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# Prediction of Nitrite Content of Pickled Okra with *Lactobacillus plantarum* Using Sensors and by Numerical Simulation Based on Artificial Intelligence

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Numerical simulation methods are combined with experimental data to predict the completion of fermentation processing. Through the control of fermentation process parameters, numerical simulation, and model estimation, the nitrite content is reduced to ensure product safety. In this study, using fresh okra as the primary raw material, the effect of Lactobacillus plantarum fermentation on the nitrite content of pickled okra was studied. Sensors for measuring temperature and concentration and a data collection system based on Raspberry Pi were set up, and the optimal fermentation process parameters for controlling and reducing the nitrite content were found by artificial intelligence simulation. The results showed that compared with natural fermentation, the nitrite content of pickled okra produced by Lactobacillus plantarum fermentation was lower and the maturity period was shorter. For the theoretical optimal parameters (inoculation with 3.6% LP, fermentation temperature of 20 °C, fermentation time of 2.6 days, and saltwater concentration of 7.8%), the average nitrite content of pickled okra was 2.2 mg/kg, which was lower than the national limit of nitrite in pickled vegetables ( $\leq 20$  mg/kg). These results suggest that Lactobacillus plantarum fermentation should be used for the production of pickled okra. This study provides a reference for the advantages of Lactobacillus plantarum fermentation in the industrial production of pickled okra.

# 1. Introduction

Many machine learning models use a multilayer perceptron classifier (MLP) as the kernel. An MLP has multiple neurons, each of which is activated using a set of activation functions such as the ReLU, sigmoid, and tanh functions. MLP was a state-of-the-art multiple regression model with kernels. Many researchers have used the regression MLP kernel as an estimation model in machine learning. Krithik *et al.*<sup>(1)</sup> reported a novel MLP model that improved a mixture model of a random forest classifier with a multilayer perceptron regressor. The model was used for real-

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time rainfall prediction and the prediction accuracy was improved to 0.92537. In addition, Maheshwari and Lamba<sup>(2)</sup> compared the differences in estimates of honey production among different regression algorithms. Mahajan and Kotecha<sup>(3)</sup> demonstrated a hybrid model for network traffic prediction and wireless mesh networks. They also compared the prediction results of several different algorithms. Maheshwari and Lamba<sup>(2)</sup> used the MLP model to predict air quality. Their experimental results indicated the accuracy of the model. Aboubakar *et al.*<sup>(4)</sup> used different algorithms to compare power consumption estimation models and verified the predictive performance and accuracy of different models from experimental values.

Okra is a tropical to subtropical crop that is sensitive to frost and prone to browning, fiber aging, and loss of food value.<sup>(5)</sup> Okra is also known as lady's finger, coffee yellow sunflower, green ginseng, and beauty finger.<sup>(6–8)</sup> Okra pods and okra seeds are rich in polyphenolic compounds, mainly composed of flavonol derivatives and oligocatechins, including rutin, catechin, and epicatechin. The unique viscous substances in okra pods are composed of fibers and polysaccharides such as water-soluble pectin, galactan, and gum arabic, which make the meat tender and smooth in taste and give food a special flavor. Okra stimulates the central nervous system and accelerates blood circulation,<sup>(9)</sup> promotes metabolism, and can combine with cholesterol and bile acids to promote the transfer of harmful substances into the liver for metabolism.<sup>(10)</sup> As a dietary vegetable, okra has the functions of strengthening the stomach, protecting the liver, strengthening the kidneys, anti-oxidation, lowering blood lipids, and lowering cholesterol.<sup>(11)</sup> Okra has long been considered to have special nutritional value.<sup>(12,13)</sup>

Nitrite is an anion widely distributed in the nitrogen cycle. Nitrite compounds are found in water, soil, microorganisms, plants, and animals.<sup>(14)</sup> Recent research has indicated that nitrite is harmful, causing fatal methemoglobinemia upon its consumption and having possible links to some human cancers.<sup>(15,16)</sup> Nitrite compounds are also added as preservatives in some foods, such as cured meats, exposing consumers to health risks.<sup>(17,18)</sup> Nitrite is often present in pickled vegetables. Nitrite has been found to have adverse effects on health, including changing the normal form of hemoglobin and promoting the formation of carcinogenic nitrosamines. Changes in the nitrite content in pickled vegetables likely result from differences in the characteristics, processing technique, and storage condition of the pickled vegetables.<sup>(19)</sup>

Fermented food has a long history, low production cost, and simple production process. Most traditional homemade production is based on a natural fermentation process.<sup>(20)</sup> This process occurs in a complex microbial ecological environment, which has a long production cycle and also cannot guarantee the quality and safety of products.<sup>(21,22)</sup> The nitrite content in fermented foods remains high, which has been a food safety issue and a focus of consumer attention, limiting the use of the traditional approach in fermented food production.<sup>(23,24)</sup> A previous study showed how processing vegetables by fermentation or acidification may affect the nitrite content:<sup>(25)</sup> during the fermentation of vegetables, the nitrite content decreased and then reached a stationary level, whereas the nitrite content initially increased and then decreased when the pH was lower than 4.5.<sup>(26,27)</sup>

In this study, okra was used as the main raw material, which was fermented by artificial inoculation combined with a natural fermentation process, and *Lactobacillus plantarum* (LP) fermentation was used to produce pickled okra, to study the changes in nitrite content, and to

study the effect of fermentation conditions on the nitrite content. Through control of the fermentation process parameters, numerical simulation, and model estimation, the nitrite content was reduced to ensure product safety. The research provides a theoretical basis for the industrialized and standardized production of nutritious, safe, and healthy pickled okra. In addition, a simple and low-cost okra processing and storage method for farmers is reported.

# 2. Materials and Methods

# 2.1 Preparation of samples

Fresh okra, salt, sugar, Chinese prickly ash, chili, ginger, and garlic were purchased from a local supermarket in Longyan city, China. The fresh okra was washed and kept at 4 °C before further use. The sodium borate, sodium nitrite, zinc acetate, potassium ferrocyanide, p-sulfanilic acid, naphthalene ethylenediamine hydrochloride, and glacial acetic acid used in this study were all AR grade. The strain LP CICC 21805 was purchased from Shanghai Biotechnology Center. LP was activated then transferred into 10 mL of de Man, Rogosa, and Sharpe (MRS) liquid medium and expanded for 2–3 days at 37 °C. The number of viable bacteria in the fermented seed liquor was required to reach 10<sup>8</sup>–10<sup>9</sup> CFU/mL. To effectively control the entire fermentation process, sensors for measuring temperature and concentration and a data collection system based on Raspberry Pi were set up. The final data were different factors and nitrite contents. We converted the formula based on the results of the sensor to calculate the nitrite content.

# 2.2 Effects of LP on nitrite content in pickled okra

Our experiment was divided into two groups. In one group, the expanded LP culture medium with a bacterial concentration of 10<sup>8</sup> CFU/mL was inoculated. The other group was a natural fermentation group without lactic acid bacteria (LAB) under the same conditions, which was used as the control group (CK). Each group contained the same amounts of auxiliary materials (prickly ash, pepper, ginger, and garlic) and fermentation brine (salt 6%, sugar 2%, cooking wine 2%). The fermented okra was left at room temperature, and its nitrite content was measured every 24 h.

# 2.3 Determination of nitrite content in pickled okra using naphthalene ethylenediamine hydrochloride method

On the basis of the approach of Ding *et al.*<sup>(28)</sup> and the guidelines for naphthalene ethylenediamine hydrochloride in China National Quality Standard (GB/T 5009.33-2003: *Determination of nitrite and nitrate contents in food*), we investigated the effect of the amount of added naphthylethylenediamine hydrochloride on light absorbance. We weighed 5 g of each pickled okra sample, then placed it in a 200 mL stoppered flask and added 80 mL of water and 1 mL of 1 mol/L potassium hydroxide solution, then ultrasonically extracted and mixed the substances obtained from pickled okra for 30 min. Then, we agitated the mixture every 5 min to

ensure that the solid phase remained dispersed and placed it in a water bath at 75 °C for 5 min. Then, we removed the mixture from the water bath and allowed it to cool to room temperature, transferred it to a 100 mL volumetric flask, added water to the 100 mL mark to dilute it, and mixed the solution well. After the solution was filtered with filter paper, part of the solution was centrifuged at 10000 rpm/min for 15 min, and the supernatant was taken for later use. We placed 0.40 mL of sodium nitrite standard solution and 2.00 mL of sulfanilic acid in a 25 mL colorimetric tube. We then added 0.60 mL of hydrochloric acid, 0.60 mL of naphthalene, and 1.0 mL of ethylamine. After standing for 15 min, the standard solution without sodium nitrite was used as the blank control. Then, the absorbance was measured at 538 nm under the experimental conditions.

# 2.4 Control of content, numerical simulation, and parameter prediction for pickled okra

The MLP is a supervisory learning approach that identifies the similarity between the input and output after training. The weights and bias are updated using the backpropagation algorithm, and the errors are measured as the root mean square error (RMSE). The MLP output depends on the strength of interconnections as shown in Eq. (1).

$$y = \varphi\left(\sum_{i=1}^{n} w_i x_i + b\right) = \varphi\left(w^T x + b\right)$$
(1)

Here, w denotes the vector of weights, x is the vector of inputs, and b is the bias.

In this study, the hyperparameters used in the algorithm were as follows: 64 hidden layers, the ReLU function used as the activation function of each hidden layer, the Adam optimizer used as a solver, and a maximum of 2000 iterations.

# 3. Results and Discussion

#### 3.1 Effect of LP on nitrite content in pickled okra

In this study, the LP was inoculated with fermented pickled okra, and naturally fermented okra was used as the CK. Figure 1 shows that the maximum nitrite content was reached on the fourth day after fermentation for the CK group. Compared with the LP group, the nitrite peak for the CK group appeared later and the nitrite peak was higher. The main reason was that traditional fermented food takes longer to ferment. The nitrite peak for the LP group appeared on the second day after fermentation and then decreased rapidly. Figure 1 shows that the nitrite content of the CK group after 7 days is still higher than that of the LP group at 4 days. It can be seen that LP fermentation can make the nitrite peak appear earlier, shorten the fermentation period, and reduce the amount of nitrite.<sup>(29)</sup>

The result in Fig. 1 demonstrated that the content of nitrite in food was related to different fermentation groups. LP are common bacteria in the natural fermentation of food and are considered to be safe in probiotic foods. Their metabolites impart aromatic flavor and good taste



Fig. 1. (Color online) Changes in nitrite content in pickled okra for different fermentation groups.

to foods and lower the pH of the fermentation environment, thereby inhibiting the growth of harmful bacteria. There is also an ideal strain for naturally degrading nitrite.<sup>(29)</sup> Many studies have shown that pure LP fermentation produces a lower nitrite content than natural fermentation. Because LP metabolizes to produce enzymes, acids, and other substances, which change the fermentation environment, nitrite can be efficiently degraded. Moreover, LP can shorten the maturity period of fermented vegetables, inhibit the growth of miscellaneous bacteria, and change the sensory quality of fermented vegetables, thereby improving the overall quality of fermented vegetables.<sup>(30,31)</sup> Therefore, the result illustrated that the main reason for inoculation in LP fermentation is to make LP the dominant bacteria from the beginning of fermentation, which not only inhibits the growth of other miscellaneous bacteria but also reduces the pH value of the lactic acid generated, even if nitrite is produced. Moreover, the LP reduced the nitrate content, and the degradation of nitrite can also be accelerated under acidic conditions.<sup>(32)</sup>

# **3.2** Effects of inoculum concentration and fermentation time of pickled okra fermented with LP on nitrite content

Under the same conditions as when using the fermented brine formula (salt 6%, sugar 2%, cooking wine 2%, appropriate amounts of spices), we inoculated 1, 2, 3, 4, and 5% of expanded LP culture solution ( $10^8$  CFU/mL) that had been fermented at 25 °C for 4 days. Figure 2 indicates that a low inoculum concentration resulted in the low ability of LP to degrade nitrite and a high residual nitrite content in the product. When the inoculum concentration was  $\geq 3\%$ , LP degraded the nitrite. Upon smelling and tasting the fermented pickled okra, we found that flavor development was also influenced by the microbial populations during the ripening of the fermented pickled okra. The primary contribution of LAB to flavor development has been attributed to the generation of organic acids.<sup>(33,34)</sup> When the inoculum concentration reaches a certain limit, the nitrite content no longer decreases; thus, the inoculation of 2 to 3% expanded LP culture solution is preferable. Essid and Hassouna<sup>(35)</sup> found that the inoculum concentration also affects the flavor of samples. When the inoculum concentration was too small, the product



Fig. 2. Effect of inoculum concentration on nitrite content in pickled okra.

was insufficiently sour and the taste and aroma were poor. When the inoculum concentration was too large, the product was too sour and the flavor was inconsistent.<sup>(36–40)</sup>

# 3.3 Effects of fermentation temperature of LP-fermented pickled okra on nitrite content

Under the same conditions as the fermented brine formula (salt 6%, sugar 2%, cooking wine 2%, appropriate amounts of spices), we inoculated 3% LP expansion medium ( $10^8$  CFU/mL). Fermentation was carried out at 25 °C for 1, 2, 3, 4, 5, and 6 days. It can be seen from Fig. 3 that the nitrite content of pickled okra peaked after 2 days of fermentation. When the fermentation exceeded 4 days, the rate of decrease in the okra content became slow.<sup>(41,42)</sup> Therefore, it can be seen from the experimental results that, considering the cost of the production cycle, a fermentation time of 4–5 days is appropriate.<sup>(43,44)</sup>

Using the same fermented brine formula (salt 6%, sugar 2%, cooking wine 2%, appropriate amounts of spices), we inoculated 3% expanded LP culture medium ( $10^8$  CFU/mL) and carried out fermentation at 20, 25, 30, 35, and 40 °C for 4 days. The experimental results in Fig. 4 show that when the fermentation temperature was 20 °C, the slow growth of LP led to a low cell number, low acid production, little inhibitory effect on bacteria, and low ability to degrade nitrite, and the nitrite content in the final pickled okra was high.<sup>(45)</sup> When the temperature was 30 and 35 °C, the growth and reproduction of LAB reached a reasonable speed, and the nitrite content was significantly reduced. A high temperature inhibited the growth of LP. An ideal fermentation temperature is 30-35 °C.<sup>(46)</sup>

In the metabolic process, the degradation of nitrite by LAB is mainly reflected in the production of lactic acid and a series of enzymes. At the same time, when LAB become the dominant bacteria, they can inhibit the growth of other miscellaneous bacteria, thereby inhibiting the regeneration of nitrite.<sup>(47)</sup> Nitrite degradation methods are mainly divided into physical degradation, chemical degradation, and biological degradation.<sup>(48)</sup> The physical degradation method mainly involves high-temperature treatment, which can not only inhibit the



Fig. 4. Effect of fermentation temperature on nitrite content in pickled okra.

activity of nitrate reductase in plants but also kill nitrite-producing and nitrate-reducing bacteria.<sup>(49)</sup> Chemical degradation mainly involves adding antioxidants, such as ascorbic acid and erythorbic acid. The chemical method has a strong ability to degrade nitrite; however, the antioxidants used are easily oxidized, making this method not conducive to application in complex food systems.<sup>(49)</sup> Biodegradation of nitrite is an efficient and healthy method, and the most effective microorganisms for nitrite degradation are LAB, mainly including LP.<sup>(50)</sup>

### 3.4 Effects of salt water concentration on nitrite content in pickled okra fermented by LP

We performed an experiment to evaluate the production components of okra subjected to different levels of salt water and to evaluate the preservation by lactic fermentation of okra produced under salt water. As shown in Fig. 5, the pickled okra was inoculated with a 3% expanded LP culture solution (10<sup>8</sup> CFU/mL) and fermented at 25 °C with the same formula of fermented brine as before apart from the varied salt water concentration (sugar 2%, cooking



Fig. 5. Effect of salt water concentration on nitrite content in pickled okra.

wine 2%, appropriate amounts of spices) for 5 days to investigate the effect of the salt water concentration (0, 2, 4, 6, 8, 10, and 12%) on the nitrite content in pickled okra. As can be seen from Fig. 5, the nitrite content decreased as the salt water was increased from 0 to 4% in fermented pickled okra. A large number of miscellaneous bacteria grew and multiplied faster than LP and secreted nitrate reductase, resulting in a high nitrite content in the fermented pickled okra. At a salt water concentration of 4–8%, the bacteria were inhibited from producing nitrite.<sup>(51)</sup> Therefore, our results suggest that a suitable salt water concentration is 4–8%, at which LP reduced the nitrite content in the fermented pickled okra. In the experiment, the okra was unsalted, and no lactic fermentation occurred. However, under appropriate brine and fermentation conditions, the nitrite content was effectively reduced in the pickled okra fermented by LP.<sup>(52)</sup>

#### 3.5 Numerical simulation and model estimation of pickled okra fermented by LP

In the production of pickled okra, the nitrite content can be estimated from the interaction between the inoculum concentration, fermentation temperature, fermentation time, and saltwater concentration. Because it is impractical to examine all the combinations of these four parameters, we performed a numerical simulation and model estimation to establish a model for predicting the nitrite content for different combinations of the parameters and for estimating their optimal values.

The results of the numerical simulation are shown in Fig. 6. Using the cubic spline fitting method, all possible results within the range of the experimental values were inversely estimated by numerical simulation. Five hundred data points in the concentration range (x axis) were used to estimate the nitrite content (y axis), which was consistent with the inoculum concentration plot in Fig. 2. In the same way, 500 data points each for the other three factors were obtained. If the estimated original 2000 data points had been directly used for modeling, the results would not have been ideal because the correlation between the four factors had not been established. Therefore, numerical simulations were estimated on a per-parameter basis, so different



Fig. 6. (Color online) Numerical simulation results of cubic spline method for inoculum concentration.

Out[34]: 6250000

Fig. 7. (Color online) Calculation process.

parameters had different nitrite values. However, the model used the same nitrite concentration value to correspond to the four parameters. Moreover, the four parameters must be corrected for the nitrite concentration before model training. After recalculating the data, the data volume became 1303 points. In this study, the MLP regressor was used as the core of the model estimation. After nearly 2000 calculations, the R<sup>2</sup> value of the model was 0.99, which means that the parameter fit of the model was very high. The calculation process is shown in Fig. 7. To find

Out[34]:	6250000
In [35]:	<pre>xmatrix = np.array(testv) xmatrix.shape xtranslated = sc_X.transform(xmatrix) xtranslated</pre>
Out[35]:	array([[-0.59830413, 0.74626876, -0.03374551, 1.07986447], [-0.59830413, 0.74626876, -0.03374551, 1.11346654], [-0.59830413, 0.74626876, -0.03374551, 1.11346654], [.2.83092733, 5.71644461, 2.89572501, 2.65916206], [ 2.83092733, 5.71644461, 2.89572501, 2.69276413], [ 2.83092733, 5.71644461, 2.89572501, 2.72636621]])
In [36]:	<pre>y_pred=reg.predict(xtranslated) y_pred</pre>
Out[36]:	array([4.20719158, 4.18432588, 4.16146018,, 4.76748182, 4.79893274, 4.82786565])
In [37]:	<pre>#min_value = min(y_pred) # Get the indices of minimum element in numpy array result = np.where(y_pred == np.amin(y_pred)) print('Returned tuple of arrays :', result) print('List of Indices of minimum element :', result[0]) print('result:',y_pred[result[0]]) print('the matrix value:',xmatrix[result[0]]) #salt—*amount-*ferm_time—*ferm_temper</pre>
	Returned tuple of arrays : (array([4080800]),) List of Indices of minimum element : [4080800] result: [2.19875902] the matrix value: [[ 7.83673469 3.6122449 2.63265306 20. ]]

Fig. 8. (Color online) Optimal parameters for model estimation.

the best combinations of the experimental parameters, four loops were used for each parameter, and the nitrite content was predicted by the model using parameters. It can be seen from Fig. 7 that there was a total of 6250000 different combinations of parameters, which were input to the model to calculate the lowest nitrite content. As shown in Fig. 8, the optimal conditions obtained from the numerical simulation were an inoculum concentration of 3.6%, a fermentation temperature of 20 °C, a fermentation time of 2.6 days, and a saltwater concentration of 7.8%, for which the nitrite content was 2.2 mg/kg. However, it is necessary to verify the difference between this result and the experimental value.<sup>(53)</sup>

# 4. Conclusions

The research combined numerical simulations and actual fermentation experiments. An MLP kernel-based model was proposed to estimate the optimal parameters for the fermentation process. The use of LP to ferment pickled okra not only advanced the nitrite peak and shortened the fermentation period, but also reduced the nitrite content. To ferment pickled okra using LP and reduce the production of nitrite, we found that the theoretical optimal fermentation parameters were an inoculum amount of 3.6%, a fermentation temperature was 20 °C, a fermentation time of 2.6 days, and a saltwater concentration of 7.8%. The average nitrite content of pickled okra was 2.2 mg/kg, which was lower than the national limit of nitrite in pickled vegetables (20 mg/kg). Through future experiments, we plan to further automate the control of the fermentation process.

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