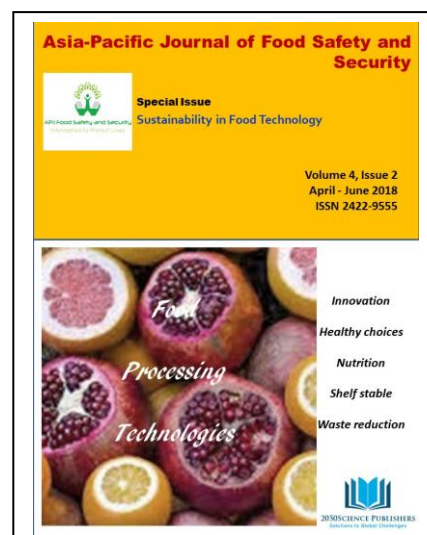**Theme:** *Food Security*

*Research paper*

## Multivariate Data Analysis of Pesticide Residue on Spinach Leaves Based on FT-IR Spectroscopy

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**Highlights**

- Fourier transform-infrared spectroscopy (FT-IR) was used as a non-destructive and rapid measurement method to trace residues of malathion on spinach
- Two types of multivariate data analysis (MVA) which are principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) successfully separated washed and non-washed samples of spinach after sprayed with malathion
- FT-IR combined with MVA can serve as a practical and reliable method for safety diagnosis of agricultural produce such as vegetables

**About Authors**

**Zanariah Hashim** (PhD) is a Senior Lecturer and researcher at the Department of Bioprocess & Polymer Engineering, Faculty of Chemical & Energy Engineering, Universiti Teknologi Malaysia. Previously she worked in metabolomics focusing on the metabolism and the roles of transcription factors in baker's yeast. Her current research interests include application of metabolomics and chemometrics in the authentication and quality control of food samples, as well as encapsulation technology in food and pharmaceuticals.

**Abstract**

Vegetables are susceptible to diseases and pest attack; hence pesticides are widely used by farmers to avoid or reduce pests. The use of pesticides may leave some residues on the vegetables after harvesting and not safe for consumption. Thus, it is essential to monitor the levels and concentrations of pesticide residues in vegetables. Currently, gas chromatography and high-performance liquid chromatography are used as a method for detecting the components of pesticide residues in vegetables. However, these methods are destructive and often time consuming. Therefore, in this study, Fourier transform infrared (FT-IR) spectroscopy combined with multivariate data analysis was selected as a rapid, non-destructive method to obtain spectral information from pesticide residues. Spinach leaves were sprayed with malathion followed by washing treatments. The results showed that washed and non-washed samples can be clearly distinguished, with non-washed samples showing a high absorbance intensity at 970 cm$^{-1}$ which belongs to the ester functional group that can be used to determine malathion. This approach can be further developed as a routine analysis tool for quantification of the safe range of pesticide residue concentrations in vegetables.

**Keywords:** Pesticide residue; malathion; FT-IR; multivariate data analysis

1. **Introduction**

Vegetables are an important part of a healthy diet and provide a source of many nutrients, including potassium, fiber, folate and vitamins A, E and C. These nutrients are vital for health and maintenance of our body, and eating a diet rich in vegetables may reduce the risk for stroke, cancer, heart diseases and type-2 diabetes. However, as vegetables are susceptible to infection and pest attacks, farmers usually use pesticides at the plantation to control the infection from spreading. These pesticide residues may still be left on the vegetables after harvesting and being sold in the market. Thus, we need to ensure that the pesticide residues are within the permissible limit and that the vegetables are safe to be consumed. Often, proper washing with water before eating the vegetables is enough to get rid of harmful residues.

Fourier transform-infrared spectroscopy (FT-IR) is a technique to obtain an infrared spectrum of absorption, emission, or photoconductivity of a solid, liquid or gas. The spectrum indicates specific chemical bonds or functional groups that enable us to deduce the chemical properties of the measured sample. FT-IR has advantages over other methods; it is non-destructive, reproducible, rapid (within a few seconds), with minimal or no sample preparation required and no use of harmful chemical solvents (van de Voort et al., 2001).

Multivariate data analysis (MVA) refers to any statistical technique used to analyze data that arises from more than one variable. Previous studies have demonstrated the utility of FT-IR combined with MVA for a rapid evaluation of a large volume of spectral data for quality control (Hashim et al., 2017). In this research, spinach *Spinacia oleracea* leaves were sprayed with malathion, a type of commonly used pesticide, and subjected to FT-IR and MVA to distinguish washed and non-washed samples. The

results show that washed and non-washed samples can be differentiated with our method. Thus, FT-IR combined with MVA can serve as a practical and reliable method for safety diagnosis of agricultural produce such as vegetables.

## 2. Materials and Method

Organic spinach was purchased from Aeon Supermarket, Skudai, Johor, Malaysia. Malathion 57% was obtained from Wesco Agencies (M) Sdn. Bhd., Selangor, Malaysia. Mature leaves of similar size were washed with running tap water for 10 s and pat dried with tissue papers. Next, the leaves were sprayed evenly with diluted malathion (0.04 mL malathion in 20 mL water) and left to dry for 2 h (Harinathareddy et al., 2014). Then, the leaves were washed with two methods: (1) Samples were washed with running tap water for 10 s then dried with tissue paper. (2) The samples were soaked in 2% of salt water (20 g of salt in 1 L of water) for 10 minutes (Harinathareddy et al., 2014) then washed with running tap water and dried with tissue paper. All samples were left in a fume chamber to dry completely before FT-IR measurement. Non-washed leaves were used as a control.

FT-IR spectra were recorded as reported previously (Hashim et al., 2017), using the IRTracer-100 (Shimadzu, Kyoto, Japan) equipped with an attenuated total reflectance (ATR) accessory. Leaf sample (one at a time) was placed on the measurement plate and measurements were taken at 40 scans and resolution of 2 cm$^{-1}$ with Happ-Genzel apodisation. A proprietary Lab Solutions software (Shimadzu, Kyoto, Japan) was used for FTIR data collection and processing. All spectra were recorded from 4,000 to 400 cm$^{-1}$ and read as absorbance in triplicate before taking the averaged value.

Multivariate data analysis of FTIR spectra was performed using MetaboAnalyst 3.0 (Xia et al., 2015). Peak intensities were scaled to unit variance.

## 3. Results and Discussion

Figure 1 shows representative FT-IR spectra from malathion standard (0.04 mg/20 mL-water), control (unwashed spinach leaf sprayed with malathion 0.04 mg/20 mL-water) and washed spinach leaves. Several peaks can be identified as summarized in Table 1.

Based on the FT-IR spectra (Figure 1), although we can see a clear difference in spectral pattern between malathion standard and the leaves, the control leaf and washed leaves cannot be distinguished by just observing the spectra. Thus, we proceeded to perform multivariate data analysis using principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) as these two methods are able to detect subtle differences among samples from spectral data or other multivariable measurements. From the PCA scores plot (Figure 2a), non-washed samples can be clearly separated from washed samples. Red clusters represent non-washed sample (control, NW), green for salt water-washed technique (SW) and blue for tap water-washed technique (TW). The SW and TW samples
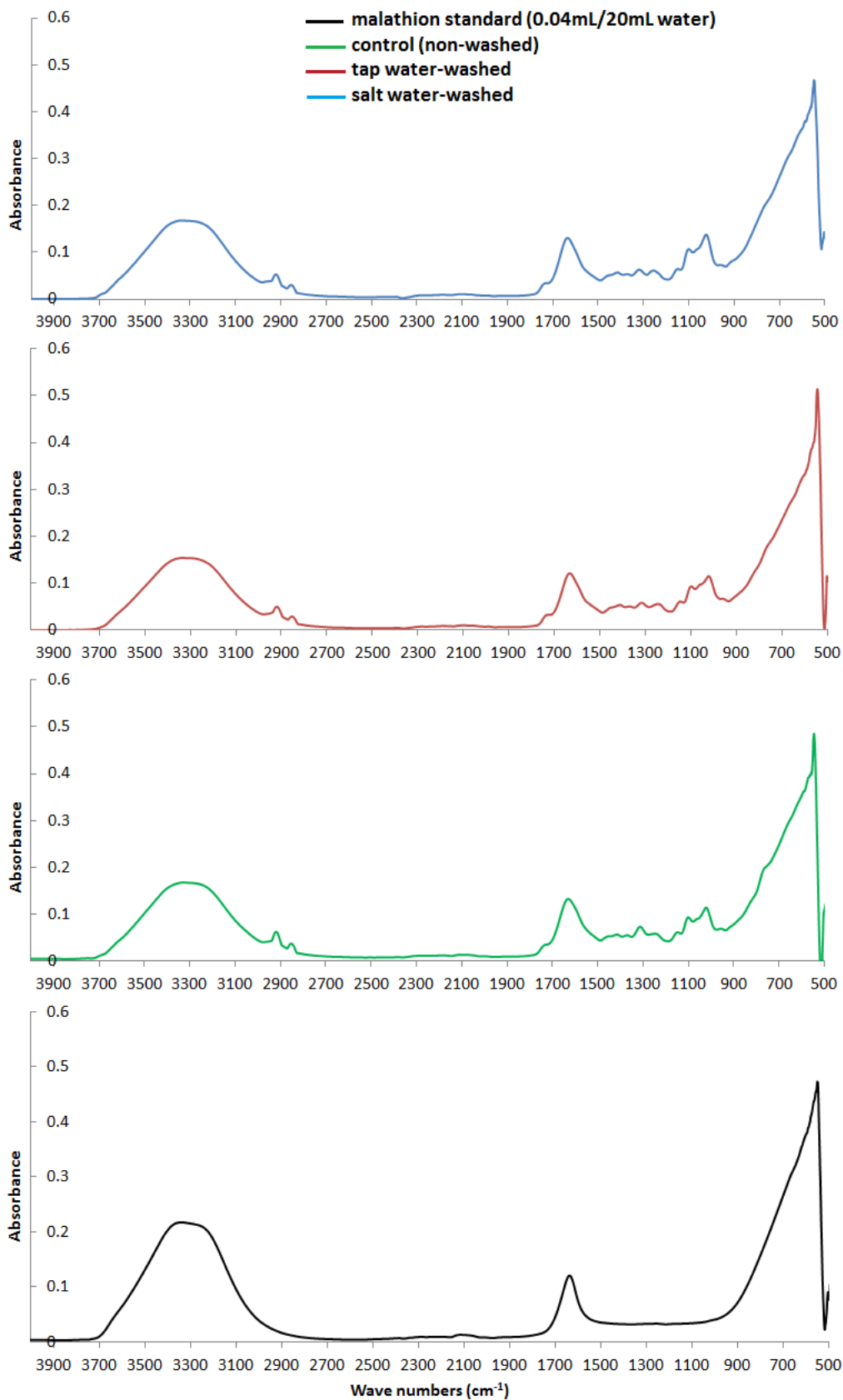
**Figure 1.** Representative FTIR spectra of malathion standard (0.04 mg/20 mL-water), control (un-washed spinach leaf sprayed with malathion 0.04 mg/20 mL-water) and washed spinach leaves.

are overlapped but not NW samples, indicating that tap-water technique alone is sufficient in eliminating malathion residues on spinach leaves.

**Table 1.** Major peaks identified from the FTIR spectra of washed and non-washed spinach leaves sprayed with malathion 0.04 mL/20 mL-water

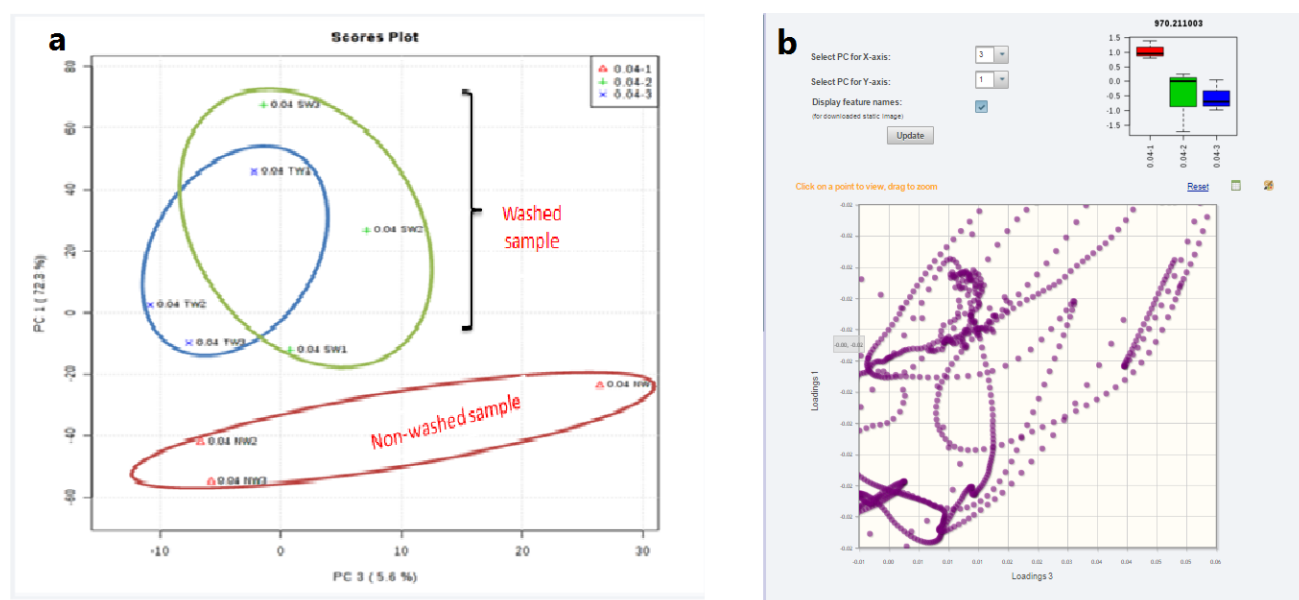| Peak | Frequencies/ Wave numbers (cm$^{-1}$) | Functional Group | Reference |
|------|----------------------------------------|------------------|-----------|
| 1 | 3500-3300 | N-H primary amine, amide and secondary amine, amide | Socrates, 2004 |
| 2 | 1680-1640 | C=C olefins | Socrates, 2004 |
|   | 1665 | C=N from oxime | |
| 3 | 1100-900 | P-O-C bond | Socrates, 2004 |



**Figure 2.** PCA scores plot (a) and loading plot (b) for non-washed and washed samples. Red clusters refer to non-washed sample (control, NW), green for salt water-washed technique (SW) and blue for tap water-washed technique (TW).

Identification of the functional groups that contribute to the differences observed on PCA score plot can be conducted by examining the PCA loading plot. The higher the absorbance intensity, the higher content of that functional group component in the samples. From the PCA loading plot (Figure 2b), it was found that NW (red) has a higher absorbance intensity at 970 cm$^{-1}$ compared to SW (green) and TW (blue). According to previous research, the frequencies of 940-2000 cm$^{-1}$ which belongs to the

ester (P-OR) functional group can be used to determine malathion (Quintás et al., 2004). Therefore, it can be concluded that the non-washed samples still have high pesticide residue whereas the washed samples have low or no pesticide residue.

Similarly, PLS-DA also demonstrated a clear separation between washed and non-washed samples (Figure 3a), and there was no distinction between salt water- and tap water-washed samples. The FT-IR frequency at 967 cm$^{-1}$ showed different intensities between non-washed and washed groups (Figure 3b).
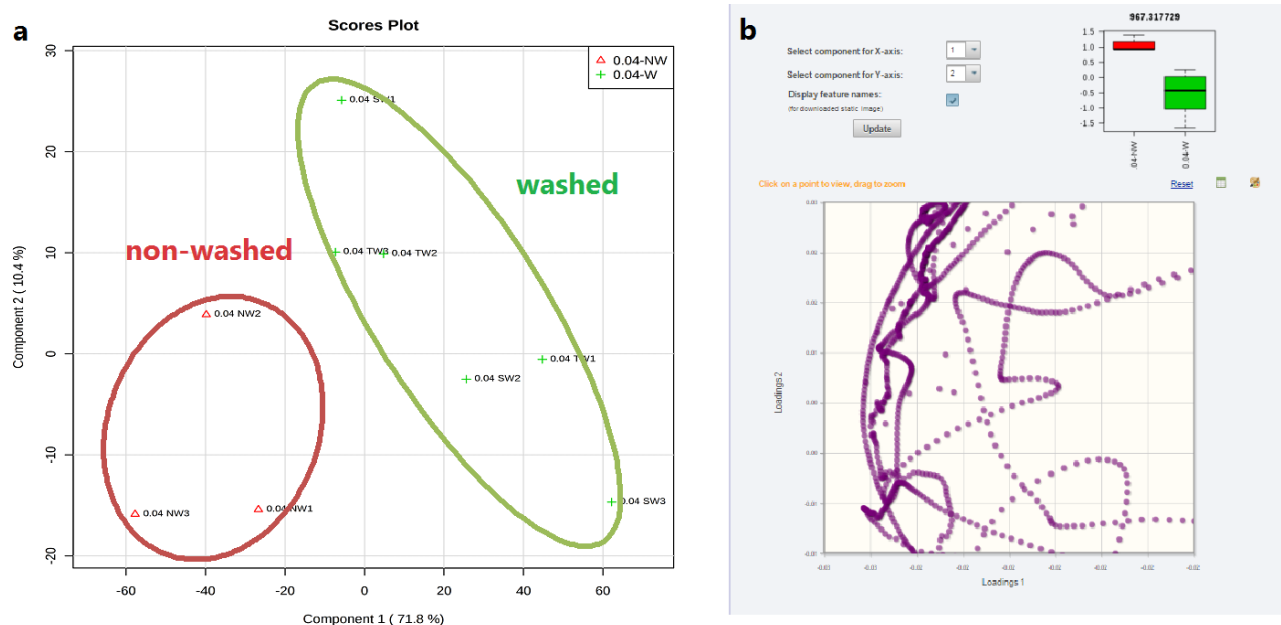


**Figure 3.** PLS-DA scores plot (a) and loading plot (b) for sprayed samples with 0.04 mL/20 mL malathion: non-washed (NW, red) and washed (green) with salt water, SW and tap water TW.

### 4. Conclusion

As a conclusion, FT-IR spectroscopy can quickly and easily identify malathion residues on spinach leaves with minimum sample preparation. FT-IR combined with multivariate data analysis can be developed to trace pesticide residues as a non-destructive method and to ensure that the pesticide concentrations are within a safe range.

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