

Ability of Fourier Transform-Near Infrared Spectroscopy to Detect Organophosphate (OP) Pesticides and Reduction of OP using a Washing Process

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Abstract

Since December 2020, the Thai Department of Agriculture (Thai DOA) placed chlorpyrifos on the banned chemical list, based on evidence the organophosphate (OP) class of pesticides was shown adverse effects on human health and environment. However, the use of this organophosphate class of pesticides have been found contamination in vegetable and fruit such as grave imported from China. The Fourier transform-near infrared (FT-NIR) spectroscopy combined with the dry extract system for infrared (DESIR) technique have been succeeded on detecting chlorpyrifos and ethion residues in spiked Chinese kale, head cabbage and green chili spur pepper employed as a vegetable model. Chinese kale, head cabbage and green chili spur pepper was spiked with chlorpyrifos and ethion at 0.44 - 111.29 mg/kg were detected using the quick, easy, cheap, effective, rugged and safe (QuEChERS) method coupled with gas chromatography-mass spectrometry (GC-MS). Partial least squares regression (PLSR) was used to develop a calibration equation. FT-NIR combined with DESIR and PLSR produced an equation that showed good performance and provided the best calibration equations based on values for R^2 (0.88 - 0.94), SEP (7.65 - 11.36 mg/kg), RMSEP (7.68 - 11.80 mg/kg), bias (-3.83 to 3.32 mg/kg) and RPD (3.03 - 4.10). These statistics revealed no significant difference between FT-NIR-predicted values and actual values at a confidence interval of 95 %. After applying ozonated water (1 mg/L) and EO water (available free Cl_2 at 70 mg/L) for 10 min resulted in 67 % reduction in chlorpyrifos and ethion levels in Chinese kale, head cabbage and green chili spur pepper. Since this developed method was limit to predict the amounts of residues (≤ 30 mg/kg) but it could be applied to monitor pesticide residues at harvesting, or inspection of incoming raw materials before entering food chain to ensure the safety of the vegetable for human consumption.

Keywords: NIR spectroscopy, DESIR, Organophosphate, Pesticide residues, Washing, Food safety

Introduction

Among leafy vegetable Chinese kale (*Brassica oleracea* var *alboglabra*) is a common green vegetable in Thai dishes, which the total production area in 2016 was 8,907 ha with the yield of 70,152 tons [1]. Head cabbage (*Brassica oleracea* var. *capitata*) and green chili spur pepper (*Capsicum annuum* Linn. var *acuminatum* Fingerh) are also in the top-ten vegetable by demand in Thailand. Head cabbage production and yield in 2016 were 7,590 ha and 153,956 tons, while

chili (all varieties) was as high as 40,409 ha and 282,047 tons [2]. Many vegetables and fruits both export or import such as exported chili has been reported contamination with OP pesticides [3], while imported grape from China has been found contaminated with many types of pesticide including chlorpyrifos [4]. Chlorpyrifos is the top residues found in European Union (EU) testing and 7th most common residues found in The United States agricultural commodities;

consequently, growers there were no longer permitted to possess or use this product after December 31, 2020 due to human health effects [5].

In 2021 and 2020 the Rapid alert system for food and feed (RASFF) reported that the amounts of chlorpyrifos in dill, lime leaves and holy basil leaf imported from Thailand to the EU, the amount were 0.2, 0.034 and 0.12 mg/kg, respectively [3]. Sankom *et al.* [6] revealed the OP pesticide residues, carbamate and pyrethroid pesticide groups were contaminated in Thai fresh vegetable (Chinese kale and chili spur pepper) by

0.01 - 52.7 mg/kg (**Table 1**). Even though chlorpyrifos was banned since 2020 but recently Thai Pesticide Alert Network (Thai-PAN) Shine issued a warning that Muscat grapes imported from China was being contaminated with chlorpyrifos exceeded the maximum permissible limit [7]. The presence of pesticide residues in fresh produce poses a health hazard to Thais particular erodes customer trust, resulting in market losses. The pesticide residue contaminated in fresh produce heightens food safety concern Thai consumers.

Table 1 Pesticide residues in vegetable samples from farms and retail shops in year 2016.

Sampling Location	Produces	Amount of sample	Pesticide residues (mg/kg)		
			OP group	Carbamate group	Pyrethroid group
Farm	Chinese kale	4	omethoate (0.08) chlorpyrifos (6.4, 43.19), diazinon (0.01), profenofos (52.71)	carbofuran (1.40)	bifenthin (0.03) lambda-cyhalothin (0.09), cypermethrin (0.08, 8.14)
	Green chili spur pepper	4	chlorpyrifos (1.15, 3.39, 14.40), ethion (0.09, 2.45), profenofos (1.42, 3.05, 8.49)	methomyl (0.01) carbofuran (0.02)	lambda-cyhalothin (0.04), cypermethrin (0.02, 0.04, 1.36), bifenthin (0.01)
Retail shop	Chinese kale	4	profenofos (0.02, 0.02, 0.03), chlorpyrifos (0.01, 0.02, 3.30)	ND	cypermethrin (0.04, 0.19) lambda-cyhalothin (0.02)
	Green chili spur pepper	4	profenofos (0.05, 0.10) chlorpyrifos (0.02)	carbofuran (0.01, 0.02), carbaryl (0.02), methomyl (0.03)	bifenthin (0.01), lambda-cyhalothin (0.01, 0.02), deltamethrin (0.01)

Note: ND = Not Detected; LOD at 0.005 mg/kg.

Organophosphate (OP) pesticides are a group of insecticides that are approved for application in vegetable production (particularly in Chinese kale, cabbages and chili) in Thailand [8]. The imported quantities of chlorpyrifos and ethion in 2017 were high at 3,324.8 tons (18.9 million USD) and 220 tons (1.5 million USD) indicating their common use in agricultural production. Since 2018, the quantity of chlorpyrifos imported to Thailand declined to 1,961 tons and in 2019 to 896 tons resulting from reconsideration by Thai authorities on limiting the use of this chemical [9]. In 2020, the Thai Department of Agriculture (Thai DOA) placed chlorpyrifos on the banned chemical list based on evidence of its adverse effects on human health and the environment [10]. Ethion is also the OP group, but is still approved only for chili/pepper cultivation,

with a maximum residue limit (MRL) not more than 3 mg/kg. Therefore the inspection and method of detecting the OP group in vegetable residue has become an issue of concern to ensure legal limits are not exceeded.

OP pesticides contain relatively small molecules that act as acetylcholinesterase inhibitors. Their acute toxicity and widespread use have attracted worldwide concern regarding trace amounts of residues on agricultural products that might cause adverse long-term health effects [11]. To ensure high crop yields, these pesticides are used during the production of agricultural commodities such as rice, Chinese kale and chili in Thailand [12-14]. Since OP pesticides are commonly used for pest control in a wide variety of crops, residual OP pesticide levels over the safety limits have been found often in vegetable supplied from Thai markets and

this has raised food safety awareness in household consumption [15,16].

Consequently, there is a need for the development of a method to detect, monitor and prevent pesticides contaminating vegetable from entering the food supply chain. The traditional pesticides detection technologies such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) though standard have shortcomings such as a longer detection cycle and complex testing process including the use of toxic solvents [17-19]. Near-infrared (NIR) spectroscopy is a potential alternative technique for safe, fast, reliable and low-cost analytical methods for the determination of pesticide residues including avoid the use of toxic solvents [6,17-19]. Therefore, NIR spectroscopy has been applied to the quality control of food in particular with fruits such as tomatoes, peppers, longans, navel oranges and olive [18,20-23]. The NIR spectroscopy technique has potential as a less/non-destructive, routine inspection method to segregate accepted and rejected products [24]. In Thailand, there have been some reports of using NIR spectroscopy methods to investigate the feasibility of pesticides inspection in food and agricultural commodities [6,25-27].

Not only are consumers aware of the chemical contamination on fruit and vegetable, industries also perform strict inspections on incoming raw materials before they undergo further processing. Washing is the most common process applied to fresh vegetable for pesticide residues reduction. It appears that washing removes pesticide residues on the surface of fruits and vegetable and decreases the pesticide concentration in the final products. Many studies have reported success at removing pesticides using oxidizing agents such as ozone and acidic electrolyzed oxidizing water (EO water) in the washing water [28-31]. Nevertheless, in most of the recent published works, little attention has been given to the quantification of OP in wash vegetable using the NIR spectroscopy technique.

Therefore, the aim of the present work was to examine the ability of FT-NIR spectroscopy to determine the levels of OP pesticides (particularly

chlorpyrifos and ethion) in 3 different types of vegetable: Chinese kale, head cabbage and green chili spur pepper. The current situation regarding pesticide residues on Chinese kale and chili spur pepper in Thailand and the reduction of OP pesticides using the washing process were also investigated. The detection methods will be useful not only the local agriculture commodities but the imported goods as well. Additionally, the accuracy and performance of the FT-NIR calibration equations were verified according to ISO 12099 on the guidelines for the application of near infrared spectroscopy in animal feeding stuffs, cereals and milled cereal products.

Materials and methods

Spiked vegetable samples with OP pesticides

Chinese kale, head cabbage and green chili spur pepper samples (pesticide residue free) were purchased from a supermarket in Bangkok, Thailand. These vegetable samples were confirmed that no chlorpyrifos and ethion traces were found according to the test by using the reference method (GC-MS) prior spiking. The Chinese kale leaves were trimmed to 20×20 cm² (approx. 20 ± 2 g per sample). The white inner head cabbage leaves with stem were trimmed into 12×12 cm² (approx. 20 ± 2 g per sample). Two green chili spur peppers was chosen 10 - 12 cm in length and 10 ± 2 g per pod. The placenta with seed was removed, only fruit with skin was used. All vegetable were individually spiked on the surface with drops of chlorpyrifos (C₉H₁₁Cl₃NO₃PS) and ethion (C₉H₂₂O₄P₂S₄) solutions (standard grade, purity > 98 %, Dr. Ehrenstorfer, DE) to obtain at different concentrations of 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 50.0, 70.0 and 100.0 mg/kg [6,18]. Then, dried at 25 ± 2 °C for 2 h, before each sample (20 ± 2 g) was blended separately in a blender, followed by pesticide analysis. Each blended sample was divided into 2 portions (a 10 g sample for FT-NIR analysis and another 10 g sample for GC-MS analysis as the reference method). The number of samples and concentrations of chlorpyrifos and ethion in the vegetable samples are described in **Table 2**.

Table 2 Number of samples and concentrations of chlorpyrifos and ethion in vegetable for calibration and validation sample sets obtained from the GC-MS method.

Vegetable	Parameter	Chlorpyrifos		Ethion	
		Calibration set	Validation set	Calibration set	Validation set
Chinese kale	Number of samples	65	33	61	32
	Maximum (mg/kg)	100.39	95.67	96.54	94.66
	Minimum (mg/kg)	0.44	0.54	0.65	0.65
	Average (mg/kg)	32.34	31.68	28.96	28.90
	Standard deviation (mg/kg)	31.73	30.84	31.38	29.53
Head cabbage	Number of samples	63	35	63	36
	Maximum (mg/kg)	103.90	99.99	111.29	105.63
	Minimum (mg/kg)	0.57	0.61	0.46	0.63
	Average (mg/kg)	32.61	31.35	38.79	36.33
	Standard deviation (mg/kg)	31.09	31.17	35.65	34.11
Chili spur pepper	Number of samples	61	36	63	36
	Maximum (mg/kg)	110.47	102.46	104.33	101.79
	Minimum (mg/kg)	0.50	0.51	0.54	0.55
	Average (mg/kg)	38.41	36.22	31.19	31.85
	Standard deviation (mg/kg)	36.31	34.49	30.30	32.62

Note: Recovery (%) \pm relative standard deviation (%) from spiked vegetable samples (Chinese kale, head cabbage and chili spur pepper) contained 2 levels of chlorpyrifos and ethion residues at 0.50 and 5.00 mg/kg using the QuEChERS and GC-MS method were 84 ± 7 to 110 ± 6 %, respectively. The correlation coefficients (R^2) of analytical curves were 0.9967 - 0.9996 with detection (LOD) and quantification (LOQ) limits were 0.11 - 0.13 and 0.38 - 0.44 mg/kg, respectively.

Sample preparation for the FT-NIR measurement

Spiked samples (10 g) were extracted using 10 mL acetone solvent in a glass vial (analytical grade, Merck, DE). Then each sample was shaken for 1 min using a vortex mixer (model 232, Fisher Scientific, UK), kept at 25 ± 2 °C for 10 min and passed through a filter paper (model No.1, Whatman, UK). The dry-extract system for infrared (DESIR) technique was performed by placing a glass microfiber filter paper (model GF/A, Whatman, UK) on a 55 mm diameter glass petri dish

(Pyrex, UK). The embedded 2 mL of extract sample in the filter paper was then evaporated in a vacuum-drying oven (model VD 23, BINDER GmbH, DE at 35 ± 2 °C for 60 min. The DESIR samples were kept in a desiccator (vacuum with plate 30 cm, Duran, GM) at 25 ± 2 °C for 30 min before the FT-NIR (model multi-purpose analyzer, Bruker Optic, DE) measurement [6,18,22]. The sample preparation scheme based on the DESIR technique and the FT-NIR spectroscopy measurement is shown in **Figure 1**.

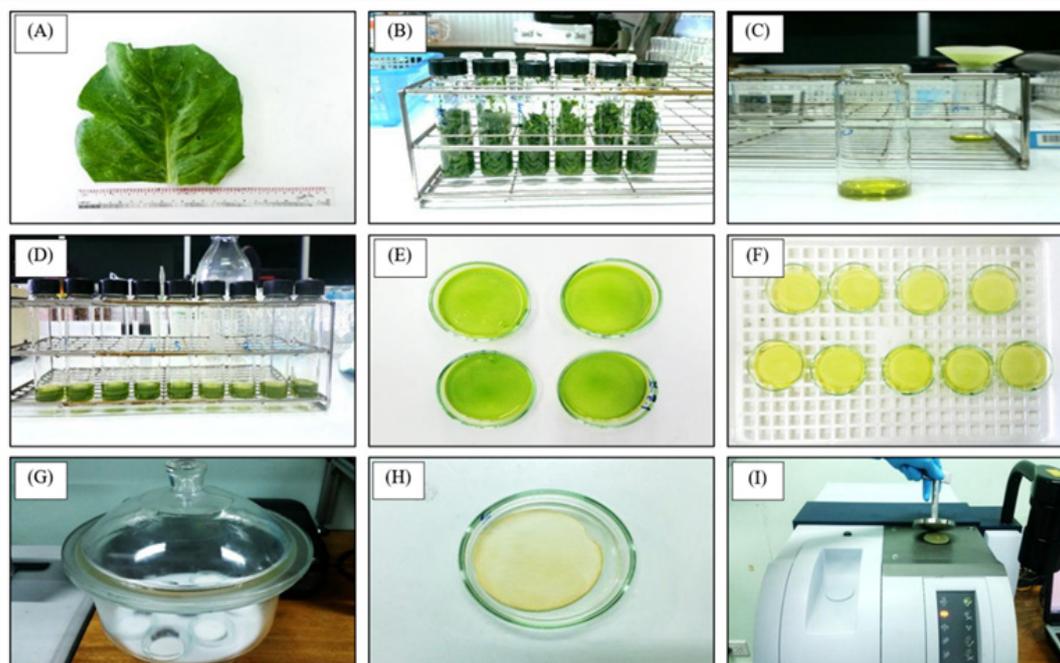


Figure 1 Sample preparation method for the FT-NIR spectroscopy measurement (A) was spiked samples then blended (B), extracted and passed through a filter paper (C), extracted samples (D), embedded 2 mL of extract sample in the GF/A (E), dried at 35 ± 2 °C for 60 min (F), equilibrated for 30 min (G), DESIR samples (H) and the FT-NIR in transreflectance mode measurement (I).

FT-NIR spectra collection

Each DESIR sample was placed using clean forceps on the measuring window, covered with the reflector (stainless steel, diameter 8.00 cm) and scanned using the FT-NIR spectrometer (model multi-purpose analyzer or MPA, Bruker Optic, DE) at wavenumber ranges of $12,500 - 4,000 \text{ cm}^{-1}$ in transreflectance mode at a resolution of 16 cm^{-1} , the average was obtained from 32 scans. An integrating sphere equipped with a lead sulfide (PbS) detector was used for spectrum measurement. The reference spectrum were measured by scanning the internal reference gold-coated device with spacer before scanning every samples [6]. To prevent cross contamination of pesticides on samples, the reflector and measuring window were cleaned thoroughly between consecutive measurements using 99 % acetone.

Reference method

Sample extraction and clean-up method based on the QuEChERS (quick, easy, cheap, effective, rugged and safe) with the GC-MS was used as the reference method for the chlorpyrifos and ethion residues analysis [32-34].

The homogenized sample (10 g) was placed in a 40 mL Teflon centrifuge tube and 10 mL acetonitrile (analytical grade, Fisher Scientific, UK) was added before capping tightly and then shaking vigorously for 1 min using a vortex mixer (model 232, Fisher Scientific, UK). Then, 4 g anhydrous magnesium sulfate (MgSO_4 , Applichem GmbH-an ITW company, DE) and 1 g sodium chloride (NaCl , Merck KGaA, DE) were added and mixed immediately for 1 min, before the extract was centrifuged (model 5804R, Eppendorf, DE) at 5,000 rpm and 25 ± 2 °C for 5 min. A 1 mL aliquot of the upper acetonitrile layer was transferred into a 1.5 mL micro-centrifuge vial containing 25 mg primary secondary amine (PSA) sorbent (Agilent Technology, USA) and 150 mg anhydrous MgSO_4 , capped tightly and shaken in the vortex mixer for 30 s. Following centrifugation for 1 min at 6,000 rpm (mini centrifuge model D1008, Scilogex, USA), 0.5 mL of extract was transferred into a sample vial. The extract samples were dried in a vacuum-drying oven (model VD 23, BINDER GmbH, DE) and then re-dissolved by mixing with ethyl acetate: cyclohexane (1:1, v/v; pesticide analysis grade, Fisher Scientific, UK) for GC/MS [32-34].

The analysis was performed in an isocratic system using a GC-MS (model QP2010, Shimadzu, JP) fitted with a capillary column (liquid phase, TC1701, GL Sciences, DE). The injection mode was splitless and used 1 μL with helium gas as the mobile phase. The system was composed of a 2.0 μL sample at 220 $^{\circ}\text{C}$ and 97.9 kPa with a gas flow rate of 1.5 mL/min. The mass spectrometer was operated initially in the SCAN mode, sweeping between 25 and 400 m/z. Lower concentration could be detected in the SIM mode by selecting the characteristic ions (m/z); chlorpyrifos: 197, 97 and 314 and ethion: 231, 384 and 125, respectively [6,33,35]. Retention time (min) for chlorpyrifos and ethion were presented at 13 and 17.5 min, respectively. The factors considered in the validation included recovery, precision (relative standard deviation), determination coefficient (R^2), linearity, detection (LOD) and quantification (LOQ) limits.

Data analysis and calibration equation development

Pre-processing and chemometrics methods were performed to develop the FT-NIR spectroscopy calibration equation for predicting the amounts of chlorpyrifos and ethion residues. Mathematical treatments such as derivative, standard normal variate (SNV) and their combination were applied to correct the baseline drift and the scattering effect [17,19]. The partial least squares regression (PLSR) was performed using the OPUS software (version 6.5, Bruker Optics, DE). Samples were randomly divided into 2 sets of the calibration and validation sets using an independent test set with 2:1 ratio: 1) the calibration sample sets (61 to 65 samples); and 2) the validation sample sets (32 to 36 samples). Samples with minimum and maximum of chlorpyrifos and ethion residues were collected into the calibration sample sets in order to ensure that the range covered the content for samples in the validation sample sets [6]. The average and standard deviation of content in calibration sample sets should be similar to the ones in validation sample sets (**Table 2**). The calibration equations were developed from the calibration sample sets and the accuracy of the equation was tested against values in the validation sample sets (**Table 2**).

The optimization procedures and the number of optimum components were selected based on the

minimum root mean square error of prediction value (RMSEP). The predictive capability of the calibration equation was assessed using the statistics coefficient of determination (R^2), standard error of prediction (SEP), bias and the ratio of performance deviation (RPD) [36,37].

Statistic for performance measurement

The accuracy and performance of the optimized calibration equation were evaluated using the validation sample set according to the procedure described in ISO 12099 [38].

Bias

The significance of the bias was checked based on a pair t-test to compare the obtained data from the experiment with the predicted data from the FT-NIR equation. The performance of the equation was based on comparing the bias values (obtained from the FT-NIR equation) and the bias confidence limits. The bias confidence limits (BCLs) or T_b were used to determine the limits for accepting or rejecting equation performance, as defined in Eq. (1):

$$T_b = \pm \frac{t_{(1-\alpha/2)} \cdot \text{SEP}}{\sqrt{n}} \quad (1)$$

where α is the probability of making a type I error, t is the appropriate Student's t-value for a 2-tailed test with the degrees of freedom associated with the SEP and the selected probability of type I error, n is the number of independent samples and SEP is the standard error of prediction. If the bias value was less than the T_b value, the t-test indicated no significant difference and the performance of the equation was acceptable.

SEP

The SEP was used to express the accuracy of the routine NIR results corrected for the mean difference (bias) between the routine NIR and reference methods. The SEP can be verified using the unexplained error confidence limits (UECLs or T_{UE}) value, which expressed the accuracy of the routine NIR results, as defined in Eq. (2):

$$T_{UE} = \text{SEC} \sqrt{F_{(\alpha, v, M)}} \quad (2)$$

where SEC is the standard error of calibration, α is the probability of making a type I error, $v = n - 1$ is the numerator degrees of freedom associated with the SEP of the test set, n is the number of samples in the validation process, $M = n_c - p - 1$ is the denominator degrees of freedom associated with the SEC, in which n_c is the number of calibration samples and p is the number of terms or PLSR factors in the model. A SEP value less than T_{UE} indicated that SEP could be accepted.

Slope

A t -test was used to verify the hypothesis that the slope $b = 1$. The t_{obs} value was considered as different from 1 when $t_{obs} \geq t_{(1-\alpha/2)}$ (3): where t_{obs} was the observed t -value, as defined in Eq. (3):

$$t_{obs} = |b - 1| \sqrt{\frac{S_{\hat{y}}^2(n-1)}{S_{res}^2}} \quad (3)$$

where n is the number of independent samples, $S_{\hat{y}}^2$ is the variance of the n predicted values and S_{res} is the residual standard deviation as defined in the following Eq. (4):

$$s_{res} = \sqrt{\frac{\sum_{i=1}^n (y_i - (a + b\hat{y}_i))^2}{n-2}} \quad (4)$$

where a is the intercept equation; $a = \bar{y} - b\bar{\hat{y}}$, whereas \bar{y} is the mean of the reference values; b is the slope; $\bar{\hat{y}}$ is the mean of the predicted values; \hat{y}_i is the i^{th} predicted value obtained when applying the multivariate NIR model. The slope, b , is considered as different from 1 when $t_{obs} \geq t_{(1-\alpha/2)}$, where t_{obs} is the observed t -value, calculated according to Eq. (3); $t_{(1-\alpha/2)}$ is the t -value obtained from table t -distribution for a probability of $\alpha = 0.05$.

Washing process with oxidizing agents and determining residue using the FT-NIR with DESIR technique

Chinese kale, head cabbage and green chili spur pepper (pesticide free) were kept at 4 ± 2 °C until use

(within 24 h). Preparation of samples was similar to the procedure described above. Each 100 g sample was spiked by dropping with standard solutions of chlorpyrifos and ethion to achieve approximately 5 and 10 mg/kg, followed by air-drying for 2 h in a laminar air flow cabinet (model no. CF43S, Gelman Science, UK) at 25 ± 2 °C before the washing process.

EO water (electrolyzed water generator, model: ROX-10 WA-E, Oshizaki Electric Co., Ltd., JP) with free available chlorine (Cl_2) 70 mg/L and ozonated water 1 mg/L (ozone generator, model OZVA 1021E, ProMinent Fluid Controls Co. Ltd., TH) were used as the washing water according to Sankom [31]. N, N-diethyl-p-phenylenediamine with a Dulcotest DT1 photometer (model DULCOTES DT1, ProMinent Fluid Controls Co. Ltd., TH) were used to determine free available chlorine (DPD no. 1 tablet) and ozone concentration (DPD no. 4 tablet). The pH and oxidation-reduction potential (ORP, mV) in the washing water were measured using a pH/ORP meter (model Cyberscan pH 510, Eutech Instrument, Possess Science Co., Ltd., TH).

Reduction of the pesticide levels on the fresh vegetable was determined after washing with EO water (free available Cl_2 at 70 mg/L, pH 2.7 and ORP 1,190 mV) and ozonated water (1 mg/L, pH 7.4 and ORP 880 mV) after a contact time of 10 min compared to tap water as the control. Comparisons were made on the residues from the 4 treatment groups with 5 replications: (I (no washing), II (washing with tap water), III (EO water) and IV (ozonated water)). Each 100 g sample of spiked fresh vegetable was washed with 2 L of wash water; soaked without shaking. After washing for 10 min, each sample was rinsed with tap water for 1 min) except for the no washing treatment and air-dried for 2 h under a laminar air flow (0.5 m/s) at 25 ± 2 °C before analysis. The levels of chlorpyrifos and ethion residues were determined based on the QuEChERS coupled with GC-MS technique [32-34]. Data analysis and a calibration equation were developed similar to the procedure described above. Six selected FT-NIR-PLSR calibration equations were chosen for predicting the levels of chlorpyrifos and ethion residues in vegetable samples after the washing process. The prediction accuracy of each selected FT-NIR-PLSR calibration equation was determined based on the values for R^2 ,

SEP, RMSEP, bias and RPD (OPUS software, version 6.5, Bruker Optics, DE).

Results and discussion

Ability of NIR spectroscopy for detecting OP pesticide residues in vegetable

Data analysis and calibration equation development

Data preprocessing was applied on the FT-NIR spectra prior to the computation of the regression method in order to correct for interference not related to the 2 pesticides. Mathematical formulations such as derivatives, the standard normal variate and their combinations have been applied to correct for baseline drift and the scattering effect [6,17,19]. The PLSR method was used for regression of the FT-NIR spectra against the pesticide residue levels in the vegetable (Chinese kale, head cabbage and green chili spur pepper) at concentrations of 0.44 to 111.29 mg/kg.

All the optimized FT-NIR-PLSR calibration equations for detecting chlorpyrifos and ethion residues in Chinese kale, head cabbage and green chili spur pepper are shown in **Table 3**. The best FT-NIR-PLSR calibration equation produced calibration equations that performed well in detecting chlorpyrifos and ethion residues in the validation sample dataset, based on

acceptable values for R^2 and RPD of 0.88 - 0.94 and 3.03 - 4.10, respectively. In addition, these calibration equations had the lowest values of SEP, RMSEP and bias at 7.65 - 11.36, 7.68 - 11.80 and 3.83 - 3.32 mg/kg, respectively. These results were consistent with the sensitivity of detection of euparin-spiked tomato fruits fungicide at 2 - 90 mg/kg reported by Saranwong and Kawano [18] when using the NIR spectroscopy with DESIR that achieved an SEP of 7.89 mg/kg.

The values of R^2 and RPD are the most meaningful statistics for appraising analytical efficiency using the NIR spectroscopy [37,39]. They considered the values of R^2 in the range 0.83 - 0.90 could be used with caution in most applications, including research, while value of R^2 over 0.90 indicated excellent capacity for quantitative analysis. Thus, in the study the values R^2 from validation sample sets of 0.88 - 0.94, would generally excellent for quantitative analysis. Furthermore, Williams and Norris [37]; Nicolai *et al.* [39] considered minimum RPD values of 2.0 - 3.0 were required for adequate screening, while values of 3.0 - 5.0 would produce acceptable predictability. Thus, the RPD values in the current study were in the range 3.03 - 4.10 indicated the acceptable calibration equation for prediction.

Table 3 FT-NIR-PLSR calibration and validation results for OP residues (mg/kg) in Chinese kale, head cabbage and chili spur pepper.

Pesticide	Vegetable	Pre-processing	Number of factor	Wavelength region (c^{-1})	Calibration set		Validation set				
					R^2	SEC	R^2	SEP	RMESP	Bias	RPD
Chlorpyrifos	Chinese kale	1 st derivative + SNV	8	9,403 - 7,498, 6,102 - 4,242	0.86	11.41	0.91	8.99	9.37	3.07	3.43
	Head cabbage	1 st derivative + SNV	6	6,102 - 4,242	0.89	9.82	0.90	9.96	10.80	3.32	3.16
	Chili spur pepper	1 st derivative + SNV	3	6,804 - 6,094, 5,454 - 4,242	0.88	12.00	0.89	11.36	11.80	-3.83	3.03
Ethion	Chinese kale	SNV	8	5,454 - 4,242	0.91	8.81	0.94	7.65	7.68	-1.53	4.10
	Head cabbage	SNV	5	5,778 - 5,446, 4,605 - 4,242	0.87	12.42	0.88	10.35	11.20	-3.52	3.30
	Chili spur pepper	1 st derivative	5	7,506 - 4,242	0.89	9.56	0.90	10.03	9.95	-1.14	3.17

Note: R^2 : Coefficient of multiple determination; SEC: Standard error of calibration; SEP: Standard error of prediction; RMSEP: Root mean square error of prediction; bias: Average of differences between reference values and NIR values; RPD: Ratio of performance to deviation. Unit: mg/kg. SNV: Standard normal variate.

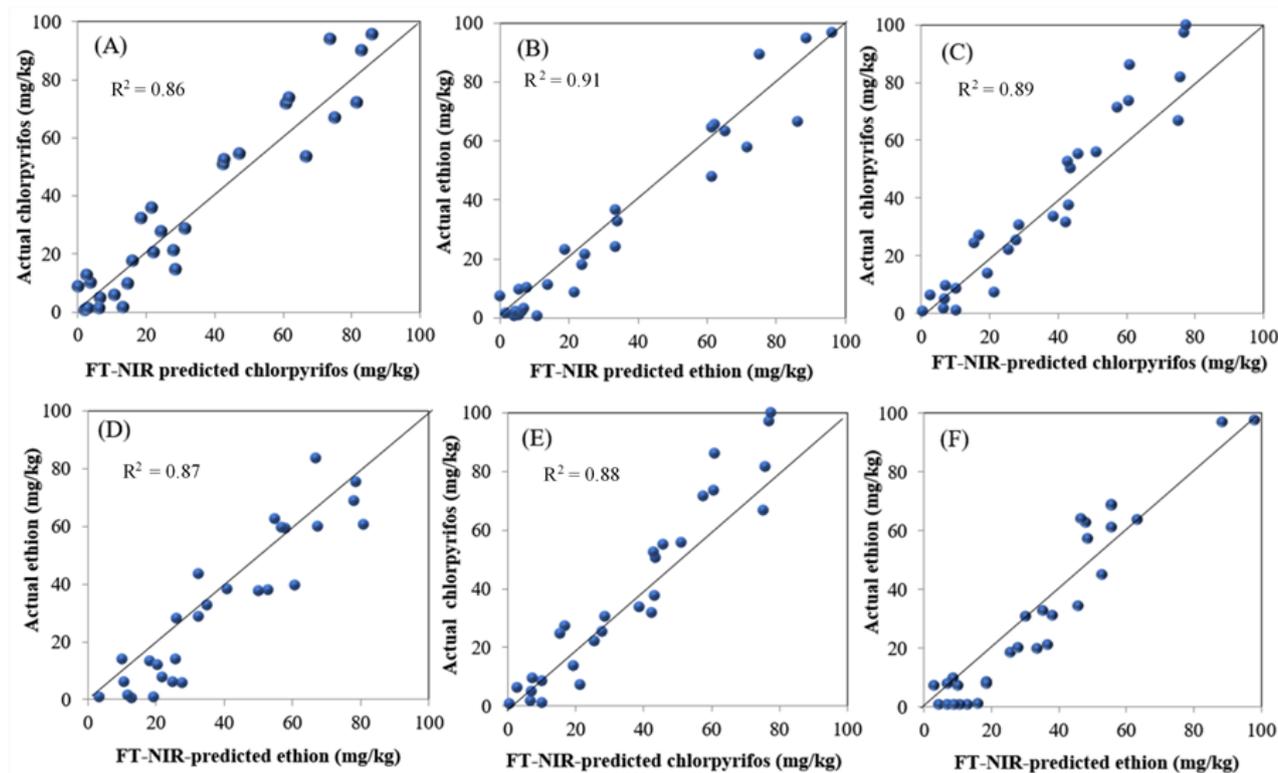


Figure 2 Scatter plots of validation sample sets for predicting chlorpyrifos and ethion residues in Chinese kale (A,B), head cabbage (C,D) and green chili spur pepper (E,F).

Figure 2 indicates that the best FT-NIR-PLSR-calibration equations had good scatter plot correlation (R^2 values 0.88 - 0.94) and that the predicted OP (chlorpyrifos and ethion) residue values (data from FT-NIR method) agreed well with the actual chlorpyrifos and ethion residues values (data from GC-MS method) for all 3 vegetable. **Figure 3** presents the regression coefficient plot of the FT-NIR-PLSR calibration equations for detecting chlorpyrifos and ethion residues in vegetable samples. The purpose of regression coefficient plots were primarily used to consider the importance wavenumber (X-variable) from the optimized equations, which it was described or correlated to the reference value (Y-variable). A wavenumber at a peak with high regression coefficient

values indicates that the variable is high correlated with the reference value [40]. **Figure 3(A)** shows that the regression coefficient plots of the calibration equation for predicting chlorpyrifos in Chinese kale presented the high regression coefficient values at wave number regions of 9,200 - 7,500 and 6,100 - 4,200 cm^{-1} . Based on the structure of chlorpyrifos, some variables located at wavenumber regions around 8,755, 8,747, 5,924, 5,916, 4,651 and 4,643 cm^{-1} pertained to the C-H bond in the aromatic compounds [41]. In addition, the variable at 5,376 cm^{-1} could have been due to the C-Cl bond in the chlorpyrifos structure (**Figure 4(A)**). Some variables observed at 8,369, 5,785, 4,335 and 4,312 cm^{-1} were correlated to the CH_2 and/or CH_3 bond in aliphatic compounds [41].

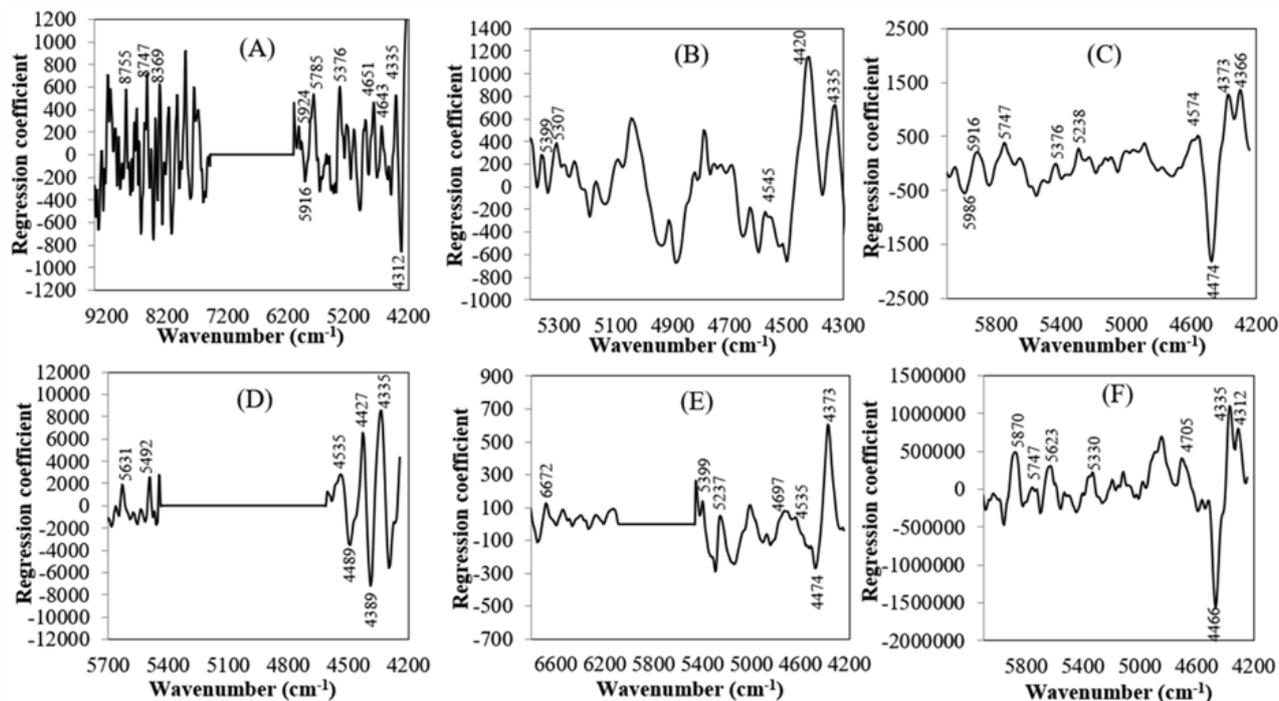


Figure 3 Regression coefficient plots of NIR-PLSR calibration equations for predicting chlorpyrifos and ethion residues in Chinese kale (A,B), head cabbage (C,D) and chili spur pepper (E,F).

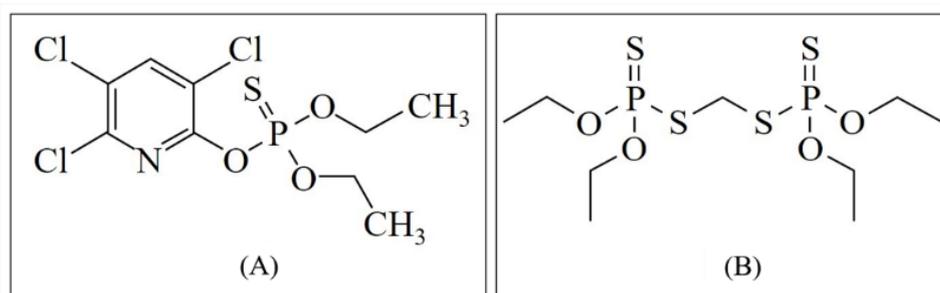


Figure 4 Chemical structure of chlorpyrifos (A) and ethion (B).

Noticeably, **Figure 3(C)** shows the highest regression coefficient at around $4,366\text{ cm}^{-1}$ which was associated with the C-H group in the aromatic compound contained in the chlorpyrifos structure in head cabbage extract. Some variables located at around $5,986, 5,916, 5,882 - 5,555, 4,574, 4,474$ and $4,373\text{ cm}^{-1}$ were associated with the C-H group, the CH in aromatic compounds and the CC + CH group in aromatic hydrocarbon compounds [41-44]. Additionally, 2 variables were detected at $5,376$ and $5,238\text{ cm}^{-1}$ which could have been due to the C-Cl group and P-OH (phosphate compound) in the chlorpyrifos structure (**Figure 4(A)**) [41]. Similarly, the results for green chili spur pepper (**Figure 3(E)**) indicated the highest regression coefficient was at $4,373\text{ cm}^{-1}$ which was

specified to the C-H group in the aromatic compound [41]. Some high regression coefficient presented at $5,520 - 5,300\text{ cm}^{-1}$ could have been due to the existence of an organophosphorus group [41]. In particular, the low regression coefficient of the P-OH group in a phosphate compound was observed at $5,237\text{ cm}^{-1}$ [41]. Some variables were observed in FT-NIR regions between $6,672$ and $4,761 - 4,444\text{ cm}^{-1}$ corresponding to the peak of the C-H group, while the variables around $4,697$ and $4,535\text{ cm}^{-1}$ referred to the CC + CH group in the aromatic compound (**Figure 4(A)**) [41-44]. The summarized band assignments of chlorpyrifos in vegetable by FT-NIR regions are presented in **Table 4**.

The regression coefficient plots of the FT-NIR-PLSR calibration equations for detecting ethion in

Chinese kale, head cabbage and green chili spur pepper are presented in **Figures 3(B) - 3(F)**, respectively. The main regression coefficient plots at 4,335, 4,420 and 4,545 - 4,500 were clearly related to absorption of the CH bond and CH₂/CH₃ in aliphatic compounds (**Figure 4(B)**) [18,41]. An interesting FT-NIR-PLSR regression coefficient was evident at around 5,399 and 5,307 cm⁻¹, indicating an organophosphorus group (**Figure 3(B)**) [45].

Figure 3(D) shows that the highest regression coefficient for ethion residues in head cabbage was at 4,335 cm⁻¹ which could be specified with the CH₂ group in aliphatic hydrocarbon compounds [41]. Moderate regression coefficients were observed around 5,631, 4,535, 4,489, 4,427 and 4,389 cm⁻¹ which were associated with the CH and CH₃ groups in hydrocarbon compounds (**Figure 4(B)**) and aliphatic hydrocarbon

compounds [19,41-43]. Other variables were observed at 5,492 cm⁻¹ and at around 5,520 - 5,300 cm⁻¹ that could be related to organophosphorus groups [45]. Likewise, **Figure 3(F)** reveals the main regression coefficients suitable for detecting ethion residues in green chili spur pepper. For example, at 4,466, 4,335 and 4,312 cm⁻¹ these variables were associated with the CH₂ and CH₃ groups in aliphatic hydrocarbon compounds [19,41,42]. At around 5,870, 5,747, 5,623 and 4,705 cm⁻¹, according to Workman and Weyer [41]; Tang *et al.* [43] all these variables were correlated to the CH, CH₂ and CH₃ group in aliphatic hydrocarbon compounds. In addition, the low value of the regression coefficient at around 5,330 cm⁻¹ could be correlated to the organophosphorus group (**Figure 4(B)**) [45]. The summarized band assignments of ethion in vegetable under the FT-NIR regions are shown in **Table 5**.

Table 4 Band assignments of chlorpyrifos in vegetable by FT-NIR regions.

Chinese kale		Head cabbage		Chili spur pepper	
Band	Functional group	Band	Functional group	Band	Functional group
8755	CH in aromatic [41]	5986	CH in aromatic [41]	6672	CH group [44]
8747	CH in aromatic [41]	5916	CH in aromatic [42,43]	5520 - 5300	Organophosphorus group [45]
8369	CH ₃ in aliphatic [41]	5882 - 5555	CH ₃ , CH in aromatic [42,43]	5399	Organophosphorus group [45]
5924	CH in aromatic [41]	5376	C-Cl [41]	5237	P-OH in phosphate compounds [41]
5916	CH in aromatic [41]	5238	P-OH in phosphate compounds [41]	4761 - 4444	CH group [42]
5785	CH ₃ in aliphatic [41]	4574	CC + CH in aromatic [41]	4535	CC + CH in aromatic [41]
5376	C-Cl [41]	4474	CH group [42]	4373	CH in aromatic [41]
4651	CH in aromatic [41]	4373	CH in aromatic [41]		
4643	CH in aromatic [41]	4366	CH in aromatic [41]		
4335	CH ₂ in aliphatic [41]				
4312	CH ₃ in aliphatic [41]				

Table 5 Band assignments of ethion in vegetable by FT-NIR regions.

Chinese kale		Head cabbage		Chili spur pepper	
Band	Functional group	Band	Functional group	Band	Functional group
5399	organophosphorus group [45]	5631	C-H aliphatic [43]	5870	C-H ₃ in aliphatic [41]
5307	organophosphorus group [45]	4535	CH groups [42,47]	5747	CH in aliphatic [43]
4545 - 4500	CH ₃ [41]	5492	Organophosphorus group [45]	5623	C-H ₂ [43]
4420	CH ₃ absorption [47]	4489	CH group [42]	5520 - 5300	Organophosphorus group [45]
4335	CH ₂ in aliphatic [41]	4427	CH ₃ absorption [47]	4705	CH group [41]
		4389	CH groups [42]	4466	CH group [42]
		4335	CH ₂ in aliphatic [41]	4335	CH ₂ in aliphatic [41]
				4312	C-H ₂ in aliphatic [41]

Although the DESIR technique combined FT-NIR detection with chemometric methods provide the maximum amount of chemical information, but this technique did not provide an accurate value for the OP (chlorpyrifos and ethion) residues in vegetable at below ≤ 30 mg/kg, based on the scatter plot data. This agreed with the low sensitivity of the signal (which is a shortcoming of NIR spectroscopy) resulting in a limitation on substance determination if the concentration is below 0.1 % (1,000 mg/kg). Thus, this technique is questionable for detection of analytes at parts-per-million (mg/kg) levels [6,18,42]. Regarding the DESIR technique, a liquid containing the extracted constituent of interest is dried into a solid substrate with low IR absorptivity. DESIR method was employed for pesticide residues analysis which may increase the concentration of analyte [6,18,46,47]. The scatter plot from using the DESIR technique with FT-NIR (**Figure 2**) shows clear improvement in the sensitivity of the FT-NIR spectroscopy for detecting pesticide residues in vegetable at the mg/kg level and in particular at ≥ 30 mg/kg. However, the DESIR technique coupled with the FT-NIR spectroscopy had advantages of its rapid and using less-harmful chemicals in the analysis. In this study, total time of a single test for the DESIR coupled with the FT-NIR spectroscopy method is approximately 100 min/test with the machine warm-up for 1 h, while total time of a single test for the QuEChERS with GC-MS method is approximately 60 min/test with the

machine warm-up for 12 h. Moreover, detection of OP pesticides (chlorpyrifos and ethion) in vegetable based on the FT-NIR spectroscopy could reduce the measurement time from 30 min/test (GC-MS method) to only 1 min/test (FT-NIR spectroscopy method).

Performance measurement statistics

It is necessary to test the equation performance of NIR method before routine application. Consequently, the accuracy and precision of the calibration equation were checked to confirm the utility of the FT-NIR method following the approach of ISO12099 [38]. The equation performance checking was based on the bias, SEP and slope from the best equations and verified following the criteria in ISO 12099 (**Table 6**). All statistics obtained from the predicted equations for the chlorpyrifos and ethion residues in the 3 types of vegetable passed these tests based on the criteria. The bias was lower than T_b confirming it was not significantly different from 0. The SEP index was lower than T_{UE} , thus indicating that it was low enough to make it acceptable in a practical sense. In addition, t_{obs} was lower than $t_{(1-\alpha/2)}$, at $\alpha = 0.05$, indicating the slope was not significantly different from 1. Therefore, the calibration equation developed could be used to predict the concentrations of OP pesticide (chlorpyrifos and ethion) residues 0.44 - 111.29 mg/kg in Chinese kale, head cabbage and green chili spur pepper. There was no significant differences between the FT-NIR-predicted

values and the actual values (data from GC-MS) at a confidence interval of 95 %, indicating these calibration equations were acceptable for routine FT-NIR screening measurement. Moreover, further analysis should be carried on in order to standardize the FT-NIR method

for detecting chlorpyrifos and ethion in each vegetable types by increasing the varieties of vegetable and number of samples to get better accuracy of measurement.

Table 6 Performance measurement of FT-NIR-PLSR calibration equations for detecting OP pesticide residues in fresh vegetable following ISO 12099.

Pesticide	Vegetable	Parameter	Calculated value	Criterion	Result
Chlorpyrifos	Chinese kale	Bias	3.07 mg/kg	$T_b = \pm 3.18$	Accepted
		SEP	8.99 mg/kg	$T_{UE} = 14.65$	Accepted
		t_{obs}	0.07	$t_{(1-\alpha/2)} = 2.03$	Accepted
	Head cabbage	Bias	3.32 mg/kg	$T_b = \pm 3.42$	Accepted
		SEP	9.96 mg/kg	$T_{UE} = 12.57$	Accepted
		t_{obs}	0.04	$t_{(1-\alpha/2)} = 2.03$	Accepted
	Chili spur pepper	Bias	-3.83mg/kg	$T_b = \pm 3.84$	Accepted
		SEP	11.36 mg/kg	$T_{UE} = 15.31$	Accepted
		t_{obs}	0.04	$t_{(1-\alpha/2)} = 2.03$	Accepted
Ethion	Chinese kale	Bias	-1.53 mg/kg	$T_b = \pm 2.85$	Accepted
		SEP	7.65 mg/kg	$T_{UE} = 11.49$	Accepted
		t_{obs}	0.14	$t_{(1-\alpha/2)} = 2.04$	Accepted
	Head cabbage	Bias	-3.52 mg/kg	$T_b = \pm 3.55$	Accepted
		SEP	10.35 mg/kg	$T_{UE} = 15.88$	Accepted
		t_{obs}	0.05	$t_{(1-\alpha/2)} = 2.03$	Accepted
	Chili spur pepper	Bias	-1.14 mg/kg	$T_b = \pm 3.39$	Accepted
		SEP	10.03 mg/kg	$T_{UE} = 12.20$	Accepted
		t_{obs}	0.03	$t_{(1-\alpha/2)} = 2.03$	Accepted

Note: SEP: Standard error of prediction; T_b : Bias confidence limits; T_{UE} : Unexplained error confidence limits value; $t_{(1-\alpha/2)}$ t -value obtained from table t -distribution for a probability of $\alpha = 0.05$; t_{obs} : Observed t -value for slope testing.

Efficacy of oxidizing agents in washing process to reduce OP pesticide residues on fresh vegetable

Vegetable are washed or rinsed with water upon harvest to remove mud and dirt on the surface, and best practice requires consumers to wash them again, with or without detergent, before consumption. In the current study, the initial concentrations of the chlorpyrifos and ethion residues on the 3 fresh vegetable were approximately 5 and 10 mg/kg, respectively (5.18 - 6.80 and 9.24 - 9.86 mg/kg, respectively). Washing with either EO water or ozonated water revealed their great

ability to reduce the levels of chlorpyrifos and ethion residues on all 3 fresh vegetable (Table 7). Ozonated water at 1 mg/L for 10 min produced the best results for chlorpyrifos reduction (67 %) among the 3 vegetable samples ($p < 0.05$). There were different chlorpyrifos residues on the fresh vegetable at 2 levels: 2.16 - 3.07 mg/kg (55 - 67 % reduction) and 3.52 - 3.78 mg/kg (60 - 64 % reduction) and similar results were recorded using EO water, with levels of 2.83 - 3.21 mg/kg (51 - 57 % reduction) and 3.54 - 4.60 mg/kg (51 - 63 % reduction).

There was no significant ($p < 0.05$) difference regarding the efficacy of using either EO water or ozonated water as oxidizing agents on the reduction of ethion in vegetable. Both washing treatments of EO water and ozonated water reduced ethion on all fresh vegetable by 51 - 67 and 58 - 67 %, respectively, with the remaining levels being 1.95 - 2.49 and 3.24 - 3.73 mg/kg, respectively. Washing fresh vegetable with oxidizing water produced a significantly ($p < 0.05$) notable improvement on pesticides reduction (67 %) compared to tap water, with the reduction being 17 - 31 %.

Dissolved ozone generates hydroxyl radicals that are highly effective at decomposing organic molecules such as in pesticide residues. The oxidation reaction takes place via the radicals generated in the decomposition of ozone (hydroxyl, superoxide, ozonide and hydroperoxide radicals) reacting with organic compounds [48]. When ozone reacts with organic pesticides, it disrupts unsaturated aromatic hydrocarbons and aliphatic hydrocarbons in the molecular structure of pesticides by breaking carbon chains and opening benzene rings, and it also oxidises dichlorovinyl, nitro, methoxy, amino and other functional groups. Oxidative cleavage radically changes the molecular structure of organic pesticides and causes them to lose their potency. In addition, the small-molecule compounds produced further by the reaction between ozone and unsaturated carbon chains in pesticide molecules, such as acids, alcohols, amines, carbonyls, carboxylates and their oxides, are primarily water-soluble [49,50]. Therefore, OP molecule that involved phosphorus atom might be broken, replaced with oxygen atom, and formed to simple esters of phosphoric acids. The main reaction for OP molecule with P=S group was taken place from P=S bonds to P=O bonds. Consequently, they can be washed away with tap

water, leading to the degradation and removal of pesticide residues [49,50].

The application of ozone for the oxidation of residual pesticides on agricultural products should be considered due to its strong oxidation ability. Ozone is classified as a generally recognized as safe (GRAS) compound and has been used with many types of fruit and vegetable. The removal of pesticides residues after washing with ozonated water depended on the ozone dosage and contact time, which ozone at 1 - 4 mg/L for 5 - 60 min could reduce pesticides (such as chlorothalonil, tetraconazole, chlorpyrifos, profenofos and dichlorvos) on fruits and vegetable (such as Chinese kale, head cabbage, Chinese cabbage, chili spur pepper, coriander, grapes, strawberry and tomato) by 30 - 86 % [29,31,51,52].

EO water consists of hydrogen ions (H^+), hypochlorous acid (HClO) and hydrochloride (HCl) and has a low pH (2.3 - 2.7). The high oxidation reduction potential or ORP (1,000 - 1,200 mV) with a high concentration of dissolved chlorine and dissolved oxygen causes an oxidation reaction [53]. Our results agreed with those of Hao *et al.* [28] who demonstrated that EO water at pH < 2.7, a high ORP (> 1,100 mV) and a high free available chlorine concentration (30 - 300 mg/L) could eliminate > 60 % of pesticides (acephate, omethoate and dichloroviny phosphate) from fresh spinach. These organic phosphorous pesticides contain P=O and C=O bonds and when the oxidation occurs, the double bond is broken down under acid and alkaline conditions, resulting in then biomolecular nucleophilic substitution [28]. Similarly, Qi *et al.* [30] reported that EO water) pH 2.8, ORP 1,151 mV (with free available chlorine at 120 mg/L for 15 min could remove diazinon (37 - 59 %), cyprodinil (32 - 50 %) and phosmet (50 - 86 %) from spinach, snap beans and grapes.

Table 7 Efficacy of oxidizing agents on reduction of chlorpyrifos and ethion residues on fresh vegetable.

Pesticide	Vegetable	Initial residue (mg/kg)	% Reduction		
			Tap water	EO water (free available Cl ₂ 70 mg/L)	Ozonated water (mg/L)
Chlorpyrifos	Chinese kale	6.60 ± 0.62	23.61 ± 3.05 ^c	57.13 ± 6.44 ^b	67.23 ± 3.23 ^a
		9.86 ± 0.75	31.41 ± 1.11 ^c	53.30 ± 2.91 ^b	61.63 ± 2.71 ^a
	Head cabbage	6.80 ± 0.50	21.21 ± 3.49 ^b	52.77 ± 5.00 ^a	54.92 ± 5.22 ^a

Pesticide	Vegetable	Initial residue (mg/kg)	% Reduction		
			Tap water	EO water (free available Cl ₂ 70 mg/L)	Ozonated water (mg/L)
	Chili spur pepper	9.55 ± 1.26	23.45 ± 5.02 ^b	62.94 ± 4.98 ^a	63.09 ± 4.28 ^a
		6.04 ± 0.66	21.34 ± 3.52 ^c	50.56 ± 3.09 ^b	57.06 ± 6.53 ^a
		9.24 ± 1.19	25.60 ± 3.28 ^c	51.34 ± 4.20 ^b	60.36 ± 3.12 ^a
Ethion	Chinese kale	5.18 ± 0.69	16.85 ± 4.88 ^b	51.85 ± 5.83 ^a	57.77 ± 6.60 ^a
		9.67 ± 1.00	27.57 ± 6.63 ^b	61.44 ± 6.56 ^a	63.17 ± 4.07 ^a
	Head cabbage	6.39 ± 0.92	23.93 ± 5.23 ^b	67.04 ± 7.89 ^a	64.64 ± 7.44 ^a
		9.44 ± 0.57	20.70 ± 4.49 ^b	63.25 ± 6.48 ^a	63.67 ± 7.94 ^a
	Chili spur pepper	5.83 ± 0.27	20.76 ± 2.81 ^b	64.77 ± 5.61 ^a	66.49 ± 5.75 ^a
		9.35 ± 0.24	22.12 ± 5.40 ^b	66.10 ± 2.30 ^a	64.26 ± 4.73 ^a

Note: Means (from 5 replicates) within the same row with different lowercase superscripts are significantly ($p \leq 0.5$) different based on Duncan's multiple range test.

The prediction accuracy performance in all calibration equations of non-washed ($n = 10$) and washed vegetable ($n = 30$) was in the range 0.5 - 10 mg/kg. The scatters plots from the FT-NIR-PLSR calibration equations showed a very poor relationship. Consequently, poor results were observed with very low values of R^2 (0.03 - 0.06) and RPD (0.05 - 0.17) and very high values of SEP, RMSEP and bias (data not shown) and consequently, these equations were unusable [37,39].

The sensitivity of the NIR spectroscopy technique for detecting at the mg/kg level pesticide residues in fruits and vegetable has been investigated. For example, it was successful on fresh mango [46], apple and tomato [53] but only with a notably level of high active ingredient (g/L) with a high spike concentrations (0.1 - 0.5 %) from the same sample set. However, the very large amounts of residues that provide good detection performance may not be realistic in routine situations. Furthermore, the sensitivity of this method may vary with the concentration of residues and the intact sample.

Similar to Zhou *et al.* [54]; Wu *et al.* [55] also succeeded to detect chlorpyrifos residues at low amount in minced white radish (0.01 - 5.5 mg/kg) and spinach juice (0.1 - 4 mg/kg) with good performance indexes of RMSEP at 0.1 and 0.0485 mg/kg, but quiet low sample numbers was observed with require validation thus calibration equation robustness was still questionable. The feasibility of NIR spectroscopy technique for

qualification detection of pesticide residues in food by classifying presence/absence or safe and unsafe (based on MRL) of pesticides in vegetable (peppers and cucumber) was success [20,22]. The percentage of correctly classified samples in calibration and prediction sets (PLSR-discriminant analysis) were 75 - 97.5 %, respectively. However, the authors concluded that these results were not adequate to carry out the development of robust equation that can be established directly at an industrial level [20,22]. Lately, Sankom *et al.* [6] reported on the ability of the FT-NIR combined with the DESIR technique, demonstrating its strong potential for detecting profenofos residues (0.53 - 106.28 mg/kg) in vegetable such as Chinese kale, head cabbage and chili spur pepper. The best FT-NIR-PLSR equation provided good profenofos detection in all vegetable based on values for R^2 (0.88 - 0.97), SEP (5.27 - 11.07 mg/kg), RMSEP (5.25 - 11.00 mg/kg), bias (-1.39 - 1.30 mg/kg) and RPD (2.91 - 5.22). These statistics revealed no significant differences between the FT-NIR predicted values and actual values at a confidence interval of 95 %, with agreeable results presented at pesticide residue levels over 30 mg/kg. In summary, these studies indicated that NIR spectroscopy techniques were not satisfactory to quantify pesticide residues in fresh produce at low levels (≤ 100 mg/kg).

Conclusions

The proposed combination of the FT-NIR spectroscopy with DESIR and chemometric methods was useful for screening detection of OP pesticide residues on fresh vegetable at concentrations over 30 mg/kg. However, the FT-NIR spectroscopy technique had advantages of its rapid, low cost and using no or less-harmful chemicals in the analysis and so it could be of value for high throughput screening rather than using HPLC or GC-MS analysis. Consequently, this method will be useful to monitor pesticide residues during crop cultivation and to inspect raw vegetable before harvesting. For the food industry, this method offers an ideal solution for testing pesticide authenticity and detecting adulteration, such as identifying banned pesticides (such as chlorpyrifos) in imported fresh produce and agricultural raw materials before further processing and marketing. In addition, application of an oxidizing agents such as ozonated water at 1 mg/L and EO water with free available chlorine at 70 mg/L for 10 min in the washing process could reduce the levels of pesticide residues on fresh vegetable by up to 67 %. Thus, integrated pesticide management incorporating Good Agricultural Practices (GAP) standards, along with methods for detecting and monitoring pesticide residues and optimizing the washing process should be implemented in vegetable production. These processes help ensure product quality and enhance the food safety of vegetable consumption.

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