Detection of Tetracycline in Milk using NIR Spectroscopy and Partial Least Squares

Nan WU[1], Chenshan XU[1], Renjie YANG[1*], Xinning JI[2], Xinyuan LIU[1], Fan YANG[1], and Ming ZENG[3]

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

[2] College of Horticulture and Landscape, Tianjin Agricultural University, Tianjin 300384, China

[3] College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin 300457, China

Corresponding author's e-mail address: rjyang1978@163.com

Abstract. The feasibility of measuring tetracycline in milk was investigated by near infrared (NIR) spectroscopic technique combined with partial least squares (PLS) method. The NIR transmittance 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 pure milk and tetracycline adulterated milk samples were properly assigned to the categories with 100% accuracy in the calibration set, and the rate of correct classification of 96.3% was obtained in the prediction set. For the quantitation of tetracycline in adulterated milk, the root mean squares errors for calibration and prediction models were 0.61 mg/L and 4.22 mg/L, respectively. The PLS model had good fitting effect in calibration set, however its predictive ability was limited, especially for low tetracycline concentration samples. Totally, this approach can be considered as a promising tool for discrimination of tetracycline adulterated milk, as a supplement to high performance liquid chromatography.

1. Introduction

With the improvement of people's living standard, the consumption of milk continues to grow all over the world today. Meanwhile, the harmful substance (e.g. antibiotics, melamine, and urea) in milk had attracted much attention of consumer, with the increase of public attention to food safety.

Tetracyclines are broad-spectrum antibiotics exhibiting activity against a wide range of gram-positive and gram-negative bacteria, which are extensively used in clinical treatment and livestock industry [1]. As one of the most effective drugs, tetracyclines are commonly used for the treatment of dairy cow mastitis, which probably results in the presence of tetracycline residues in milk. Tetracycline residues in milk may provoke allergic symptoms in humans, or may lead to monetary losses in the dairy industry by inhibiting starter cultures in food technological processes [2]. At present, the analysis methods for antibiotic residues in milk are available, such as traditional microbiological method, high-performance liquid chromatography with UV (HPLC-UV) or fluorescence (HPLC-FLD) detection method, and high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) [3]. The detection limits of the above method are lower (from ppb to ppm level), but the sample pretreatments are relatively complex and not suitable for the on-site tests and fast detection.

Near infrared (NIR) spectrum detection technology has the characteristics of fast speed, low cost, and no need for complicated sample pretreatment [2]. Combined with chemometric methods, effective spectral information in samples can be extracted for qualitative and quantitative analysis, such as the determination of tetracyclines, and urea in milk and milk products [2, 4].

In this paper, the qualitative and quantitative analysis models are constructed using near infrared spectroscopic technique combined with multivariate calibration method, in order to study the feasibility of measuring tetracycline residues in milk.

2. Materials and Methods

2.1. Chemicals and Materials

Pure milk (Yili brand) samples were purchased from the local supermarket. Tetracycline hydrochloride powders (>99 %) were obtained from Solarbio (Beijing, China). Distilled water was obtained from a Molgene pure water system. Standard tetracycline stock solutions (100 mg/L in water) were prepared and stored at 4 °C. Working standard solutions of different concentrations were prepared by diluting the stock solutions with water. Then, 10 μL of working standard solutions was added to 10 mL of milk sample so that in all cases the change in the volume of the milk sample was negligible. Two replicates were prepared for each concentration. As a result, 40 tetracycline adulterated milk samples with different concentrations (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) were obtained.

2.2. Spectra Measurements

NIR spectral measurement was preformed at room temperature using a Spectrum GX Fourier transform infrared Spectrometer (Perkin-Elmer, USA). The spectrometer is equipped with an InGaAs detector and a quartz beam splitter. The NIR transmittance spectra of all the samples were obtained by taking the average reading of 16 scans in the range from 4000 to 10 000 cm^{-1} with a spectral resolution of 4 cm^{-1}. Forty pure milk samples and 40 tetracycline adulterated milk samples were scanned. In order to eliminate the influence of instrumental drift, the spectra of distilled water were collected as the background after each sample measurement to correct the spectra of milk samples.

2.3. Data processing

The raw spectra of samples obtained from the NIR spectrometer were processed by Nicolet Omnic. Then the Unscrambler software was used to construct PLS model based on raw spectra of pure and adulterated milk. Chemometric analyses were performed in MATLAB (The Mathworks Inc., Natic, MA).

3. Results and Discussions

3.1. Near infrared spectrum characteristics of pure and adulterated milk

Figure 1 shows the absorption features of pure milk and tetracycline adulterated milk in the near infrared range of 4000~10000 cm^{-1} spectra. Obviously, the spectral shape and peak positions of pure and adulterated milk samples were almost the same in the whole range of the spectra. It was evident that the differences were not strong enough to directly distinguish whether the milk sample was adulterated with tetracycline or not.

The interpretation of the complex NIR spectra of milk samples was difficult because the diverse components (e.g. lipids, proteins, vitamins, and minerals as well as somatic cells and bacteria) resulted in band overlapping, and a specific band in the spectra could contain information from more than one type of molecular vibration [2]. As the spectra show similar basic NIR spectral patterns, further discrimination analysis is quite necessary, where the whole spectral region was selected.

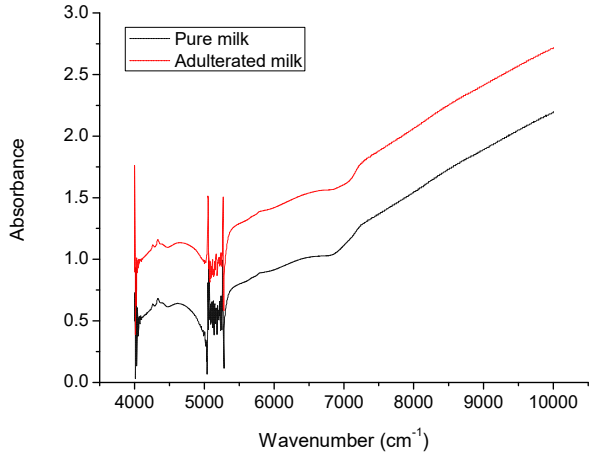

Figure 1. FT-NIR spectra of pure milk and tetracycline adulterated milk.

3.2. Qualitative analysis of adulterated milk

In present work, the partial least squares discriminant analysis (PLS-DA) was used to build a discrimination model. Eighty samples were randomly divided into calibration and prediction sets (Table 1). The calibration set consisted of two-thirds of samples (53 samples including 27 pure milk samples, and 26 tetracycline adulterated milk samples), and the remaining one-third of the samples were used to construct the prediction set. In the calibration set and prediction set, the pure and tetracycline adulterated milk samples were labeled as '0' and '1', respectively.

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

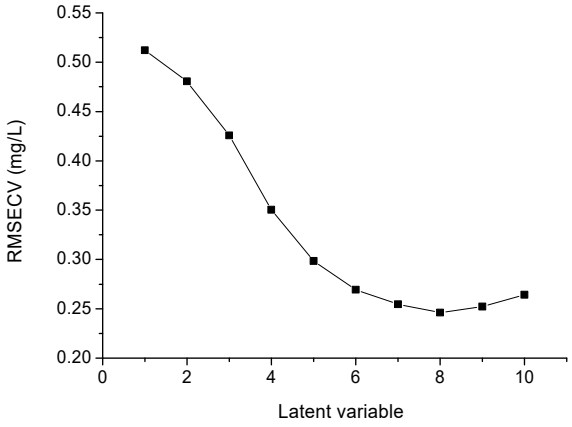

Figure 2. The effect of factor number on RMSECV of PLS-DA model.

Choosing the appropriate number of factors is a crucial step in PLS-DA model. The root mean square error of cross-validation (RMSECV) values of PLS-DA model at each number of factors were

calculated, which are shown in Figure 2. The minimum of RMSECV value (0.25 mg/L) was achieved with eight factors, which was chosen for optimal factor number.

Figure 3a shows the predicted results for calibration set using PLS-DA model. In the PLS-DA models used in this study, the threshold was set to 0.5. Above the threshold, the sample was estimated as 1 and was assigned to the adulterated milk with tetracycline. Below the threshold, the sample was estimated as 0 and was assigned to the pure milk. Samples 1 to 27, which represent the pure milk samples, belong to class 1. Samples 28 to 53, which represent the milk samples adulterated with tetracyclines, belong to class 2. As shown in Figure 3a, the prediction values of all pure samples were below 0.5, and the prediction values of all adulterated samples were over 0.5. Therefore, the pure milk and adulterated milk samples were properly assigned to the categories with 100% accuracy in the calibration set.

The predicted results of samples in the prediction set are shown in Figure 3b. Obviously, all the pure milk samples were correctly recognized. There was only one milk sample adulterated with tetracycline, which failed to be correctly recognized. Thus, the rate of correct classification of 96.3% was obtained in the prediction set.

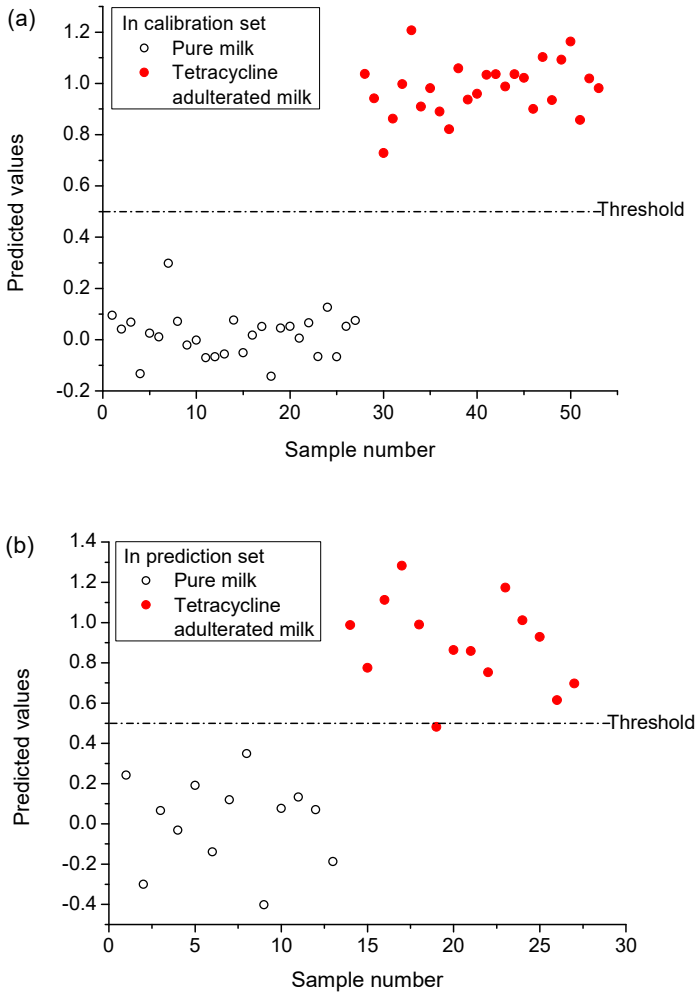

Figure 3. Predicted results of pure milk and tetracycline adulterated milk samples in (a) calibration set and (b) prediction set by PLS-DA model.

3.3. Quantitative analysis of adulterated milk

After qualitative discrimination, 40 adulterated milk samples were further quantitatively analyzed. A mathematical model for the determination of tetracycline in milk was established by partial least squares (PLS) method using the Unscrambler software. In order to evaluate the stability and fitting effect of the model, the internal interaction of the model is verified. Figure 4 presents the linear relationship between actual values (C_{actual}) and predicted values ($C_{predict}$) of tetracyclines in milk. The prediction equation was $C_{predict}$=0.003+0.997×C_{actual}, and the correlation coefficient R is 0.998. The root mean square error of cross validation (RMSECV) was 0.61 mg/L. The results indicate that the model has good stability and fitting effect in calibration set.

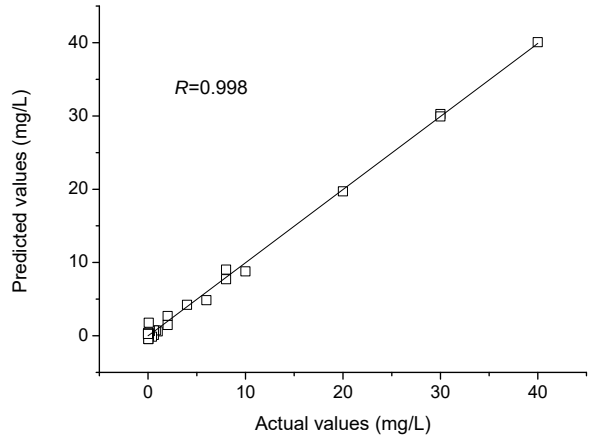

Figure 4. Relationship between actual values and predicted values of tetracycline content in calibration set.

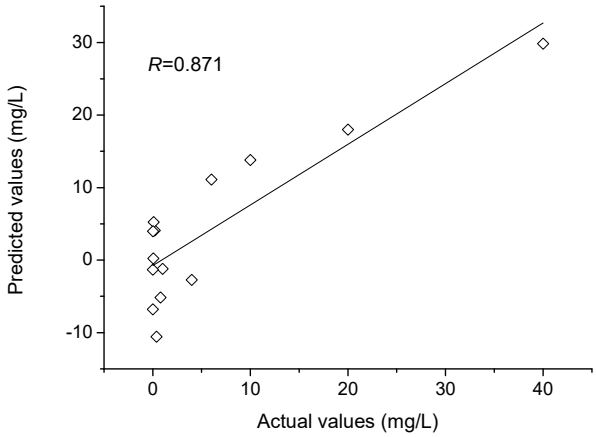

Figure 5. Relationship between actual values and predicted values of tetracycline content in prediction set.

External verification method is used to evaluate the actual prediction effect of PLS model. Figure 5 presents the linear relationship between actual values (C_{actual}) and predicted values ($C_{predict}$) of tetracyclines in prediction set. The prediction equation was $C_{predict}$=-0.750+0.836×C_{actual}, and the correlation coefficient R was 0.871. The root mean square error of prediction (RMSEP) was 4.22 mg/L. According to the results, the relative errors between the predicted and actual values were relatively large, especially for low concentration samples. The results indicate that the established model cannot meet the requirements of precise quantitative test well, especially for adulterated milk with low tetracycline concentrations (e.g. <1mg/L). The feasibility of quantitative model in tetracyclines prediction might be limited by trace tetracycline contents and full spectra modelling.

When spectral range is large, full spectra modeling can lead to information redundancy, model complexity, as well as the decrease of model accuracy. Moreover, due to the diversity and trace of adulterants and overlapping characteristic peaks between adulterants and milk, it is difficult to extract the feature information of adulterants in milk using conventional one dimensional spectrum [5].

4. Conclusions

This research demonstrated the potential of NIR spectroscopy to determine tetracycline contents in milk. The pure milk and adulterated milk samples were properly assigned to the categories with 100% accuracy in calibration set, and the rate of correct classification of 96.3% was obtained in prediction set. For the quantitation of tetracycline concentration in adulterated milk, the root mean squares errors for calibration and prediction models were 0.61 mg/L and 4.22 mg/L, respectively. The PLS model had good fitting effect in calibration set, however its predictive ability was limited, especially for low tetracycline concentration samples. Totally, the method based on NIR spectroscopy is able to discriminate tetracycline adulterated milk as a supplement to high performance liquid chromatography, which can improve the detection efficiency.

Acknowledgments

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