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Growth Modeling Kinetics of *Aspergillus flavus* in Dried Jujube at Different Temperatures

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Abstract

Dried jujube is a characteristic fruit of Xinjiang. Aspergillus is one of the main pathogens that causes mold on dried jujube, and *A. flavus* is a toxin-producing species, the aflatoxin produced by *A. flavus* is extremely toxic and carcinogenic. In this study, the growth kinetic models of *A. flavus* isolated from red jujube at different temperatures and times were fitted to Huang model and linear equation respectively, the Cardinal model was used to describe the growth rate and lag time of *A. flavus* on dried jujube agar. It turned out that 30–35 °C was the optimal temperature for growing *A. flavus*, so dried jujube should avoid storing in this temperature range. The kinetic model established in this study will help to understand the growth characteristics of *A. flavus*, and lay a foundation for evaluating the quality of stored dried jujube and predictions of shelf life, which are conducive to optimizing storage methods for dried jujube. It can be judged according to the value of A_f and B_f, the Huang model had a better fitting effect than the Baranyi model, The two models all had the highest growth rate at 35 °C, and *A. flavus* grew more vigorously and the lag period shortened as the temperature was increased. The secondary Cardinal model had a good fitting effect on the growth rate and lag time, and the secondary Ratkowsky model had a good fitting effect on the growth rate. This study may have theoretical and application value to strengthen the safety of jujube storage in the future.

Keywords: Primary growth model, secondary model, growth rate, lag time

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INTRODUCTION

Dried jujube is a characteristic fruit of Xinjiang, China, as Xinjiang's climate is suitable for growing jujube. Jujube is sweet and delicious, which can be eaten fresh or dried, and it is also valuable.[1] The planting area for Xinjiang jujube has increased rapidly in recent years, and it has become the fastest growing and most profitable industry in Xinjiang.[2-4] As Xinjiang jujube is nutritional rich, it is extremely vulnerable to contamination by harmful microorganisms, which causes various problems, such as an imbalance between sweet and sour, reduced nutritional value, and plant diseases during storage. During storage of jujube, mold on the surface of the jujube secretes pectinase and cellulase that break down cellulose, pectin, and other substances in the cell wall of the jujube into simple small molecules, and gradually invades the inside pulp. As a result, the protein, pectin, starch, organic acids and sugars in the jujube are destroyed and the jujube fruit rots.[5,6]

Aspergillus is one of the most important pathogens that causes mold of dried jujube. Several studies have isolated and identified mold strains on dried jujube in China. Chen and Shanawaer[7-10] reported that *Aspergillus* is the main fungal species on dried jujube, and Shanawaer et al[10] isolated *Aspergillus flavus*. *A. flavus* is a kind of toxin-producing fungi. Some strains of *A. flavus* produce aflatoxin. Aflatoxin is a metabolite of *A. flavus* and *Aspergillus parasiticus*, which not only poisons and kills livestock and poultry, but also causes carcinogenesis. Aflatoxin is a carcinogen. Aflatoxin has a damaging effect on liver tissues of humans and other animals. In severe cases, aflatoxin causes liver cancer and death. Therefore, it is of great significance to clarify the growth characteristics of *A. flavus* on dried jujube.

Research on dried jujube has mainly focused on physiological and biochemical aspects, storage conditions, and technical measures after harvest.[11,12] However, a predictive model of *A. flavus* has not been reported for dried jujube. Several mold models have been proposed. Peromingo et al[13] studied the effects of temperature and water activity on aflatoxin and built a growth model. Somjaipeng and Ta-Uea[14] studied the effects of temperature and water activity on the growth rate of *Aspergillus* in stored rice, and built a growth model. Marín et al[15] used the modified Gompertz model to assess the effects of temperature and water activity on the lag phase and generation time of some *Aspergillus* in corn extract medium. Sautour et al[16] used potato dextrose agar (PDA) plates to study the relationship between water activity and growth of several molds to build a model, and evaluated minimum water activity, optimal water activity, and maximum water activity. Pitt[17] proposed a model of the effects of environmental conditions on mold growth and toxin production by collating some literature data, and provided a theoretical explanation for the temperature change during toxin production. Yue et al[18] constructed a model of the effects of major ecological factors on the growth of *A. flavus* in stored corn. This model predicts the effects of temperature and water activity on the specific growth rate and lag time of *A. flavus*, and the equation obtained can be used to predict the growth of *A. flavus* in stored corn.

A microbial predictive model is used to predict changes in microorganisms in different environments and to prevent food safety accidents. In this study, the mold fungus *A. flavus* was isolated from dried jujube and purified. The growth of *A. flavus* in dried jujube was analyzed at different temperatures. Predictive microbiological methods were used for fitting primary and secondary models and for estimating their parameters. The results will provide data to control the growth of *A. flavus* on dried jujube and provide an *A. flavus* risk assessment.

METHODS

Jujube samples and growth medium

Twenty-eight Xinjiang dried jujube samples were purchased from a retail market in Shihezi and maintained in PDA and Rose Bengal agar (Qingdao Hi-Tech Park Haibo Biotechnology Co., Ltd., Qingdao, China).

Using laboratory-made jujube medium. A 250 g portion of washed dried jujube was added to 500 mL of distilled water and boiled for 20 min. The mixture was placed in a juicer with the cores removed and mixed until evenly viscous. Distilled water was added to 1000 mL. A 20 g portion of agar was added and the mixture was dispensed into a triangular bottle while hot and sterilized at 121 °C for 20 min (refer to PDA medium)

Chen et al[7]. The water activity of the jujube medium was 0.823 as measured with a water activity meter.

Fungal activation, isolation and purification

The surface of 28 dried jujube dried fruit samples was cleaned with 70% alcohol cotton balls. The diseased and healthy pulp of the jujube was cut out on an ultra-clean worktable using a sterile blade and shredded. A 25 g portion of dried jujube pulp was randomly selected from the 16 samples, and was placed in a sterile bag containing 225 mL of sterile potato dextrose water and shaken for 30 min. The suspension was placed in an incubator for 24 h, until the fungi were activated[7].

A 100 µL aliquot of the activated solution was added to Bengal Red medium and cooled to about 50 °C. A coating inoculation was applied with a coating rod. Each sample was repeated three times, and cultured in an incubator at 28°C for 5–7 d.

After the colony began to grow well on the plate, a small number of spores were selected on a "Z" type plate using the plate streak separation method[9,19]. A small amount of the fungal hyphae containing the sample was selected aseptically by regular streaks on the surface of Rose Bengal agar. The strains were purified and isolated several times, and finally a purified single colony was cultured at 28 °C.

Fungal preservation and preliminary identification

The pure colonies obtained after isolation and purification were stored under sterile conditions in single colony plate medium. A few spores were picked with an inoculating loop and inoculated on oblique PDA medium. The plates were cultured in an incubator at 28 °C for 2–3 d [13].

A small amount of mold mycelia was picked from the edge of the mold colony with a dissecting needle, and immersed in 50% ethanol for a few seconds to elute the spores. The mycelia were dispersed with a needle on a clean glass slide containing a drop of physiological saline (0.85% NaCl), covered with a cover glass, and observed under a microscope[20].

rDNA-ITS Sequencing

DNA was extracted using liquid nitrogen[21]. The mycelia were ground in liquid nitrogen in a precooled mortar and then transferred to a new 1.5 mL centrifuge tube. The lysate was added before thawing the mycelial powder for DNA extraction. Genomic DNA was extracted with a fungal DNA extraction kit[22].

By referring to the method of White et al[23] to amplify the rDNA-ITS sequence. The universal primers ITS1 (5'-GTAGTCATATGCTTGTCTC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used with genomic DNA as the template[10].

The polymerase chain reaction (PCR) system was 25 µL of PCR Taq Mix, 2 µL of upstream primer (10 µmol/L), 2 µL of downstream primer (10 µmol/L), 1 µL of genomic DNA, and 20 µL of ddH_2O to a final volume of 50 µL. The ITS gene PCR amplification program was pre-denaturation at 94°C for 4 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min for a total of 30 cycles, and a final extension at 72 °C for 10 min.

The amplified products were electrophoresed on a 1.5% agarose gel in 0.5× TBE running buffer. A gel imaging system was used to detect and record the displayed image of the amplified products on the agarose gel. The amplified products were purified and sent to the detection department for sequencing[10].

According to the microscopy and BLAST results, the mold species was identified as *A. flavus*.

Growth experiments

A. flavus was cultured on PDA medium in an incubator at 25 °C for 7 d. A mycelial suspension was prepared by submerging the surface of the plate medium in sterile 0.05% Tween-80 saline solution (0.9% NaCl) and scraping to remove the hyphae through a four-layered sterile gauze filter. The number of spores was counted with a blood cell counting plate, and the quantity of spores was adjusted to 10^7 spores/mL, and then to 10^3 spores/mL. Place a 4 mm groove in the center of the jujube medium with a pipette tip, shake well when taking samples, take 40 µL of the adjusted spore suspension, and point to the center of the plate[24].

The jujube medium was placed in a constant temperature incubator at 20, 25, 28, 30, 35, and 37 °C for at least 120h, and diameters were measured every 12 h with a Vernier caliper[24]. Samples were run in three groups in parallel and data were recorded.

Primary growth model

Using the United States Department of

Agriculture (USDA) IPMP 2013 software, three primary growth models, the Huang model and the Baranyi model, were used to fit the growth of *A. flavus* on dried jujube at different temperatures and to obtain the corresponding model fitting parameters.[25]

$$Y_{(t)} = Y_O + Y_{max} - \ln\left[e^{Y_O} + (e^{Y_{max}} - e^{Y_O}) \times e^{-\mu_{max}B_{(t)}}\right] \quad ...(1)$$

$$B_{(t)} = t + \frac{1}{\alpha} ln \frac{1+e^{-\alpha(t-\lambda)}}{1+e^{\alpha\lambda}} \quad ...(2)$$

Where $Y_{(t)}$ is the diameter of *A. flavus* (mm) at time t; Y_0 is the initial diameter of *A. flavus* (mm); Y_{max} is the maximum diameter (mm) reached by *A. flavus*; μ_{max} is the maximum specific growth rate of *A. flavus* (mm/h); λ is the lag period (h); and α is the lag phase transition coefficient of 4.00.

Baranyi model[26]

D_0, D_{max}, $D_{(t)}$ are the bacterial population, in natural logarithm of microorganism counts, at initial, maximum, and time t. μ_{max} is the specific growth rate. h_0 is the physiological state of the microorganism under consideration. All four parameters, including Y_0, Y_{max}, h_0, and μ_{max}, will be estimated using nonlinear regression.

$$D(t) = D_0 + \mu_{max}A(t) - ln\left\{1 + \frac{exp[\mu_{max}A(t)]-1}{\exp(Y_{max}-Y_0)}\right\} \quad ...(3)$$

$$A(t) = t + \frac{1}{\mu_{max}} ln[\exp(-\mu_{max}t) + \exp(-h_0) - \exp(-\mu_{max}t - h_0)] \quad ...(4)$$

Secondary model

Using USDA IPMP 2013 software, the secondary growth model was used to fit the growth of *A. flavus* on dried jujube at different temperatures.[27]

$$\mu_{max} = \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{[(T_{opt}-T_{min})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)](T_{opt}-T_{min})} \quad ...(5)$$

$$\frac{1}{\lambda_{max}} = \frac{(\frac{1}{\lambda_{opt}})(T-T_{\max})(T-T_{min})^2}{[(T_{opt}-T_{min})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)](T_{opt}-T_{min})} \quad ...(6)$$

Where μ_{max} is the maximum growth rate at each temperature T, μ_{opt} is the optimal growth rate at the optimal temperature (T_{opt}), and T_{min} and T_{max} are the minimum growth temperature and the maximum growth temperature. The Cardinal model is only suitable for the full temperature range. In Eq. (5), λ_{max} is the maximum lag time at each temperature T, and the others are the same as in Eq. (4).

Full temperature range Ratkowsky square-root model.[28]

$$\sqrt{\mu} = a(T - T_{min})\left[1 - e^{b(T-T_{max})}\right] \quad ... (7)$$

In this equation, μ s the growth rate (time-1); a and b are coefficients; T is temperature; T_{min} is the nominal/notational minimum temperature; T_{max} is the estimated maximum growth temperature.

***B_f*, *A_f*, RMSE values of the models**

The bias factor (B_f) can be used to determine the structural deviation of a model; that is, the degree of underestimation or overestimation of the estimation. The accuracy factor (A_f) can be used to determine the accuracy of the model, and the root mean square error (RMSE) is used to evaluate the change in the data error[29]. The expressions are as follows:

$$B_f = 10^{\frac{\sum \lg(\frac{N_{predicted}}{N_{observed}})}{n}} \quad ... (8)$$

$$A_f = 10^{\frac{\sum\left|\lg(\frac{N_{predicted}}{N_{observed}})\right|}{n}} \quad ... (9)$$

$$RMSE = \sqrt{\frac{\sum(N_{observed}-N_{predicted})^2}{n}} \quad ...(10)$$

Where $N_{predicted}$ and $N_{observed}$ are the predicted and observed growth diameters (mm) of *A. flavus*, respectively, and n is the number of samples.

RESULTS AND DISCUSSION

Modeling primary growth of *A. flavus*

Fitting of the first growth model of *A. flavus*

The experimental data of *A. flavus*

growth on jujube medium showed typical mould growth curves, with clear adaptation time and linear growth phases, absence of stationary growth phase (during the evaluated time), and temperature-dependent growth rates. For the higher temperatures (25,28, 30, 35 and 37 °C), the maximum diameter of the plate was reached by the *A. flavus,* whereas at 20 °C, no stationary phase was observed over the experimental time.

All the evaluated primary models were able to describe very well the growth of *A. flavus* on jujube medium. The growth rate (μ_{max}) and lag time (λ) of *A. flavus* were determined from the primary growth models, as shown in Table 1. The B_f and A_f values for the Huang and Baranyi models fitted to the experimental data at 20, 25, 28, 30, 35 and 37 °C are shown in Table 2. The typical mold growth characteristics have important reference significance. The two models all had the highest growth rate at 35°C. And *A. flavus* grew more vigorously and the lag period shortened as the temperature was increased.

According to the analysis in Table 1, the primary Huang model for *A. flavus* on jujube medium had the best estimation effect, the B_f values of the Huang model were 0.941–0.993, and the A_f value were 1-1.124. The fitting effect of the Baranyi model was not as good as that of the Huang model.

Secondary modeling of *A. flavus*

Fitting of the secondary model of *A. flavus*

Fig. 2 showed the effect of the temperature on the growth of *A. flavus* on dried jujube medium after fitting the second-level models. According to Fig. 2, the growth rate of *A. flavus* was maximum at 30–35 °C, the reciprocal lag time tended to increase with increasing temperature.

Tables 3 and 4 showed the parameters from fitting of the secondary model. According to Table 3, the two different primary Huang and Baranyi models were fitted to obtain the secondary Cardinal and Ratkowsky model. For the fitting of Huang model to Cardinal model, the optimal growth temperatures was 33.31°C, the best growth rates was 4.687. For the fitting of Baranyi model to Cardinal model, the optimal growth temperatures was 32.790°C , the best growth rates was 3.992. According to Table 5, for the fitting of Huang model to Cardinal model, the optimal growth temperatures was 37.941 °C , the best reciprocal lag time was 0.054. For the fitting of Baranyi model to Cardinal model, the optimal growth temperatures was 39.356°C , the best growth rates was 0.032, as shown in Table 5. The

Table 1. Values of parameters μ_{max} and λ for the Huang and Baranyi models fitted to the growth data of *A. flavus* diameter on jujube at 20, 25, 28, 30, 35 and 37 °C

Parameter	Model	Temperature					
		20	25	28	30	35	37
μmax	Huang	0.6170	1.027	1.641	1.855	1.912	1.408
	Baranyi	0.735	1.233	1.487	1.650	1.649	1.374
λ	Huang	118.3	57.73	57.38	43.77	50.20	38.79
	Baranyi	136.054	81.103	47.381	22.957	26.187	14.887

Table 2. B_f and A_f values of the fitting of the Huang and Baranyi models to the growth data of *A. flavus* diameter on jujube at 20, 25, 28, 30, 35 and 37 °C

Statistical Index	Model	Temperature					
		20	25	28	30	35	37
Bf	Huang	0.989	0.992	0.941	0.98	0.98	0.993
	Baranyi	1.014	0.988	0.916	1.053	0.959	1.002
Af	Huang	1.078	1.047	1.087	1.061	1.124	1.086
	Baranyi	1.173	1.204	1.222	1.12	1.346	1.107

smaller the RMSE, and the better the fit. Therefore, the primary model and the secondary model had better fits.

In this study, *A. flavus* was isolated on dried jujube, and the effects of *A. flavus* on stored dried jujube at different temperatures (20, 25, 28, 30, 35, and 37 °C) were studied. The growth of *A. flavus* on stored dried jujube changed with temperature. *A. flavus* growed fastest in jujube medium at 35 °C. *A. flavus* displayed the maximum growth rate at 30–35 °C.

The Huang model and the Baranyi model were used to obtain the first-level growth model of *A. flavus* on the dried jujube medium

Table 3. T_{min}, T_{max}, T_{opt}, μ_{opt} and RMSE values of *Aspergillus flavus* on jujube medium by fitting the Cardinal model

Model	Parameter				
	T_{min} (°C)	T_{max} (°C)	T_{opt} (°C)	μ_{opt} (mm/h)	RMSE
fitting of Huang model to Cardinal model	10.29	38.86	33.31	4.687	0.2860
fitting of Baranyi model to Cardinal model	6.095	40.53	32.79	3.992	0.02700

Table 4. T_{min}, T_{max} and RMSE values of *A. flavus* on jujube medium by fitting the Ratkowsky model

Model	Parameter				
	T_{min} (°C)	T_{max} (°C)	a	b	RMSE
fitting of Huang model to Ratkowsky model	15.79	38.83	0.3020	0.3840	0.3370
fitting of Baranyi model to Ratkowsky model	13.05	40.38	0.2450	0.2290	0.03000

Table 5. T_{min}, T_{max}, T_{opt}, $1/\lambda_{opt}$ and RMSE values of *A. flavus* on jujube medium by fitting the secondary model

Model	Parameter				
	T_{min} (°C)	T_{max} (°C)	T_{opt} (°C)	$1/\lambda_{opt}$ (1/h)	RMSE
fitting of Huang model to Cardinal model	15.199	60.683	37.941	0.054	0.008
fitting of Baranyi model to Cardinal model	12.029	39.354	39.356	0.166	0.032

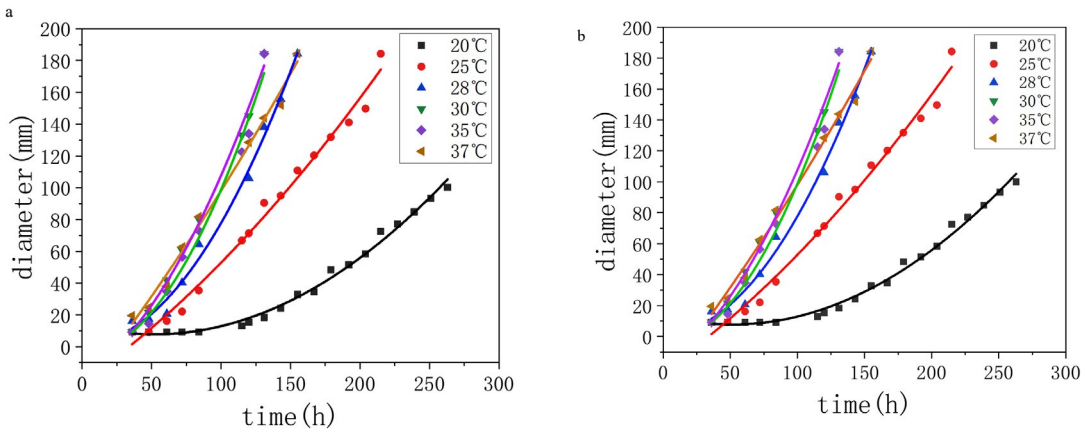

Fig. 1. Growth curve of *A. flavus* on jujube medium by fitting the model a, Huang model; b, Baranyi model.

at different temperatures. The parameters of the first-level growth model were compared to obtain the maximum specific growth rate μmax (mm/h) and the lag period λ (h), fitting factors A_f and B_f based on the Huang and Baranyi model at different temperatures. Both the Huang model and the Baranyi model were suitable for describing the growth of *A. flavus* on dried jujube, and the Huang model fitted the data better, the fitted model was in good agreement with the actual growth curve. The growth rate μmax (mm/h) and lag time λ (h) of *A. flavus* at different temperatures was fitted with the first-level model through a second-level model, and the effect of temperature on the growth of *A. flavus* on dried jujube was established. The secondary Cardinal model indicated the optimal growth temperature T_{opt} (°C) and the optimal growth rate μ_{opt} (mm/h). The

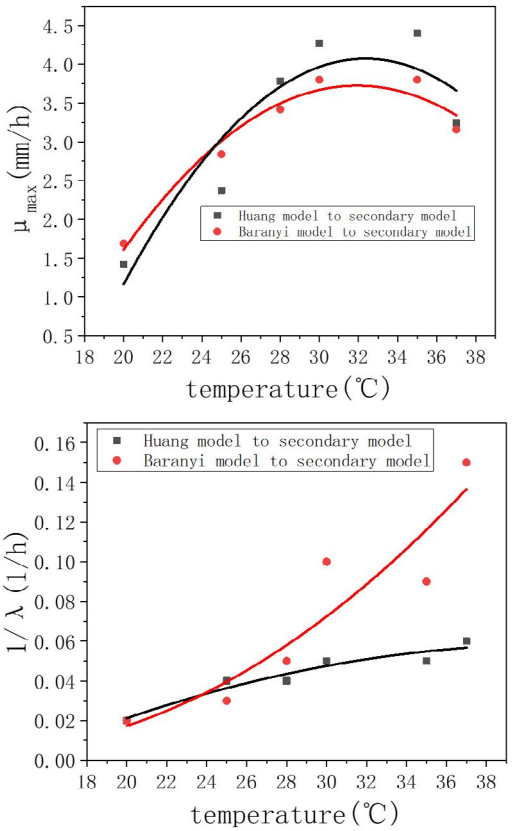

Fig. 2. Effect of temperature (T) of *A. flavus* on jujube medium on the growth rate (μ_{max}) and the reciprocal lag time (1/λ) by fitting the secondary model fitted by the primary model.
Note: secondary: Cardinal model / Ratkowsky model

optimal growth temperatures of the secondary Cardinal model fitted by the two primary models (Huang model and Baranyi model) were 33.31 and 32.79 °C, and the optimal growth rates were 4.687 and 3.992, respectively; The secondary Cardinal model indicated the optimal growth temperature T_{opt} (°C) and the optimal inverse lag time 1/λ (1/h). The optimum growth temperatures as indicated by the secondary Cardinal model fitted by the two different primary models (Huang model and Baranyi) were 37.941 and 39.356°C, and the best inverse lag times were 0.054 and 0.166, respectively. The temperature of the model affected the growth rate of *A. flavus*. The secondary Cardinal model obtained by the primary Huang model had the best fit for the inverse lag time 1/λ in the temperature models. To sum up, *A. flavus* in dried jujube had an optimum growth temperature of 30–35°C, as predicted by the primary and secondary models, so dried jujube should be avoid storing in this temperature range.

The assessed model and the jujube medium used in this study were suitable to describe the effect of temperature on growth of mold in jujube. Previous studies have isolated and purified molds, but few studies have researched mold in dried jujube or studied mold dynamics by constructing a models for dried jujube. In this study, a kinetic model was constructed by measuring the growth diameters of the fungus. Studies on mold growth and models are just beginning[24]. In the present study, *A. flavus* was modeled on dried jujube and verified separately. Performance of the model was good, indicating its suitability for predicting the effect of temperature on growth of mold in dried jujube.

CONCLUSIONS

Aspergillus is one of the main pathogens that causes mold on dried jujube, and *A. flavus* is a toxin-producing species. The aflatoxin produced by *A. flavus* is extremely toxic and carcinogenic. The kinetic model established in this study will help to understand the growth characteristics of *A. flavus*, and lay a foundation for evaluating the quality of stored dried jujube and predictions of shelf life, which are conducive to optimizing storage methods for dried jujube. This study may have theoretical and application value to strengthen the safety of jujube storage in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both the authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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