly reduce the shelf life of RTE chicken breast, leading to substantial economic losses and posing serious food safety risks due to microbial contamination, thereby exacerbating challenges faced by the meat product industry.

Generally, many spoilage bacteria would be eliminated by thermal sterilization technologies, while some microorganism (e.g., *Paraclostridium* and *Bacillus*) can survive in the form of dormant spores in meat products[6]. *Paraclostridium bifermentans*, as an anaerobic, spore-forming, gas-producing bacterium, is widely distributed in raw and processed foods[6]. The metabolically dormant spores formed from *P. bifermentans* have strong resistance to high temperature and pressure, radiation, toxic chemicals, and other environmental stress factors[6]. *P. bifermentans* still exhibited survival ability after thermal process, and can readily cause the spoilage of meat products. Additionally, improper storage temperature could induce the rapid germination of residual spores in cooked meat[7]. Then, various metabolic activities of the germinated spores lead to the spoilage of meat products and potential safety hazard[8–9]. Though proper storage temperature can be feasible to delay the spore germination, outgrowth, and

multiplication, it cannot completely prevent their growth. Therefore, highly efficient antimicrobial agents are crucial to develop for inhibiting the germination and multiplication of spores, and further extend the shelf life of meat products.

Chemical preservatives have been used to exterminate the spoilage microorganisms in meat products, while they have potential toxicogenic and carcinogenic characteristics[10]. Therefore, natural antibacterial preservatives such as plant extracts, are regarded as substitutes of chemical preservatives due to their safety and high efficiency[11–12]. Many studies have confirmed that plant extracts were conductive to extend the shelf life and improve the flavor quality of chicken products. Wang et al.[1] discovered that after 15 days of storage at 4 °C, the total viable count of chicken breast treated with cinnamon essential oil nanoemulsions decreased by 1.00–3.95 (lg (CFU/g)). The grape seed extracts showed inhibitions to the growth of spoilage microbes and lipid oxidation during the refrigerated storage of chicken breast[13]. Barido et al.[14] found that the black garlic extract was favorable to reduce the cooking loss and improve the water-holding capacity of chicken breast. Licorice is rich in bioactive substances (e.g., triterpenoids and flavonoids), which have great potential antibacterial properties[15]. Nevertheless, there is still a lack of comprehensive knowledge on the effect of licorice extract on the growth of *P. bifermentans* spores from RTE chicken breast during storage.

Predictive models play a vital role in evaluating product shelf-life and ensuring food safety. They are instrumental in conducting hazard analyses, establishing critical control points, and formulating risk assessment plans[16–17]. Primary (Gompertz, Logistic, and Hill) and secondary (Ratkowsky) models offer a rapid, efficient, and cost-effective approach to estimating or predicting microbial growth under specific conditions, eliminating the need for lengthy, costly, and labor-intensive analytical methods requiring specialized expertise[16,18]. In contrast, traditional microbial enumeration techniques are limited and impractical for assessing microbial growth under fluctuating environmental scenarios[16]. Predictive models, however, excel in forecasting microbial growth in foods during processing and storage, enabling real-time decision-making. They have been utilized to characterize the growth patterns of spoilage microorganisms, where the critical parameter is the time required to reach a specific target level under varying temperature conditions.

This study aimed to investigate the inhibition of different concentrations of licorice extract on the growth of *P. bifermentans* spores from RTE chicken breast during the storage period, and further evaluate the licorice extract on the quality of RTE chicken breast. The antibacterial properties of licorice extract were preliminarily evaluated by colony counts and growth curves at different times and temperatures, followed by developing growth models (primary and secondary models) to predict the growth potential, maximum cell numbers, specific growth rate and lag phase of *P. bifermentans* spores. Furthermore, pH values, surface color, thiobarbituric acid reactive substances (TBARS) value and total volatile basic nitrogen (TVB-N) content were investigated to evaluate the quality changes of RTE chicken breast during storage. This study would provide a theoretical basis for controlling secondary contamination of *P. bifermentans* spores remained in RTE chicken breast by licorice extract.

2 Materials and methods

2.1 Materials and reagents

Licorice root (*Glycyrrhiza uralensis* Fisch) was obtained from Yonggang Co., Ltd. (Haozhou, China). RTE chicken breast samples were obtained from Nanjing Huangjiaoshou Food Science and Technology Co., Ltd. (Nanjing, China). Briefly, fresh chicken breasts were blanched and boiled in bittern soup (mainly contained NaCl and spices) at 90 °C for 40 min, then vacuum-packaged in aluminum foil bag and heated at 110 °C for 40 min. Reinforced clostridium medium (RCM), reinforced clostridium agar (RCA) and Duncan-strong sporulation media (DSSM) were prepared as the description of Song et al.[6].

2.2 Growth conditions

P. bifermentans (NCBI accession number: ON078501) was isolated from the vacuum-packaged RTE chicken breast with "blown pack" spoilage[6]. The strain was inoculated into RCM and incubated overnight in an anoxia workstation (Bugbox M, Ruskinn, UK) at 37 °C. The system was regulated by three gases: N_2, a mixture of 5% H_2 and N_2, and CO_2, where the CO_2 content was set at 5%. Then transferred the culture broths into RCM broth (contained 25% glycerol) and stored at −80 °C[19].

2.3 Preparation and purification of spores

Spore suspensions of *P. bifermentans* were prepared and purified according to Alnoman et al.[20]. Briefly, 100 μL activated cultures of *P. bifermentans* were streaked on DSSM agar plates and incubated in anoxia workstation at 37 °C for 7 days. The cultures were scraped into cold sterile water and centrifuged at 8 000 r/min for 15 min at 4 °C. Repeated the centrifugation process for 3 times to obtain the precipitates (spores). The remaining precipitates were washed by cold sterile water (4 °C) for 3 times and then preserved at 4 °C for 24 h. The spores were inactivated by heat shocked (85 °C, 20 min). The spores were stained green and the purity of spore suspensions were observed over 99% by the upright fluorescence microscope. The purified spores were plated in RCA by gradient dilution method, then incubated at 37 °C for 24 h under anaerobic conditions. The amounts of purified spores were determined using a colony meter and adjusted to 10^6 CFU/mL for subsequent experiments.

2.4 Preparations of licorice extract and chicken breast

The ethanol-soluble extract of licorice was prepared by the method of Sohail et al.[21]. The licorice powder was added to 75% ethanol (1:10, *V/V*) and extracted by ultrasound-assisted methods (40 kHz, 40 min, 1 h). Then, the licorice extract was obtained by freeze-dried and kept at −20 °C for further analysis. Licorice extract was dissolved in sterile water at final concentrations ranging from 0 to 100 mg/mL.

RTE chicken breast was cut into pieces of (10.0 ± 0.2) g and sterilized at 121 °C for 30 min, and then packed in sterile vacuum bags at −20 °C for further analysis.

2.5 Detection of total colony counts

RTE chicken breast (10 g) was smearing with 100 μL of different concentrations of licorice extract (1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg/mL) and massaging for 1 min under aseptic condition. The control group was smeared without licorice extract. Each group contained 6 RTE chicken breasts. These samples were sealed by vacuum packaging machine and incubated at 37 °C for 24 h. The above treated samples were added into sterile homogenization bags (12 cm × 18 cm) that contained 90 mL sterile saline (0.85 g/100 mL NaCl), and homogenized for 2 min by sterile homogenizer. Subsequently, 100 μL dilutions with appropriate concentrations were plated onto RCA and incubated at 37 °C for 24 h under anaerobic condition, total colony counts were determined by a colony meter and expressed as CFU/g[7].

2.6 Storage experiment

According to the results of the total colony counts, three concentrations (12.5, 25, and 50 mg/mL) of licorice extract were selected for further experiments. The samples of RTE chicken breast were prepared by incubating with spore suspensions and smearing with different concentrations of licorice extract (0 mg/mL, control group; 12.5, 25, and 50 mg/mL, treatment groups). Briefly, the 100 μL of spore suspensions (10^6 CFU/mL) was inoculated into 10 g sterile RTE chicken breast, followed by an evenly distributed massage. Then, licorice extract solutions (100 μL) with different concentrations were evenly smeared on the RTE chicken breast by manually massaged for 1 min. These samples were stored at 4, 15, 20, and 25 °C, respectively. Six pieces of RTE chicken breast from each group were used for the calculation of microbial counts and analysis of physicochemical indicators.

2.7 Establishment of the growth prediction model of *P. bifermentals* spores

2.7.1 Establishment of the primary models

Gompertz, Logistic, and Hill equations were used according to the growth curves that measured by storage experiments at 15, 20, and 25 °C, and further established the predictive models for *P. bifermentans* spore growth[16]. The optimal primary models, namely Gompertz, Logistic, and Hill models, were determined at different storage temperatures using equations (1) to (3), respectively:

$$N(t) = N_0 + a_1 \exp\left[-\exp\left(b_1 - c_1 t\right)\right] \quad (1)$$

$$N(t) = N_0 + \frac{a_2}{1 + \exp\left(b_2 - c_2 t\right)} \quad (2)$$

$$N(t) = N_0 + \frac{a_3}{1 + \left(t/b_3\right)^{c_3}} \quad (3)$$

Where t represents time (day); $N(t)$ represents the bacterial count at t; N_0 represents the initial bacterial count (lg (CFU/g)); a, b, and c were undetermined coefficients.

2.7.2 Establishment of the secondary model

The square root model was used to describe the linear relationship between the square root of maximum growth rate ($\sqrt{\mu_{max}}$) or reciprocal of the lag phase ($\sqrt{1/\lambda}$) of microorganisms and temperature. Therefore, the Ratkowsky equation[22] was used in the following equations (4) and (5), respectively:

$$\sqrt{\mu_{max}} = b_1 \left(T - T_{min1}\right) \quad (4)$$

$$\sqrt{1/\lambda} = b_2 \left(T - T_{min2}\right) \quad (5)$$

Where T represents temperature (°C); μ_{max} represents the maximum growth rate at temperature T (d^{-1}); λ represents the lag phase (day); b_1 and b_2 are coefficients to be determined; T_{min1} and T_{min2} are the minimum temperatures (°C) for microbial growth.

2.7.3 Model validation

The fit goodness of the models was evaluated by coefficient of determination (R^2), sum of squared errors (SSE), root mean square error (RMSE), and Akaike information criterion (AIC)[23].

$$\mathrm{SSE} = \sum_{i=1}^{n} \left(y_i - \hat{y}_i\right)^2 \quad (6)$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} \left(y_i - \hat{y}_i\right)^2} \quad (7)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(y_i - \hat{y}_i\right)^2}{\sum_{i=1}^{n} \left(y_i - \bar{y}_i\right)^2} \quad (8)$$

$$\mathrm{AIC} = 2k + n\ln\frac{\sum_{i=1}^{n} \left(\hat{y}_i - \bar{y}_i\right)^2}{n} \quad (9)$$

Where n represents the number of data; y_i represents the observed value; $\hat{y}_i$ represents the predicted value; $\bar{y}_i$ represents the mean value; k represents the number of parameters.

2.8 Determination of quality changes in RTE chicken breast

2.8.1 Determination of surface color of RTE chicken breast

The surface color of the RTE chicken breast was determined by using a CR-400 colorimeter (Minolta, Osaka, Japan). Three random locations on the surface of each RTE chicken breast were selected to measure under indoor lighting conditions, and the results were recorded as lightness value (L^*), redness value (a^*), and yellowness value (b^*)[5].

2.8.2 Measurement of pH value of RTE chicken breast

RTE chicken breast (1 g) was mixed with ultrapure water (10 mL) and homogenized (Ultra Turrax T25, IKA, Germany) for 30 s. The pH value was determined by a pH meter (Hanna HI9025c, Amorim, Portugal). The pH meter was calibrated using pH standard solutions (pH 4.0, 7.0, and 10.0) before measurement[24].

2.8.3 Measurement of TBARS value of RTE chicken breast

The TBARS value reflected the level of lipid oxidation of RTE chicken breast, and was evaluated by the method of Sogut et al.[24]. Briefly, 0.5 g of RTE chicken breast was mixed with 3.5 mL of trichloroacetic acid (TCA, 7.5 g/100 mL) solution and homogenized at 10 000 r/min for 1 min (2 × 30 s with 10 s interval). Subsequently, the homogenate solution was centrifuged at 13 000 r/min (4 °C, 10 min), and then 2 mL of supernatant was mixed with 2 mL of 0.02 mol/L thiobarbituric acid and incubated at boiling water bath for 30 min. The blank group composed of 2 mL of 0.02 mol/L thiobarbituric acid and 2 mL of 7.5 g/100 mL TCA. After natural cooling, the absorbance of the mixture was measured at a wavelength of 532 nm by a 96-Well Plate Reader M200 (Tecan, Austria). The TBARS values were calculated according to a 1,1,3,3-tetramethoxypropane standard curve, and expressed as milligrams of malondialdehyde (MDA) of RTE chicken breast (kg).

2.8.4 Determination of TVB-N content in RTE chicken breast

TVB-N content in the RTE chicken breast was measured according to Zhang et al.[25]. Briefly, 4 g of RTE chicken breast was mixed with 30 mL of ultrapure water and shocked (200 r/min) at room temperature for 30 min. After filtering, 1 g magnesium oxide was added to 10 mL filtrate and determined using a fully automatic Kjeldahl nitrogen tester.

2.9 Statistical analysis

All data reported were averages of triplicate experiments. One-way analysis of variance procedure (SAS 8.1) was used for statistical analysis. Duncan's multiple range test was used for detecting the significant differences of physicochemical indicators (total colony count, pH value, TBARS value, and TVB-N content) of different treatments at a level of 5%. The primary models (Gompertz, Logistic, and Hill equations) of growth prediction were developed based on the changes in total colony counts at different storage temperatures, with the data being analyzed and processed using MATLAB R2018a. The growth rates at different temperatures obtained from Gompertz model were used to develop a secondary model (Ratkowsky equation).

3 Results and discussion

3.1 Selection of licorice extract concentration

The inhibitory effect of different concentrations of licorice extract on the growth of *P. bifermentans* spores in RTE chicken breast was investigated. As shown in Figure 1, licorice extract significantly ($P < 0.05$) inhibited the growth of *P. bifermentans* spores at concentrations of ≥ 12.5 mg/mL, and showed no inhibition ($P > 0.05$) on spores when the concentrations < 12.5 mg/mL. After 24 h of incubation, a reduction of over 1.5 (lg (CFU/g)) in *P. bifermentans* spores was observed in RTE chicken breast treated with licorice extract at concentrations of > 50 mg/mL. Specifically, the total colony count decreased from 6.83 (lg (CFU/g)) in the control group to 5.19 (lg (CFU/g)) in the treatment group with a concentration of 100 mg/mL after 24 h of incubation. When the concentration of licorice extract exceeded 50 mg/mL, the changes in total colony counts were not statistically significant ($P > 0.05$). As for the control group, the total colony counts in RTE chicken breast significantly ($P < 0.05$) increased from 3.62 (lg (CFU/g)) at 0 h to 6.83 (lg (CFU/g)) at 24 h. These findings indicated that concentration of licorice extract ≥ 12.5 mg/mL could inhibit the growth of *P. bifermentans* spores and further help to extend the shelf life of chicken products. Therefore, the three concentrations (12.5, 25, and 50 mg/mL) of licorice extract were selected for subsequent storage experiments.

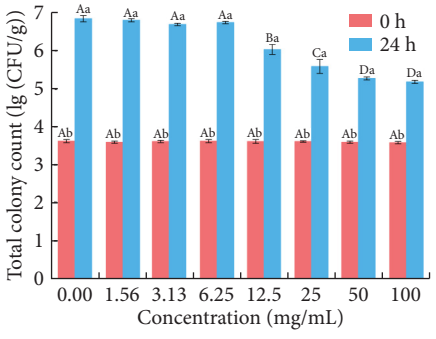
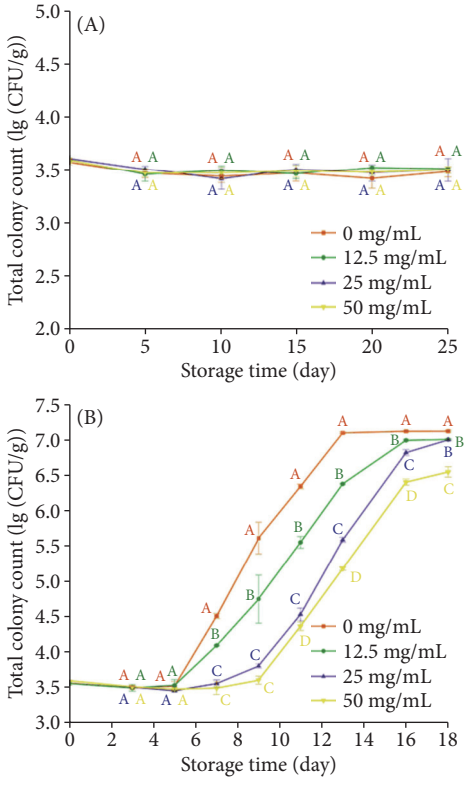


Figure 1 Growth of *P. bifermentans* spores in RTE chicken breast treated with different concentrations of licorice extract. Different uppercase letters at the same time represent significantly difference ($P < 0.05$) among different concentration groups. Different lowercase letters at the same concentration indicate significant difference ($P < 0.05$) among different time groups.

3.2 Analysis of total colony counts

The growth profiles of *P. bifermentans* spores in RTE chicken breast treated with or without licorice extract at different temperatures are shown in Figures 2A–D. The counts of *P. bifermentans* spores among four groups of RTE chicken breast showed no significant differences within 25 days of storage at 4 °C (Figure 2A). Similar reports have been discovered in cooked beef smeared with grape seed extracts, the counts of *Clostridium perfringens* spores showed no variation at 4 °C within 28 days[11]. This could be explained that the low temperature is not conducive to the growth of spores. After storage at 15 °C for 5 days (Figure 2B), the counts of *P. bifermentans* spores in RTE chicken breast smeared with licorice extract (concentration ≤ 12.5 mg/mL) significantly ($P < 0.05$) increased. Until the 7 days, the spore count in RTE chicken breast treated with licorice extract (concentration ≥ 25 mg/mL) began to increase. Similarly, *C. perfringens* in vacuum-packed cooked beef started to grow after 5 days at 15 °C[26]. As shown in Figures 2C and D, the colony counts of untreated group rapidly reached to 5 (lg (CFU/g)) at 40 and 30 h when the storage temperatures were 20 and 25 °C, respectively; while the three treatment groups significantly delayed the time required for colony numbers to reach 5 (lg (CFU/g)). Results showed that the addition of licorice extract markedly ($P < 0.05$) inhibited the growth of spores. This could be attributed by that licorice extract (mainly contained glycyrrhizic acid and flavonoids) can inhibit the germination of *P. bifermentans* spores by blocking the germinant sensing and breaking the inner membrane of outgrowing spores[6]. Compared with other groups, the chicken breast treated with 50 mg/mL licorice extract showed the lowest level ($P < 0.05$) of *P. bifermentans* spores when storage at 15, 20, and 25 °C. According to GB 2726–2016, the total bacterial count in cooked meat product should not exceeded 5 (lg (CFU/g)), the 50 mg/mL of licorice extract nearly exceeded 2-fold shelf life of RTE chicken breast when storage at 15 and 20 °C. Thus, licorice extract could be used as protective agent for inhibiting the germination of *P. bifermentans* spores in chicken breast products.



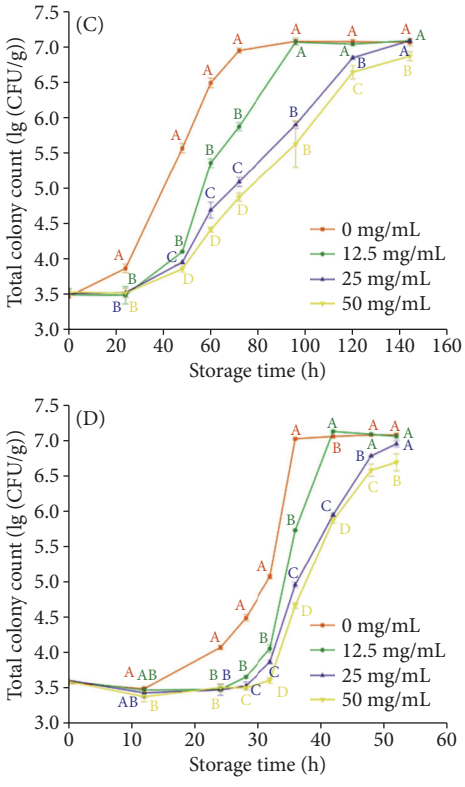


Figure 2 Growth of *P. bifermentans* spores at (A) 4, (B) 15, (C) 20, and (D) 25 °C in RTE chicken breast added licorice extract. Different uppercase letters at the same time represent significantly difference ($P < 0.05$) among different concentration groups.

3.3 Growth models

Growth models are used to assess the growth of foodborne pathogens in specific substrates to ensure food safety, and are important tools for estimating shelf life, identifying critical control points, and conducting hazard analysis and risk assessment[22]. In our study, there was no significant differences of *P. bifermentans* spores among 4 groups at 4 °C, and growth data of *P. bifermentans* spores at 15, 20, and 25 °C were selected for establishing models. As listed in Table S1, both the Gompertz and Logistic models could fit well the growth of *P. bifermentans* spores in the RTE chicken breast at 15 °C, with $R^2 > 0.98$. According to the description of Huang et al.[23], low values of RMSE, SSE and AIC showed the prediction accuracy of models. However, the Hill model could only fit the growth of *P. bifermentans* spores in RTE chicken breast with 25 and 50 mg/mL licorice extract. At 20 and 25 °C, both Gompertz and Logistic models also fit well with the growth of *P. bifermentans* spores in the RTE chicken breast with $R^2 > 0.98$; while the Hill model could not fit the growth of *P. bifermentans* spores in RTE chicken breast with licorice extract. The results indicated that Gompertz and Logistic models could be used as primary model of the growth of *P. bifermentans* spores during storage.

The R^2 values of the Gompertz and Logistic models were above 0.98, respectively, indicating the good fitting effects. Therefore, the fitting curves of these two models were plotted. As shown in Figure 3, all of the fitting curves showed "S" shaped, which was consistent with the previous study reported by Wang et al.[27]. They successfully fitted the growth of *C. perfringens* with the Gompertz model, and the curves showed typical S-shape. Tarlak et al.[16] used the Logistic model to predict well with the growth of *Pseudomonas* spp. on sliced mushrooms stored at 4–28 °C. Zhu et al.[17] used a modified Gompertz model to predict the effects of spices essential oils on the growth of *C. perfringens*. Therefore, both of two models showed excellent accuracies in predicting the growth curve of *P. bifermentans* spores.

Based on the levels of R^2, RMSE, SSE and AIC, the Gompertz model showed more ideal fitting curves compared with Logistic model. Therefore, the experimental data obtained by the Gompertz

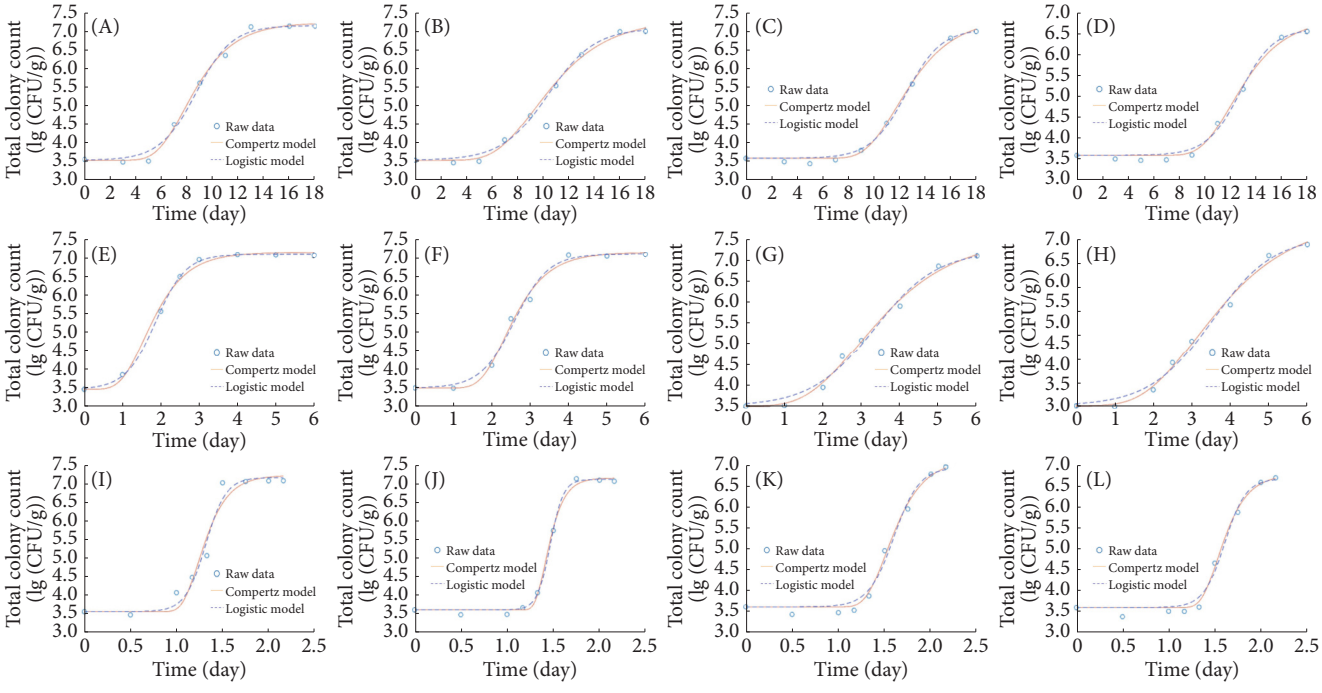


Figure 3 Primary models of *P. bifermentans* spores growth in RTE chicken breast. Growth prediction models of *P. bifermentans* spores in RTE chicken breast added with 0, 12.5, 25, and 50 mg/mL licorice extract and stored at (A–D) 15, (E–H) 20, and (I–L) 25 °C, respectively. (A, E, I) 0 mg/mL; (B, F, J) 12.5 mg/mL; (C, G, K) 25 mg/mL; (D, H, L) 50 mg/mL.

model was used to establish the secondary model. The secondary model mainly used for exploring the effect of temperature on the growth of *P. bifermentans* spores in RTE chicken breast. As shown in Table S2 and Figure 4, the Ratkowsky model could fit well the correlation between temperature and $\sqrt{\mu_{max}}$ at licorice extract concentrations of 0, 12.5, and 25 mg/mL, as well as the relevancy between temperature and $\sqrt{1/\lambda}$ at licorice extract concentrations of 0 and 12.5 mg/mL. Previous study showed that the Ratkowsky model could fit well with the growth of *C. perfringens* spores in RTE chicken meat, as all sample data had R^2 values over 0.9 for fitting $\sqrt{\mu_{max}}$ and $\sqrt{1/\lambda}$[27]. In our study, all R^2 values at different temperatures of the Gompertz model were over 0.98, which further supported the reliability for the establishment secondary model.

The μ_{max} of *P. bifermentans* increased with increasing temperature while the λ extended, as listed in supplementary Table S3. This result was consistent with the previous study by Wang et al.[27], who reported that the μ_{max} of *C. perfringens* spores raised with increasing temperature, while the lag phase was shortened. Among different temperatures, the maximum counts of *P. bifermentans* spores in RTE chicken breast treated with 50 mg/mL licorice extract were lower than the untreated group, and μ_{max} were also lower than that of the untreated group, while λ was longer than the untreated group, respectively. This result indicated that 50 mg/mL licorice extract reduces the counts of *P. bifermentans* spores by delaying their growth rate, and further extend the lag phase of *P. bifermentans* spores. As for the other two concentrations of licorice extract, their inhibitory effects on *P. bifermentans* spores in RTE chicken breast under different temperatures were also be observed; while 50 mg/mL licorice extract showed the highest effect in inhibiting the growth of *P. bifermentans* spores. Similarly, 1% ginger essential oil and grape seed extract added to RTE chicken meat significantly extended the lag phase and reduced the growth rate of *C. perfringens* spores[27]. Thus, according the analysis of primary and secondary models, the addition of licorice extract exhibited effective inhibition on *P. bifermentans* spores in RTE chicken breast and helped to extend the shelf life. Thus, the above models could be applied for references of critical control points in storage temperature and licorice extract addition to ensure product safety during industrial process of chicken breast.

3.4 Quality changes in RTE chicken breasts

3.4.1 *pH value and surface color of RTE chicken breasts*

The pH value plays a crucial role in depending the quality and freshness of meat products, it often correlates with microbial activities and various chemical reactions[28]. Therefore, the pH values of RTE chicken breasts inoculated with *P. bifermentans* spores and smeared with different concentrations of licorice extract were measured, as listed in Tables 1–4. After smeared RTE chicken breasts with different concentrations of licorice extract, determining the pH values immediately. All the pH values in these samples were over 6.0 and showed no significant differences under four storage temperatures, indicating that licorice extract did not affect the pH values substantially. The pH values of all samples were increased with the increase of storage time. During the storage period, the pH values of chicken breasts decreased with the increase of concentration of licorice extract; the pH values of chicken breasts treated with 50 mg/mL licorice extract showed the lowest level ($P < 0.05$) compared with other treatments. These results were in accordance with the changes in RTE chicken breasts reported by Qiu et al.[29]. They found that the pH values of RTE chicken breast patties increased with storage time in both control and treated groups, however, the increase of pH values in the chicken patties that coated with plant essential oils was significantly slower than control group. The change of pH values was correlated with the acidity in meat, while the gradual rise in acidity could be attributed to the acid metabolites (nitrogenous compounds such as ammonia, histamine and trimethylamine) from microbial activity[30]. In our study, the pH values in treatments groups showed lower levels in contrast to control group, which could be attributed to the inhibition of licorice extract on the growth of *P. bifermentans* spores.

Color (L^*, a^*, and b^*), as one of the most important indicators for sensory attributes of meat products, directly affects the appetite of consumers[28]. Compared with the variations of L^* and a^*, b^* showed differences among the 4 groups. After smearing of licorice extract, L^* of chicken breast increased, especially b^* of chicken breast; while b^* gradually decreased with the extension of storage time, and the difference between the control group and treatment group gradually diminished. Additionally, the color of RTE chicken breast remained unchanged as the protein underwent thermal

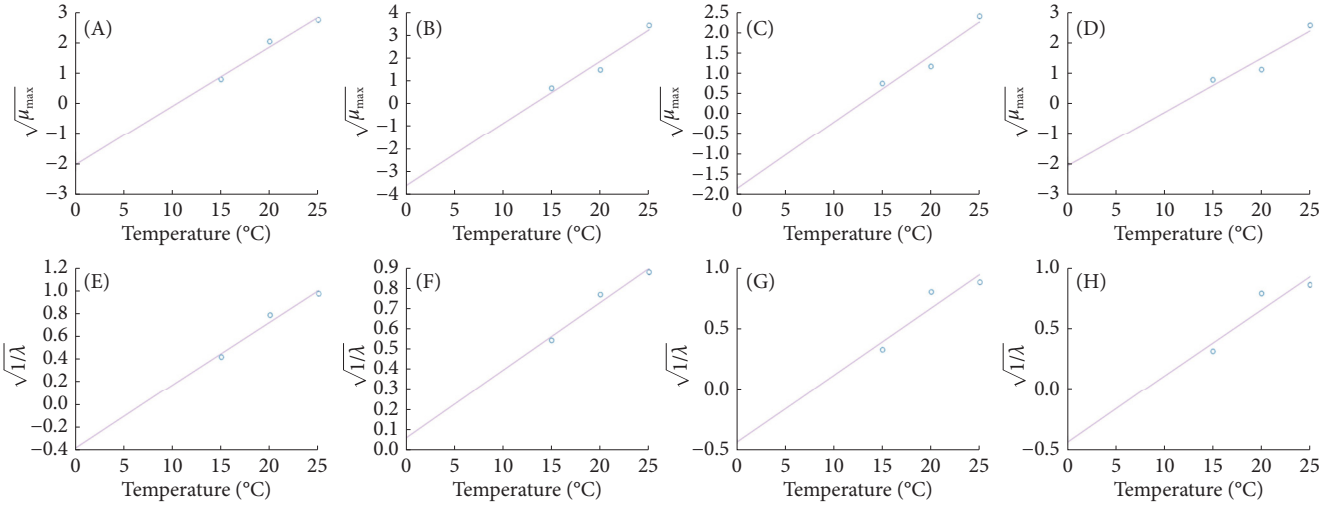


Figure 4 Secondary model of *P. bifermentans* spores growth in RTE chicken breast. (A–D) The relationship between temperature and $\sqrt{\mu_{max}}$ of *P. bifermentans* spores. (E–H) The relationship between temperature and the $\sqrt{1/\lambda}$ of *P. bifermentans* spores. (A, E) 0 mg/mL; (B, F) 12.5 mg/mL; (C, G) 25 mg/mL; (D, H) 50 mg/mL.

Table 1 Effect of licorice extract on the pH value and surface color of RTE chicken breast during storage at 4 °C.

Index	Concentration (mg/mL)	Storage time (day)					
		0	5	10	15	20	25
pH	0	6.11 ± 0.03^{Abc}	6.09 ± 0.03^{Ac}	6.09 ± 0.03^{Ac}	6.12 ± 0.03^{Ab}	6.13 ± 0.02^{Ab}	6.18 ± 0.01^{Aa}
	12.5	6.09 ± 0.01^{Ac}	6.09 ± 0.03^{Ac}	6.09 ± 0.05^{Ac}	6.10 ± 0.05^{ABc}	6.13 ± 0.04^{Ab}	6.16 ± 0.04^{Aa}
	25	6.09 ± 0.02^{Abc}	6.07 ± 0.02^{Ac}	6.08 ± 0.05^{Ac}	6.09 ± 0.03^{Bbc}	6.11 ± 0.03^{ABb}	6.14 ± 0.03^{ABa}
	50	6.11 ± 0.03^{Aab}	6.08 ± 0.04^{Ac}	6.08 ± 0.04^{Ac}	6.09 ± 0.03^{Bbc}	6.11 ± 0.09^{ABab}	6.13 ± 0.05^{Ba}
L^*	0	71.65 ± 1.28^{Aa}	72.91 ± 5.87^{Aa}	73.31 ± 1.08^{Aa}	70.60 ± 3.11^{Aa}	72.35 ± 1.47^{Aa}	72.49 ± 0.55^{Aa}
	12.5	71.71 ± 2.61^{Aa}	73.03 ± 0.66^{Aa}	71.00 ± 2.98^{Aa}	72.98 ± 0.61^{Aa}	73.28 ± 1.37^{Aa}	72.24 ± 0.42^{Aa}
	25	72.50 ± 0.35^{Aa}	72.35 ± 1.80^{Aa}	72.86 ± 3.01^{Aa}	72.41 ± 0.12^{Aa}	72.73 ± 0.82^{Aa}	72.20 ± 0.80^{Aa}
	50	72.63 ± 0.23^{Aa}	72.14 ± 0.55^{Aa}	72.25 ± 0.34^{Aa}	70.09 ± 0.86^{Ab}	72.38 ± 1.19^{Aa}	72.24 ± 0.33^{Aa}
a^*	0	4.30 ± 0.42^{Abc}	5.19 ± 0.16^{Aa}	4.55 ± 0.22^{Abc}	4.64 ± 0.25^{Ab}	4.10 ± 0.31^{Ac}	4.49 ± 0.29^{Abc}
	12.5	4.54 ± 0.13^{Aa}	4.28 ± 0.28^{Bab}	3.89 ± 0.26^{Bbc}	3.98 ± 0.17^{Bbc}	3.74 ± 0.23^{Ac}	4.02 ± 0.14^{Bbc}
	25	4.49 ± 0.47^{Aa}	4.39 ± 0.31^{Bab}	3.84 ± 0.28^{Bbc}	4.01 ± 0.25^{Babc}	3.84 ± 0.32^{Abc}	3.69 ± 0.08^{Cc}
	50	4.57 ± 0.44^{Aa}	3.83 ± 0.17^{Cb}	4.00 ± 0.27^{Bb}	3.95 ± 0.18^{Bb}	3.70 ± 0.33^{Ab}	3.69 ± 0.18^{Cb}
b^*	0	22.81 ± 0.48^{Dbc}	23.60 ± 1.15^{Bab}	24.65 ± 0.87^{Ba}	22.19 ± 0.56^{Cbc}	21.86 ± 0.55^{Bc}	23.41 ± 1.64^{ABab}
	12.5	25.93 ± 0.63^{Ca}	25.87 ± 0.52^{Aa}	25.81 ± 1.55^{Ba}	23.66 ± 0.61^{Bb}	22.98 ± 0.68^{Bb}	23.01 ± 1.37^{Bb}
	25	27.66 ± 0.83^{Ba}	26.69 ± 0.79^{Aa}	25.31 ± 0.61^{Bb}	25.26 ± 0.62^{Ab}	25.52 ± 0.12^{Ab}	24.03 ± 0.54^{ABc}
	50	29.45 ± 0.86^{Aa}	27.35 ± 0.48^{Ab}	28.11 ± 0.67^{Ab}	25.38 ± 0.40^{Ac}	25.14 ± 1.41^{Ac}	24.89 ± 0.65^{Ac}

Note: Results were expressed as means ± standard deviations. Values with different superscript uppercase letters were significantly different ($P < 0.05$) at the same time point in different concentration groups. Values with different superscript lowercase letters were significantly different ($P < 0.05$) in same concentration groups at the different times.

Table 2 Effect of licorice extract on the pH value and surface color of RTE chicken breast during storage at 15 °C.

Index	Concentration (mg/mL)	Storage time (day)								
		0	3	5	7	9	11	13	16	18
pH	0	6.09 ± 0.03^{Ah}	6.19 ± 0.06^{Ag}	6.26 ± 0.02^{Afg}	6.33 ± 0.03^{Aef}	6.43 ± 0.01^{Ade}	6.50 ± 0.01^{Ad}	6.61 ± 0.02^{Ac}	6.72 ± 0.03^{Ab}	6.84 ± 0.02^{Aa}
	12.5	6.09 ± 0.02^{Ae}	6.11 ± 0.04^{Bd}	6.15 ± 0.02^{Bd}	6.18 ± 0.02^{Bd}	6.23 ± 0.02^{Bcd}	6.30 ± 0.01^{Bc}	6.36 ± 0.02^{Bbc}	6.41 ± 0.03^{Bb}	6.51 ± 0.01^{Ba}
	25	6.09 ± 0.03^{Ad}	6.10 ± 0.05^{Bd}	6.12 ± 0.01^{Bd}	6.15 ± 0.02^{Bcd}	6.20 ± 0.01^{BCc}	6.24 ± 0.01^{BCbc}	6.30 ± 0.02^{BCb}	6.39 ± 0.02^{Cab}	6.45 ± 0.02^{Ca}
	50	6.09 ± 0.02^{Ac}	6.09 ± 0.01^{Bc}	6.11 ± 0.02^{Bc}	6.13 ± 0.02^{Bc}	6.16 ± 0.04^{Cbc}	6.19 ± 0.02^{Cbc}	6.24 ± 0.02^{Cb}	6.29 ± 0.02^{Dab}	6.35 ± 0.01^{Da}
L^*	0	71.25 ± 0.78^{ABc}	71.46 ± 0.52^{Ac}	73.70 ± 1.40^{Ab}	75.70 ± 0.38^{Aab}	76.84 ± 1.11^{Aa}	74.86 ± 1.79^{Aab}	74.74 ± 0.45^{Aab}	74.86 ± 1.79^{Aab}	74.39 ± 1.22^{Aab}
	12.5	70.47 ± 0.59^{Bc}	71.31 ± 0.37^{Ac}	73.93 ± 0.04^{Ab}	75.61 ± 2.11^{Aab}	77.32 ± 1.65^{Aa}	75.33 ± 0.78^{Aab}	75.43 ± 1.09^{ABab}	75.33 ± 0.78^{Aab}	74.99 ± 0.63^{Aab}
	25	72.68 ± 0.94^{Acd}	71.59 ± 0.34^{Ad}	73.63 ± 0.58^{Ac}	75.72 ± 0.70^{Aab}	76.97 ± 1.07^{Aa}	76.37 ± 0.50^{Aa}	74.84 ± 0.62^{Bb}	76.37 ± 0.50^{Aa}	76.62 ± 1.82^{Aa}
	50	72.54 ± 0.81^{Ab}	72.48 ± 1.08^{Ab}	74.46 ± 0.72^{Aab}	76.09 ± 0.30^{Aa}	77.13 ± 1.78^{Aa}	77.27 ± 1.78^{Aa}	76.68 ± 0.78^{Aa}	76.27 ± 3.25^{Aa}	76.37 ± 1.58^{Aa}
a^*	0	4.24 ± 0.21^{Adef}	4.50 ± 0.27^{Abcd}	4.91 ± 0.07^{Aa}	4.43 ± 0.02^{Acde}	4.15 ± 0.13^{BCef}	4.53 ± 0.16^{ABbcd}	4.02 ± 0.17^{Bf}	4.67 ± 0.32^{Aabc}	4.80 ± 0.13^{Aab}
	12.5	4.32 ± 0.16^{Acd}	4.12 ± 0.23^{ABd}	4.43 ± 0.16^{Bc}	4.52 ± 0.25^{Abc}	4.85 ± 0.05^{Aa}	4.81 ± 0.11^{Aab}	4.57 ± 0.17^{Aabc}	4.80 ± 0.13^{Aab}	4.55 ± 0.14^{Babc}
	25	4.40 ± 0.29^{Aabc}	3.89 ± 0.12^{Be}	3.89 ± 0.18^{Cde}	4.05 ± 0.10^{Bcde}	4.28 ± 0.21^{Babc}	4.41 ± 0.25^{ABabc}	4.61 ± 0.25^{Aa}	4.57 ± 0.19^{Aab}	4.20 ± 0.09^{Cbcd}
	50	4.38 ± 0.13^{Aabc}	4.15 ± 0.09^{Abcd}	3.41 ± 0.20^{De}	3.87 ± 0.03^{Bd}	3.95 ± 0.12^{Cd}	4.32 ± 0.26^{Babc}	4.43 ± 0.24^{ABab}	4.64 ± 0.33^{Aa}	4.03 ± 0.15^{Ccd}
b^*	0	22.88 ± 1.20^{Ccd}	23.79 ± 0.67^{Cabc}	23.46 ± 0.16^{Babc}	24.72 ± 0.51^{Ba}	22.51 ± 1.00^{Ccd}	24.29 ± 0.17^{ABab}	23.24 ± 0.69^{Abc}	21.90 ± 0.31^{Cd}	22.50 ± 0.88^{Bcd}
	12.5	25.99 ± 1.00^{Bb}	25.65 ± 0.06^{Bab}	25.66 ± 0.87^{Aab}	26.84 ± 0.64^{Aa}	23.21 ± 0.99^{BCc}	23.48 ± 0.97^{Bc}	24.33 ± 0.33^{Abc}	23.73 ± 0.45^{Bc}	23.12 ± 0.58^{ABc}
	25	27.93 ± 0.55^{Aa}	25.40 ± 0.63^{Bb}	26.54 ± 0.14^{Ab}	25.44 ± 0.12^{Bb}	25.33 ± 0.52^{Ab}	25.35 ± 1.48^{Ab}	23.25 ± 0.86^{Ac}	23.73 ± 0.71^{Bc}	24.06 ± 0.22^{Ac}
	50	29.43 ± 0.81^{Aa}	27.60 ± 0.09^{Ab}	26.28 ± 0.65^{Abc}	26.68 ± 0.07^{Ab}	24.39 ± 0.36^{ABde}	22.87 ± 0.57^{Be}	24.63 ± 2.17^{Ad}	24.91 ± 0.57^{Acd}	24.26 ± 0.49^{Ade}

Note: Results were expressed as means ± standard deviations. Values with different superscript uppercase letters were significantly different ($P < 0.05$) at the same time point in different concentration groups. Values with different superscript lowercase letters were significantly different ($P < 0.05$) in same concentration groups at the different times.

Table 3 Effect of licorice extract on the pH value and surface color of RTE chicken breast during storage at 20 °C.

Index	Concentration (mg/mL)	Storage time (h)							
		0	24	48	60	72	96	120	144
pH	0	6.08 ± 0.01^{Af}	6.25 ± 0.02^{Ade}	6.36 ± 0.03^{Ad}	6.49 ± 0.01^{Acd}	6.52 ± 0.02^{Ac}	6.62 ± 0.04^{Abc}	6.70 ± 0.03^{Ab}	6.80 ± 0.06^{Aa}
	12.5	6.08 ± 0.03^{Ae}	6.16 ± 0.03^{Bde}	6.21 ± 0.01^{Bd}	6.25 ± 0.01^{Bcd}	6.31 ± 0.02^{Bc}	6.39 ± 0.02^{Bbc}	6.47 ± 0.01^{Bab}	6.54 ± 0.05^{Ba}
	25	6.08 ± 0.02^{Af}	6.13 ± 0.04^{BCef}	6.18 ± 0.03^{BCe}	6.23 ± 0.02^{Cde}	6.26 ± 0.01^{Ccd}	6.30 ± 0.01^{Cc}	6.39 ± 0.04^{Cb}	6.48 ± 0.03^{Ca}
	50	6.08 ± 0.02^{Ae}	6.09 ± 0.03^{Cde}	6.13 ± 0.01^{Cd}	6.19 ± 0.02^{Ccd}	6.22 ± 0.01^{Cc}	6.26 ± 0.02^{Cb}	6.30 ± 0.02^{Dab}	6.39 ± 0.03^{Da}
L^*	0	72.21 ± 1.44^{Ac}	74.42 ± 0.44^{Aab}	74.85 ± 0.58^{Aab}	76.03 ± 0.60^{Ba}	73.66 ± 1.48^{Bbc}	74.73 ± 1.24^{Aab}	74.57 ± 1.37^{Aab}	75.39 ± 0.66^{Aab}
	12.5	72.5 ± 1.02^{Ac}	73.88 ± 0.81^{Abc}	73.87 ± 1.20^{Abc}	74.13 ± 1.57^{Babc}	74.97 ± 0.79^{ABab}	76.05 ± 0.94^{Aa}	75.98 ± 0.51^{Aa}	75.65 ± 1.16^{Aab}
	25	72.52 ± 0.96^{Ac}	73.90 ± 0.74^{Abc}	75.03 ± 0.63^{Aab}	75.89 ± 1.25^{ABa}	76.10 ± 0.16^{Aa}	76.46 ± 1.34^{Aa}	76.12 ± 0.83^{Aa}	75.15 ± 1.55^{Aab}
	50	72.41 ± 1.01^{Ac}	73.64 ± 1.18^{Abc}	74.77 ± 0.70^{Aab}	76.25 ± 0.92^{Aa}	75.78 ± 0.99^{Aab}	75.67 ± 2.36^{Aab}	76.08 ± 1.41^{Aab}	75.41 ± 1.05^{Aab}
a^*	0	4.44 ± 0.08^{Ab}	4.13 ± 0.18^{Cc}	3.88 ± 0.07^{Cd}	3.66 ± 0.06^{Dd}	3.82 ± 0.13^{Cd}	4.19 ± 0.14^{Bc}	4.71 ± 0.06^{Aa}	4.41 ± 0.19^{Ab}
	12.5	4.48 ± 0.17^{Abc}	4.77 ± 0.06^{Aab}	5.03 ± 0.09^{Aa}	4.82 ± 0.10^{Aa}	4.43 ± 0.25^{ABc}	4.04 ± 0.12^{Cd}	4.43 ± 0.22^{Bc}	4.52 ± 0.19^{Abc}
	25	4.63 ± 0.17^{Aa}	4.51 ± 0.15^{Bab}	4.72 ± 0.14^{Ba}	4.27 ± 0.08^{Bc}	4.14 ± 0.12^{BCc}	3.86 ± 0.06^{BCd}	3.72 ± 0.09^{Cd}	4.35 ± 0.16^{Abc}
	50	4.41 ± 0.19^{Aa}	3.67 ± 0.12^{Db}	3.77 ± 0.13^{Cb}	3.94 ± 0.08^{Cb}	4.57 ± 0.19^{Aa}	4.48 ± 0.19^{Aa}	4.69 ± 0.08^{Aa}	4.47 ± 0.28^{Aa}
b^*	0	22.75 ± 0.61^{Db}	22.58 ± 1.00^{Cb}	23.95 ± 1.15^{Cab}	23.93 ± 1.44^{Bab}	22.64 ± 1.16^{Bb}	24.61 ± 0.26^{Aa}	23.78 ± 0.57^{BCab}	23.25 ± 0.67^{Bab}
	12.5	25.76 ± 0.38^{Ca}	25.56 ± 0.13^{Ba}	25.95 ± 0.62^{Ba}	23.65 ± 1.19^{Bcd}	24.95 ± 0.39^{Aab}	23.58 ± 0.19^{Acd}	22.80 ± 0.65^{Cd}	24.33 ± 0.54^{Abc}
	25	27.63 ± 0.83^{Ba}	26.79 ± 0.68^{ABab}	27.24 ± 0.50^{ABa}	25.52 ± 0.87^{ABcd}	25.90 ± 0.63^{Abc}	24.26 ± 0.67^{Ae}	23.84 ± 0.57^{Be}	24.60 ± 0.07^{Ade}
	50	29.48 ± 0.22^{Aa}	27.77 ± 0.63^{Ab}	27.76 ± 0.47^{Ab}	26.44 ± 0.58^{Ac}	25.70 ± 0.08^{Ac}	23.88 ± 0.73^{Ad}	25.71 ± 0.20^{Ac}	24.56 ± 0.20^{Ad}

Note: Results were expressed as means ± standard deviations. Values with different superscript uppercase letters were significantly different ($P < 0.05$) at the same time point in different concentration groups. Values with different superscript lowercase letters were significantly different ($P < 0.05$) in same concentration groups at the different times.

Table 4 Effect of licorice extract on the pH value and surface color of RTE chicken breast during storage at 25 °C.

Index	Concentration (mg/mL)	Storage time (h)								
		0	12	24	28	32	36	42	48	52
pH	0	6.10 ± 0.03^{Ag}	6.18 ± 0.02^{Af}	6.32 ± 0.03^{Aef}	6.40 ± 0.01^{Ade}	6.45 ± 0.01^{Ade}	6.58 ± 0.02^{ADd}	6.69 ± 0.02^{Ac}	6.77 ± 0.01^{Ab}	6.89 ± 0.03^{Aa}
	12.5	6.09 ± 0.04^{Ag}	6.11 ± 0.04^{Bg}	6.19 ± 0.03^{Bfg}	6.25 ± 0.01^{Bf}	6.33 ± 0.02^{Be}	6.42 ± 0.04^{Bd}	6.52 ± 0.01^{Bc}	6.64 ± 0.01^{Bb}	6.78 ± 0.02^{Ba}
	25	6.10 ± 0.01^{Ae}	6.11 ± 0.04^{Be}	6.16 ± 0.03^{BCde}	6.22 ± 0.02^{BCd}	6.29 ± 0.02^{BCcd}	6.34 ± 0.02^{Cc}	6.43 ± 0.03^{Cb}	6.58 ± 0.02^{Cab}	6.65 ± 0.03^{Ca}
	50	6.10 ± 0.02^{Ae}	6.11 ± 0.05^{Be}	6.13 ± 0.03^{Cde}	6.18 ± 0.02^{Cd}	6.23 ± 0.01^{Ccd}	6.26 ± 0.01^{Dc}	6.35 ± 0.02^{Dbc}	6.41 ± 0.02^{Db}	6.52 ± 0.01^{Da}
L^*	0	71.08 ± 1.01^{Bc}	72.66 ± 0.83^{Abc}	72.99 ± 1.22^{Bbc}	73.49 ± 1.15^{Bbc}	76.07 ± 2.20^{Aa}	76.23 ± 0.26^{Aa}	74.11 ± 2.37^{Aab}	73.23 ± 1.65^{Bbc}	73.33 ± 0.68^{Bbc}
	12.5	72.45 ± 1.52^{ABe}	72.78 ± 0.84^{Ade}	74.84 ± 1.22^{ABbcd}	75.06 ± 0.56^{ABbcd}	77.67 ± 1.41^{Aa}	76.06 ± 0.99^{Aab}	75.39 ± 1.87^{Abc}	73.53 ± 0.62^{Bcde}	73.88 ± 1.40^{Bcde}
	25	72.54 ± 0.09^{ABc}	72.47 ± 0.86^{Ac}	75.39 ± 0.82^{Aab}	75.74 ± 1.44^{Aab}	76.63 ± 0.41^{Aab}	76.68 ± 1.68^{Aab}	77.19 ± 3.34^{Aa}	74.03 ± 0.56^{Bbc}	75.06 ± 0.80^{ABabc}
	50	73.12 ± 0.55^{Ac}	73.45 ± 1.05^{Ac}	74.03 ± 1.04^{ABbc}	75.70 ± 0.78^{Aab}	76.81 ± 1.65^{Aa}	76.13 ± 0.99^{Aab}	75.29 ± 2.06^{Aabc}	76.38 ± 0.28^{Aa}	76.05 ± 1.16^{Aab}
a^*	0	4.51 ± 0.18^{Ab}	3.69 ± 0.08^{Cd}	4.59 ± 0.16^{Ab}	4.66 ± 0.33^{Ab}	5.00 ± 0.16^{Aa}	5.10 ± 0.08^{Aa}	4.45 ± 0.14^{Abc}	4.36 ± 0.16^{Abc}	4.15 ± 0.09^{Bc}
	12.5	4.50 ± 0.25^{Aa}	4.18 ± 0.12^{Bbc}	3.53 ± 0.26^{Bd}	3.57 ± 0.15^{Cd}	3.75 ± 0.08^{Dd}	4.46 ± 0.15^{Bab}	4.62 ± 0.08^{Aa}	4.32 ± 0.07^{ABabc}	4.04 ± 0.21^{Bc}
	25	4.49 ± 0.22^{Abc}	4.72 ± 0.09^{Aab}	4.53 ± 0.16^{Abc}	4.84 ± 0.11^{Aa}	4.29 ± 0.05^{Bcd}	3.90 ± 0.12^{Cef}	3.75 ± 0.08^{Bf}	4.13 ± 0.09^{Bde}	4.60 ± 0.22^{Aab}
	50	4.28 ± 0.31^{Abcd}	4.61 ± 0.33^{Aab}	3.68 ± 0.05^{Be}	4.13 ± 0.10^{Bcd}	4.04 ± 0.05^{Ccd}	3.98 ± 0.20^{Cde}	4.52 ± 0.20^{Aab}	4.34 ± 0.09^{ABbc}	4.79 ± 0.12^{Aa}
b^*	0	22.62 ± 1.00^{Dde}	23.77 ± 0.40^{Cbc}	24.47 ± 0.07^{Bb}	21.80 ± 0.39^{Ce}	23.99 ± 0.48^{Bb}	23.90 ± 0.47^{Bb}	25.63 ± 0.12^{Aa}	24.26 ± 0.56^{Ab}	22.89 ± 0.63^{Bcd}
	12.5	25.74 ± 0.84^{Cab}	26.06 ± 0.38^{Ba}	24.55 ± 0.84^{Bcd}	23.83 ± 0.69^{Bd}	24.81 ± 0.66^{Bbcd}	25.26 ± 0.75^{Aabc}	23.66 ± 0.06^{Bd}	21.92 ± 0.55^{Be}	23.68 ± 0.12^{ABd}
	25	28.00 ± 0.33^{Ba}	27.61 ± 0.95^{Aa}	27.98 ± 0.68^{Aa}	24.99 ± 0.76^{Bcd}	26.22 ± 0.63^{Ab}	25.92 ± 0.47^{Abc}	23.48 ± 0.24^{Be}	24.83 ± 0.63^{Acd}	24.18 ± 0.52^{Ade}
	50	29.74 ± 0.39^{Aa}	27.80 ± 0.70^{Ab}	27.54 ± 0.90^{Ab}	26.34 ± 0.68^{Ac}	24.86 ± 0.50^{Bde}	25.77 ± 0.63^{Acd}	23.25 ± 0.47^{Bf}	24.40 ± 0.47^{Ae}	24.56 ± 0.99^{Ae}

Note: Results were expressed as means ± standard deviations. Values with different superscript uppercase letters were significantly different ($P < 0.05$) at the same time point in different concentration groups. Values with different superscript lowercase letters were significantly different ($P < 0.05$) in same concentration groups at the different times.

denaturation, making it less susceptible to negative effects. Furthermore, the evolutions of meat color could be associated with the dynamic changes of physicochemical compositions and microbial activities. Previous research reported similar results that licorice extract supported the caiman meat color and quality for 120 days by antioxidant and antimicrobial capacities[31]. Therefore, in present study, the addition of licorice extract has no adverse effect against the color quality of RTE chicken breasts during the storage period.

3.4.2 *TBARS value of RTE chicken breasts*

TBARS value represents the contents of secondary product of lipid oxidation, which is normally used to evaluate the oxidation degree of lipid[32], while the lipid oxidation is closely correlated with the quality of meat products. As shown in Figures 5A–D, the TBARS values of chicken breast increased with the extension of storage time, as well as the rise of storage temperature. The TBARS values of control group were over 0.4 mg/kg when the storage temperature

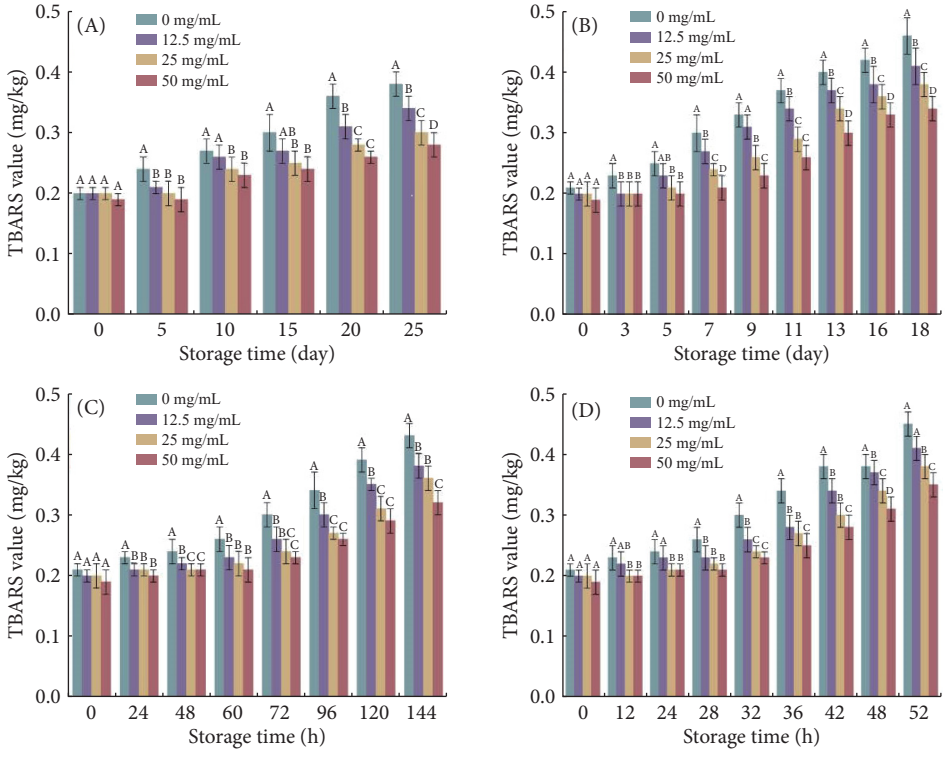


Figure 5 Effects of different concentrations of licorice extract on the TBARS values of RTE chicken breast under storage at (A) 4, (B) 15, (C) 20, and (D) 25 °C. Different uppercase letters at the same time represent significantly difference ($P < 0.05$) among different concentration groups.

over 15 °C, while TBARS values of three treated groups showed significant lower ($P < 0.05$) than that of control group under different temperatures. This indicated that the licorice extract could inhibit the lipid oxidation of chicken breast during the storage. Similar results have been observed in caiman meat[31] and precooked pork patties[33]. The antioxidant effect could be attributed to the bioactive compounds from licorice extract, as glycyrrhizic acid and flavonoids[34]. In our previous study, the main components of licorice extract were composed of glycyrrhizic acid, liquiritin, liquiritin apioside, and licoisoflavone A[6]. These components could be favorable in inhibiting the lipid oxidation. Interestingly, the chicken breast smeared with 50 mg/mL licorice extract showed the lowest level ($P < 0.05$) of TBARS values in contrast with other groups. This could be explained by that higher addition of licorice extract provided more bioactive components. Additionally, the TBARS values in our study were lower than reports of Zhang et al.[5] (0.8–1.5 mg/kg in chicken leg meat) and Huang et al.[35] (0.8–2.2 mg/kg in carbonated chicken leg), this could be the lower fat content in chicken breasts. In summary, the results indicate that licorice extract has great potential as a natural antioxidative additive in extending the shelf-life and keeping the quality of RTE chicken breast.

3.4.3 TVB-N content in RTE chicken breasts

TVB-N content can be used for assessing the freshness of meat products. It mainly consist of ammonia, dimethylamine, trimethylamine, putrescine, and cadaverine compounds that generated from the decomposition of protein and non-protein nitrogenous compounds by spoilage microbial activities[1]. In our study, licorice extract showed significant effect in reducing the TVB-N contents of RTE chicken breast during storage at different temperatures, as shown in Figures 6A–D. Similar effect of licorice extract on TVB-N values has been observed in sea bass fillets at 4 °C[36]. The inhibition effect on protein degradation by microbes was increased with the increase of concentration of licorice extract. Compared with other treatments, 50 mg/mL licorice extract exhibited the lowest values ($P < 0.05$) of TVB-N contents under different storage temperatures. This can be explained by that 50 mg/mL of licorice extract contained more antibacterial active substances compared with other groups[6]. Furthermore, when storage at 4 °C for 25 days, the TVB-N contents of four groups were below 12 mg/100 g; while storage at 25 °C for 28 h, the TVB-N contents of these groups over 10 mg/100 g. This indicated that proper temperature promoted the growth of microbes, and further accelerated the degradation of muscle proteins. Therefore, the licorice extract showed significant effect in reducing the TVB-N formation by inhibiting microbe growth, and provided realistic basis for supporting the chicken breast storage.

3.5 Pearson correlation analysis

Pearson correlations between *P. bifermentans* spore counts and physicochemical parameters of RTE chicken breasts that smeared with licorice extract were shown in Figures 7A–D. *P. bifermentans* viable counts were showed significantly positive correlation ($P < 0.01$) with pH value, TBARS value, and TVB-N content when storage at 15, 20, and 25 °C, the metabolism of *P. bifermentans* promoted the protein hydrolysis and lipid oxidation of RTE chicken breast, and further led to the increase of corresponding indicators. At 4 temperatures, there were positive correlations ($P < 0.01$) among pH value, TBARS value, and TVB-N content; while the b^* of the RTE chicken breast showed negative relationship with pH value, TBARS value, and TVB-N content, this could be due to the oxidation of muscle protein by *P. bifermentans*. Therefore, the growth of *P. bifermentans* would affect the quality of chicken breast, while licorice extract could be used as natural antibacterial agent in preserving meat safety.

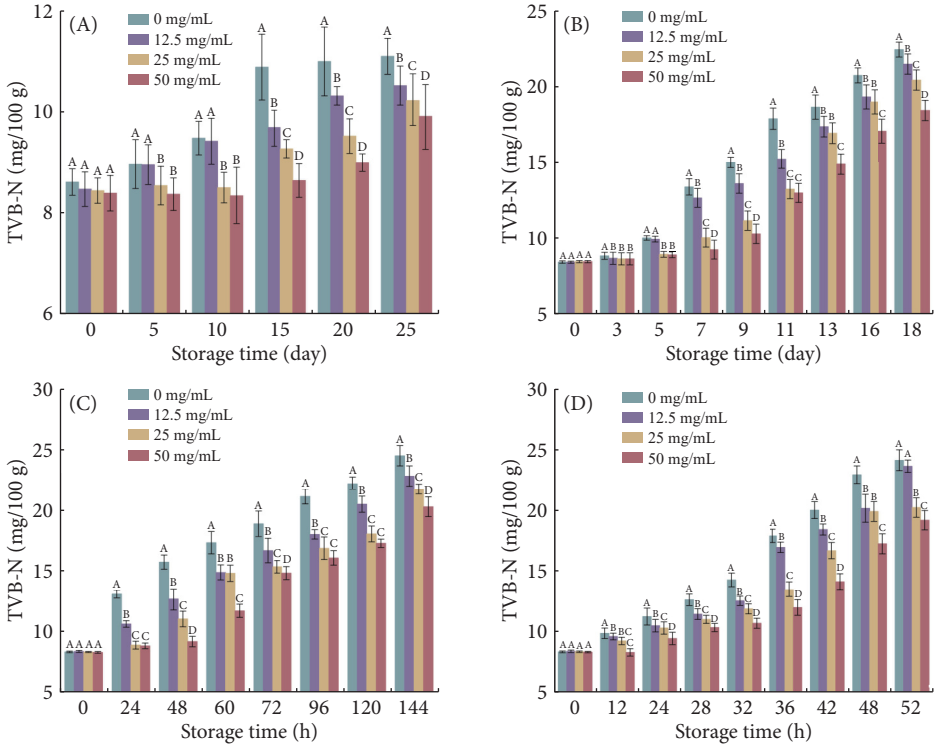


Figure 6 Effects of different concentrations of licorice extract on the TVB-N contents of the RTE chicken breast under storage at (A) 4, (B) 15, (C) 20, and (D) 25 °C. Different uppercase letters at the same time represent significantly difference ($P < 0.05$) among different concentration groups.

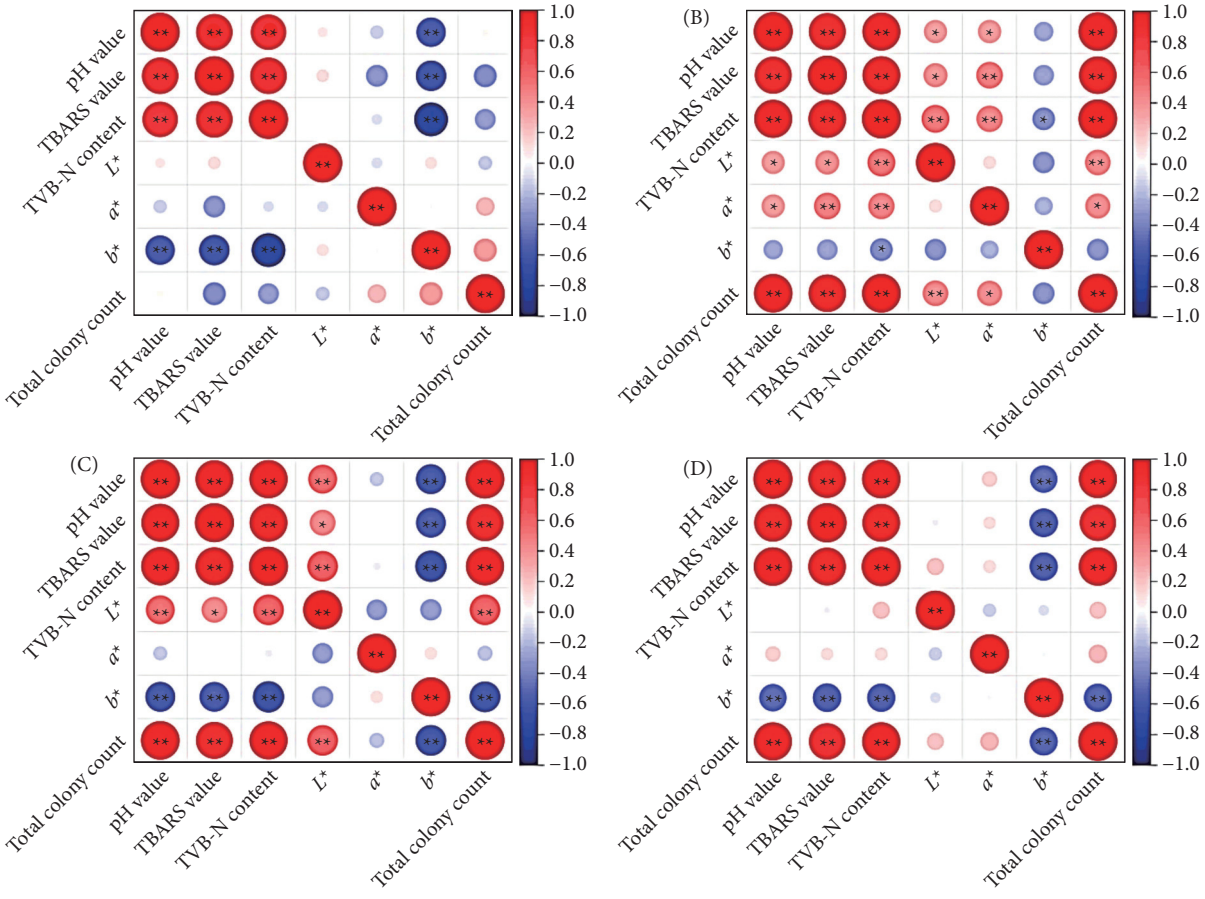


Figure 7 Correlation of microbiological and physicochemical parameters of the RTE chicken breast treated by different concentrations of licorice extract storage at (A) 4, (B) 15, (C) 20, and (D) 25 °C, respectively. * $P < 0.05$; ** $P < 0.01$.

4 Conclusion

Licorice extract showed significant inhibitory on the growth of *P. bifermentans* spores in RTE chicken breast. The inhibition effect was enhanced with the increasing concentrations of licorice extract across various temperatures. The Gompertz and Logistic models fitted well ($R^2 > 0.98$) with the growth curves of *P. bifermentans* spores stored at 15, 20, and 25 °C. Additionally, Ratkowsky model effectively described the relationships among temperature, $\sqrt{\mu_{max}}$, and $\sqrt{1/\lambda}$. These primary and secondary models may serve as valuable references for identifying critical control points during the industrial processing of chicken, as they provide help in optimizing storage conditions (temperature and time) to ensure the safety and quality of meat product. Across all storage temperatures, licorice extract effectively preserved the color of chicken breast, reduced pH value, and inhibited the increase of TBARS value, and TVB-N content. Consequently, licorice extract emerges as a promising natural antimicrobial agent capable of maintaining quality and extending the shelf life of RTE chicken breast.

Conflict of 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.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSAP.2025.9240115.

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