

Original Research Paper

Characterization of the Changes in Opened Sufu Bottles During Storage with Mathematical Model

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Abstract: Artificial Neural Network (ANN) is a type of nonlinear empirical model, which can clarify the complex relation between the inputs and outputs that allows it to approximate any nonlinear function for making predictions. The objective of this study is to monitor the Biogenic Amines (BAs) content and selected physicochemical properties of sufu (a traditional Chinese fermented soybean product) along time. Simultaneously, based on initial values, a grey model and an ANN were developed to predict the influence of storage process parameters on the quality changes during storage. Results revealed that the total amounts of BAs in newly opened bottles of white, red and grey sufu were 419.61, 311.52 and 603.10 mg kg⁻¹, respectively, no sufu samples posed the total biogenic amines tolerance level (over 1000 mg kg⁻¹). Results showed that slight changes in the individual BAs were detected at 4°C, 15°C, 25°C and the formation of BAs was promoted at 35°C in grey sufu. Furthermore, grey model was developed with average relative errors within ±7%, the statistical parameters (R²) of pH, water activity and amino nitrogen was all above 0.90. In the ANN, the number of neurons in the hidden layer was optimized, ten neurons revealed a positive correlation between the values obtained experimentally and those predicted values (R² = 0.99). So ANN with highest R² was selected to predict biogenic amines and our results demonstrated that grey sufu were not edible on the 25th days at 4°C (BAs > 1000 mg kg⁻¹) and it would be better if white and red sufu are consumed within 40 days. We envision that our works can be used for proving a reference for consumers and offer new perspectives by mathematical model to avoid difficult, costly and time-consuming quality inspection, particularly in the field of storage.

Keywords: Biogenic Amines, Sufu, Storage Conditions, Grey Model, Artificial Neural Networks

Introduction

Sufu is a traditional fermented soy curd in China that is popular in oriental diets due to its unique flavor and rich nutrients. It has been consumed for more than 1000 years in China and is also called as Chinese cheese (Han *et al.*, 2001). The manufacturing process of sufu varies in different regions of China, among which mold-fermented sufu is the most common type. The procedure for making mold-fermented sufu generally consists of four steps: Preparation of tofu via salt

precipitation from boiled soymilk; preparation of pehtze through a pure culture bacterial fermentation; salting; and ripening in dressing mixture, which typically does not last for more than three months (Chao *et al.*, 2008). During the manufacture and storage, the proteins are hydrolyzed to a range of intermediate-sized peptides, which are then hydrolyzed to shorter peptides and amino acids. Thus, proteolysis is the main biochemical event, the extent of which can change the flavor, texture and color of the sufu (Han *et al.*, 2001). This process leads to the accumulation of free amino acids,

some of which are the precursors of biogenic amines (Schirone *et al.*, 2013).

BAs are organic, basic and low-molecular-weight nitrogenous compounds that are formed mainly by the decarboxylation of amino acids (Guan *et al.*, 2013). Their presence in foods can cause varying degrees of harm to susceptible consumers, including headaches, vertigo and increased arterial blood pressure (Komprda *et al.*, 2008; Elsanhoty and Ramadan, 2016). For this reason, the FDA also recommended that 1000 mg kg⁻¹ of total BA should be the tolerance level in fish (USFDA, 2001). The accumulation of BAs in fermented foods is a complex process affected by multiple factors and their interactions (Schirone *et al.*, 2013). Also they are often used as good chemical indicators of products' hygienic quality. Sufu has been comprehensively examined throughout the different stages of its manufacture. The concentrations of BAs have also been determined in brines (Sun *et al.*, 2010), tofu (Liang *et al.*, 2013; Tripathi and Misra, 2005; Li *et al.*, 2014) and post-fermentation (Han *et al.*, 2004). However, no one has studied the changes of biogenic amines during storage.

During storage, the microorganisms play a significant role, either directly by means of their metabolic activity, or indirectly via enzymes released into the sufu matrix. Given that sufu is consumed as a condiment which may be kept in opened bottles for quite a time, it is vulnerable to the microbial contamination in the environment, thereby affecting sufu quality and safety. For this reason, we aim to monitor the evolution of biogenic amines and physicochemical properties in sufu along time under different storage conditions and to our knowledge, it is the first report regarding focusing on the quality changes in an opened bottle. We envision that our works can be used for proving a reference for consumers about the preservation of sufu.

Grey predication Model (GM) is based on grey theory, which was developed for handling incomplete and rough datasets, this process is called grey prediction. The prediction is performed by solving an ordinary differential equation, which contains model coefficients being updated on line in order to characterize dynamic process. A grey prediction model was used to provide pH control that is widely used in the food industry (Chen *et al.*, 2005; Chung *et al.*, 2010). ARTIFICIAL NEURAL NETWORK (ANN) arises as a powerful and flexible tool with excellent predicting abilities, even when involving a complex systems. Artificial neural network is which is composed of three layers: The input layer links the network to the control variables, the hidden layer supplies the nonlinear functions for conversing input space to hidden space with high dimensionality, the output layer eventually provides the response of the input layer. As a more accurate

alternative to predict, the use of ANN has notoriously increased in different scientific fields. Previous studies have shown the benefits of using ANN in prediction compared to other statistical methods. For instance, artificial neural network could be used to describe and predict the effects of temperature, pH and storage time on food microbiology (Fang *et al.*, 2015; Lou and Nakai, 2000). Llave *et al.* (2012) applied ANN to predict cold spot temperature in retort sterilization of starch-based foods. ANN is applied for description of food quality changes during storage (Liu *et al.*, 2015). However, there are few reports on the utilization of ANN for predicting quality of sufu during storage. Therefore, with separation of the initial values into training, validation and test sets as 70:15:15 ratios, the mathematical model was established to predict future quality in sufu.

In this research, we aim to contribute to improvement of the knowledge of quality changes in sufu for proving a reference for consumers about the preservation of sufu. Meanwhile based on experimental data, we develop linear modeling (through mathematical equations) and nonlinear modeling (with ANN) to predict the influence of storage process parameters on the Biogenic Amines (BAs) content and selected physicochemical properties of sufu, thus, the complex, costly and time-consuming laboratory determination would be reduced and it might be able to provide a potential tool in modeling food quality changes for the factory.

Materials and Methods

Chemicals and Reagents

Standard putrescine, cadaverine, spermine, spermidine, tryptamine, 2-phenylethylamine, histamine, tyramine and dansyl chloride (purity >99%) were purchased from Sigma-Aldrich. Acetonitrile (High-Performance Liquid Chromatography (HPLC grade) and methanol (HPLC grade) were obtained from Tedia (USA). The other reagents were of analytical grade. Ultra-pure water (18.2 MΩ cm) was produced using the Simplicity water purification system from Millipore (Bedford, MA, USA). The solutions of the BAs were prepared with 0.1 M HCl.

Sufu Samples

On the basis of the dressing mixtures, sufu was classified into three types: White, red and grey sufu. A total of 108 bottles of sufu samples were purchased in local supermarkets. The samples of the three species were stored at 4°C, 15°C, 25°C and 35°C, because consumers typically store sufu in a refrigerator (4°C) or at room temperature. Moreover, 15°C, 25°C and 35°C represent the room temperatures in different seasons of the year. In view of the formation rates of BAs at different storage temperatures, the concentrations of BAs

in the samples were determined for the following time intervals: 0, 7 and 14 days at 4°C and 0, 5, 10 and 15 days at 15°C, 25°C and 35°C. The longest time of determination is 15 days, from then on, there will be obvious rotten smell of the preserved sufu after opening. Each sufu sample was analyzed in duplicate ($n = 3$).

Preparation of Standard Solution

BA stock solutions were individually prepared in 0.1 M HCl at a given concentration of 1 mg mL⁻¹. All BA solutions were refrigerated at 4°C and they remained stable at least for 20 days. In total, 10 mg mL⁻¹ dansyl chloride solution was prepared in acetone.

Determination of BA Content

The BAs were extracted and derivatized using the method developed by Saarinen (2002). Briefly, the BA contents were extracted by homogenizing a 2 g sample (each) with 20 mL of 0.1 M HCl. Then, the extract was centrifuged for 30 min at 6000 × *g* and the supernatant was filtered. Next, 1 mL of the filtrate or standard was mixed with 0.3 mL of saturated sodium bicarbonate and 0.2 mL of 2 M NaOH. The mixture was derivatized by 1 mL of dansyl chloride. Then, the mixture was vortexed for 1 min and incubated for 45 min at 42°C. Afterward, the remaining dansyl chloride was consumed by the addition of 0.1 mL ammonia (25% v/v). After 30 min of reaction (protected from light), the samples were filtered through a 0.45 μm membrane prior to the HPLC analysis.

The BAs were determined by HPLC. The Waters HPLC system consisted of a Waters 1525 Binary HPLC pump, a Waters 2707 autosampler injector, a CTO-20AC oven, a DGU-20A5R degasser and a Waters 2489 ultraviolet-visible detector. Waters data processing was performed on a Waters Millennium data station. The chromatographic column (5 μm, 4.6×250 mm; Agilent Co., USA) had a column temperature of 40°C. The binary mobile phase consisted of 0.1 mol L⁻¹ ammonium acetate (A) and acetonitrile (B). The mobile phases were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA). The chromatograms were analyzed at 254 nm and the injection volume was 20 μL. The following gradient program was performed at a flow rate of 1 mL min⁻¹: 0.0-7.0 min 55.0-50.0% B, 7-25 min 50.0-90.0% B and 25.0-32 min 90-55% B.

Determination of Physicochemical Properties

The pH values of the samples were determined using a pH meter (Model 340, Mettler Toledo GmbH, Schwerzenbach, Switzerland). For each sample, a 5.0 g portion was collected, cut into pieces and homogenized with 40.0 mL of deionized water for 30 min. The suspension was centrifuged at 6000 × *g* for 10 min. The supernatant was used to determine the pH value. The

water activity (a_w) was determined at 25°C by using a water activity meter (Novasina AG, Switzerland).

Amino nitrogen was measured by formalin titration method (Xia *et al.*, 2014) with slight modifications. Approximately 10 mL of the supernatant was mixed with 50 mL of water and titrated to pH 8.2 with 0.05 M NaOH. Then, 10 mL of 36% (w/v) formalin solution was added. The mixture was titrated to pH 9.2 with 0.05 M NaOH. The volume of the consumed NaOH for increasing pH (from 8.2 to 9.2) was recorded to determine the amino nitrogen content.

Grey Model

Generally, the procedure of grey model is explained as follows:

Step1: The data of BAs in white sufu stored for 0, 5, 10 and 15 days at 35°C were selected as the prediction data segment and the original data were accumulated to generate a cumulative data sequence:

$$x^{(0)} = (x^{(0)}(1), x^{(0)}(2), \dots, x^{(0)}(4)) \quad (1)$$

Step2: Accumulation is a method of graying process, which plays an important role in the grey system. By accumulating and generating, the integral characteristics or laws contained in the chaotic original data can be fully revealed:

$$x^{(1)}(1) = x^{(0)}(1) \quad (2)$$

$$x^{(1)}(k+1) = [x^{(1)}(k) + x^{(0)}(k+1)] \\ = \sum_{i=1}^{k+1} x^{(0)}(i), k = 1, 2, \dots, n-1 \quad (3)$$

The background value is selected by taking the weighted average of accumulated data as the background value and α is the determining parameter:

$$z^{(1)}(k+1) = \alpha x^{(1)}(k+1) + (1-\alpha)x^{(1)}(k), (k = 1, 2, 3) \quad (4)$$

Step3: Establishment of a model for the albino differential equation of GM:

$$\frac{dx^{(1)}}{dt} + ax^{(1)} = u \quad (5)$$

where, a is the developed grayscale and u is the endogenously controlled grayscale.

Step4: Establishing a first-order grey differential equation:

$$x^{(0)}(k) + az^{(1)}(k) = u, (k = 2, 3, 4) \quad (6)$$

$$Y = B \begin{bmatrix} a \\ u \end{bmatrix} = BA \quad (7)$$

$$Y = (x^{(0)}(2), x^{(0)}(3)), B = \begin{bmatrix} -z^{(1)}(1) & 1 \\ -z^{(1)}(2) & 1 \\ \vdots & \vdots \\ -z^{(1)}(4) & 1 \end{bmatrix}, A = \begin{bmatrix} a \\ u \end{bmatrix} \quad (8)$$

Step5: Conversion to time response function, least squares method was used to obtain the estimators (\hat{a}, \hat{u}) for the parameters. Then, the solution of the grey differential equation was acquired:

$$\hat{A} = \begin{bmatrix} \hat{a} \\ \hat{u} \end{bmatrix} = (B^T B)^{-1} B^T Y \quad (9)$$

The solution of the differential equation:

$$\hat{x}^{(1)}(k+1) = \left(x^{(1)}(1) - \frac{u}{a} \right) e^{-ak} + \frac{u}{a} \quad (10)$$

$$\hat{x}^{(0)}(k+1) = -a \left(x^{(0)}(1) - \frac{u}{a} \right) e^{-ak} \quad (11)$$

Notice that the symbol $\hat{\cdot}$ indicates a predicted value.

Artificial Neural Network (ANN)

Artificial Neural Network (ANN) is a nonlinear operational model used for classification and prediction. As its name implies, a typical ANN is composed of a large number of interconnected neurons to simulate the structure and function of the brain's nervous system, it can automatically induct rules from the known data, so we could get the inherent laws of these data and it has strong nonlinear mapping ability. These neurons are distributed on three layers: An input layer, an output layer and a hidden layer. The input layer links the network to the control variables, the hidden layer supplies the nonlinear functions for conversing input

space to hidden space with high dimensionality and the output layer eventually provides the response of the input layer. The number of hidden layers and neurons in hidden layer could be regarded as design parameters. In this study, a one-hidden-layer network was performed as showed in Fig. 1. The input layer was composed of the following variables: Temperature and storage time and the output layer consisted of four neurons which indicated pH values, water activity, amino nitrogen and biogenic amines. An optimal choice of the number of hidden layers was performed, 5, 6, 7, 8, 9, 10, 11, 12 or 13 neurons were compared. The neural network distributed the database into three sets: (training, validation and test), account for 70%, 15% and 15%, separately.

Evaluation of Grey Model and ANN

To assess the accuracy of these two models in prediction, in the ANN, the Mean Square Error (MSE) was used to analyze the model precision and the regression coefficients R^2 was selected to ensure the minimum difference between the experimental value and the predicted value in training, validation and test data. In the Grey model, the regression coefficients R^2 and the relative error between predicted and experimental value were proposed.

Statistical Analysis

Each experiment was conducted in three bottles and the analyses were performed in triplicate for each bottle. The results were presented as means \pm standard deviation. Analysis of variance was accomplished using Tukey's multiple range tests ($p < 0.05$) to compare the means for different sampling days during the evolution of sufu. These statistical analyses were performed using the IBM SPSS Statistics Package version 22.0 (SPSS Inc., Chicago, IL, USA). The Grey Model (GM) was established to predict changes in BA levels. The model was created using MATLAB R2012a (MathWorks, Inc., Natick, Massachusetts, USA).

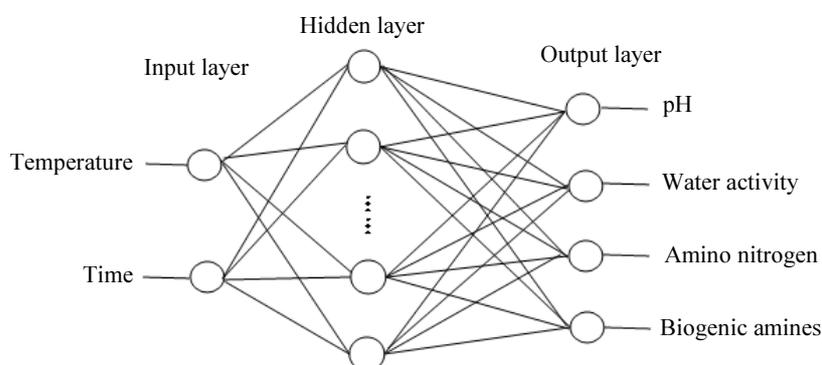


Fig. 1: Artificial neural networks for predicting quality changes of sufu during storage

Results and Discussion

BA Concentrations in Just Opened Sufu Samples

The BA concentrations (mg kg^{-1}) of different sufu samples are shown in Supplementary Material. The total contents of BAs varied markedly for different types of sufu, ranging from $311.52 \text{ mg kg}^{-1}$ to $603.10 \text{ mg kg}^{-1}$. The maximum content of BAs was found in grey sufu, which was twice of that in red sufu ($311.52 \text{ mg kg}^{-1}$). These results might be due to the various flavors in the sufu. Red sufu contains red kojic rice and yellow wine, which exert inhibitory effects on the microorganisms. These results were consistent with the findings of García-Marino *et al.* (2010) who observed that grey sufu had higher levels of BAs than white and red sufu.

Among BAs, histamine is one of the most potentially harmful. Excessive histamine can cause hypotension, headaches, chemical poisoning and other human health problems (Kung *et al.*, 2007). In the present study, the most abundant BAs in all samples were histamine and tyramine (Supplementary Table S1). The high levels of histamine and tyramine in tofu have also been reported by Han *et al.* (2004). The content of histamine varied markedly among the samples, ranging between 50.96 mg kg^{-1} and $195.67 \text{ mg kg}^{-1}$. This result slightly differed from the findings of Kung *et al.* (2007), who detected histamine in excess of 50 mg kg^{-1} in only one red sample. This discrepancy might be attributed to the differences in the manufacturing processes and microorganism starter in the production process of sufu.

Putrescine and cadaverine were present in all sufu and the concentration of putrescine in red sufu ($20.07 \pm 1.45 \text{ mg kg}^{-1}$) was significantly lower ($p < 0.05$) than that in white ($42.20 \pm 1.31 \text{ mg kg}^{-1}$) and grey sufu ($196.07 \pm 10.56 \text{ mg kg}^{-1}$). These findings agreed with those reported by Han *et al.* (2001). Han *et al.* (2001) detected putrescine and cadaverine in all sufu samples. Putrescine and cadaverine were the primary amines in all sufu products, which could be associated with poor hygienic conditions (Guan *et al.*, 2013). In addition, the presence of cadaverine has been associated with the action of enterococci and coliform bacteria (Bover-Cid *et al.*, 2003).

For the rest of the BAs, only two samples presented lower levels of spermine ($0.75\text{-}2.48 \text{ mg kg}^{-1}$). Spermidine, phenylethylamine and tryptamine had very low contents in most of the analyzed sufu.

Changes of BAs and Physicochemical Properties During Storage

The changes of eight BAs in sufu stored at 4°C , 15°C , 25°C and 35°C are summarized (Supplementary Table S1-3).

In white sufu, the total amount of BAs did not exhibit a clear and significant trend during storage. The initial value of total BAs was $419.61 \text{ mg kg}^{-1}$, which rapidly increased to 908.73 , 536.82 and $466.12 \text{ mg kg}^{-1}$ at the end of storage at 4°C , 15°C and 35°C . At 25°C , the level of total BAs was slightly changed. The same trend was observed in histamine possibly because histamine is the dominant BA and its changes will affect the total BAs. This result also revealed that the production of BAs in white sufu was hardly related to temperature. This result contradicted the findings of other investigators (Bakar *et al.*, 2010). Bakar *et al.* (2010) found that histamine gradually increased in barramundi slices during storage at different temperatures. The concentrations of phenylethylamine and spermidine fluctuated along time under all conditions, their amounts were so low that they did not affect the total concentrations. Unlike white sufu, the initial values of total BAs in red sufu was $311.52 \text{ mg kg}^{-1}$, which rapidly increased to 398.72 and $338.41 \text{ mg kg}^{-1}$ at the end of storage at 4°C and 15°C , respectively (Supplementary Table S1-2). The histamine concentrations significantly increased from the fifth day to the tenth day under all conditions, similar to that in white sufu.

For grey sufu, slight differences were observed under the different conditions of storage. The total amount of BAs significantly increased ($p < 0.05$) with higher storage temperatures. At 35°C , the total amount of BAs in grey sufu reached 985 mg kg^{-1} , which is twice that of white sufu (Supplementary Table S4).

In general, the BAs in the three types of sufu did not exhibit a common trend. Nonetheless, the histamine concentration significantly increased from the fifth day to the tenth day in the three types of sufu. This finding was consistent with Chin and Koehler (1986), who observed that the content of histamine increased during miso fermentation. Moreover, the different storage conditions employed in this experiment did not affect the BA profiles, except that the BAs were higher in grey sufu at 35°C . Similarly, most BAs only slightly changed during their storage at different temperatures in closed red wine bottles (González Marco and Ancín Azpilicueta, 2006).

Supplementary Fig. S1 showed the water activity changes of sufu during storage. The pH value varied markedly among the three kinds of samples, $5.93\text{-}7.77$ (Supplementary Fig. S2), which was similar to the value reported by Han *et al.* (2001). The pH value in white sufu increased over time (15°C , 25°C and 35°C), whereas the grey and red sufu demonstrated an opposite trend. The difference was due to the variation of the lactic acid bacteria population in sufu (Li *et al.*, 2014).

At 4°C , the amino nitrogen in the white sufu increased significantly ($p < 0.05$) over time, which may be attributed to the restriction of the degradation degree of

the water-soluble protein by alcohol. Alcohol is added in white sufu and it exerts an inhibitory effect on the activity of microorganism-induced protease, which would restrict the degradation of soybean proteins during the sufu ageing period (Chou and Hwan, 1994). However, during storage at 15°C, 25°C and 35°C, the amino nitrogen increased slightly and then gradually decreased (Supplementary Fig. S3). Similar trends in amino nitrogen were observed by Andic *et al.* (2010).

The Grey Model

The dataset of pH, water activity, amino nitrogen and total biogenic amines in white sufu during storage were collected to establish the grey model. The corresponding criterion of the relative error was listed in Table 1. It can be seen from Table 2 that the average relative error of pH, water activity, amino nitrogen and total biogenic amines was 0.51%, 0.21%, 3.80% and 21.78%, respectively. According to Table 1, a majority of the prediction errors are controlled below 10%, which indicates a reasonable prediction in the model. The relative error of total biogenic amines exceed 10%, the result may be caused by chaotic data and the normalization can prevent the overflow error caused by

independent variables. In this experiment, we also consider the regression coefficients R^2 between the experimental value and the predicted value, the results were presented in Fig. 2. As seen in these figures, there is good correlation between the predicted and experimental data. The predicted values are very close to the observed values, the statistical parameters (R^2) of pH, water activity and amino nitrogen were all above 0.90. According to previous works, the accuracy of the GM(1,1) model used for predicting the drying process based on four-point data was better than the other exponential models (Chen *et al.*, 2010) and Chung *et al.* (2008) has shown that grey-based Taguchi method have effectively improved and solved optimization problems of a fermentation process with multiple performance characteristics.

Table 1: Criterion of the relative error in grey model

Forecasting power	Relative error
Excellent Table 1	<1%
Good	1%-5%
Reasonable	5%-10%
Incorrect	>10%

Table 2: Experimental values, grey model predicted values, and relative errors of pH, water activity, amino nitrogen and biogenic amines of sufu during storage at different temperatures

Temperature (C°)	Storage time (days)	pH			Water activity			Aminonitrogen			Biogenic amines		
		Experimental value	Predicted value	Relative errors (%)	Experimental value	Predicted value	Relative errors (%)	Experimental value	Predicted value	Relative errors (%)	Experimental value	Predicted value	Relative errors (%)
4	0	6.40	6.40	0.00	0.88	0.88	0.00	0.19	0.19	0.00	419.61	419.61	0.00
	7	7.02	7.01	0.01	0.88	0.88	0.00	0.17	0.17	0.08	193.43	139.24	38.92
	14	6.82	6.82	0.01	0.89	0.89	0.00	0.19	0.19	0.09	908.73	509.89	78.22
15	0	6.40	6.40	0.00	0.88	0.88	0.00	0.19	0.19	0.00	419.61	419.61	0.00
	5	6.69	6.74	0.81	0.88	0.88	0.44	0.17	0.17	0.57	292.50	415.33	29.57
	10	7.07	6.96	1.50	0.87	0.86	0.92	0.14	0.15	0.72	705.50	506.49	39.29
25	15	7.13	7.18	0.71	0.84	0.85	0.48	0.12	0.12	0.80	536.82	617.67	13.09
	0	6.40	6.40	0.00	0.88	0.88	0.00	0.19	0.19	0.00	419.61	419.61	0.00
	5	6.62	6.67	0.82	0.89	0.89	0.13	0.18	0.18	4.05	422.98	538.74	21.49
35	10	7.02	6.91	1.51	0.88	0.88	0.27	0.15	0.16	10.55	764.51	528.02	44.79
	15	7.10	7.15	0.71	0.87	0.87	0.14	0.16	0.15	5.25	396.73	517.51	23.34
	0	6.40	6.40	0.00	0.88	0.88	0.00	0.19	0.19	0.00	419.61	419.61	0.00
35	5	6.73	6.76	0.43	0.89	0.88	0.18	0.17	0.16	6.76	495.71	544.95	9.04
	10	6.98	6.92	0.82	0.88	0.89	0.35	0.13	0.15	19.78	632.44	531.30	19.04
	15	7.07	7.09	0.39	0.89	0.89	0.17	0.15	0.14	8.74	466.12	517.99	10.01

Table 3: Changes of MSE and R^2 between experimental and predicted values of training, validation and testing data in different hidden neurons in the ANN

Neurons	R^2 (TR)	R^2 (VA)	R^2 (TE)	MSE (TR)	MSE (VA)	MSE (TE)
5	0.83	0.98	0.94	0.13	0.02	0.06
6	0.99	0.86	0.94	0.01	0.13	0.17
7	0.99	0.81	0.95	0.01	0.20	0.05
8	0.94	0.87	0.71	0.06	0.31	0.45
9	0.99	0.82	0.89	0.01	0.42	2.64
10	0.99	0.99	0.98	0.01	0.01	0.02
11	0.99	0.95	0.88	0.01	0.06	1.05
12	0.98	0.80	0.89	0.02	0.54	0.08
13	0.89	0.93	0.91	0.96	0.78	2.56

“TR” indicates the training data. “VA” indicates that the

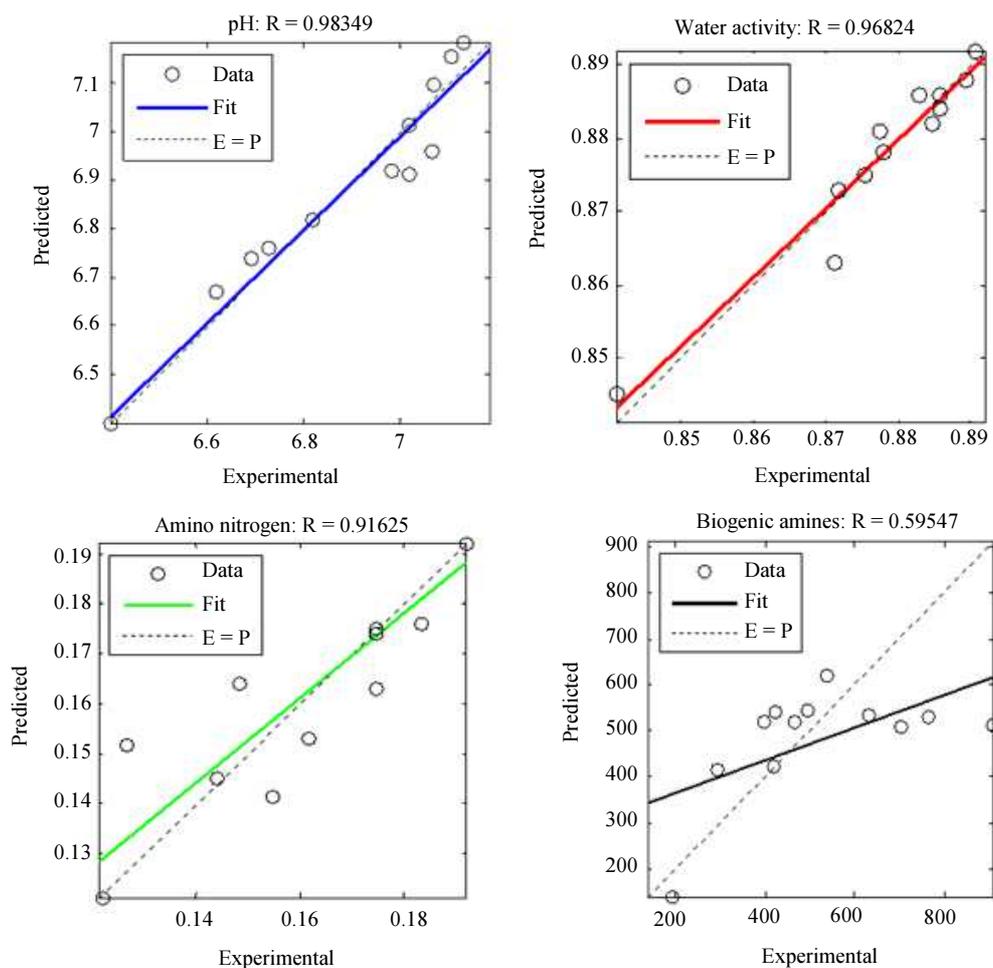


Fig. 2: Comparison between experimental and predicted values of pH, water activity, amino nitrogen, and biogenic amines in grey model. “fit” indicates the best linear fit. “E = P” indicates that the experimental values is equal to the predicted values

Model Development and Validation of Artificial Neural Network (ANN)

In our model, the sample data is preprocessed in order to clean chaotic data before the execution of ANN. A min–max normalization method has to be employed to transform data measured on different scales to a notionally common scale. The normalization can prevent the overflow error caused by independent variables when calculating the parameters of our model.

In this study, experimental data of white sufu during storage at 4°C, 15°C, 25°C and 35°C was used for modeling with a distribution as follows: 70% for the training, 15% for the validation and 15% for the test.

The number of neurons in the hidden layer has been optimized so that the influence of temperature, time of conservation on quality changes could be accurately predicted. The optimal network was chosen following the values of the mean square error (MSE) and the coefficient (R^2). The lowest MSE and the highest R^2

were expected for the optimal network. The results obtained in this optimization (Table 3). It can be seen from Table 3 that ten neurons in the hidden layer presented the lowest MSE (MSE = 0.01 for training; MSE = 0.01 for validation and MSE = 0.02 for testing) and highest R^2 ($R^2 = 0.99$ for training; $R^2 = 0.99$ for validation and $R^2 = 0.98$ for testing), good agreement between experimental and predicted data was obtained. The ANN model accurately predicts the influence of agglomeration process parameters on physical and chemical properties of cocoa powder mixtures ($R^2 = 0.949$) (Benkovic *et al.*, 2015). Furthermore, radial basis function neural networks (RBFNNs) were developed to predict quality of brined carp fillets during frozen storage with relative errors all within $\pm 5\%$ (Kong *et al.*, 2016). Therefore, in this study, ten hidden neurons was chosen to obtain a proposed model. A log-sigmoidal activation function and a linear activation function were performed in the hidden layer and output layer, respectively.

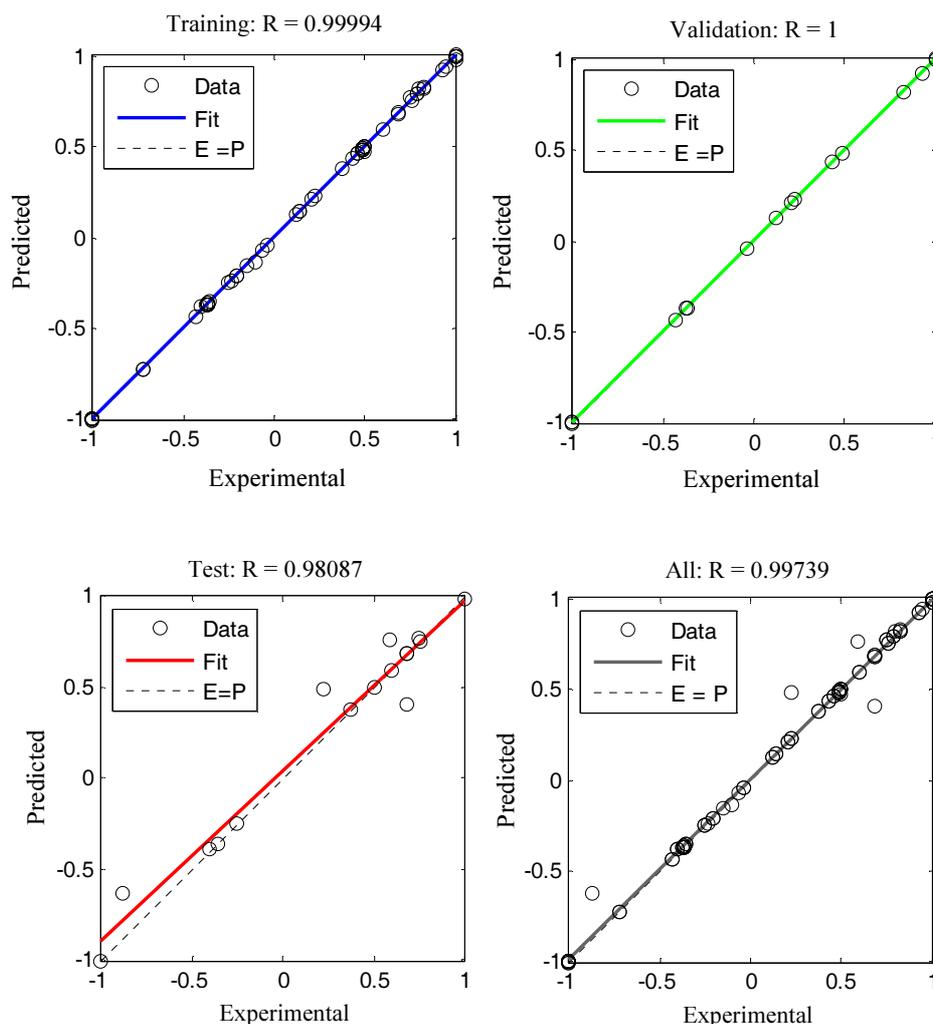


Fig. 3: Comparison between experimental and predicted values of training, validation and testing data in ANN. “fit” indicates the best linear fit. “E = P” indicates that the experimental values is equal to the predicted values

Based on the ANN with ten hidden neurons, highest R^2 (Fig. 3) ($R^2 = 0.99$ for training; $R^2 = 1.0$ for validation; $R^2 = 0.98$ for testing and $R^2 = 0.99$ for all) was found to estimate quality changes in sufu.

We predict the changes of biogenic amines in sufu over the next 40 days and based on our prediction results, if uncapped grey sufu was stored in the refrigerator (4°C), it is recommended that it should be eaten within 25 days, otherwise the content of biogenic amines will exceed 1000 mg kg^{-1} , for other storage temperatures, grey sufu should be consumed in a shorter period of time (<25 days) (Supplementary Table S5).

Conclusion

During storage, the denaturation of proteins led to the decrease in amino nitrogen, this process also leads to the accumulation of free amino acids, some of

which are the precursors of biogenic amines, which is related to water activity and pH value. For monitoring quality changes, artificial neural network with two different inputs (temperatures and time) was designed. A good correlation was observed between the values predicted and those obtained experimentally. Furthermore, in grey model the predicted values are very close to the observed values, the statistical parameters (R^2) of pH, water activity and amino nitrogen were all above 0.90. These results offer new prediction tool, particularly in the field of food storage, since they could be a potential tool in modeling food quality changes during storage.

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Author's Contributions

Jingjing Liang: Has contributed in a whole experiment, data analysis, paper writing and publication.

Dawei Li and Ruiqin Shi: Have assisted in the experiment.

Yanli Ma, Jie Wang and Ke Xiong: Has reviewed and revised the manuscript.

Ethics

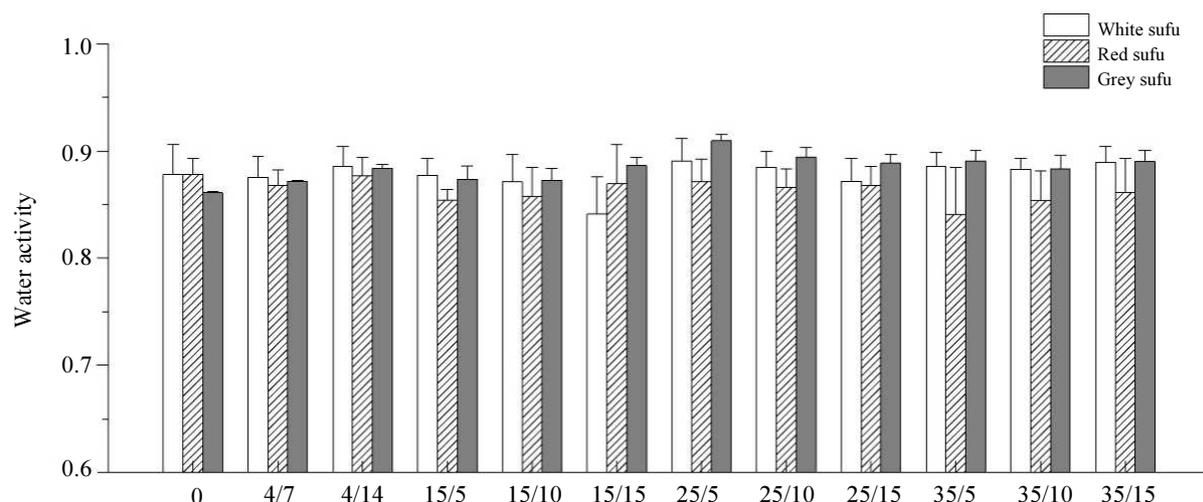
All authors read and approved the final version of this manuscript. There are not any ethical issues to declare that could arise after the publication of this manuscript.

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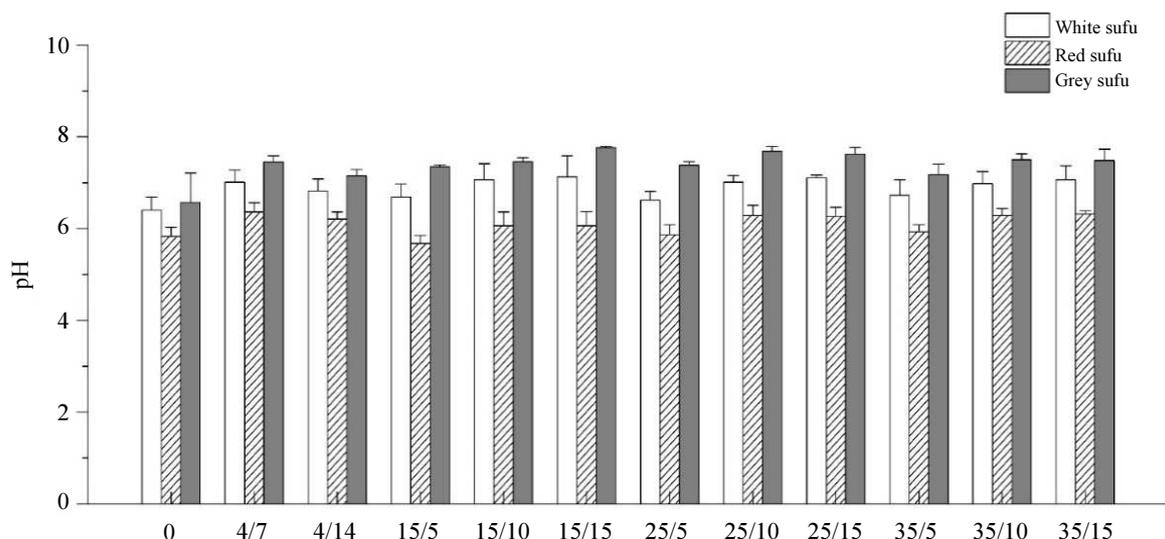
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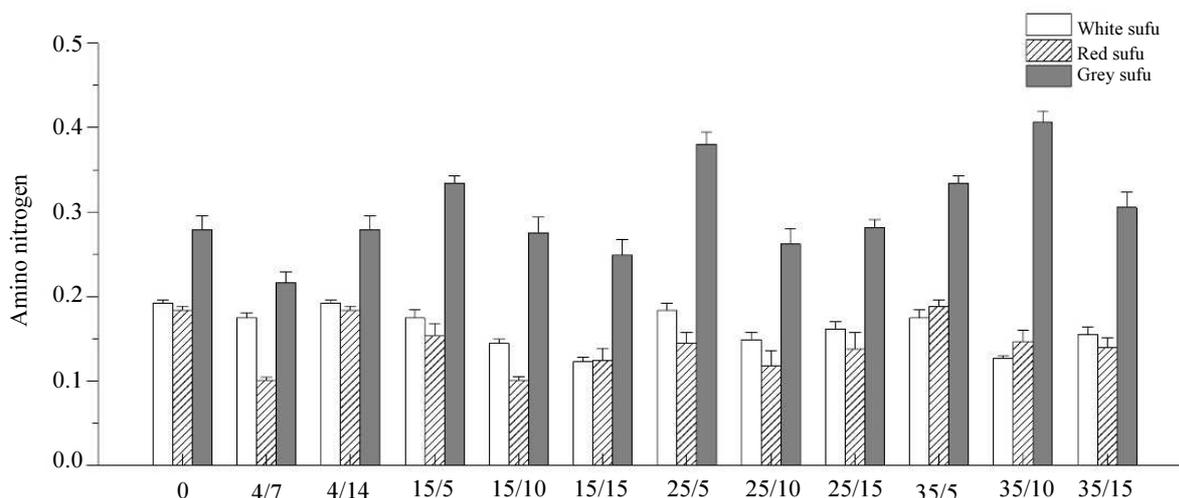
Annex



Supplementary Fig. S1: Effect of storage condition on the water activity of different sufu. Error bars represent the standard deviations of mean values. a/b: a indicates the temperature; b indicates the storage time(days)



Supplementary Fig. S2: Effect of storage condition on the pH value of different sufu. Error bars represent the standard deviations of mean values. a/b: a indicates the temperature; b indicates the storage time(days)



Supplementary Fig. S3: Effect of storage condition on the amino nitrogen of different sufu. Error bars represent the standard deviations of mean values. a/b: a indicates the temperature; b indicates the storage time(days)

Supplementary Table S1: Evolution of biogenic amine concentrations in the three types of sufu at 4°C

Storage time (days)	Biogenic amines (mg kg ⁻¹)								
	PUT	CAD	HIS	TYR	PHE	TRY	SPD	SPM	TOTALS
White sufu									
0	42.20±1.31c	38.43±1.99a	158.17±8.99b	146.41±12.45a	8.95±0.77b	10.42±0.45c	14.91±0.85ab	ND	419.61
7	66.31±5.78b	31.81±2.36b	68.59±4.14c	57.93 ± 3.45b	5.29±0.77c	66.51±3.87a	15.07±1.69a	ND	193.43
14	127.81±7.89Aa	24.39±0.90Bc	336.59±18.26Aa	57.49±2.73Bb	20.48±1.99a	24.7±1.12 Ab	11.60±0.91Bc	ND	908.73
Red sufu									
0	20.07±1.45a	24.11±2.41a	50.96± 2.56b	34.46±1.76a	6.86±0.35a	49.83±2.49a	7.16±0.46c	ND	311.52
7	7.86±0.49c	15.43±0.83b	57.09±2.46b	2.61±0.29c	4.02±0.25b	27.27±1.61b	8.59±0.69b	ND	122.89
14	11.37±1.12Cb	17.62±0.88Cb	339.63±19.52Aa	33.36±1.01Cab	4.12±0.26b	7.8±0.42Bc	13.48±0.17ABa	ND	398.72
Grey sufu									
0	196.07±10.56a	30.60±2.66ab	195.67±8.62c	298.33±19.70a	49.51±2.50a	125.37±6.10a	13.21±0.72ab	ND	603.1
7	24.54±1.23c	21.41±1.93c	226.23±14.09b	76.28±3.53c	19.00±1.26b	16.95±0.86b	14.18±1.69ab	ND	427.42
14	85.14±5.47Bb	31.28±0.79Aa	330.97±23.09Aa	105.71±5.16Ab	16.29±0.94b	4.74±0.20Cc	15.72±0.98Aa	ND	589.89

Biogenic amines content is expressed in mg kg⁻¹ (mean ± SD).

“a-d” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the same type of sample according to the Tukey’s multiple range tests.

“A-C” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the different type of sample stored for 15 days according to the Tukey’s multiple range tests.

Supplementary Table S2: Evolution of biogenic amine concentrations in the three types of sufu at 15°C.

Storage time (days)	Biogenic amines (mg kg ⁻¹)									
	PUT	CAD	HIS	TYR	PHE	TRY	SPD	SPM	TOTALS	
White sufu										
0	42.20±1.31c	38.43±2.96a	158.17±8.00c	146.41±8.02a	8.95±0.57b	10.42±0.45d	14.91±0.85a	ND	419.61	
5	19.59±0.92d	30.88±1.01c	70.39±3.90d	87.57±5.15d	6.88±0.35c	66.64±3.72a	10.52±0.82c	ND	292.50	
10	165.92±9.21a	34.11±1.35b	294.32±15.78a	141.16±10.45b	4.56±0.21d	53.93±4.62b	11.48±0.75c	ND	705.50	
15	128.10±6.17Ab	31.51±0.84Abc	184.78±10.59Bb	119.36±5.70Cc	11.22±0.63Ba	47.12±2.27Bc	13.99±0.81Aab	0.75±0.11	536.82	
Red sufu										
0	20.07±2.45c	24.11±2.41b	50.96±2.82d	34.46±1.76c	6.86±0.45b	49.83±2.41b	7.16±0.46d	ND	311.52	
5	29.67±2.08a	7.51±0.36d	150.65±9.68b	34.48±1.45c	2.47±0.13c	70.52±4.48a	11.60±1.15bc	ND	306.90	
10	8.33±0.31d	27.67±1.45a	169.04±8.57a	184.89±9.14a	2.77±0.23c	12.55±1.15d	14.08±0.70a	ND	419.33	
15	23.43±1.86Cb	19.88±0.96Ac	102.57±5.48Cc	145.44±9.46Bb	10.44±0.58Ba	24.39±1.73Cc	12.26±0.90ABb	ND	338.41	
Grey sufu										
0	196.07±10.56a	30.60±2.66b	195.67±9.62c	298.33±16.97b	49.51±2.50a	125.37±6.10a	13.21±0.72b	ND	603.10	
5	19.60±0.92d	6.61±0.27d	127.40±7.40d	123.16±10.68d	12.49±1.23c	49.42±2.60d	19.05±1.51a	ND	357.72	
10	28.73±1.72c	39.40±1.93a	454.66±48.44a	306.31±15.53a	8.15±0.42d	114.88±5.83b	10.82±0.46bc	ND	962.94	
15	84.02±6.20Bb	23.92±1.48Ac	366.71±30.76Ab	193.27±13.34Ac	27.69±2.67Ab	67.52±4.93Ac	11.98±2.02Bbc	ND	775.09	

Biogenic amines content is expressed in mg kg⁻¹ (mean ± SD).

“a–d” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the same type of sample according to the Tukey’s multiple range tests.

“A–C” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the different type of sample stored for 15 days according to the Tukey’s multiple range tests.

Supplementary Table S3: Evolution of biogenic amine concentrations in the three types of sufu at 25°C

Storage time (days)	Biogenic amines (mg kg ⁻¹)									
	PUT	CAD	HIS	TYR	PHE	TRY	SPD	SPM	TOTALS	
White sufu										
0	42.20±1.31d	38.43±1.96ab	158.17±10.00c	146.41±9.02a	8.95±0.78ac	10.42±0.45d	14.91±0.85a	ND	419.61	
5	108.71±6.54c	17.50±1.18d	170.86±9.69b	80.77±5.61d	9.49±0.61a	23.69±1.94c	11.97±0.78bc	ND	422.98	
10	189.11±10.34a	29.58±1.56c	357.94±18.49a	120.36±10.49b	9.18±0.35ab	46.68±2.78b	11.67±1.16bc	ND	764.51	
15	132.32±7.86Ab	40.16±1.19Aa	56.57±3.58Bd	97.98±8.02Bc	6.47±0.49Bd	50.95±3.16Aa	12.29±1.05Ab	ND	396.73	
Red sufu										
0	20.07±1.45b	24.11±2.41a	50.96±3.82b	34.46±1.76a	6.86±0.35a	49.83±2.41a	7.16±0.46d	ND	311.52	
5	108.43±5.18a	14.94±0.95c	22.45±1.50c	11.21±0.61c	6.82±0.35ab	15.07±0.75b	10.17±0.51c	ND	189.09	
10	10.91±1.03c	18.89±5.21b	255.28±14.22a	20.37±1.40b	4.6±0.29d	13.19±0.65c	14.85±0.99a	ND	338.09	
15	3.75±0.26Cd	14.5±0.96Cc	23.60±1.80Cc	2.08±0.07Cd	5.92±0.34Bc	11.77±1.46Bc	13.39±0.97Aab	2.48±0.71	77.50	
Grey sufu										
0	196.07±10.56a	30.60±2.66c	195.67±11.62d	298.33±16.97a	49.51±2.50a	125.37±6.10a	13.21±0.94a	ND	603.10	
5	112.45±5.44c	22.64±0.87d	343.16±15.56c	201.51±13.99c	36.10±1.31b	6.41±0.37d	12.22±0.72ac	ND	734.86	
10	145.72±8.29b	83.58±5.17a	522.04±31.24a	259.35±10.68b	8.30±0.42c	12.27±0.46b	12.8±0.99ab	ND	1044.06	
15	103.88±5.88Bd	34.12±2.4Bb	415.08±24.92Ab	195.7±9.95Ad	9.96±0.46Ac	12.08±0.71Bbc	10.69±0.64Ac	ND	781.51	

Biogenic amines content is expressed in mg kg⁻¹ (mean ± SD).

“a–d” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the same type of sample according to the Tukey’s multiple range tests.

“A–C” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the different type of sample stored for 15 days according to the Tukey’s multiple range tests.

Supplementary Table S4: Evolution of biogenic amine concentrations in the three types of sufu at 35°C.

Storage time (days)	Biogenic amines (mg kg ⁻¹)									
	PUT	CAD	HIS	TYR	PHE	TRY	SPD	SPM	TOTALS	
White sufu										
0	42.20±1.31d	38.43±3.96bc	158.17±10.00b	146.41±8.02a	8.95±0.45d	10.42±0.45d	14.91±0.85b	ND	419.61	
5	144.48±10.85a	28.93±1.25d	146.78±11.11d	121.25±7.48c	17.72±1.11abc	23.74±1.20c	12.81±1.38bc	ND	495.71	
10	97.52±10.19b	38.76±2.75b	287.6±16.14a	135.95±9.27b	17.69±1.81a	35.3±3.44b	19.64±1.13a	ND	632.44	
15	73.78±8.87Bc	50.15±3.57Aa	153.72±13.11Bc	109.37±8.66Bd	18.17±3.42Ba	52.73±3.13Ba	8.21±0.67Bd	ND	466.12	
Red sufu										
0	20.07±1.45a	24.11±2.41bc	50.96±5.82c	34.46±3.76a	6.86±0.75a	49.83±2.41a	7.16±0.46b	ND	311.52	
5	4.62±0.24d	11.44±0.69d	4.53±0.55d	0.73±0.01bc	4.63±0.34c	8.87±0.66c	11.25±0.53a	ND	46.07	
10	6.71±0.35b	30.08±1.77a	258.64±13.74a	2.86±0.35b	6.76±0.72ab	9.18±0.55c	0.83±0.06	ND	315.06	
15	4.72±0.24Cc	25.91±2.26Cb	107.68±8.71Cb	1.28±0.07Cbc	5.41±0.42Cc	18.95±0.95Cb	3.24±0.36Cc	ND	167.21	
Grey sufu										
0	196.07±15.56a	30.60±2.66d	195.67±18.62d	298.33±16.97a	49.51±3.50a	125.37±6.10a	13.21±0.72c	ND	603.1	
5	69.59±6.64d	49.68±4.72a	317.22±17.11c	173.46±13.23d	18.55±0.99d	5.6±0.21c	9.63±0.71d	ND	643.72	
10	121.87±18.36b	42.98±5.78b	446.9±21.27a	246.25±12.35bc	34.79±2.96b	6.88±0.41c	84.31±6.37a	ND	983.99	
15	106.47±9.49Ac	36.08±0.68Bc	421.61±20.96Ab	249.5±15.38Ab	34.26±3.35Abc	91.43±7.54Ab	46.32±4.6Ab	ND	985.65	

Biogenic amines content is expressed in mg kg⁻¹ (mean ± SD).

“a–d” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the same type of sample according to the Tukey’s multiple range tests.

“A–C” Different superscripts in the same column indicate significant statistical differences (p<0.05) in the different type of sample stored for 15 days according to the Tukey’s multiple range tests.

Supplementary Table S5: Predicted the content of total biogenic amines in the next 20-40 days by artificial neural networks. Biogenic amines content is expressed in mg kg⁻¹

Storage time(days)	4°C			15°C			25°C			35°C		
	White sufu	Red sufu	Grey sufu	White sufu	Red sufu	Grey sufu	White sufu	Red sufu	Grey sufu	White sufu	Red sufu	Grey sufu
20	634.24	409.33	878.76	556.98	376.88	890.76	743.64	876.54	903.45	789.34	247.34	523.76
25	711.72	476.54	1006.65	998.34	455.09	906.11	994.22	1022.47	1126.33	985.37	487.45	578.32
30	823.35	656.65	1078.51	754.77	568.98	1062.83	862.43	1065.24	1324.24	1123.84	589.65	687.32
35	845.71	665.25	1154.12	878.42	976.54	1149.61	935.33	1189.40	1053.32	1222.87	765.99	712.22
40	876.34	805.64	1223.87	966.43	983.83	1042.95	988.65	922.32	1032.43	1015.35	996.76	1174.43