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## Research Paper

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FEASIBILITY STUDY ON THE QUALITY OF SAUSAGE BY SYNCHRONOUS  
FLUORESCENCE COUPLED WITH PARAFAC, ANN AND SVMYanhui Sun<sup>1</sup>, Shulan Liu<sup>1</sup>, Shuangfang Li<sup>1</sup>, Wenjuan Miao<sup>1</sup>  
and Haiyang Gu<sup>1,2\*</sup>

\*Corresponding Author: Haiyang Gu, ✉ 1323992481@qq.com

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Sausage before and after exposure to vitamin C had been analyzed in solid phase using synchronous fluorescence method. A series of Excitation Emission Matrix (EEM) produced by scanning synchronous fluorescence intensity and providing a unique fingerprint data for the specific analytes. Parallel factor analysis (PARAFAC) was carried out to reduce high-dimensional EEM data from three dimensional data to two dimensional data. Artificial Neural Network (ANN) and Support Vector Machine (SVM) were applied to build the classification models based on the reduced-dimensional data. Results indicated that the EEM data was reduced by the selection of emission data at  $\Delta\lambda = 60$  nm based on the analysis of loading values. In contrast to ANN, SVM obtained an relative better classification result. The best classification models were constructed by Grid-SVM and PSO-SVM with 94.44% and 91.67% for train set and test set, respectively. This study suggested that synchronous fluorescence coupled with ANN method or SVM method is a helpful method for the quality evaluation of sausage and perhaps other food sensitivity to oxidation environment.

**Keywords:** Sausage, Vitamin C, Parallel factor analysis, Artificial neural network, Support vector machine

## INTRODUCTION

Sausage is obviously an important product widely consumed worldwide for both personal concerns and industrial. It mainly consisted of meat and solid fat dispersed into water (liquid phase) with a moderate heat treatment that form a stable mass (Mercadante *et al.*, 2010). Fat is an important component in sausage sample, which is important in the textural, sensory characteristics, and processing of sausage product. The fat quality can be changed by the feed ingredients and the fat sources during product formulation that makes fat is one of the most variable components. Besides the source influence, the oxidation influence is considered to be one of the most important factors for the changes of sausage quality during the process and storage.

As a consequence, it is of great interest to investigate the oxidation station of sausage before and after exposure to antioxidant during the storage.

A number of traditional methods have been developed to evaluate the state of sausage freshness, such as the detection of chemical changes associated with fat oxidation in sausage (Fuentes *et al.*, 2014), amperometric biosensors for determination of nitrite (Li *et al.*, 2014), near-infrared spectroscopy for the non-destructive and rapid analysis of chemical compositions in pork sausage (Ritthiruangdej *et al.*, 2011), and color evaluation for assessing sausage oxidation based on the color of sausage surface with respect to the interest regions (Jridi *et al.*, 2015). Despite these successes with prior oxidation evaluation methods, a series

<sup>1</sup> School of Bio & Food Engineering, Chuzhou University, Chuzhou, China 239000, India.

<sup>2</sup> School of Food Science and Engineering, Hefei University of Technology, Hefei, China 230000, India.

of problems are found in the requirement of more time, destruction of sample, and sensitivity to the interference from the environmental changes.

In the past decades, synchronous fluorescence spectra is developed as a helpful analytical method for food industry. For example, Konstantina I. Poulli and George A. Mousdis (Poulli *et al.*, 2007) applied synchronous fluorescence spectra in the quality evaluation of virgin olive oil, and they demonstrated that this method is helpful for the discrimination of olive-pomace, corn, sunflower, soybean, rapeseed and walnut oil in virgin olive oil at levels of 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% (w/w), respectively. Therefore, the detection and analysis of synchronous fluorescence spectra might be used for the quality control and quality assurance of sausage for food industrial.

This study was therefore intended to develop a rapid detection method based on synchronous fluorescence spectra for rapid evaluation of sausage freshness.

## MATERIALS AND METHODS

### Sample Preparation

Pork was selected because of its high nutrition, low price, popularity and availability throughout the year. Hind leg meat of pork purchased in a local market was used in the study. Pork meat was then cleaned, stripped and sliced to produce sausage with the addition of white sugar, sodium nitrite, carrageenan, and vitamin C. All samples were storage at room temperature to investigate oxidation influence on sausage at different days. The experiments of fluorescence spectrum were carried out for 17 days.

### Synchronous Fluorescence Data

Synchronous fluorescence data was obtained by the Cary Eclipse spectrofluorometer. This instrument is fully controlled by a computer with a double-grating monochromator for emission and excitation monochromator. Synchronous fluorescence spectra was obtained emission wavelength in the range from 320 to 600 nm with the offsets of  $\Delta\lambda = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110$  and 120 nm between excitation and emission monochromators. The sausage samples were moved to a front-surface sample-holder and incidence angle was set at  $56^\circ$  to avoid the influence of depolarization phenomena, reflected light, and scattered radiation (Sahar *et al.*, 2009). All synchronous fluorescence experiments were carried out at room temperature.

### Parallel Factor Analysis (PARAFAC)

PARAFAC is a generalization of principal component analysis (i.e., PCA) to decompose high dimensional data to two-dimensional data (Burdick, 1995). The PARAFAC Toolbox was obtained from the website: <http://www.models.kvl.dk>. The synchronous fluorescence data was a three-dimensional data composed of samples, excitation wavelength, and emission spectrum. The number of PARAFAC components is an important parameter to reconstruct the synchronous fluorescence data (Rutledge and Jouan, 2007). In this study, the appropriate number of PARAFAC components is evaluated by the sum of squared error. When the sum of squared drops from a high value to a relative low value, the appropriate number of components will be determined. Then, the smallest emission loading in the appropriate number of components is applied to decompose three-dimensional data to be two-dimensional data for the further analysis.

### Support Vector Machine (SVM)

SVM is performed to find a linear relation between the dependent variables and the classification method similar to the other classification methods (i.e., ANN and PLS). It is a statistical learning theory that was proposed by Vapnik and Chervonenkis (1971). SVMs are generally performed as a regression tool and principles can easily be extended to classification method. The dependent variable (i.e.,  $y_i$ ) is predicted by  $\hat{y}_i$ , and the specific regressor is represented by  $x_i$ , and the  $w$  and  $b$  are slope and the offset of the regression line. The equation for input data and output data is described as  $(\hat{y}_i = wx_i + b)$  where the line minimising a certain cost function is used to define the best line. The following equation 1 is a cost function that is minimized to define the best regression line.

$$Q = \frac{1}{2} W^T W + C \sum_{i=1}^N (\xi_i + \xi_i^*) \quad (1)$$

The above cost function consisted of a set of constraints and the error weight,  $C$  which were 2-norm penalty on the regression coefficients. This cost function is carried out to minimize both the prediction errors and the coefficients size, as shown in Equation 2.

$$\begin{cases} y_i - wx_i - b \leq \varepsilon + \xi_i \\ wx_i + b - y_i \leq \varepsilon + \xi_i^* \\ \xi_i, \xi_i^* \geq 0 \end{cases}$$

## Artificial Neural Network

Artificial Neural Network (ANN) is a powerful method to process complex multivariable data. The comprehensive mechanism of neural network from an engineering perspective was presented by Haykin (Sun *et al.*, 2014) to use multi-layer perceptron neural network of with NNG to select variable information. A traditional ANN method consists of an input layer, a hidden layer and an output layer. The input data was used to train ANN to extract the statistical properties of the input data for constructing a classification model. Then, the input and output variables can be established a meaningful relationship during the learning process. In this paper, the input data was extracted from synchronous fluorescence data by PARAFAC method.

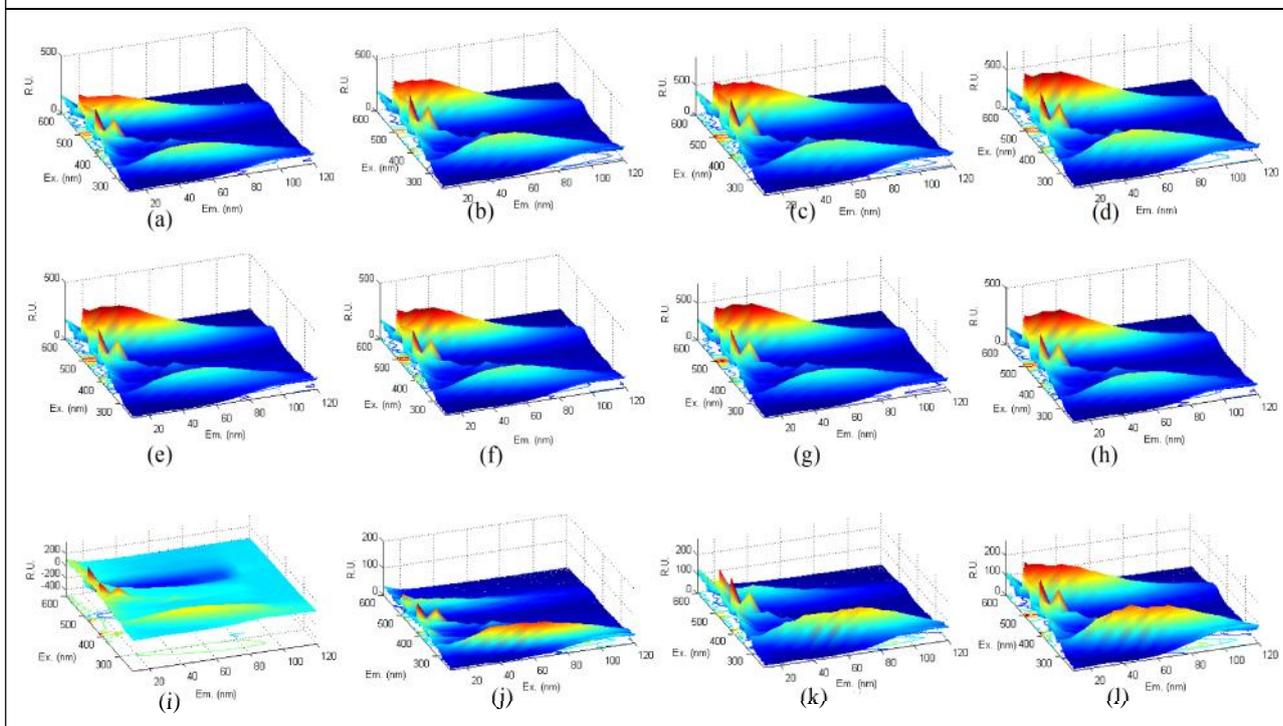
## RESULTS AND DISCUSSION

### Synchronous Fluorescence Spectrum

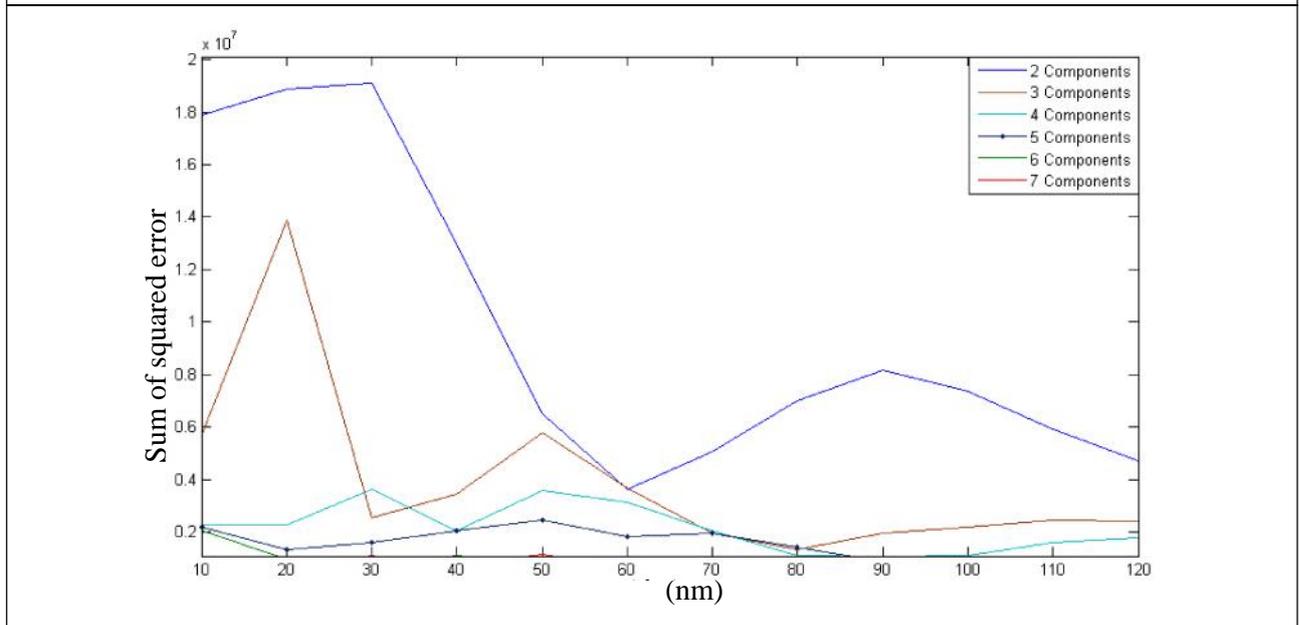
As shown in Figure 1a, the synchronous fluorescence spectra of the sausage before exposure to vitamin C at day 1 serves as a starting point for investigating the influence of storage days and Antioxidants. The synchronous

fluorescence spectra of original sausage for four different storage days (i.e., day 1, day 5, day 9, and day 13) were shown in Figures 1a-1d. Besides the sausage before exposure to vitamin C, the sausage after exposure to vitamin C were similarly displayed in Figures 1e-1h. These eight images show that Excitation Emission Matrices (EEM) changed with the variety and intensity of the chemical components from the sausage during the storage and vitamin C added in the sausage. The chemical components of sausage changed as a result of oxidation of lipids decreases the nutritional value after the sausage was produced (Skrivan *et al.*, 2012). At the first stage of storage, the complex mixtures of ketones, aldehydes, hydrocarbons, alcohols, esters, lactones and furans were produced via a free radical chain mechanism (Moore and Roberts, 1998). Then, the secondary products react with amino acids, proteins or other components with the decrease of sausage freshness. Therefore, synchronous fluorescence spectra measured at this stage was significantly different from that at the first day. A similar pattern of EEM changes was observed for samples measured between day 9 and day 13. However, it is interesting to notice that the synchronous fluorescence

**Figure 1: Three-Dimensional Fluorescence Spectra for Sausage Before and After Exposure to Vitamin C, Sausage Before Exposure to Vitamin C at Day 1(a), Day 5(b), Day 9(c), and Day 13(d), After Exposure to Vitamin C at Day 1(e), Day 5(f), Day 9(g) and Day 13(h), Whereas Difference Images Between Before and After Exposure to Vitamin C at Day 1(i), Day 5(j), Day 9(k) and Day 13(l)**



**Figure 2: The Sum of Squared Errors Calculated by PARAFAC Method at Different Component Models**



spectra of the sausage after exposure to vitamin C (i.e., Figures 1e-1h) have no significant change during the storage. The reason may be that the addition of vitamin C prevents the oxidation reaction in the sausage. The difference EEM images, which were obtained by subtracting the after-exposure data from the before-exposure data, indicated more changes on the image for each (a) sample. Therefore, it makes the EEM images more convenient to discriminate the influence of vitamin C on the sausage storage.

### The Analysis of Components

The objective of PARAFAC is to decompose the synchronous fluorescence data from three dimensional data to be two dimensional data present in the spectral data set of sausage, that is, to establish a classification model for the further analysis. The EEM spectra of sausage samples obtained before and after exposure to vitamin C at different storage times were analyzed using PARAFAC method. To ensure that our PARAFAC results were reliable, the suitable number of components is very important before analyzing EEM results (Holbrook *et al.*, 2006). It is well reported that the result of residuals was used to determine the appropriate component (Stedmon *et al.*, 2003; Stedmon and Bro, 2008; and Yu *et al.*, 2010). To investigate which component is suitable, the sum of squared error for components varying from 2 to 7 were shown in Figure 2. It is interesting to notice that the sum of squared error for each component reduces

following the increase of component number. It is easily to find that the sum of squared error is much smaller when the component number is more than 5. Therefore, the model with 6 component was selected for the further analysis of emission loading.

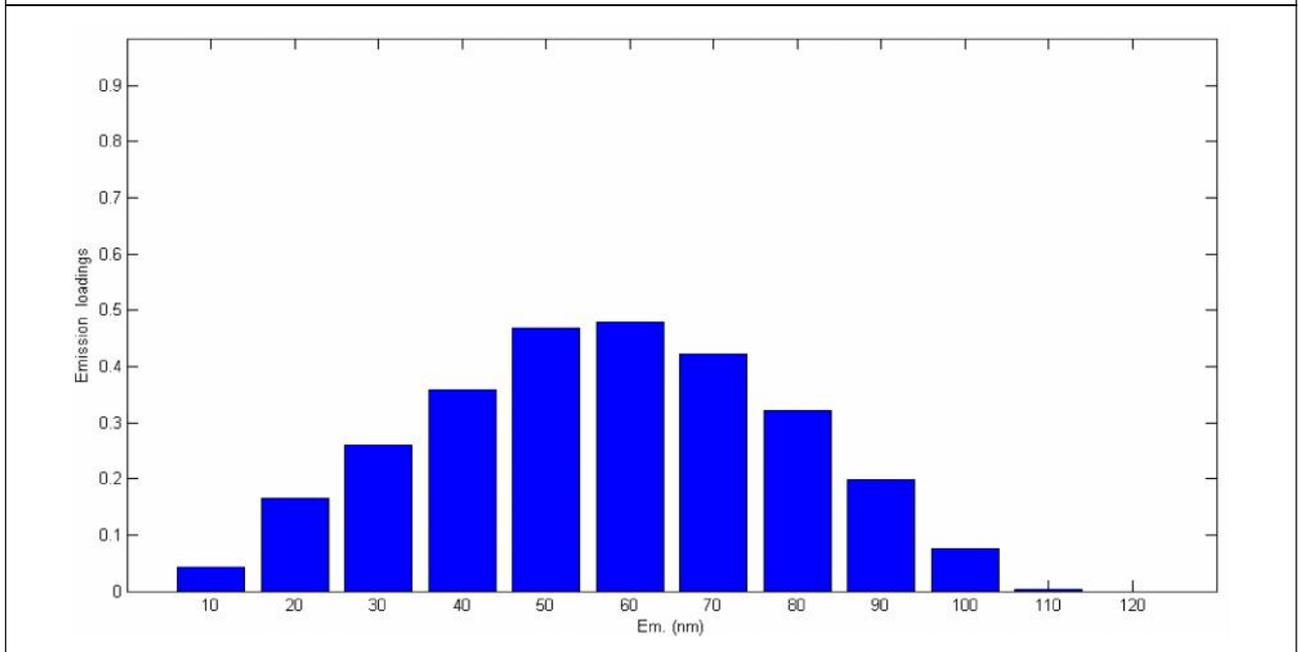
### Emission Loading

The loading values in emission wavelength were calculated to find the concentration mode for synchronous fluorescence data by PARAFAC method. As shown in Figure 3, the loading values are displayed in such a way that can easily be discriminated that one of the two dimensional datas (i.e., three dimensional data of synchronous fluorescence data at different  $\Delta\lambda$ ) is can be used to represent the three dimensional data. It is generally accepted that the analysis of emission loading provides an easy mechanism for the reduce of three dimensional data to be two dimensional data. It is important to notice that the emission at  $\Delta\lambda = 60$  nm possesses the largest loading value, followed by the emission at  $\Delta\lambda = 40, 50, 70$  and  $80$  nm, whereas the emission at other  $\Delta\lambda$  possess relative smaller loading value.

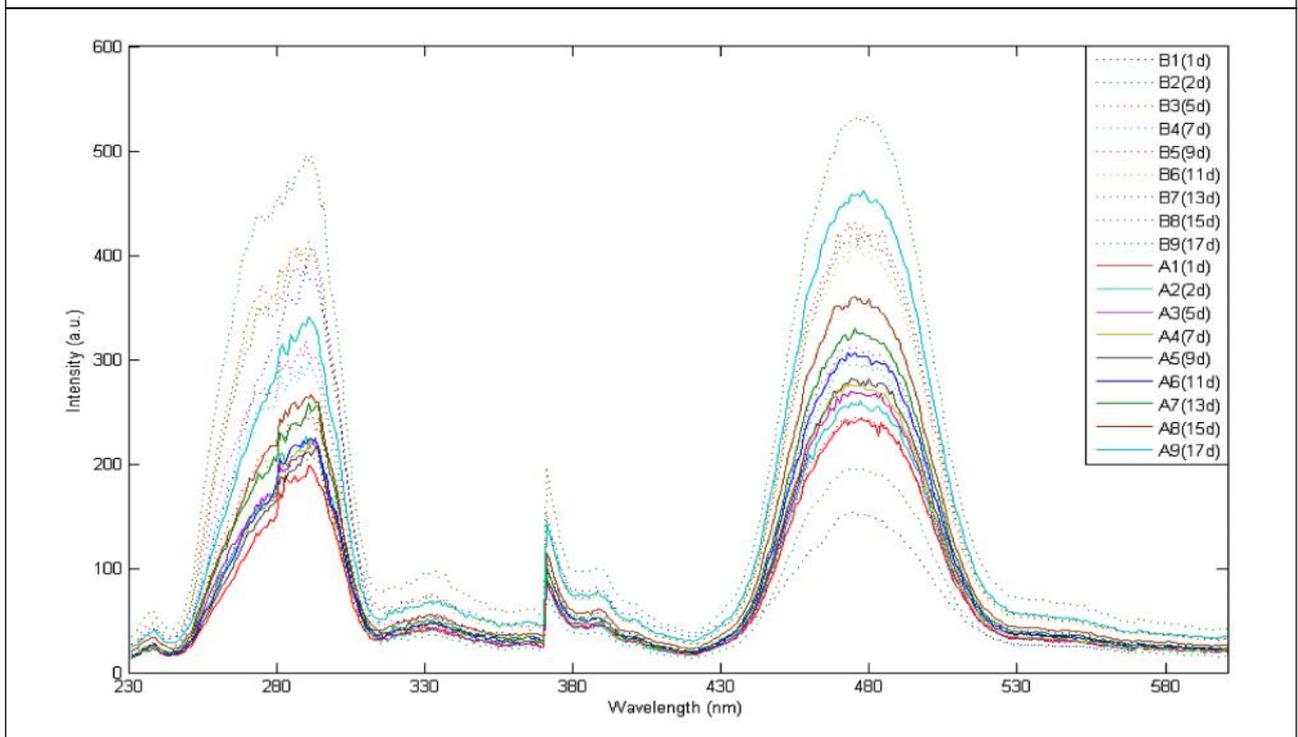
### Two Dimensional EEM Image

Figure 4 shows the two dimensional spectrum of excitation wavelength at  $\Delta\lambda = 60$  nm in sausage samples obtained by decomposition of the EEM spectra by the comparison of emission loading using the six component PARAFAC model.

**Figure 3: The Emission Spectra Loadings of 6 Component Derived from PARAFAC Model**



**Figure 4: xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx**



The two dimensional spectrum possesses the similar peak shape for all sausage samples before and after exposure to vitamin C at different storage day.

### Classification Results

The two dimensional data obtained from EEM data decomposed by the PARAFAC method was classified by

**Table 1: The Classification of Sausage Samples Before and After Exposure to Vitamin C by SVM Methods**

Models	Best c	Best g	Train Samples	Train Accuracy	Test Samples	Test Accuracy	CV Accuracy
GA-SVM	31.73	0.0012	72	94.44%	36	88.89%	94.44%
Grid-SVM	68.59	0.0011	72	94.44%	36	91.67%	93.06%
PSO-SVM	39.81	0.01	72	94.44%	36	91.67%	93.06%

**Table 2: The Classification of Sausage Samples Before and After Exposure to Vitamin C by Artificial Neural Network**

Output Class	Train Confusion Matrix			Test Confusion Matrix			Validation Confusion Matrix		
	Group 1	Group 2	Classification Accuracy (%)	Group 1	Group 2	Classification Accuracy (%)	Group 1	Group 2	Classification Accuracy (%)
Group 1	26	0	100	11	1	91.7	11	1	91.7
Group 2	5	33	86.8	1	9	90	0	10	100
Classification Accuracy (%)	83.9	100	92.2	91.7	90	90.9	100	90.9	95.5

ANN method and SVM method into two groups, corresponding to sausage before and after exposure to vitamin C.

#### SVM Results

The GA-SVM, Grid-SVM, and PSO-SVM algorithm were applied to discriminate sausage before and after exposure to vitamin C. As seen from Table 1, the optimized c and g showed that the best c and best g for GA-SVM, Grid-SVM, and PSO-SVM were (31.73, 0.0012), (68.59, 0.0011) and (39.81, 0.01), respectively. After the selection of best c and best g, it can be easily to find that the all SVM methods possess the similar train accuracy at 94.44%. However, the test accuracy of GA-SVM was 88.89%, which was smaller than that from Grid-SVM and PSO-SVM methods at 91.67%. Besides the train accuracy and test accuracy, CV accuracy also had the important influence on the classification results. Results showed that the GA-SVM had the largest accuracy with 94.44%, followed by Grid-SVM and PSO-SVM with 93.06%, respectively.

#### ANN Results

The results of the classification models by ANN method were shown in Table 2. As seen from this table, the sausage samples were classified into two groups, corresponding to sausage samples before exposure to vitamin C (group 1), and sausage samples after exposure to vitamin C (group 2). The train classification accuracy, test classification accuracy, and validation classification accuracy were 92.2%, 90.9%,

and 95.5%. The ANN method had lower classification accuracies for samples from group 2 at train set and test set. It misclassified 5 samples for the group 2 into group 1 for train set, and 1 for the group 2 into group 1 for test set. This is partly because the sausage samples after exposure to vitamin C are more sensitivity to oxidation environment; some sausage samples oxidated faster than other that made the samples in group 2 misclassified into group 1. Comparing to the classification results from SVM method, the train accuracy and test accuracy possessed smaller classification accuracy. The results maybe that the SVM method is more suitable for the small samples, and ANN method is always used to process the large samples.

#### CONCLUSION

This paper investigated the sausage samples before and after exposure to vitamin C based on synchronous fluorescence spectrum. ANN, GA-SVM, Grid-SVM, and PSO-SVM classification models produced acceptable accuracy and precision in the train set and test set. The relative larger classification accuracy of 94.44% and 93.06% were achieved by PSO-SVM and Grid-SVM methods. Results suggest that synchronous fluorescence spectrum method coupled with ANN method and SVM method displayed a well performance in rapid and non-destructive discrimination of vitamin C in sausage. This method may be also helpful for other types of food before and after exposure to vitamin C. Further study is needed to improve the classification accuracy for predicting sausage freshness.

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