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Research Article

Study on Detection and Classification of Tetracycline Residue in Duck Meat Using Synchronous Fluorescence Spectra and Support Vector Machine

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Abstract

To the rapid detection of whether the tetracycline residues are excess in duck meat, the optimum characteristic wavelength difference □λ was determined by synchronous fluorescence analytical method. The recognition model of different residual levels of tetracycline was established by using support vector machine classification algorithm. Firstly, the optimum wavelength difference □λ for duck meat samples was determined as 70nm, and synchronous fluorescence spectra of different samples under the condition of □λ 70nm were collected. Secondly, original synchronous fluorescence spectra were preprocessed by using standard normal variables change (SNV). Finally, 18 wavelength variables were selected from 121 wavelength variables of pretreatment spectra by using competitive adaptive reweighted sampling (CARS). Then the radial basis function (RBF) was selected as the kernel function of support vector classification (SVC), and the optimal kernel function factor C and g were determined as 2.83 and 1, respectively, which were obtained by using grid searching combined with 5-fold cross validation. The classification model of SNV-CARS-SVC was established, and the classification accuracy rate of the model was 95.7% for prediction sets samples. The results showed that the synchronous fluorescence analysis method could identify tetracycline different residual levels quickly and accurately, and a feasible method was provided for identifying the quality of duck meat.

Keywords: synchronous fluorescence spectra, competitive adaptive reweighted sampling, support vector machine, tetracycline, duck meat.

1. Introduction

Duck meat is rich in high protein, low cholesterol and low fat. Generally，duck meat contains more vitamin B, vitamin E and nicotine acid than other meat products, and has rich nutritional value of nourishing stomach and reinforcing kidney [1]. Tetracycline, which is a kind of broad-spectrum sterilization and antibacterial antibiotics, has been widely used as feed additives for promoting growth and keeping health, and are also used to prevent and treat infectious diseases because of low cost, strong sterilization ability and wide antibacterial [2]. A series of problems has emerged as a result of such extensive applications in animal industry, for example a large number of tetracycline residues were found in poultry products. Furthermore, the tetracycline residues can affect meat quality and endanger human body health through the food chain.

Conventional detection methods of tetracycline in poultry products include microbiological method [3], HPLC [4], spectrophotometry [5], TLC [6] and etc. For example, Yantu Zhang et al [7] used high performance liquid chromatography with chemiluminescence to detect tetracycline residues in milk. Although the methods are sensitive and have low limits of detection, they are time-consuming, and can't achieve requires of rapid detection because of the complicated and expensive sample preparation procedures. Xiaorong Huang et al [8] developed a method based on microorganism for detecting antibiotics residues in food, but the enrichment process was time-consuming. These conventional methods tend to time-consume and complicate sample preparation processes. In general, it was difficult to achieve requirements of rapidly detecting whether tetracycline residues were excess in duck meat. However, the application of tetracycline residues in duck meat by synchronous fluorescence was rarely reported. Synchronous fluorescence spectrometry [9], [10], [11] is a simple method for the detection of complex background interference analytes, and can narrow band and reduce the scattering interference. Therefore, a recognition model, which was used to distinguish whether tetracycline residues were excess in duck meat, was established by using synchronous fluorescence spectrometry [12] combined with SVM classification algorithm in this paper. Synchronous fluorescence spectra of 70 duck samples containing different concentrations of tetracycline were measured using solid collection device of the Cary Eclipase instrument. The characteristic variables for the spectra of the tetracycline in duck meat were selected by using CARS [13], [14]. Finally, SVM [9], [15] was used to build the classification model in order to achieve the goal of rapidly identifying the quality of duck meat.

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2. Materials and Methods

2.1. Reagents and Instruments

Duck meat was purchased from the vegetable market of Jiangxi Agricultural University; Tetracycline (98.0%) was obtained from standard substances network of china; Sodium hydroxide; Ultrapure water (Kertone Co., Ltd.) was used for the preparation of all aqueous solutions. Unless stated otherwise, all chemicals were of analytical reagent grade and used without further purification.

Fluorescence spectra were recorded using Cary Eclipase spectrofluorophotometer (Varian, USA) with the solid collection device; JK-50B ultrasonic cleaner was purchased from Hefei Jinnike Machinery Co., Ltd; FA1004B electronic weigher (accuracy 0.1mg) was purchased from Shanghai Shangping instrument Co., Ltd; FD-1-50 vacuum freeze-drying machine (Beijing Boyikang instrument Co., Ltd); HH-6 digital thermostat water bath (Shanghai Pudong physical optical Instrument Factory).

2.2 Preparations of Samples

(1)The breast meats were removed from ducks and the membranes were removed from the breast meats, they were frozen for a moment in the refrigerator. The breast meats were cut into thin slices, and then frozen for some time. (2)Water was removed from the breast meats of frozen by vacuum freeze dryer for 30 hours, and 70 circular samples were made into the diameter of 1.3cm and the thickness of 2mm. (3)500 mg/L tetracycline solution was prepared by dissolving 100 mg of standard substance in 200mL ultrapure water, working solutions were freshly prepared from stock solution by appropriate dilution with ultrapure water. (4)1.5mol/L NaOH solution was prepared by dissolving 3 g of sodium hydroxide in 50mL volumetric flask with ultrapure water. (5) Different concentrations of tetracycline solution were added into 70 brown volumetric flasks of 10 mL, meanwhile, the same volume of NaOH solution was added. And the mixed solution was degraded in boiling water for 30min. (6) Duck samples were soaked into the different concentrations of tetracycline degradation solution for half an hour, 70 duck samples containing different concentrations of tetracycline were obtained after they were dried on the condition of the room temperature. (7)The synchronous fluorescence spectra of duck samples were collected by using spectrophotometer with the solids collection device, parameters were set as follows: The wavelength difference □λ was 20～120nm, and the interval was 5nm; The voltage was set at 680V; Excitation and emission slits were set at 10nm and 5nm, respectively; Emission filter was set at 295～1100nm; The spectra was scanned with middle scanning speed in the wavelength range of 240～360nm.

In the production process of the samples, the tetracycline content of each sample was obtained by the analysis, and the concentration range was 0.081mg/kg～180.7mg/kg. The detection sensitivity was slightly lower than the solution state because the spectra of the samples were directly detected. 11 samples of the concentration range between 0.081mg/kg and 3.2mg/kg were classified as low degree of residues. 27 samples of the concentration range between 6.9mg/kg and 95.9mg/kg were classified as moderate residues. 32 samples of the concentration range between 104.2mg/kg and 180.7mg/kg were classified as higher degree of residues. The relevant data is shown in Table 1.

2.3 Analysis Methods of Data

Firstly, the preprocessing of spectra was considered in the classification model whether the tetracycline residues are excess in duck meat. The performances of four preprocessing methods, which were multiplicative scatter correction (MSC), smooth handling, standardized processing and standard normal variable transformation (SNV), respectively, were compared, and the appropriate method of pretreatment was determined through the level of classification accuracy of the classification model. Then, synchronous fluorescence spectra of 70 samples were preprocessed by the optimal pretreatment method. The characteristic wavelength variables of tetracycline were selected by CARS, and the algorithm principles and methods reference [13], [14]. Finally, the selected variables were used as the input variables of the SVM classification algorithm, and the recognition model of tetracycline residues levels was established. The best parameters of SVM classifier were obtained by using grid searching combined with the 5-fold cross validation method. According to the principle of division of 2:1, 47 samples were used to train SVM classifier, and the remaining 23 samples were used to predict. The relevant procedures were completed by MATLAB 2010b (The Math Works, USA) software.

Table 1: Statistical result of tetracycline of duck meat in data sets

Classes	Number of samples	Minimum mg/kg	Maximum mg/kg	Average mg/kg
low degree of residues	11	0.08	3.20	1.59
moderate residues	27	6.90	95.9	51.3
higher degree of residues	32	104.2	180.7	138.6

3. Results and discussion

3.1 Synchronous Fluorescence Spectra of Samples

As shown in Fig.1, the 3D synchronous fluorescence spectra were scanned for duck meat samples containing tetracycline. In most of synchronous fluorescence studies for different systems, the selection of wavelength difference □λ is very important. The influence of □λ can be substantial on the spectral shape, width, signal strength and spectral degree of overlap. In this paper, in order to obtain the best value of □λ, 3D synchronous fluorescence spectra were studied between 20nm and 120nm, and three-dimensional data was made up of the fluorescence intensity, excitation wavelength and the wavelength difference (□λ). It can be observed that the fluorescence peaks of the samples, as shown in Fig.1, the duck meat has background fluorescence peaks at the excitation wavelength of about 290nm. And shown in Fig.1 (b), the background fluorescence peaks of duck meat and tetracycline peaks were well separated, and the fluorescence peak of tetracycline in duck meat was located the excitation wavelength of about 334nm.

Three dimensional synchronous fluorescence spectra contained the abundance of characteristic peaks, and scanning of the spectra was time-consuming, thus, that is not conducive to rapidly analysis. According to the analysis, the results showed that the signal intensity of fluorescence peaks was higher when the wavelength difference □λ was equal to

70 nm. The synchronous fluorescence spectra of 70 samples were selected on the condition of □λ 70nm for data analysis and processing.

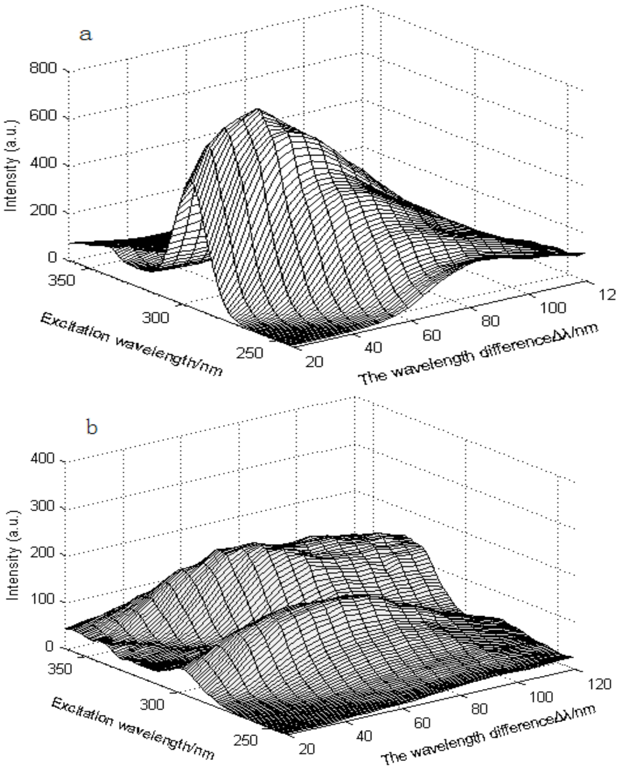

Fig.1. The 3D synchronous fluorescence spectroscopy of duck meat (a) and duck meat containing tetracycline (b)

3.2 Preprocessing of Synchronous Fluorescence Spectra

The information of synchronous fluorescence spectra not only contained the substance signals, but also the noise which was caused the instrument or the environment. In order to minimize error and improve the classification accuracy of SVM classifier, the original synchronous fluorescence spectra of samples were pretreated by using MSC, Smooth, Normalization and SNV, respectively, and the four pretreatment methods had a different impact on the classification forecast model.

Table 2: Results of CARS-SVC model with different pretreatment methods

Modeling methods	Number of correct classify /prediction samples	Classification accuracy%
SNV-CARS-SVC	22/23	95.7
MSC-CARS-SVC	21/23	91.3
Smooth-CARS-SVC	21/23	91.3
Normalization -CARS-SVC	21/23	91.3
None-CARS-SVC	19/23	82.6

The characteristic variables associated with tetracycline, which were selected from spectrum pretreatment data by CARS, were determined as the inputs of SVM classifier. Different classification results of tetracycline residual level in duck meat were shown in Table 2. The model was built up by CARS-SVC. The highest classification accuracy of the CARS-SVC model was 95.7% after the spectra were preprocessed by SNV pretreatment method. The other three spectra preprocessing classification accuracy were all 91.3%, and the spectra of none preprocessing classification accuracy was lowest, only 82.6%. Therefore, the SNV were selected for spectra pretreatment method in subsequent data processing and analysing

3.3 CARS Variable Selection Method

The SVC model was taken more time to calculate, and would affect the classification accuracy because the full band spectra contain a large number of irrelevant information and background noise signal. Characteristic variables were selected from the full band spectra before modeling. In this paper, a new variable selection method called CARS [13] was used to collect informative variables and remove unimportant variables. The method imitated the principle of “survival of the fittest”, each wavelength of the spectrum as an individual. Major wavelength points of the PLS model were selected by using adaptive re-weighted sampling technique, and small weight wavelength points were removed. Thesubset with the lowest root mean square error of cross-validation was considered as the best variable subset. The sampling number was 50, and the PLS cross-validation was 5-fold. The classification model was established when 18 wavelength variables were selected by CARS as the inputs of the model. As shown in table 3, the CARS method was used to extract wavelength variables, the model was not only simple and quick, but also classification accuracy increased to 95.7% from 87.0%.

Table 3: Results of SVC model using CARS methods

Modeling methods	Number of correct classify /prediction samples	Classification accuracy%
SNV-SVC	20/23	87.0
SNV-CARS-SVC	22/23	95.7

3.4 SVC Model

Support vector machines (SVM) [9] based on the structural risk minimization principle. It can provide good generalization performance in the classification problem, and have the characteristics of versatility and robustness. Solving ability of model is reflected in the small sample size, the local minimum point and non-linear problem. SVM [15] can obtain the optimal classification results in the linear separable problems. The nonlinear separable problem can map to be divided into high-dimensional space by a nonlinear mapping. An inner product function was defined as non-linear mapping that was the kernel function. Radial basis function selected can help reduce the complexity of training process of classification model, because the residual tetracycline samples belong to the non-linear separable problems.

Kernel function types and the choice of the relevant parameters (penalty parameter C and kernel function parameters g) were important for the SVM classification model. The C parameters are related to the size of the error penalty score, g parameters are related to the function of the regression error and the parameters even affect the initial value of the feature vector and the characteristic value. Grid search combined with 5-fold cross-validation were usually employed to find the optimal parameters of the model for the training samples. The process of optimization parameters was as follows: Firstly, the kernel parameters C and g were rough chosen, the initial value, the step, the end values were set at [-10 0.8 10]. Then, the parameters (C, g) of each pair of values were searched by using the grid search method, the best parameters were found by using the 5-fold cross-validation method which could make the cross-validation accuracy rate highest. The parameters were carefully chosen based on the results of a rough choice. The initial value, the step, the end values of C and g were changed at [-2 0.5 4] and [-4 0.5 4], respectively. And the best parameter pairs (C&g) were selected. As shown in Fig.2, a rough selection and choiceness search results of the figure were obtained, the abscissa and ordinate expressed the log2C and log2g of parameters, respectively. A contour plot of cross-validation accuracy of the SVM classification model was described in Fig.2. It was shown in Fig.2 that the cross-validation of the model prediction accuracy rate was up to 82.98% at C=2.83 and g=1.

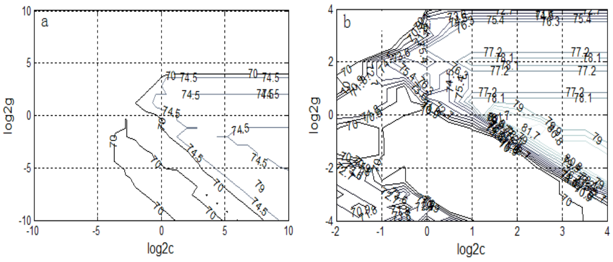

Fig.2. The contour plot of cross-validation prediction accuracy rate of SVM model by roughing (a) and choiceness (b)

3.5 Prediction Results of SVC Model

The C=2.83 and g=1 as the best parameters of the kernel function RBF were found by using grid search combined with 5-fold cross-validation method, and the SVM classifier was trained for training samples, then, SVC model of tetracycline residues in different levels was established. The classification accuracy rate was 95.7% for prediction samples. The plot of actual classes and predictive classes of SVC model was shown in Fig.3. A sample of the second class was classified as the third class mistakenly, and other samples were predicted correctly.

4. Conclusion

The wavelength difference □λ=70nm was selected by analyzing three dimensional synchronous fluorescence spectra of duck samples containing tetracycline. 18 wavelength variables related to tetracycline were selected by using CARS from synchronous fluorescence spectra after SNV spectral preprocessing. Variable numbers were reduced from 121 to 18, and the compression rate was 85.1%. SVC was employed to establish classification model of tetracycline residual level in duck meat, the classification accuracy of SNV-CARS-SVC prediction model was 95.7%. A new method was provided for the rapid and accurate detection of whether the tetracycline residues are excess in duck meat.

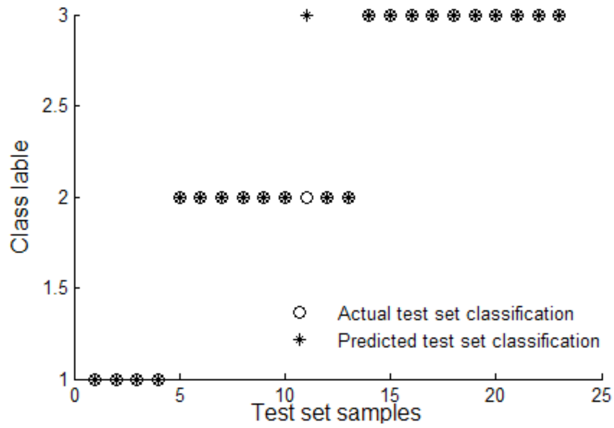

Fig.3. The plot of actual classes and predictive classes of SVC model

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