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Quantitative Determination of Potassium Sorbate in Muddy Flesh by Near-infrared Spectroscopy

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In current study, near-infrared spectroscopy (NIRS) was used to measure the amount of potassium sorbate in muddy flesh (pork). Partial least squares (PLS) and principal component regressions (PCR) were used to establish quantitative models. The partial least squares model parameters were investigated using raw spectra and different processing methods, such as the multiplicative scatter correction and the first- and second-derivative and Savitzky-Golay smoothing techniques. Moreover, these parameters and the prediction results were discussed in detail. The coefficient of determination and root mean square error of cross-validation of the partial least squares regression were 0.964583 and 0.00106, respectively. These results demonstrate that near-infrared spectroscopy can be used to quantify potassium sorbate, which avoids the pollution of the gas and high-performance liquid chromatography columns.

Key Words: Preconcentration, Extraction, Quantification, Near infrared.

INTRODUCTION

To inhibit the activity and multiplication of microorganisms, preservatives are added to many kinds of food products such as meat, juices and jams. Some directives and legislations are published as references for the currently allowed preservatives[1,2], of which sorbic acid and its corresponding potassium and calcium salts are some of the most widely used. However, the excessive use of antimicrobial food additives could result in adverse effects such as metabolic acidosis, convulsions and hyperpnoea in humans[3]. Thus, an effective and reliable analytical method should be developed to monitor the preservative levels in food products. Several methods are available for the analysis of sorbates in food products. Examples are thin-layer chromatography[4], ultraviolet spectroscopy[5], headspace solid-phase microextraction[6], capillary zone electrophoresis[7], capillary electrophoresis with capacitively coupled contactless conductivity detection[8], molecular imprinting-chemiluminescence[9], high-performance liquid chromatography (HPLC)[10-13] and gas chromatography (GC)[14].The GC and HPLC techniques were established as the national[15,16] and Association of Official Analytical Chemists[17] standards for detecting sorbic acid in food, respectively. However, these methods have disadvantages. They require strict pretreatment and may pollute (and even block) the GC or HPLC columns.

In recent years, near-infrared spectroscopy (NIRS) has been employed for raw material testing, product quality control and process monitoring, particularly in the pharmaceutical sector, because of its short-time and convenience[18,19]. Near infrared spectroscopy is the region in the electromagnetic spectrum that consists of radiation from 780-2500 nm (12820-4000 cm^{-1}). The energies arising from this spectral range mainly correspond to the vibrational transitions due to superior harmonics (overtones) and combination bands. Near infrared spectroscopy has been employed to determine meat products and properties, such as water, fat, protein content[20] and tenderness[21]. However, this technique is not suitable for micro- or trace analysis because the typically low molar absorptivity of NIRS signals considerably reduces its sensitivity[22]. Thus, the NIR reflectance analysis of potassium sorbate in meat has not been reported although it is fast developing for the rapid compositional analysis of food products. Moreover, the preconcentration technique was used to extract potassium sorbate and improve the NIR sensitivity of the preservative before determination[22].

This paper describes the quantitative determination of potassium sorbate in muddy flesh using NIRS. The preconcentration technique was used to purify the samples and improve the NIR sensitivity of the preservative before the determination. Two regression models were adopted as multivariate regression tools and the best regression model was found. Furthermore, considering that the NIR method can measure constituents at a low cost and avoid polluting columns, it can be another valuable tool for the quality control of processed meat[23].

EXPERIMENTAL

Reagents and adsorbent: All chemical reagents, including the potassium sorbate, were of analytical purity grade. Doubly distilled water was used to prepare the samples and the potassium sorbate was purchased from Yindaxigui Chemical Reagent Corporation (China). The HPLC-grade neutral alumina adsorbent (100-200 mesh) was purchased from Wusi Chemical Reagent Corporation (China).

Sample preparation: Thirty artificial muddy-flesh samples were prepared by adding different contents of potassium sorbate into muddy flesh (100 g). The concentrations of potassium sorbate ranged from 0.0005-0.0095 g kg^{-1}.

Each sample was washed thrice with deionized water using an oscillator and then filtered. The solution volume was concentrated to *ca.* 20 mL using a rotary evaporator. Then, a HCl solution (6.0 mol L^{-1}, 2.0 mL) was added to convert the potassium sorbate into sorbic acid and three solution samples were extracted using 15 mL of ether. The samples were then evaporated at 45 ºC using a rotary evaporator and transferred into 25 mL volumetric flasks with *n*-hexane. The adsorption process was performed using 25 mL of the aforementioned solution and *ca.* 2 g of alumina in a flask at room temperature for 0.5 h *via* ultrasonic vibration. The cloudy solutions were filtered using a vacuum pump and the solid parts were preserved in a vacuum desiccator.

NIR spectral measurement: All NIR spectra were measured using an Antaris analyzer (Thermo Nicolet, USA) equipped with an In-GaAs detector and controlled by TQ Analyst version 7.2 (Thermo Nicolet, USA) from 10000-4000 cm^{-1} in diffuse reflectance mode. A NIR integrating sphere diffuse reflectance accessory (Bruker Optics Inc., Ettlingen, Germany) was also used. The spectra were digitalized at approximately 8 cm^{-1} intervals in the Fourier transform. A reference spectrum was taken before measuring each sample. To increase the signal-to-noise ratios, both reference and sample spectra were measured with scan number 32 and each spectrum was averaged from three parallel measurements.

Quantitative modeling: The partial least squares (PLS) regression and principal component regression (PCR) were used for the modeling and prediction. The modeling was performed using TQ Analyst version 7.2 (Thermo Nicolet, USA). To build a robust PLS model, all 30 samples were divided into a calibration and a validation set. According to the NIR spectrum distribution in the principal component space, 7 samples were selected as the validation set for external validation and the remaining 23 samples were taken as the calibration set for building the PLS regression and PCR models. The statistics of the potassium sorbate content, including the content ranges, means and standard deviations of the 23 calibration samples, are shown in Fig. 4. The seven validation samples were within the concentration range of the calibration samples.

The performances of these regression models were evaluated using the correlation coefficient (R^2) and root mean square error of cross-validation (RMSECV), which were determined *via* leave-one-out cross-validation. For the validation set, the relative error of prediction was used to estimate the prediction of the developed models.

RESULTS AND DISCUSSION

Spectral analysis: The absorption of the NIR radiation by the organic molecules is due to overtone and combination bands primarily of the O-H, C-H, N-H and C=O groups, whose fundamental molecular stretching and bending absorptions are in the mid-IR region. These overtones are anharmonic, *i.e.*, they do not behave in a simple fashion, making the NIR spectra complex and not directly interpretable compared with those of the other spectral regions.

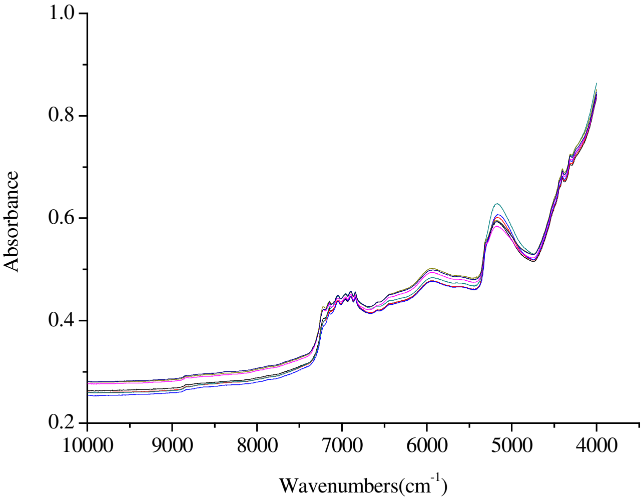

Fig. 1. NIR spectra of the potassium sorbate for external validation

The main characteristic of this spectrum is the strong water absorption bands centered at 5000 cm^{-1}. The specific absorptions that originate from the organic/biological components occur in the 5000-4000 cm^{-1} region and correspond to the combinations of fingerprint absorptions with CH, OH and NH stretching modes. Another useful spectral region is the 7692-5556 cm^{-1} region, which encompasses the bands originating from the first overtones of these X-H stretching modes. Although these absorption bands are weak and/or concealed by the strong water absorption bands, they are significantly influential in forming the basis for the quantitative analysis.

Wavelength-range options: The correlation coefficient and variance were used to determine the wavelength-range options for building the models. Fig. 2 shows the wavelength regions of 7400-4400, 5500-4000 and 7400-5900 cm^{-1}. Moreover, raw spectra without any processing method were used to build the models. The results of the different wavelength ranges are listed in Table-1. After comparing the R^2 and RMSECV, the optimal regions for sorbic acid were clearly 7400-4400 cm^{-1} of the PLS model and 5500-4000 cm^{-1} of the PCR model. Considering the calibration set and RMSECV values of the PLS and PCR models, the 7400-4400 cm^{-1} wavelength range of the PLS model is found to be better than the 5500-4000 cm^{-1} range of the PCR model.

Determination of the optimum principal component numbers: To avoid the insufficient fitting (underfitting) and rupture (overfitting) of the predictions of the other samples, the optimum number of factors for the models was determined using the LOO-CV. The relational graph of the RMSECV values against the LV numbers is shown in Fig. 3. Evidently, RMSECV

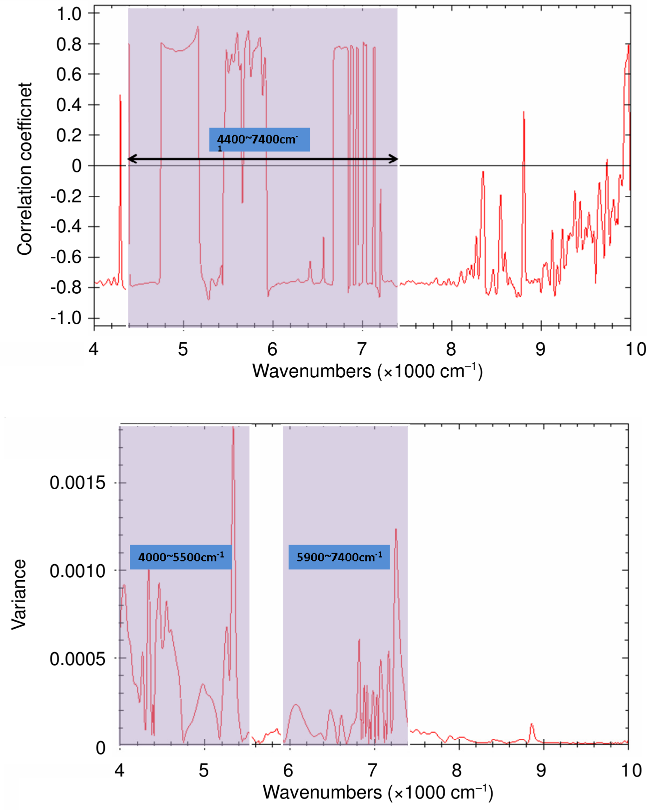

Fig. 2. Correlation coefficient and variance in relation to wavenumbers

TABLE-1
RESULTS OF THE MODELS FOR THE DIFFERENT METHODS

Methods	Region	Correlation coefficient	RMSECV (%)	RCV
PLS	All	0.96459	0.00115	0.89759
	4000-5500	0.95764	0.00122	0.88477
	5900-7400	0.95921	0.00127	0.88279
	4400-7400	0.96458	0.00106	0.91307
PCR	All	0.94833	0.00157	0.81748
	4000-5500	0.96363	0.00136	0.86248
	5900-7400	0.89299	0.00215	0.66801
	4400-7400	0.92600	0.00193	0.72645

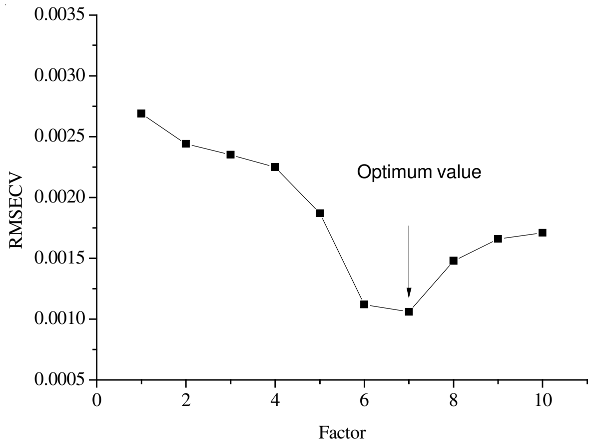

Fig. 3. Variation of the RMSECV with the principal component number for the sorbic acid model

decreased as the number of factors increased. When the principal component numbers exceeded 7, the RMSECV increased. Therefore, 7 is the optimal option for the model.

Establishment of the model using the PLS method: The PLS model selection criterion employed the cross-validation method. All 30 samples were divided into two sets, namely, a calibration (23 samples) and a prediction (7 samples) set. The calibration-set samples were used to establish the PLS model in the experiment. Each calibration data set was used as the calibration set for the prediction and the remaining data sets were employed for testing the PLS model. To extract complete and accurate information from the spectral data and, as much as possible, eliminate the influence of derivatives, a preprocessing method must be carried out. In the current study, the combined optimization processes were implemented *via* multiplicative scatter correction (MSC), standard normal variable transformation (SNV) and the first derivative (FD) and second-derivative (SD) and Savitzky-Golay (SG) smoothing techniques. To evaluate the preprocessing result of the model, RMSECV was used as a comparison criterion.

The parameters of the PLS model using different processing methods are listed in Table-2. A comparison of the R^2, RMSECV and RCV shows that the optimal processing for sorbic acid is the use of raw spectra without any processing method. The R^2, RMSECV and RCV are 0.96458, 0.00106 and 0.91307, respectively.

TABLE-2
RESULTS OF THE PLS CALCULATION OF SAMPLES FOR THE DIFFERENT PRETREATMENTS

Processing	Correlation coefficient	RMSECV (%)	Cross-validation set
Raw spectra	0.96458	0.00106	0.91307
MSC	0.94643	0.00137	0.85748
FD + SG	0.96635	0.00125	0.87782
SD + SG	0.83588	0.00172	0.74748
MSC + SD + SG	0.97773	0.00154	0.82758
SNV + SD + SG	0.83323	0.00169	0.75467

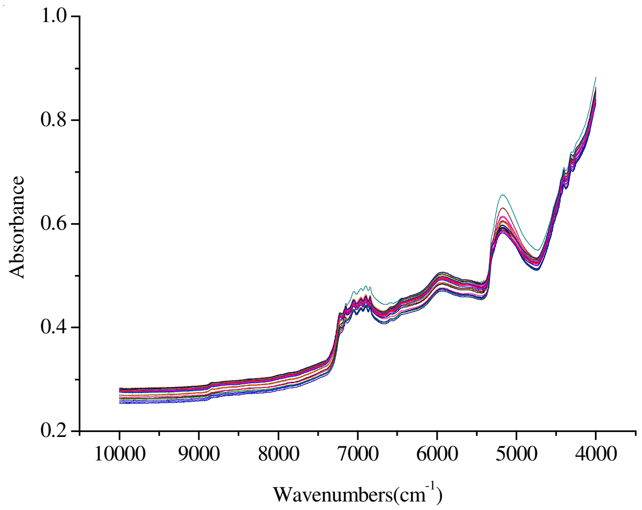

Fig. 4. NIR spectra of the potassium sorbate for establishing the model

Predicted and reference values for sorbate: The prediction set was used to test the calibration model and the relative error was used to evaluate the result. The raw spectra of the validation samples are shown in Fig. 1 and the results of the predicted values are listed in Table-3. Table-3 shows that

TABLE-3
RESULTS OF THE PREDICTED CONCENTRATIONS WITH THE REFERENCE VALUES

Number	Actual value	Predicted value	Absolute error (%)	Relative error (%)
3	0.0015	0.00161	-0.00011	-7.30
7	0.0035	0.00379	-0.00029	-8.29
14	0.0055	0.00534	0.00016	2.91
15	0.00575	0.00626	-0.00051	-8.87
22	0.0075	0.00771	-0.00021	-2.80
23	0.00775	0.00718	0.00057	7.35
27	0.00875	0.00853	0.00022	2.51

the validation samples' relative errors of the model are less than 9 %. Seven prediction samples of the validation results show that the model prediction values are accurate. Thus, the established model is reliable for predicting the potassium sorbate content in muddy flesh.

Conclusion

A new method for the quantitative analysis of potassium sorbate in muddy flesh was developed using the NIR spectrum after being preconcentrated with alumina as adsorbent. The chemometric methods, namely, PLS regression and PCR, were applied in the current research. The R^2 and RMSECV were used to evaluate the quantitative analysis model. The results show that the raw spectra, combined with the PLS method, can be efficiently utilized in the rapid and accurate quantitative analysis of potassium sorbate. The R^2 and RMSECV of the PLS model are 0.96458 and 0.00106, respectively. External validation results show that the model is accurate and that its relative errors are less than 9.0 %. Therefore, the NIR diffuse reflectance spectroscopy, combined with a preconcentration technique, can provide a feasible way for quantitatively determining potassium sorbate in meat.

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