

Quantitative of pesticide residue on the surface of navel orange by confocal microscopy Raman spectrometer

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The potential of Confocal micro-Raman spectroscopy in the quantitative analysis of pesticide (Chlorpyrifos, Omethoate) residues on orange surface is investigated in this work. Quantitative analysis models were established by partial least squares (PLS) using different preprocessing methods (Smoothing, First derivative, MSC, Baseline) for pesticide residues. For pesticide residues, the higher correlation coefficients (r) is 0.972 and 0.943, the root mean square error of prediction (RMSEP) is 2.05% and 2.36%, respectively. It is therefore clear that Confocal micro-Raman spectroscopy techniques enable rapid, nondestructive and reliable measurements, so Raman spectrometry appears to be a promising tool for pesticide residues.

Keywords: Confocal Micro-Raman spectrometer; pesticide residue; partial least squares.

1. Introduction

In recent years, fruit safety problem has caused people's extensive attention with the development of social economy and the improvement of people's living standards. On the other hand, organophosphate pesticides in fighting against pests have been used for decades to increase the yields of agriculture products.¹ Despite acute poisoning caused by an exposure to organophosphate pesticides, the use of organophosphate pesticides has been gradually

increasing over the past years, and has attracted intense public attention worldwide about microscale amounts of the residues in fruit products that might cause long-term nonfatal health influences.² However, the organophosphate pesticides are toxic substances for human even at low levels. Hence, the reliable, nondestructive and rapid detection approaches of these toxic substances are very important matters for the protection of the environment and human health.^{3,4}

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At present, concerning the quantitative detection of organophosphate pesticide residues, the main methods are based on gas chromatography (GC),^{5,6} gas chromatographic-mass spectrometry (GC-MS)^{7,8} and high-performance liquid chromatography (HPLC).⁹ However, their experimental apparatus are expensive, and sample preparations are time-consuming. In addition, narrow ranges of applications are also a major obstacle to apply these methods for food analysis.¹⁰ And the detection time of these methods is relatively longer. Therefore, the reliable, accurate and rapid detection and quantification on toxic substances is an important problem for the environment and human health.¹¹

Raman spectroscopy is an excellent non-destructive tool. It is often used since the problems that limited the use of this technology in the past have now reduced or have been conquered.^{12,13} Quantitative Raman analyses have been directly applied to liquids.^{14–16} The development of Raman spectroscopy is based on the Raman scattering. Comparing with conventional chemical analysis methods, Raman spectroscopic technique's features are nondestructive, rapid, environmentally protective, highly sensitive and require no sample preparation.¹⁷ Besides, costs of Raman detection equipment maintenance are also low.

In this work, confocal Raman micro-spectrometry method for organophosphate pesticides residue (Chlorpyrifos, Omethoate) determination was presented. Comparing different preprocessing methods (Smoothing, First derivative, Multiplicative Scatter Correction (MSC), Baseline), quantitative mathematical model was established by the partial least squares (PLS). The objective of this work was to develop reliable and rapid detection method in pesticide residue.

2. Experimental Methods

2.1. Instrumental

Laser microscopic confocal Raman spectrometer is used in experiments produced by Germany Bucker optical instrument company and equipped with Olympus BX51 optical microscope and a CCD detector which is thermoelectrically cooled. Raman spectrometer is controlled by software OPUS 6.5 developed by Bucker. All Raman spectra of samples were obtained from 90 to 3500 cm^{-1} with the same

experimental conditions (laser power, 10 mW; excitation wavelength, 785 nm; exposure time, 3 s).

2.2. Chemicals

Organophosphate pesticides reagent grade standard (Chlorpyrifos 100% w/v, Omethoate 98.6% w/v) were supplied by bwwz corporation (Beijing, China). Commercial pesticide formulations (Chlorpyrifos (48% w/w), Omethoate (40% w/w)) used in local agriculture were purchased directly for preparing the sample solutions. Methanol, reagent grade (99.5% w/w) and organic oranges in the experiment were obtained directly from the local supermarket.

2.3. Preparation of sample

Commercial pesticide formulations (Chlorpyrifos (48% w/w), Omethoate (40% w/w)) were dissolved with methanol in concentration ranging from 0.1% to 30% (w/w) to obtain 45 levels. Out of 45, the randomly selected 32 levels are used to build the calibration model and others for validation. Samples were arranged on the well-sealed reagent bottle stored in the dark. Organic oranges were cleaned carefully to ensure that no pesticide residues exist on the surface of samples. Small pieces of fruit skin from the cleaned orange were obtained, and sprayed by a little of pesticide samples solutions. It is important to ensure that the sample solutions are evenly distributed on the surface of the fruit skin. At last, filter papers were used to clean up any leftover pesticide solutions on the fruit skin, and then the fruit skin was blown dry naturally. Every fruit skin sample was placed into the substrate and analyzed in the same experimental conditions. Then, Raman spectra of five droplets were carried out for every sample in order to be averaged. Every droplet collects three spectrums and then averaged.

2.4. Data analysis

The analysis software of spectral data was Unscrambler version 10.1 (CAMO AS, Trondheim, Norway). Data preprocessing algorithms including first derivatives, MSC, baseline and Savitzky–Golay smoothing were employed in order to reduce the effect of the baseline shift and eliminate high frequency noises from the apparatus. PLS model was constructed to describe predicted variables depending on other observables, in other words, a regression

model was established by two data matrices S and Z using latent variable representation. This uses the general equation as PLS:

$$Z = SV, \quad (1)$$

In which, Z is the predicted data matrix, S is the data matrix represented using observables and V is the regression coefficients assured in the calibration step.^{18,19}

The PLS model was checked using leave-one-out cross validation, which use all samples beside one to obtain a calibration curve and repeated the above process in the dataset.²⁰ To judge the performance of the models and to compare them, the correlation coefficients (r) and root mean square errors of prediction (RMSEP) values were calculated according to the equation:

$$r = \sqrt{1 - \frac{\sum_{i=1}^n (\tilde{x}_i - x_i)^2}{\sum_{i=1}^n (\tilde{x}_i - \bar{x}_i)^2}} \quad (2)$$

In this equation, n is the number of samples, x_i is the predicted concentration of samples, \tilde{x}_i is the real concentration of samples, and \bar{x}_m is the average concentration. According to the classification of samples x_i , the correlation coefficient can be divided into the correlation coefficient of correction (R_C) and the correlation coefficient of prediction (R_P).

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (\tilde{x}_i - x_i)^2}{n}} \quad (3)$$

In this equation, n is the number of calibration samples.

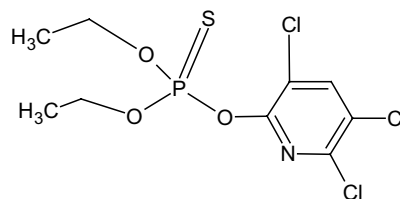
$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (\tilde{x}_i - x_i)^2}{n}} \quad (4)$$

In this equation, n is the number of prediction samples. The correlation coefficient (r) and RMSEP were used to assess the model. The higher the value of r or the lower the value of RMSEP is, the better the predictive model established is.²¹

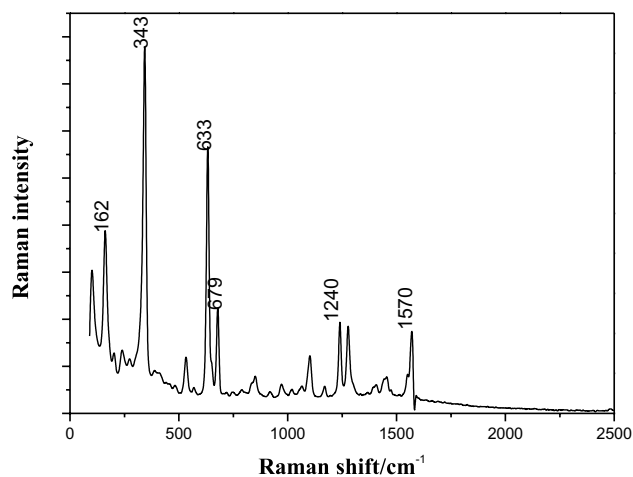
3. Results and Discussion

3.1. Raman spectra of multi-pesticide formulations and fruit

Chlorpyrifos is a broad spectrum, high efficiency organophosphate insecticide. The chemical structures of Chlorpyrifos are displayed in Fig. 1. In the



(a)



(b)

Fig. 1. (a) Chemical Structure of Chlorpyrifos; (b) Raman spectra of Chlorpyrifos solid.

chemical structure, P=S bonds show an intense band. The band is obviously distinguishable from compounds excluding benzene rings, but some vibrations, resulting from the benzene ring, overshadow it as in chlorpyrifos Raman spectrum, where two overlapping bands are 633 and 679 cm^{-1} .²² The most intense bands in the chlorpyrifos were those of N-cyclopropyl bending, located at 343 cm^{-1} , and the ring deformation at 633 cm^{-1} . Other absorption bands were located at 1570 cm^{-1} , resulting from the C=C stretching, at 162, 679 and 1240 cm^{-1} , corresponding to P-O vibration, C-H bending and ring breathing, respectively.²³

Omethoate is an efficient organophosphorous insecticide. The chemical structure of omethoate is displayed in Fig. 2. In the chemical structure, the bands were located at 3288 cm^{-1} , resulting from the NH anti-symmetric stretching, at 569, 696, 905, 1321 cm^{-1} , corresponding to C=O torsion, C-S stretching, P-O deformation and P=O stretching, respectively. The most intense bands were those of the deformation, located at 415 cm^{-1} , the vs-stretching at 770 cm^{-1} , and the anti-symmetric stretching of P-O-C at 1057 cm^{-1} .²²

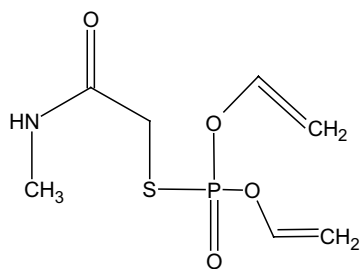


Fig. 2. Chemical structure of omethoate.

Under the measuring conditions mentioned above, the micro-Raman spectra of clean skin of navel orange are shown in Fig. 3. Raman spectra of carotene obtained with 785 nm excitation. The strongest Raman modes of carotene are located at 1531, 1158 and 1006 cm^{-1} attributed to C=C stretching, C-C stretching and C-CH₃ bending of carotene.²³ From three figures of Raman spectra, each pesticide could be distinguished using their characteristic peaks shown and these bands could be employed for determining pesticide residues on the surface of fruits.

Measurements of Raman spectra of navel orange with pesticides are performed with the same experimental condition. The Raman spectra obtained for two kinds of pesticides on the navel orange are shown in the Fig. 4. From the spectra, the locations of characteristic modes of pesticides recorded from 100 to 1100 cm^{-1} have been identified: Chlorpyrifos at 161, 343 and 633 cm^{-1} and Omethoate at 413, 697 and 1064 cm^{-1} . The Raman modes of navel orange are located at 1531, 1158 and 1006 cm^{-1} . According to this observation, the effect about the skin of the navel orange for the Raman signals of pesticides is small.

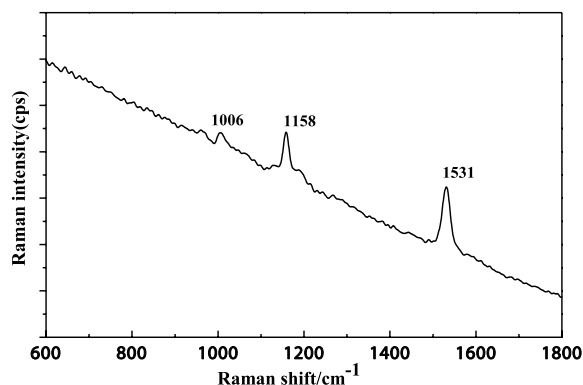


Fig. 3. Raman spectra of orange fruit.

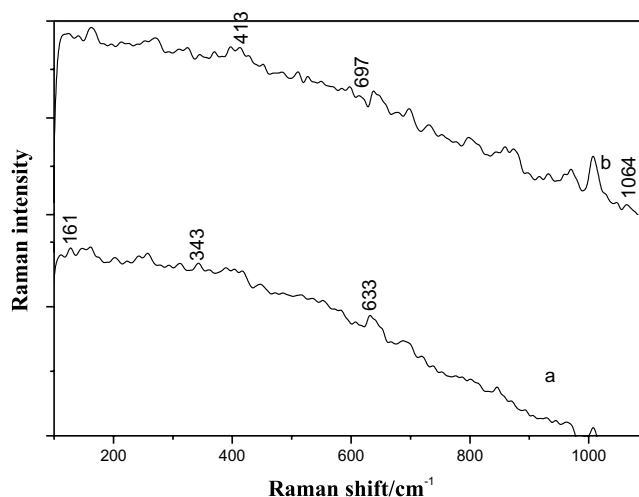


Fig. 4. Raman spectra of several pesticides left on the surface of navel orange (a) Chlorpyrifos; (b) Omethoate.

3.2. Quantitative analysis of pesticide residue

3.2.1. Quantitative analysis of chlorpyrifos residue

Table 1 shows the compared results of the calibration and validation models by PLS with different preprocessing methods for the chlorpyrifos residue on the surface of oranges. According to the comparison, the best model using Raman spectra is obtained by PLS model with MSC preprocessing, with higher R_C of 0.993, R_P of 0.972 and lower RMSEC of 0.95%, RMSEP of 2.05%.

Figure 5 is the scatter plot showing a correlation between the predicted values and reference values of chlorpyrifos residue on the surface of oranges by PLS with MSC preprocessing. The standard deviation (SD) is 0.069, and the equation of chlorpyrifos residue can be expressed as:

$$Z_1 = 0.8271S_1 + 2.3366, \quad (5)$$

Table 1. Performance comparison results for chlorpyrifos residue developed by PLS with different preprocessing methods.

Pretreatment method	Calibration set		Validation set	
	R_C	RMSEC (%)	R_P	RMSEP (%)
None	0.965	2.12	0.939	3.16
Smoothing	0.964	2.13	0.939	3.16
First derivative	0.983	1.46	0.862	4.23
MSC	0.993	0.95	0.972	2.05
Baseline	0.966	2.09	0.942	3.12

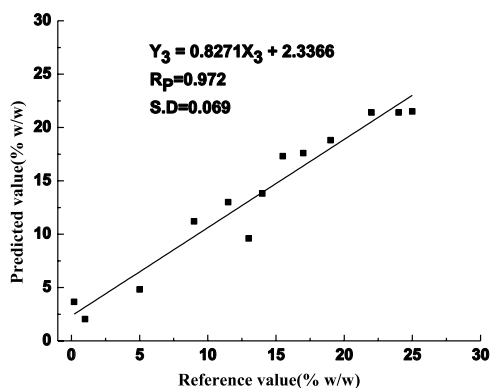


Fig. 5. Relationship of predicted values with the reference values of chlorpyrifos solutions by PLS with MSC. The straight line is obtained by linear regression.

where Z_1 is the predicted value of the chlorpyrifos residue using PLS model, and S_1 is the reference value of chlorpyrifos residue.

3.2.2. Quantitative analysis of omethoate residue

Table 2 shows the compared results of the calibration and validation models by PLS with different preprocessing methods for the omethoate residue on the surface of oranges. According to the comparison, the best model using Raman spectra is obtained by PLS model with baseline preprocessing, with higher R_C of 0.959, R_P of 0.943 and lower RMSEC of 2.03%, RMSEP of 2.36%.

Figure 6 is the scatter plot showing a correlation between the predicted values and reference values of omethoate residue on the surface of oranges by PLS with baseline preprocessing. The SD is 0.069, and the equation of omethoate residue can be expressed as:

$$Z_2 = 0.8027S_2 + 1.429, \quad (6)$$

Table 2. Performance comparison results for omethoate residue developed by PLS with different preprocessing methods.

Pretreatment method	Calibration set		Validation set	
	R_C	RMSEC (%)	R_P	RMSEP (%)
None	0.949	2.12	0.937	2.64
Smoothing	0.954	2.13	0.939	2.51
First derivative	0.960	2.36	0.924	2.52
MSC	0.912	3.08	0.880	3.45
Baseline	0.959	2.03	0.943	2.36

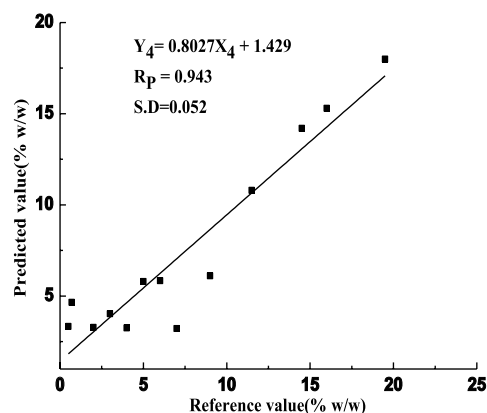


Fig. 6. Relationship of predicted values with the reference values of omethoate solutions by PLS with MSC. The straight line is obtained by linear regression.

where Z_2 is a predicted value of the omethoate residue using PLS model, and S_2 is a reference value of omethoate residue.

4. Conclusion

In this paper, the confocal micro-Raman spectroscopy is a sensitive method to study pesticide residue on the orange surface. With a few steps of simple sample preprocessing, trace amount of pesticides in oranges can be detected. The determination of pesticide residue was successfully performed based on confocal micro-Raman spectroscopy and regression methods of PLS, which is presented as an accurate quantitative analytical technique. Therefore, a rapid, nondestructive and reliable method is obtained that can be applied to the commercial pesticide residue for fruit quality and safety.

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