

Determination of Pesticide Residues on the Surface of Fruits Using Micro-Raman Spectroscopy

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Abstract. A simple, rapid and environmentally friendly method was developed for micro-Raman spectroscopy determination of pesticide residues on the surface of fruits. Raman spectra of fruits, pesticides and pericarps sprayed by pesticide solutions were acquired using a laser power of 14 mW at excitation wavelength of 780 nm. From the Raman spectra, the residual pesticides could be distinguished and determined through the characteristic Raman peaks. The overall results indicated that micro-Raman spectroscopy is a potential tool to determine the pesticide residues on the surface of fruits for fruit quality and safety control.

Keywords: micro-Raman spectroscopy; fruit; pesticide residue

1. Introduction

Pesticides are defined by the United Nations Food and Agricultural Organization (FAO) as substances or mixtures intended to prevent, destroy, repel or mitigate any pest, including insects, rodents and weeds [1]. Currently, pesticides play a critical role in protecting fruit crops. However, large amount of pesticides are used in fruits production making it unsafe and endangering humans and animals. One of the most common used pesticides are the organophosphates, which kill insects and mites by attacking the central nervous system, and consequently pose a threat to human beings and animal lives [2]. Nowadays, fruit safety and quality has been attracted more and more attention. In many countries, strict tolerance levels are set up to ensure public safety.

The current analysis methods for determining the residual pesticides in fresh fruits are based on chemical analysis, such as liquid chromatography-mass spectrometry (LC-MS) [3], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [4, 5], gas chromatography-mass spectrometry (GC-MS) [6], capillary electrophoresis-mass spectrometry (CE-MS) [7] and ultra-high-performance liquid chromatography (UHPLC) [8], etc. However, all of the methods mentioned above are time-consuming, not environmentally friendly and inconvenient enough for the process of sample preparation. Hence, it was very necessary to develop a simple, rapid and low cost

method for the determination of pesticide residuals in fruits.

Raman spectroscopy is a fast technique compared to classical chromatography. It is a powerful analytical tool that provides wonderful advantages including non-destructive, low cost and ultrasensitive characterization down to single molecular level [9]. Shende et al. [10] used surface-enhanced Raman spectroscopy (SERS) to analyze pesticides on fruit surfaces. Zhou et al. [11] recorded several spectra of fruits with pesticides using near infrared Fourier transform Raman spectroscopy (FT-Raman). Several Raman spectra of some fruits and pesticides on the surface of fruits were acquired and compared with at two excitation wavelengths of 514.5 and 1064 nm by Zhang et al. [12].

Micro-Raman has its unique characters compared to other Raman techniques, since it allows analysis of materials with a spatial resolution of several microns focused by an optical microscope. It has been developed for a rapid, environmentally friendly and low cost generation procedure, and has been applied in many fields, such as gems [13, 14], fibers [15, 16] and geology [17, 18], etc. In this paper, a new attempt to determine the residual pesticides on the surface of fruits using micro-Raman spectroscopy was reported. The objective was to record the Raman spectra of fruits and pesticides in order to study the feasibility of using micro-Raman spectroscopy to determine the residual pesticides on the surface of fruits.

2. Materials and methods

2.1. Apparatus

A Nicolet DXR Raman Microscope (Thermo Fisher Scientific Corp., Madison, WI, USA), equipped with a liquid nitrogen cooled CCD detector and a 14 mW maximum power diode laser, that emits at 780 nm, was employed for confocal micro-Raman spectra acquisition using 2 ml standard glass chromatographic vials (12 mm×32 mm) as sample cells. The laser was focused within the sample using an inverted microscope setup equipped with a 10× ultra long working distance objective. The scattered signal was then recorded at a 180° backscattering geometry and dispersed by a single monochromator using a 400 grooves/mm diffraction grating and a laser line filter used as a beam splitter to lead the laser beam into the microscope assembly and also to reject the Rayleigh scattered light on the returning path. Spectrometer was controlled with OMINIC software (Thermo Nicolet Corp., Madison, WI, USA).

2.2. Sample preparation and chemicals

The samples, including apples, pears and oranges fruits in the experiment, were purchased directly from the Nanchang fruit market. The chemicals, dimethoate standard (*O,O*-dimethyl- *S*-(*N*-methylcarbomoylmethyl) phosphorodithioate) solid (99.1% w/v), chlorpyrifos standard (*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) solid (99.5% w/v) and malathion standard

(*S*-(1,2-dicarbethoxyethyl)-*O,O*-dimethyl dithiophosphate) solution (99.1% w/v) were purchased from bwwz corporation (Beijing, China). Two emulsifiable concentrate commercial pesticide formulations, chlorpyrifos (40% w/w) and malathion (40% w/w) were obtained directly from the Nanchang pesticide market.

Before recording the micro-Raman spectra, all the fruits were cleaned carefully. Firstly, fruits were flushed several times with fresh water to get rid of the dirt left on the surface, and then wiped with cotton and alcohol. After that, small pieces of pericarps from the cleaned fruits were taken off, and then sprayed by a little amount of pesticide formulations. At last, filter papers were used to wipe off any left over pesticides from the pericarps, and let the samples dry naturally.

2.3. Micro-Raman procedure

When it was time to record the micro-Raman spectra, the resolution and accumulating time were fixed at 1.93 cm^{-1} and 100 scans per spectrum. The micro-Raman spectra of clean pericarps of apples, pears, oranges and four standard pesticides were collected, firstly. These spectra were used as standards in the comparison database. Then the spectra of pericarps sprayed by the pesticide formulations were collected and compared with those in the database to identify the trace amount of pesticides on the surface of fruits. All the spectra mentioned above were carried out in chromatographic glass vials from 3390 to 110 cm^{-1} with the diode laser power employed fixed at 14 mW.

3. Results and discussion

3.1. Raman spectra of fruits

Based on the measuring conditions mentioned above, the micro-Raman spectra of clean pericarps of apple, pear and orange fruits are shown in Fig. 1. Each of fruits has its characteristic peaks. Oranges mainly have the Raman peaks at 1530 , 1155 and 1005 cm^{-1} attributed to C=C stretching, C-C stretching and C-CH₃ bending of carotene [19]. However, both apple and pear fruits have combined peaks around 1600 , 1439 , 1136 and 850 cm^{-1} . It is estimated that these peaks are due to the combination of Raman scattering peaks by nourishment compositions of apple and pear. Because each of them contains very complicated compositions as there are usually different amount of water, sugar, vitamin, fat, protein, amino acid, as well as other components and elements such as calcium, iron and zinc, etc. At present, these fruits still can not be identified from the Raman peaks, but it has differences in the peaks attributed to the different compositions.

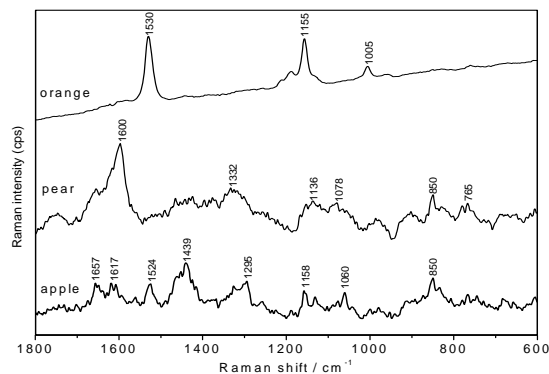


Fig. 1. Raman spectra of orange, pear and apple fruits.

3.2. Raman spectra of pesticides

Raman spectra of three standard pesticides, including dimethoate, chlorpyrifos and malathion, are shown from Fig. 2 to Fig. 4 in the 3390-110 cm^{-1} region. All pesticides have the same structure with P=S bonds, which display a strong band in the 600-700 cm^{-1} region. This band is clearly distinguishable for compounds without benzene rings, but several of the vibrations due to the benzene ring overshadow it as in chlorpyrifos spectrum, where two overlapping bands appear at 630 and 676 cm^{-1} in Fig. 2 [20]. The most intense bands in the chlorpyrifos Raman spectrum are those present at 340 and 630 cm^{-1} due to N-cyclopropyl bending and ring deformation, respectively. Other less intense bands located at 158, 676, 1276 and 1569 cm^{-1} are due to P-O vibration, ring breathing, C-H bending and C=C stretching. C-H stretching modes are observed from 2800 to 3100 cm^{-1} .

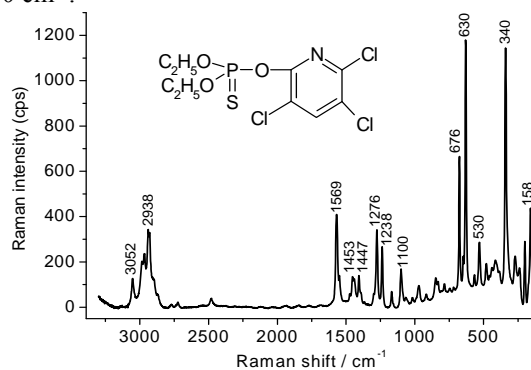


Fig. 2. Raman spectrum and molecular structure of chlorpyrifos.

In Fig. 3 of dimethoate Raman spectrum, P=S bond displays a strong band at 696

cm^{-1} . The most intense band in the spectrum is located at 575 cm^{-1} due to C-S vibration. Other less intense bands located at 171, 494, 768, 903, 1156, 1330 and 1640 cm^{-1} are due to P-O vibration, P-S vibration, S-P-O backbone mode, C-C backbone stretching, C-C stretching, N-H in-plane bending and symmetric C=O stretching. The intense bands appearing around 2936 cm^{-1} are due to CH_2 and CH_3 deformation.

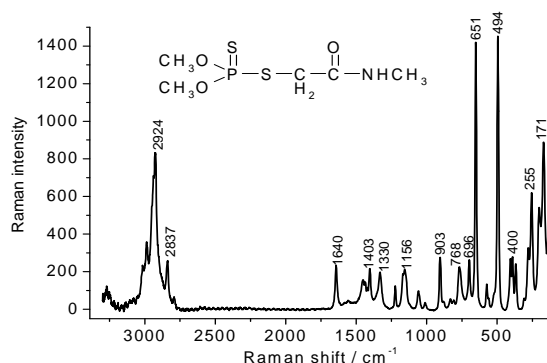


Fig. 3. Raman spectrum and molecular structure of dimethoate.

In Fig. 4 of malathion spectrum, P=S bond displays a strong band at 652 cm^{-1} . The bands located at 159, 496, 859, 1022, 1110 and 1733 cm^{-1} are due to P-O vibration, P-S vibration, C-O-C stretching, C-H in-plane deformation, C-C stretching and C=O stretching [21]. The intense bands appearing in 1451 and 2800 to 3100 cm^{-1} are due to CH_2 and CH_3 deformation and C-H stretching.

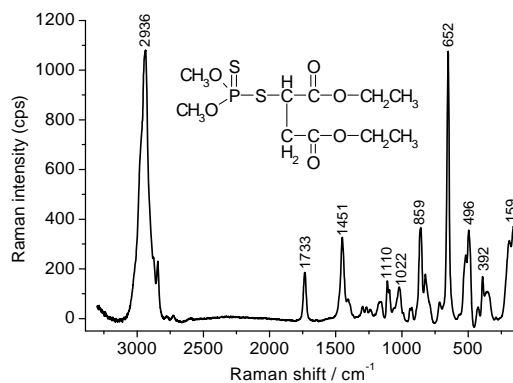


Fig. 4. Raman spectrum and molecular structure of malathion.

From the three figures mentioned above, each of pesticides could be distinguished using their characteristic peaks shown in Table 1. These bands could be employed for determining pesticide residues on the surface of fruits.

Table 1. Characteristic peaks of chlorpyrifos, dimethoate and malathion.

Pesticide	Characteristic peaks (cm ⁻¹)
Chlorpyrifos	1569, 1276, 676, 630, 340
Dimethoate	1640, 651, 494
Malathion	1733, 1451, 652

3.3. Raman spectra of pesticide residues on the surface of fruits

Acquirements of Raman spectra of fruits with pesticides were performed with the same measuring conditions as before. Fig. 5 and Fig. 6 show the Raman spectra of chlorpyrifos and malathion formulations residues on the surface of oranges. From Fig. 5, it can be obviously seen that the spectrum of chlorpyrifos residue on the surface of orange not only contains the strong peaks of orange itself, but also contains the characteristic modes of chlorpyrifos which located at 676, 630 and 340 cm⁻¹. Similarly, in Fig. 6, the characteristic modes of malathion at 1451, 859, 652 and 496 cm⁻¹ can be seen in the spectrum of its residue on the surface of orange. Based on the results, it was concluded that the method of using micro-Raman spectroscopy could be utilized for automatic and intelligent determination of the pesticide residues on the surface of fruits.

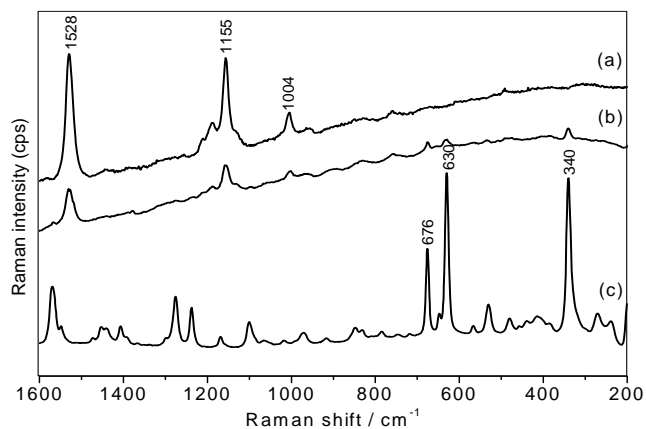


Fig. 5. Raman spectra of (a) orange's pericarp, (b) chlorpyrifos formulation (40% w/w) left on the surface of orange and (c) standard chlorpyrifos solid.

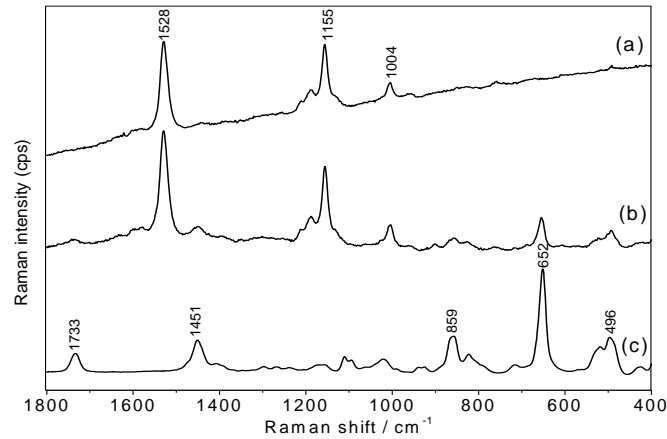


Fig. 6. Raman spectra of (a) orange's pericarp, (b) malathion formulation (40% w/w) left on the surface of orange and (c) standard malathion solution.

4. Conclusions

The micro-Raman spectra of apples, pears, oranges, several pesticides and pesticide residues on the surface of oranges were successfully recorded using a micro-Raman spectrometer. The characteristic peaks of pesticides can be seen from the spectra of pesticides left on the surface of fruits. Micro-Raman spectroscopy is a potential tool to determine the pesticide residues for fruit quality and safety control with rapid, non-destructive, low cost and environmentally friendly advantages.

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