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Determination of Tetracycline in Milk by Mid Infrared Spectroscopy

Honglu Wang[1], Nan Wu[1, a, *], Zhiwei Huang[1], Shangqi Yuan[1], Wenjing Huang[1] and Zhixing Zhang[1]

[1]College of Engineering and Technology, Tianjin Agricultural University, Tianjin 300384, China

[a]469718459@qq.com

Abstract

The feasibility of measuring tetracycline in milk was investigated by mid-infrared (MIR) spectroscopic technique combined with partial least squares (PLS) method. The MIR spectra of 40 pure milk samples and 40 tetracycline adulterated milk samples with different concentrations (from 0.005 to 40 mg/L) were obtained. The MIR spectra of all samples were collected in the range of 400 ~ 4000 cm-1. After the pretreatment of original spectral data, the PLS models were established in the full band and sub band ranges, respectively. The results show that different wave-number ranges had great impact on the prediction model of milk adulterated with tetracycline. The classification accuracies of the constructed PLS models were 100% for calibration set in the full band and sub band ranges, and the correlation coefficients of models were greater than 0.990 for prediction set, which shows that the models had good fitting effects. For the prediction set, the classification accuracies of the constructed models in the full band and in the range of 2000 ~ 4000 cm-1 were 93.9% and 90.9%, respectively.

Keywords

Antibiotics; Mid-infrared spectroscopy; Partial least squares.

1. INTRODUCTION

In recent years, the food safety problem has attracted more attention with the improvement of people's living standards. Harmful substances in milk, such as antibiotics, melamine, have attracted widespread attention of consumers, along with the increase in the consumption of milk in the world. Tetracycline, a broad-spectrum antibiotic, has been widely used in clinical treatment and animal husbandry due to its activity against various gram-positive and gram-negative bacteria. Tetracycline is usually used to treat dairy cow mastitis, which may lead to the presence of tetracycline residues in milk.

Mid-infrared (MIR) spectroscopy and near infrared (NIR) spectroscopy have the advantages of high speed, low cost and no complicated sample pretreatment [1-2]. Combined with chemometrics method, effective spectral information in samples can be extracted for qualitative and quantitative analysis, such as the detection of antibiotics, urea in milk and dairy products [3-4]. In this study, qualitative analysis models of tetracycline residues in milk were established by MIR spectroscopy combined with partial least squares discriminant analysis (PLS-DA). The feasibility of determining tetracycline residues in milk by MIR method was evaluated.

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2. MATERIALS AND METHODS

2.1. Sample Treatment

In this study, the pure milk samples were purchased in the supermarket. Tetracycline standard powder was purchased from Solarbio (purity > 99%). Tetracycline adulterated milk samples were prepared with different concentrations of 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 4, 6, 8, 10, 20, 30 and 40 mg/L. Two replicates were prepared for each concentration gradient. A total of 40 tetracycline adulterated milk samples were obtained. At the same time, 40 pure milk samples were prepared. The spectral data of 80 samples were collected by mid-infrared instrument.

2.2. Spectral Acquisition

The fourier transform infrared spectrometer of PerkinElmer Company was used, and the sample pool was the attenuated total reflection attachment of the instrument. The scanning range of mid-infrared spectrum was from 400 to 4000 cm-1, the resolution was 4 cm-1, and the scanning interval was 8 cm-1. The sample of each concentration was measured twice.

2.3. Data analysis

The spectral data were corrected by multivariate scattering correction by Unscrambler program, and the original spectral data were smoothed and preprocessed by Savitzky-Golary method in order to reduce the interference to the discriminant model. Then the discriminant model of tetracycline adulterated milk was established by using the Matlab package of PLS algorithm. The method of cross-validation was used to determine the optimal factor number of the model. The modeling correlation coefficient (R), root mean square errors of cross validation (RMSECV), and the root mean square errors of prediction (RMSEP) were used to evaluate the quality of the model.

3. RESULTS AND DISCUSSIONS

3.1. Mid infrared Spectrum Characteristics of Milk Samples

Fig. 1 shows the mid-infrared spectra of pure milk and tetracycline milk samples. In the whole spectral range, the positions of the spectral peaks of adulterated milk and pure milk were basically the same, about 1080 cm-1 and 2940 cm-1, and the obvious characteristics of tetracycline cannot be seen. This result indicates that it is difficult to distinguish whether the milk was adulterated with tetracycline or not according to the spectral shape and peak positions. So it is necessary to further use chemometrics method to distinguish the pure milk and tetracycline adulterated milk samples.

3.2. Analysis in the Full Band Range

The setting of factor number will directly affect the modeling results of PLS-DA. If the setting factor number is too high, some useless information will be introduced and the modeling time will be prolonged. If the setting factor number is too low, the ability of prediction model may be reduced due to the lack of some useful information. Because principal component analysis depends on the given data, the accuracy of the data also has a great influence on the analysis results, so the outliers in the data were eliminated before the analysis. Fig. 2 gives the variation of the RMSECV of the PLS-DA model with the factor number. It can be seen from the graph that the RMSECV of the discriminant model was the least when the factor number was five. So the factor number of the PLS-DA model of this experiment was selected as 5.

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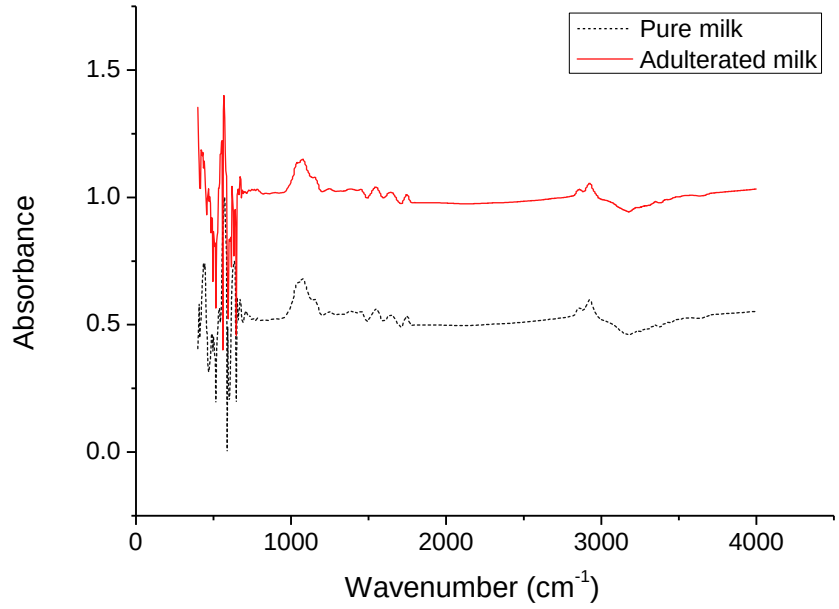

Figure 1. Mid-infrared spectra of pure milk and tetracycline adulterated milk

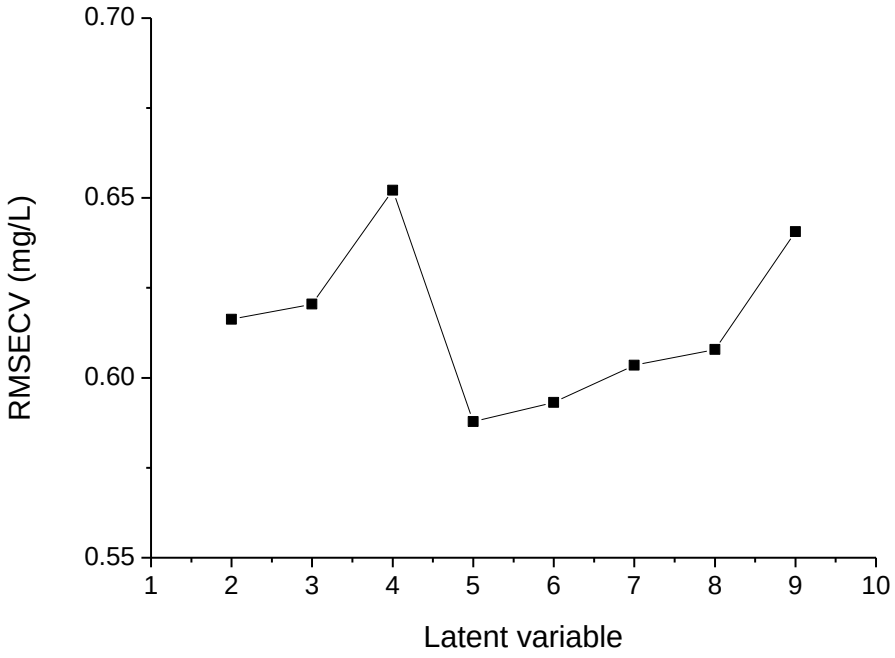

Figure 2. The effect of factor number on RMSECV of PLS-DA model

In this experiment, 80 samples were randomly divided into calibration set and prediction set, as shown in Table 1. The calibration set consists of 2/3 samples (53 samples including 27 pure milk samples and 26 tetracycline adulterated milk samples). The remaining 1/3 of the samples were used to construct the prediction set.

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Table 1. Sample division of pure milk and tetracycline adulterated milk samples for PLS-DA model

Category	Sample number in calibration set	Sample number in prediction set
Pure milk	27	13
Tetracycline adulterated milk	26	14
Total	53	27

In this experiment, a discriminant model of tetracycline adulterated milk was established based on 53 sample data in the calibration set in the range of from 400 to 4000 cm-1, as shown in Fig. 3. In PLS-DA, Y variable is a category variable. To discriminate two categories, the Y variable values of pure milk and tetracycline adulterated milk were set to 0 and 1, respectively. If the predicted value of a sample is more than 0.5, the sample is determined to be adulterated with tetracycline. If the predicted value of a sample is less than 0.5, the sample is determined to be pure milk. Fig. 3 shows the prediction results of internal samples in the calibration set by PLS-DA model. The results showed that the classification rates of pure milk and tetracycline adulterated milk were 100%.

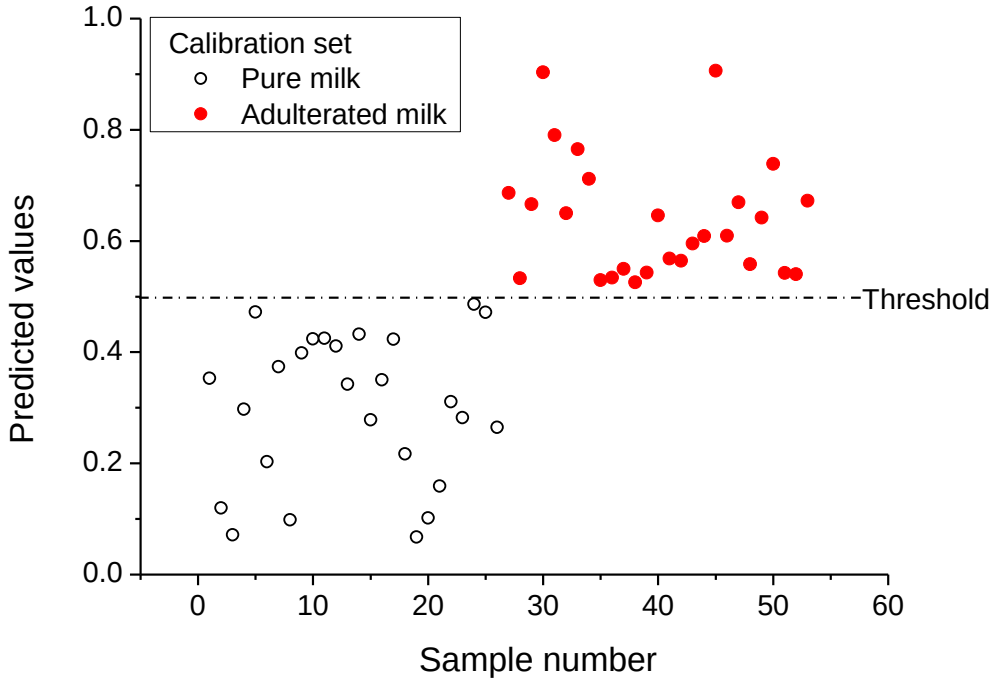

Figure 3. Predicted results of milk samples in the calibration set by PLS-DA model

In order to verify the discriminant accuracy of the established PLS-DA model, the PLS-DA model was further verified by 27 samples in the prediction set which did not participate in the modeling. Table 2 shows the predicted values and the final classification results for the prediction set samples. The results show that in the whole band (from 400 to 4000 cm-1), 2 of the 27 samples were misjudged. Specifically, two pure milk samples have been misjudged as tetracycline adulterated milk, and all the tetracycline adulterated milk samples have been correctly judged. The rate of correct classification of the established model was 93.9%.

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Table 2. Predicted results of milk samples in the prediction set by PLS-DA model

Sample	Prediction setting	Prediction values	Final classification	Sample	Prediction setting	Prediction values	Final classification
1	0	0.450	0	14	1	0.593	1
2	0	0.483	0	15	1	0.634	1
3	0	0.384	0	16	1	0.551	1
4	0	0.0373	0	17	1	0.685	1
5	0	0.463	0	18	1	0.706	1
6	0	0.675	1	19	1	0.662	1
7	0	0.692	1	20	1	0.743	1
8	0	0.245	0	21	1	0.557	1
9	0	0.057	0	22	1	0.810	1
10	0	0.332	0	23	1	0.725	1
11	0	0.064	0	24	1	0.967	1
12	0	0.327	0	25	1	0.941	1
13	0	0.250	0	26	1	0.783	1
				27	1	0.601	1

3.3. Analysis in the Sub Band Ranges

The performance and prediction accuracy of the PLS-DA model are affected by the spectral range [5], so it is very important to select the appropriate spectral band range for the establishment of the model. In this experiment, two different band ranges, i.e. 400~2000 cm^{-1} and 2000~4000 cm^{-1}, were selected to establish the PLS-DA model. As shown in Table 3, the discriminant results of the PLS-DA model established in the sub-band range and the full-band range for the correction set and the prediction set are given respectively. It can be seen that the factor number of full-band model was 5, and the factor numbers of both two sub bands were 4.

In the calibration set, the discriminating accuracies of the PLS-DA model reached 100%, regardless of the sub-band or the whole band range. When the wave number was between 400 and 2000 cm^{-1}, the correlation coefficient was 0.852, and the value was relatively low. In the sub-band (2000~4000 cm^{-1}) and the full band (400~4000 cm^{-1}), the modeling correlation coefficient values were about 0.900. When the wave number was between 2000 and 4000 cm^{-1}, the RMSECV was the lowest, which is 0.4852 mg/L.

In the prediction set, the correlation coefficients of the three different bands were all greater than 0.990, indicating that the fitting effect of the established model was good. When the band range was at 400-2000 cm^{-1}, the correlation coefficient of the model was high, but the RMSEP was lower than the other two sub bands. Moreover, when the band range was 400-2000 cm^{-1}, the discriminating accuracy of the model was 87.8%, while the discriminating accuracies in the other two sub bands were greater than 90.0%. In addition, the RMSEP of the model was the lowest (0.3342 mg/L) when the wave number was between 400 and 2000 cm^{-1}. Overall, the results indicate that the modeling effects of PLS-DA in the full band and the 2000-4000 cm^{-1} band were better than that in the 400-2000 cm^{-1} band. Different bands had a great impact on the prediction model of tetracycline adulterated milk.

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Table 3. Predicted results in the full band and sub band ranges by PLS-DA model

Wavenumber	Factor	Calibration set			Prediction set		
cm^{-1}	number	*R*	*RMSECV*	Classification accuracies%	*R*	*RMSEP*	Classification accuracies%
400~2000	4	0.852	0.5049	100	0.993	0.3342	87.8
2000~4000	4	0.901	0.4852	100	0.991	0.3452	90.9
400~4000	5	0.892	0.5878	100	0.991	0.4287	93.9

4. CONCLUSIONS

Mid-infrared spectroscopy was used to determine whether the pure milk was adulterated with tetracycline. The concentrations of tetracycline ranged from 0.005 to 40 mg/L. The classification accuracies of PLS-DA model established in the whole band range were 100.0% for 53 samples of calibration set and 93.9% for 27 samples of prediction set. The correlation coefficients of the model were above 0.990, which shows that the fitting effect of the model was good. The classification accuracies of the model established by the prediction set was 93.9% in the whole band range and 90.9% in the 2000-4000 cm^{-1} band range, respectively, which were higher than that of the 400-2000 cm^{-1} band range (87.8%). The results show that the method based on mid-infrared spectroscopy combined with PLS-DA can establish a fast and non-destructive detection method for detecting tetracycline adulterated milk. Although the tetracycline adulterated milk samples were only qualitatively analyzed in this study, as a supplement to high performance liquid chromatography, this method can preliminarily determine whether the milk is adulterated with antibiotics and improve the detection efficiency.

ACKNOWLEDGEMENTS

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