UNIVERSITI PUTRA MALAYSIA

EVALUATION OF A PORTABLE SPECTROMETER FOR DETECTION OF PESTICIDE RESIDUE ON CABBAGE

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

November 2018

DEDICATION

I dedicate this thesis to my most precious personages…

To my dearest father and mother,
Thank you for your infinite love and sacrifice,
To my sister, two little sister and little brother,
Thank you for lending me your hands when I need it at the most.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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November 2018

Chairman : Nazmi Mat Nawi, PhD
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Cabbage is one of the most popular vegetables grown in Malaysia. However, pesticides have been widely used to control the disease-born pests on the vegetable, which can lead to residue presence in agricultural products. Most of the existing technologies for detecting pesticide residues requires a destructive method for sample preparations, which is time-consuming, costly and complex process. Thus, this study investigated the potential application of non-destructive spectroscopic method by using visible shortwave near infrared (Vis-SWNIR) spectrometer to detect the presence of pesticide residues on cabbage samples. A total of 105 organic cabbages was used in the experiment. There were two types of pesticides used for this research namely deltamethrin and malathion. The samples were divided into seven batches, where the first three batches were treated with deltamethrin pesticide at different concentrations namely low (0.08% v/v), medium (0.11% v/v) and high (0.14% v/v). Another three batches were treated with malathion pesticide at different concentrations namely low (0.18% v/v), medium (0.25% v/v) and high (0.32% v/v). The seventh batch was not treated with any pesticide and used as a control sample for the experiment. The maximum residue limit (MRL) (mg/kg) obtained from gas chromatography (GC) analysis showed that the cabbage sprayed with a high concentration of pesticide gave high MRL value, whereas the low concentration of pesticide sprayed gave the low value of MRL. Based on the t-test, it shows the MRL data for both pesticides are significantly different ($p<0.05$). The spectral data focused on wavelength between 650 and 700 nm; and 740 and 940 nm to observe the trend of the spectral curve. The spectral curve of cabbage with pesticide and without pesticide shows there is a difference between the sample with and without pesticide residue. The spectral data were correlated with MRL data. The coefficient of determination (R^2) for MRL and spectral value were obtained using partial least square (PLS) analysis. The R^2 value for the calibration model of deltamethrin and malathion was 0.982 and 0.987 respectively and the R^2 value

for the prediction model was 0.948 and 0.989 respectively. ANN classifier was done using spectral data of deltamethrin and malathion pesticide with classification accuracies 66.70% and 73.30% respectively. The overall accuracy of data was increased to 93.33% and 100.00% for deltamethrin and malathion pesticide, respectively by applying the PCA. These results indicated that the portable spectroscopy was able to differentiate between cabbage samples with and without pesticide residues at different levels of pesticide concentration non-destructively. In comparison, the other method such as gas chromatography, high-performance liquid chromatography and biosensor was destructive, time consuming and high cost. In conclusion, the Vis-SWNIR spectrometer has the potential to be used for the detection of pesticide residues on cabbage samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENILAIAN SPEKTROMETER MUDAH ALIH UNTUK PENGESANAN RESIDU RACUN PEROSAK PADA KOBIS

Oleh

CHE DINI MARYANI BINTI CHE MOHAMMAD ISHKANDAR EL RAHIMIN

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Kobis merupakan salah satu daripada sayuran yang paling popular di Malaysia. Walau bagaimanapun, beberapa racun perosak telah digunakan secara meluas bagi mengawal perosak pada sayuran yang boleh membawa kepada residu racun perosak dalam produk pertanian. Kebanyakan teknologi yang sedia ada untuk mengesan residu racun perosak memerlukan kaedah pemusnahan untuk persiapan sampel yang mengambil masa, mahal dan rumit. Oleh itu, kajian ini menyiasat kemungkinan penggunaan kaedah tidak memusnahkan dengan menggunakan gelombang pendek gelombang dekat spektrometer inframerah (Vis-SWNIR) untuk mengesan kehadiran residu racun perosak pada sampel kobis. Sejumlah 105 kobis organik telah digunakan dalam eksperimen. Terdapat dua jenis racun perosak yang digunakan untuk kajian ini iaitu deltamethrin dan malathion. Sampel dibahagikan kepada tujuh kumpulan, di mana tiga kumpulan pertama telah dirawat dengan racun deltamethrin pada kepekatan yang berbeza iaitu rendah (0.08% v/v), sederhana (0.11% v/v) dan tinggi (0.14% v/v). Tiga kumpulan lagi telah dirawat dengan racun perosak malathion pada kepekatan yang berbeza menggunakan kepekatan rendah (0.18% v/v), sederhana (0.25% v/v) dan tinggi (0.32% v/v). Kumpulan ketujuh tidak dirawat dengan mana-mana racun perosak dan digunakan sebagai sampel kawalan untuk eksperimen. Keputusan maksimum residu racun perosak (MRL) (mg/kg) yang diperolehi daripada analisis gas kromatografi (GC) menunjukkan kepekatan racun perosak yang tinggi yang disembur pada kobis memberikan nilai MRL yang tinggi, namun kepekatan rendah racun serangga yang disembur memberikan nilai MRL yang rendah. Berdasarkan ujian t menunjukkan perbezaan data MRL untuk kedua-dua racun perosak adalah ketara ($p<0.05$). Data spektrum fokus pada panjang gelombang antara 650 dan 700 nm; dan 740 dan 940 nm bagi memerhatikan aliran lengkung spectrum. Garis lengkung bagi spektrum kobis dengan residu racun perosak dan tanpa residu racun perosak menunjukkan terdapat perbezaan antara sampel. Data spektrum dikaitkan dengan data MRL. Pekali penentuan (R^2) untuk MRL dan nilai spektrum diperolehi menggunakan analisis

sebahagian kecil (PLS). Nilai R^2 untuk model penentukuran deltamethrin dan malathion masing-masing adalah 0.982 dan 0.987 dan nilai R^2 untuk ramalan adalah 0.948 dan 0.989. Pengelas ANN memperolehi ketepatan tinggi untuk racun deltametrin dan malathion yang masing-masing 66.7% dan 73.3%. Ketepatan keseluruhannya telah meningkat kepada 93.3% dan 100% untuk racun deltamethrin dan malathion dengan menggunakan PCA. Keputusan ini menunjukkan bahawa spektroskopi mudah alih mampu membezakan sampel kobis dengan dan tanpa residu pestisid pada tahap kepekatan racun perosak yang berlainan tanpa memusnahkan. Dalam perbandingan, kaedah lain seperti gas komatografi, kromatografi cecair prestasi tinggi (HPLC), dan biopenderia ialah memusnahkan, memakan masa dan kos tinggi. Sebagai kesimpulan, spektrometer Vis-SWNIR mempunyai potensi untuk digunakan bagi mengesan residu racun perosak pada sampel kobis.

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I certify that a Thesis Examination Committee has met on 22 November 2018 to conduct the final examination of Che Dini Maryani binti Che Mohammad Ishkandar El-Rahimin on her thesis entitled "Evaluation of a Portable Spectrometer for Detection of Selected Pesticide Residues on Cabbages" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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I hereby confirm that:

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LIST OF ABBREVIATIONS

ANN	Artificial neural network
CV	Cross-validation
ECD	Electron capture detector
FEP	Ethylene propylene
FPD	Flame photometric detector
GC	Gas chromatography
GC-ECD	Gas chromatography with electron capture detector
GC-FPD	Gas chromatography with flame photometric detector
HPLC	High-performance liquid chromatography
k	Kappa statistic
LOD	Limit of detection
LVs	Latent variable
MAE	Mean absolute error
MRL	Maximum residue limit (mg/kg)
MSC	Multiplicative scatter correction
Mt	Mega tonne
mt	Metric tonne
NIRS	Near-infrared spectroscopy
OA	Overall accuracy
OC	Organochlorine
OP	Organophosphate
PCA	Principal component analysis
PCs	Principal component
PHI	Pre-harvest interval (day)
PLS	Partial least square
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
R^2	Coefficient of determination
RMSE	Root mean square error
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
SD	Standard deviation
SG	Savitzky-Golay
SNV	Standard normal variate
SP	Synthetic pyrethroid
Vis-SWNIR	Visible shortwave near infrared

CHAPTER 1

INTRODUCTION

1.1 General overview

Cabbage (*Brassicae oleracea var. capitata*) is a widely cultivated vegetable because of its nutritional and economic values. The world's biggest cabbage producers in 2016 were China, India, and Russia with the production around 33.8 Mt, 8.7 Mt, and 3.6 Mt, respectively (FAOSTAT, 2017). In 2016, cabbage production in Malaysia was recorded at 101,258 mt with the production value of about RM 180 million (DOA, 2016).

There are many pest attacks on cabbage such as head caterpillar (*Crocidolomia binotalis*), webworm (*Hellula undalis*), and diamondback moth (*Plutella xylostella*) which can affect the quality of the crop (Alan and Renwick, 2002). Typically, farmers used pesticides to manage the agricultural pests (Bommarco et al., 2011). However, it was reported that some farmers may overused and applied pesticides rampantly as to satisfy the high demand for vegetables (Jipanin et al., 2001). As a result, several serious problems have been reported including food poisoning, contaminated market produce, and environmental pollution.

In order to reduce health problems caused by pesticide residues in vegetables, the pesticide concentration on vegetables should not exceed the maximum residue limit (MRL) as recommended by the World Health Organisation (WHO) (Chen et al., 2012). There are several methods that have been used for pesticide residue detection. The pesticide residues were usually measured in a laboratory using expensive technologies such as gas chromatography with tandem mass spectrometry (GC-MS) (Osman et al., 2010; Páleníková et al., 2015), liquid chromatography with tandem mass spectrometry (LC-MS) (Kaczyński and Łozowicka, 2015), high performance liquid chromatography (HPLC), capillary electrophoresis (Juan-García et al., 2005), biosensor, and immunoassay (Park et al., 2004). According to Van Hoof et al. (2004), the multi-residue method was also used to detect the different analytes below or above the MRL. Unfortunately, these techniques involve more than a single stage.

In recent years, several studies have reported that the spectroscopic method was able to provide a rapid and non-destructive measurement of product quality (Temma et al., 2002; Montes et al., 2006). It is also one of the most promising non-destructive techniques used to detect pesticide residue (Jamshidi et al., 2016). The spectroscopic method requires little or no sample preparation (Peng

et al., 2012). This technique was non-contaminant and has low operating cost and fast response time compared with the conventional techniques (Montes et al., 2006). Thus, a non-destructive measurement using spectroscopic method is potentially could be used for rapid screening of pesticide presence on cabbage samples.

1.2 Problem statement

Previous studies have reported that pests and diseases were a major problem in the production of vegetables in Malaysia (Barrow et al., 2009). Farmers are highly dependent on pesticides to overcome the problem (Jipanin et al., 2001). Various types of pesticides were widely used in cabbage cultivation to kill the pests. The chemical pesticides are widely used because they are relatively cheaper than biopesticide.

Agricultural products that contain pesticide residues are toxic and lead to health risk when freshly consumed (Zawiyah et al., 2007; Dinham, 2003). Other than that, the high pesticide residue levels in cabbages may cause Malaysia to be banned from exporting their vegetable to the neighbouring countries (Grzywacz et al., 2010). Maximum residue limit (MRL) is a value used as a reference for all the countries due to its importance in international trade (FAO, 2018). MRL is the parameter to indicate the vegetables are pesticide-free and safe for consumption (Szabo et al., 2010).

Good Agricultural Practice (GAP) is a practice based on principal and standard practice for agriculture farm management whether for crop, livestock or fishery. DOA was an authority that monitoring pesticide application for GAP. The pesticide application on the crop using registered pesticide, proposed mixture rete, frequency and spray technique was required by GAP. Theoretically, if pesticide applied according the standard practice so that the pesticide residue will not exceed the MRL.

Research by Karadzic (2017) on detection of deltamethrin pesticide in cabbages obtained 0.01 to 0.20 mg/kg of MRL value which is high concentration of pesticide contain in samples. Other than that, research by Zaidon et al. (2016) reported MRL for cabbage sample was 0.00022 mg/kg and may cause health problems if it was consume in high quantity in the long terms. Research by Farina et al. (2017) for pesticide residue detection on cabbage, lettuce and mustard found that percentage of sample exceeded MRL value were 5.7%, 7.7% and 6% respectively. Besides that, research by Li et al. (2014) for the detection of acephate pesticide on cucumber, kidney bean and green pepper obtained 0.008 to 0.17 mg/kg, 0.009 to 0.013 mg/kg and 0.009 mg/kg, respectively.

The pesticide residue on cabbage was detected exceed MRL as reported by Jipanin et al. (2001). The amount of sample detected above MRL value was increased in Sabah year 1997 to 2000. As the result, the 15 vegetables including cabbage was banned to enter Brunei. Dou et al. (2015) reported the Chinese vegetable was banned in 1982 as China has exceeded the MRL. The Chinese government was set the MRL standard for pesticide in agriculture production in China. Klingbeil (2018) reported the vegetable from Lebanon contained high level of pesticide residues. The importation of vegetable from that country was banned by United Arab Emirates.

Research by Munawar and Hameed (2013) found that different types of vegetables and pesticides have different absorption rate, which is detected by the chromatographic technique. Thus, the fast detection of the different concentration of pesticide on the sample was important as it can determine the concentration of pesticide in real time. Furthermore, it is important to have a device to classify the cabbage with or without pesticide residue to ensure customer receive good quality of vegetable.

Quality sorting is considered as an important step before the cabbages are packed. Current measurement methods used to detect the presence of pesticide in agricultural product involve complex sample treatment. They usually have several processes such as crushing the vegetables, chemical adding to the sample for extracting aliquot, and analysing the aliquot through laboratory testing (Arduini et al, 2006; Li et al., 2007). These processes are destructive and time-consuming. The destructive method cause losses to the grower if the sample did not contain pesticide residue. In addition, the sample also needs to be transported back to a laboratory for future analysis which causes losses in cost and time (Ngan et al., 2013).

Spectroscopic methods have been widely studied for pesticide residue detection and determining harmful residues in foods, fruits, and vegetables. However, to date, there is still no research conducted for pesticide residue detection on cabbage using the spectroscopic method. Thus, in this research, the spectroscopic method was proposed for pesticide residue detection in cabbage as to overcome the limitation of previous methods.

1.3 Research objectives

The main objective of this research is to investigate the potential of using a portable visible shortwave near infrared (Vis-SWNIR) spectrometer for pesticide residues detection on cabbages.

The specific objectives of this research are as follow:

1. To differentiate the spectral curve between cabbages without pesticide and cabbages sprayed with pesticides at different concentration levels.

2. To determine the correlation between spectral data and maximum residue limit value of the sample using partial least square (PLS) regression.

3. To classify spectral data based on different maximum residue limit (MRL) using the artificial neural network (ANN).

1.4 Scope and limitation of the study

The study focused on the pesticide residues detection on cabbages. The cabbage variety is Tropicana (hybrid cabbage). This study also focused on the potential application of visible shortwave near infrared (Vis-SWNIR) spectrometer using Ocean Optics HR4000CG-UV-NIR for pesticide detection based on surface scanning from the top portion of cabbage. Thus, the study of spectral collected is only done for the top surface of cabbage.

1.5 Thesis layout

The thesis consists of five chapters and each chapter is divided into several sub-sections. Chapter one gives information about the overview of the research, statement of problem, objectives and the scope of the study. Chapter two is the literature review. A review on cabbage, introduction on pesticide residue and technologies for pesticide residue detection was carried out. Chapter three focused on the methodology used in this research. This chapter also gives insight on the procedure used in the common method for pesticide residue analysis. The type of data analysis was documented in this chapter. Chapter four presents the findings of the research. Spectral difference between pesticide concentration and pesticide classification using ANN were apprised in this chapter. Lastly, the conclusions and recommendations are presented in chapter five.

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LIST OF PUBLICATIONS

Conference

Ishkandar, C. D. M, Nawi, M. N., Janius, R., Mazlan, N., and Radzi, C. A. M. (2017). Detection of pesticide presence on round cabbages using visible shortwave near-infrared spectroscopy, Paper presented at International Conference on Big Data Applications in Agriculture (ICBAA 2017), Universiti Putra Malaysia, 5 - 6 December 2017.

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