DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN SEVERAL LOCAL VEGETABLES USING SOLID PHASE MICRO-EXTRACTION COUPLED WITH GAS CHROMATOGRAPHY

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By

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Thesis submitted in fulfillment of the requirements for the degree of Master of Science

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Specially dedicated to:

My dear husband (Mr. Abdul Rani Abu Bakar),

My dear parents (Mr. Sapahin & Mrs. Rohana),

My dear childrens (Zamir, Hakimi, Dania, Baby),

My dear brothers & sisters

&

All my friends

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LIST OF ABBREVIATIONS

AAC	Acidified activated carbon
ACF	Activated carbon fiber
AD	Amperometric detection
ASE	Accelerated solvent extraction
AChE	Acetylcholinesterase enzyme
CAC	Codex alimentarius commission
C18	Octadecyl bonded silica
CAD	Collision activated dissociation
CZE	Capillary zone electrophoresis
CW	Carbowax
CC	Circulation cooling
CE	Capillary electrophoresis
CAR	Carboxen
d	Dispersive
DAD	Photodiode array detection
DSC18	Polymerically bonded octadecyl
DVB	Divinylbenzene
DI	Direct immersion
ECD	Electron capture detector
EI	Electron impact
FID	Flame ionization detector
FPD	Flame photometric detector
GAP	Good agricultural practice

GPC	Gel permeation chromatography
GCB	Graphitized carbon black
GC	Gas chromatography
HP	Hewlett Packard
HPLC	High performance liquid chromatography
HS	Headspace
H_2	Hydrogen
id	Internal diameter
LOD	Limit of detection
LOQ	Limit of quantitation
LLE	Liquid- liquid extraction
LPME	Liquid phase micro extraction
LC	Liquid chromatography
LLME	Liquid- liquid micro extraction
$MgSO_4$	Magnesium sulphate anhydrous
MPa	Megapascal
Mol	Molarity
MRL	Maximum residue limit
MSPD	Matrix solid phase dispersion
MSD	Mass spectrometry detector
Min	Minute
MS	Mass spectrometry
MAE	Microwave assisted extraction
MPS	Multipurpose sampler
MIP	Molecularly imprinted polymer

NH_2	Aminopropyl
NaCl	Sodium chloride
NPD	Nitrogen phosphorus detector
n.d	Not detected
OC	Organochlorine
OP	Organophosphorus
OPPs	Organophosphorus pesticides
OPP	Organophosphorus pesticide
psi	Pound per square inch
pH	Negative log of the hydrogen ion in aqueous solution
PDMS	Polydimethylsiloxane
PA	Polyacrylate
PSA	Primary secondary amine
PV	Pressure vapour
PEG	Polyethylene glycol
rpm	Rotation per minute
RSD	Relative standard deviation
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
SBSE	Stir bar sorptive extraction
SAX	Anion exchange sorbent
SP	Synthetic pyrethroid
SIM	Selective ion monitoring
SD	Standard deviation
UV	Ultra violet

LIST OF SYMBOLS

C_O	Initial concentration of analyte in sample
°C	Degree celcius
µg	Microgram
g	Gram
kg	Kilogram
K_{fs}	Partition coefficient for analyte between coating and sample matrix
K_{ow}	Octanol water patition coefficient
µL	Microliter
L	Liter
mL	Milliliter
mg	Milligram
µm	Micrometer
m/z	Mass ratio
m	Meter
n	Number of data
nL	Nanoliter
%	Percentage
r^2	Coefficient of determination
V_f	Volume of coating
V_s	Volume of sample
v	Volume
w	Weight

PENENTUAN RESIDU PESTISID JENIS ORGANOFOSFORUS DALAM BEBERAPA SAYUR-SAYURAN TEMPATAN MENGGUNAKAN PENGEKSTRAKAN MIKRO FASA PEPEJAL DIGANDINGKAN DENGAN KROMATOGRAFI GAS

ABSTRAK

Kaedah analisis yang mudah dan tepat iaitu pengekstrakan mikro fasa pepejal (SPME) bagi menentukan 11 residu pestisid jenis organofosforus (etoprofos, sulfotep, diazinon, tolklofos-metil, fenitrotion, klorpirifos, isofenfos, metidation, etion, triazofos, leptofos) dalam tiga jenis sayur – sayuran tempatan (kobis, kailan, sawi) telah dibangunkan menggunakan alat kromatografi gas dengan pengesan fotometri nyala (GC-FPD). Parameter penting yang mempengaruhi kecekapan pengekstrakan (jenis gentian, kaedah pengekstrakan, masa pengekstrakan, penambahan garam, masa dan suhu penyaherapan) telah dikaji secara sistematik. Perbandingan empat jenis gentian yang biasa digunakan secara komersial iaitu 50/30 μm divinilbenzena / Carboxen / polidimetilsiloksana (DVB / CAR / PDMS), 65 μm polydimetilsiloksana / divinilbenzena (PDMS / DVB), 100 μm polidimetilsiloksana (PDMS) dan 85 μm poliakrilat (PA) telah dilakukan. Gentian jenis PA menunjukkan prestasi yang terbaik dan telah digunakan untuk mendapatkan keadaan yang optimum dan digunakan untuk kaedah tentusah. Keadaan optimum pengekstrakan mikro fasa pepejal ialah: masa pengekstrakan, 30 minit pada suhu bilik; kelajuan pengacauan, 1275 rpm; kandungan garam, 10% NaCl; masa dan suhu nyaherapan, 11 minit pada 260°C; dan tanpa sebarang pelarasan pH untuk ekstrak sampel. Kaedah ini telah ditentusahkan dalam julat linear diantara 1-100 μg L^{-1}. Ujian kebolehulangan adalah memuaskan, dalam julat 2.44% hingga 17.9% untuk semua

jenis organofosforus yang digunakan. Had pengesanan dan had kuantitasi adalah di antara 0.01 μg L^{-1} hingga 0.14 μg L^{-1} dan 0.03 μg L^{-1} hingga 0.42 μg L^{-1}. Kaedah ini telah diaplikasi ke atas 22 sayur-sayuran tempatan daripada tiga jenis iaitu kobis, kalian dan sawi. Racun perosak organofosforus yang paling banyak dikesan di dalam sampel yang diuji adalah klorpirifos (0.22-1.68 μg kg^{-1}). Walaubagaimanapun, nilai kepekatan yang diperolehi adalah lebih rendah daripada had maksimum residu pestisid (MRLs) yang dibenarkan di dalam Akta Makanan dan Peraturan Makanan 1985 di Malaysia.

DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN SEVERAL LOCAL VEGETABLES USING SOLID PHASE MICRO-EXTRACTION COUPLED WITH GAS CHROMATOGRAPHY

ABSTRACT

A simple analytical method based on solid-phase microextraction (SPME) followed by gas chromatography-flame photometric detection (GC-FPD) for the simultaneous determination of eleven organophosphorus pesticide residues (ethoprophos, sulphotep, diazinon, tolclofos-methyl, fenitrothion, chlorpyrifos, isofenphos, methidathion, ethion, triazophos, leptophos) in three types vegetables samples (cabbage, kale and mustard) was developed. Important parameters that influence the extraction efficiency (i.e., fibre type, extraction modes, extraction time, salt addition, desorption time and temperature) were systematically investigated. Four types of commercially available fibres namely 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 100 μm polydimethylsiloxane (PDMS), and 85 μm polyacrylate (PA) were evaluated. PA fibre exhibited the best performance and was used for the rest of the studies. The optimized extraction conditions were: extraction time, 30 min at room temperature; stirring speed, 1275 rpm; salt content, 10% NaCl; desorption time and temperature, 11 min at 260 °C; and no pH adjustment of the sample extract. The method was validated over the range 0.1–100 μg L^{-1}. Repeatabilities were satisfactory, ranging between 2.44–17.9% for all analytes. The limits of detection and quantitation ranged from 0.01–0.14 and 0.03–0.42 μg L^{-1}, respectively. The method was applied to 22 locally produced vegetables (cabbage, kale and mustard). Chlorpyrifos (0.22–1.68 μg kg^{-1}) was the

most detected pesticide in the tested samples. However, the obtained values are lower than the maximum residue limits (MRLs) as stipulated in the Food Act & Regulations of Malaysia.

CHAPTER ONE

INTRODUCTION

1.1 Pesticides in general.

Pesticide is the most effective and economical material used to kill, prevent, and destroy a broad range of specific pest organisms. It is used for controlling, preventing, destroying, repelling or mitigation any pests. It is used by farmers to control disease and pests from damaging their crops (Fenoll *et al*., 2007). These residues will penetrate inside plant tissues and remain in vegetables and fruits, constituting a possible risk to consumers (Albero *et al*., 2005). Various pesticides are being commercialized in the market to fulfill the need of the agricultural sector. It is made from a mixture of one or more biologically active substances in different compositions. They are classified based on the pest they control, chemical structure and their mode of action. The list of common group of pesticides according to the pests they control can be classified as mention in Table 1.1.

Table 1.1: The most common group of pesticides and pest organisms they control

No	Pesticide group	Pest organisms
1.	Algicide	Algae
2.	Avicide	Birds
3.	Fungicide	Fungi
4.	Insecticides	Insects, ticks and mites
5.	Herbicide	Weeds

Insecticides can be classified into groups consisting of inorganic and organic compounds. Inorganic insecticide compounds are derived from naturally occurring elements and do not contain carbon. They are non-volatile chemicals which are soluble in water. Most of these are known to be persistent and are toxic if they contain arsenic, cyanide, mercury and thallium. Boric acid, copper hydroxide and mercuric oxide are listed under this group of insecticides. Another group of insecticides are organic insecticides compounds. They are pesticides which consists of carbon, hydrogen and others elements such as sulfur, phosphorus, nitrogen, chlorine and oxygen. The compounds listed under this group are organochlorine (OC) and organophosphorus (OP) pesticides.

Pesticides are usually used to increase the food quality and agricultural production (Silva and Camões, 2010). However, the misuse in application of pesticides can give bad effect to humans and environmental quality (Hercegová *et al*., 2005). About 90% of human exposure to pesticide residues comes from contamination of foods (El-Saeid and Khan, 2009). The chronic diseases caused by the exposure to pesticides are cancer, neurological disorder, reproductive and endocrine disruption (Wang *et al*., 2013a; Sugeng *et al*., 2013).

1.1.1 Organophosphorus pesticides (OPPs)

One of the most well-known and widely employed insecticides is organophosphorus pesticides (OPPs). They are widely used due to their favourable characteristics such as biodegradable and the short persistence in the environment (Chen *et al*., 2010). OPPs protect crops by inhibiting acetilcholinesterase enzyme

(AchE) (Juhler, 1997). This pesticide group will bind to the enzyme, disrupting the nervous system and consequently resulting in paralysis and death (Bai *et al*., 2006). They are sprayed over the crops or soils and the residues can be found in a wide range of surfaces and ground waters, drinking waters, fruits, vegetables and foodstuff (Yao *et al*., 2001). In Malaysia, it is reported that the Malaysian population takes about 15% of vegetables as their daily intake food (Ding *et al*., 1981).

The wide use of OPPs can readily be seen from the data compilation of pesticides residue collected from 2004 - 2010 by the Pesticide Analytical Centre, Department of Chemistry Malaysia. The most detected OPPs in vegetables for nine (9) districts area in Perak and Cameron Highlands are chlorpyrifos, profenofos, methamidophos, dimethoate, diazinon, methidathion, phenthoate, fenthion, acephate, triazophos and malathion as shown in Fig. 1.1.

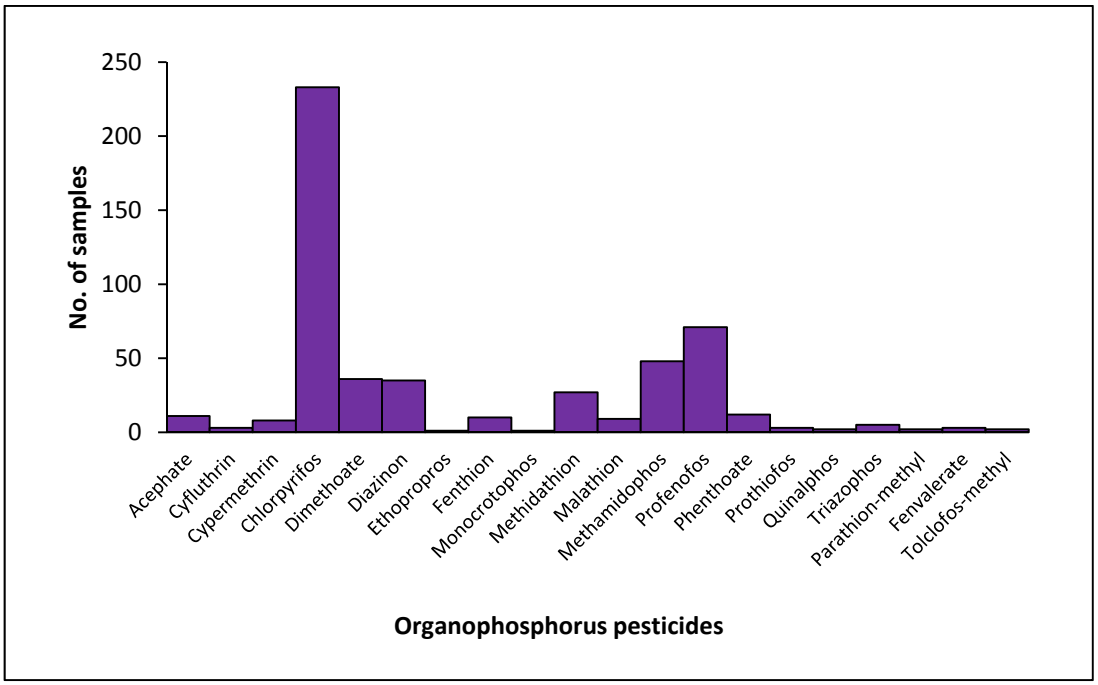
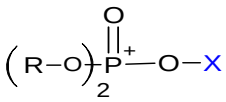
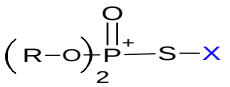
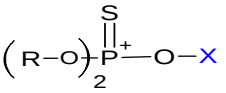
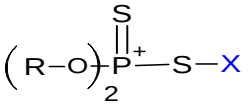
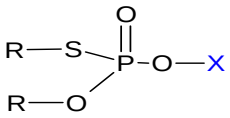
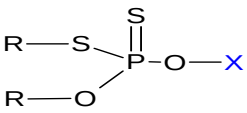
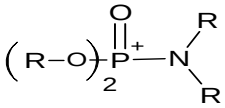
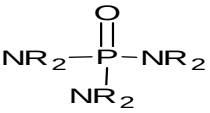
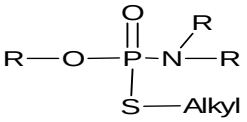
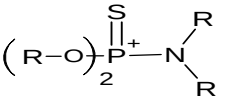
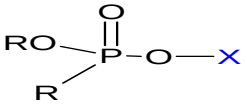
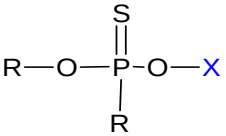


Fig. 1.1 Number of OPPs detected in samples from 2004 – 2010 analyzed by the Pesticide Analytical Centre (*Department of Chemistry Perak Branch, 2010)*

In addition, a survey on pesticides residue carried out by the Department of Agriculture Sarawak reported that 95% of the total residue violation is caused by OPPs (Kuet and Seng, 2003).

All pesticides under this group contain phosphorus and are among the chemically unstable. They are derived from phosphoric acid and are known to be among the most toxic pesticides to animals. There are three types of OPPs which are differentiated by the chemical structure such as aliphatic derivatives with a carbon chain structure, phenyl derivatives with a benzene ring attached to phosphorus moiety while heterocyclic derivatives are built by a ring structure with one or more carbon atom replaced by oxygen, nitrogen or sulfur. Table 1.2 shows the variations in the chemical structure of OPPs.

Table 1.2: The variations in the chemical structure of OPPs (Rezg *et al*., 2010)

Type of phosphorus group	Outline of structure	Common or other name
Phosphate		Triazophos
		Vamidothion
O-alkyl phosphorothioate		Chlorpyrifos, diazinon, fenitrothion, sulphotep
Phosphorodithioate		Ethion, methidathion
S-alkyl phosphorothioate		Profenofos, trifenofos
S-alkyl phosphorodithioate		Prothiofos
Phosphoramidate		Fenamiphos
Phosphorotriamidate		Triamiphos
		Methamidophos
Phosphorothioamidate		Isofenphos
Phosphonate		Trichlorfon
Phosphonothioate		Leptophos

In this study, eleven (11) OPPs were studied and a few of them are among the listed pesticides detected in vegetables samples collected by the Pesticides Analytical Centre, Department of Chemistry Malaysia. The chemical structures of the 11 OPPs are shown in Fig. 1.2.

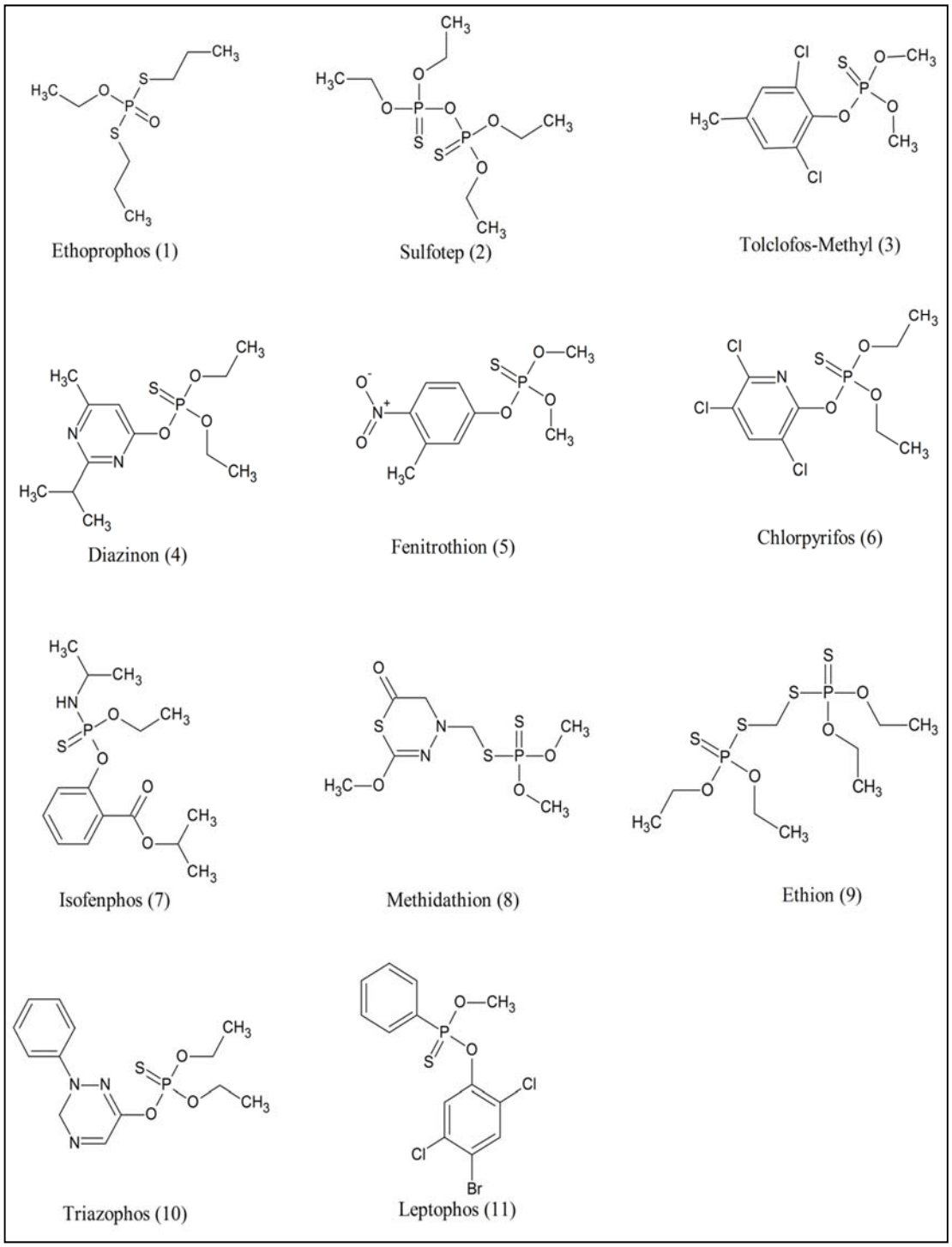


Fig. 1.2 Chemical structures of the studied OPPs

Among the eleven OPPs compounds, three compounds (ethoprophos, sulphotep and ethion) are aliphatic derivatives. They have a simple carbon chain structure and a wide range of toxicities. Meanwhile four (tolclofos-methyl, fenitrothion, isofenphos and leptophos) are the phenyl OPPs with a benzene ring structure. Generally, OPPs with a ring structure are more stable than the aliphatic structure and subsequently their residues are long lasting in the samples. Furthermore, there are four heroterocyclic derivatives OPPs (diazinon, chlorpyrifos, methidathion and trizophos). This OPPs group are complex molecules and known to be long lasting compare to aliphatic and phenyl OPPs.

1.1.2 Regulations on pesticides residues in food.

Pesticides residues, by definition are any specific substances in food, agricultural commodities or animal feed which is obtained from the use of pesticides The term includes any derivatives of a pesticide such as the metabolites, reaction products, conversion products and impurities. These substances are known to be harmful to human and environment. Therefore, a strict regulation have been established by The European Commission to protect public health and consumer's interest in this include the setting of maximum residues limits (MRLs) in food commodities.

MRLs is the maximum concentration of pesticide residues (expressed as mg kg^{-1}) legally permitted in or on food commodities and animal feeds and were fixed by The European Commission. It is based on Good Agricultural Practice (GAP) data and recommended by the Codex Alimentarius Commission (CAC). If the MRLs exceed the allowable limit, it means the violations of GAP occur. In Malaysia,

pesticides residues in food are regulated by two regulations, the Food Regulations 1985 and Codex Alimentarius. Codex Alimentarius is the international food standards adopted by the Codex Alimentarius Commission. According to Food Regulations 1985, Section 41(3) no person shall import, prepare for sale or sell any food with the following subregulations:-

(a) Containing pesticide residues in a proportion greater than the proportion specified for that food in relation to that pesticide residue as set out in the sixteenth schedule.

(b) Containing pesticide residue in a proportion greater than the proportion specified for that food in relation to that pesticide residue as recommended in the Codex Alimentarius, where the pesticide is not specified in the sixteenth schedule; or

(c) Containing more than 0.01 miligram per kilogram (mg kg^{-1}) of any pesticide residue, where the pesticide is not specified for that food in the sixteenth schedule or Codex Alimentarius.

Furthermore, both regulations list MRLs values for respective pesticide residues according to food commodities. Hence, each type of pesticides in different food commodities has their own MRLs value. As an example the MRLs values for chlorpyrifos in several commodities is shown in Table 1.3. Chlorpyrifos is the most widely used pesticide detected in various vegetables samples from 2004 - 2010 based on data collected from Pesticide Analytical Center, Department of Chemistry.

Table 1.3: MRLs values of chlorpyrifos in several types of food commodities

Pesticides	**Commodity**	**MRL (mg kg^{-1})**
Chlorpyrifos	Cabbage	0.05
	Mustards	1
	Tomato	0.5
	Chilli	0.5
	Citrus fruits	1

If MRL has not been specifically set for a commodity and not listed in both regulations, a general MRL level of 0.01 mg kg^{-1} is applicable for all cases.

1.2 Problem statement

The presence of pesticides residues (i.e., OPPs) at trace amounts in various types of samples are difficult to be detected due to sample interferences such as sugar, plant pigments and fats in vegetables samples. An evaporation step (part of clean-up) is applied after the sample preparation to pre-concentrate the OPPs. However during evaporation, degradation of pesticides can occur and this results in lower recoveries. DI-SPME at ambient temperature has manage to overcome the problem of pre-concentration where the pesticides are extracted using a small volume of solvent and mass of sample. Although, the lowest amount of OPPs (~5 µg L^{-1}) in spiked vegetables samples was used, this technique can detect a high area of analyte peak counts. Solid phase micro-extraction (SPME) is an alternative method that does not require any evaporation step during the pre-concentration process and thus will enhance the sensitivity of the method.

1.3 Research Objectives

The Chemistry Department and the Ministry of Health are the main regulatory agencies entrusted with the monitoring of food items in Malaysia. To meet the challenge of analysing large number of samples and complex matrices that have not been previously reported using SPME (i.e., mustard and kale), it is important that new approaches in the determination be introduced. Thus, the main purpose of the present studies is to evaluate the viability of the SPME technique for the selective extraction of the 11 common OPPs in Malaysia namely ethoprophos, sulphotep, diazinon, tolclofos-methyl, fenitrothion, chlorpyrifos, isofenphos, methidathion, ethion, triazophos, leptophos in three types of local vegetables samples. Hence, the main objectives of this study are:

(i) To develop a new method for simultaneous separation of 11 OPPs using gas chromatography coupled with flame photometric detector (GC-FPD).

(ii) To develop a new SPME method for the extraction of the 11 OPPs.

(iii) To apply the proposed SPME method to real vegetables samples (i.e., cabbage, mustard and kale) collected from different night markets and hypermarkets in Ipoh, Perak.

1.4 Scope of research

The determination of pesticide residues using SPME will be focused on 11 types of OPPs (ethoprophos, sulphotep, diazinon, tolclofos-methyl, fenitrothion, chlorpyrifos, isofenphos, methidathion, ethion, triazophos, leptophos) in three local vegetables samples namely cabbage, mustard and kale. These samples will be collected in several locations such as Mydin, Tesco, The Store, AEON, Giant, Econsave, Ipoh Jaya night market and Kea farm Cameron Highlands. Each location will be represented with three or four samples. The application of developed SPME method to real vegetable samples involves 6 month duration. All the samples will be go through sample preparation, extracted and analyzed using GC-FPD. The present of OPPs in sample will be confirmed by GC-MSD. The flowchart of the process is shown in figure 1.3.

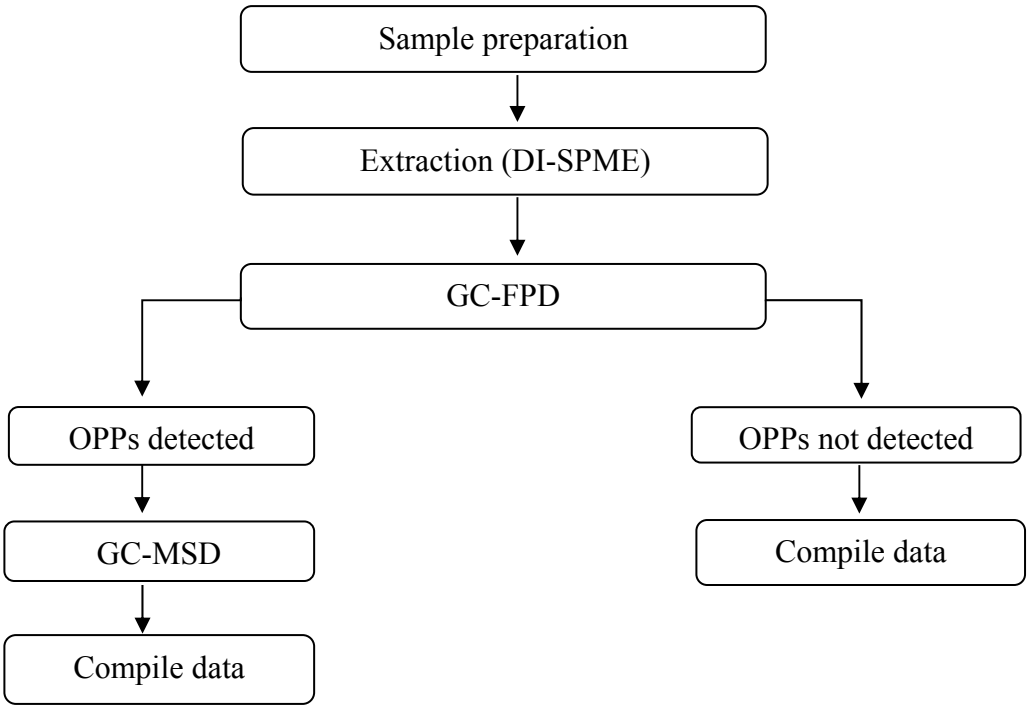


Fig. 1.3 Flowchart of the process to determine 11 OPPs in vegetable samples

CHAPTER TWO

LITERATURE REVIEW ON ANALYSIS OF ORGANOPHOSPHORUS PESTICIDES

2.1 Extraction methods for OPPs

The importance of public health and safety towards pesticide residues in food has encouraged analyst to develop analytical methods for simultaneous determination of multi-pesticide residues in food samples. Today, numerous methods have been reported for the determination of OPPs using traditional and modern extraction techniques such as liquid-liquid extraction (LLE), solid phase extraction (SPE), accelerated solvent extraction (ASE), matrix solid phase dispersion (MSPD), dispersive solid phase extraction (d-SPE), solid phase micro-extraction (SPME), stir bar sorptive extraction (SBSE), liquid phase micro-extraction (LPME) and biosensor. Analysis of OPPs usually involves sample preparation followed by instrumental analysis. Separation and quantification of OPPs are normally being carried out by chromatographic techniques either using gas or liquid. Each extraction and separation techniques will be discussed further.

2.1.1 Liquid- liquid extraction (LLE)

LLE involves the extraction and partitioning of an analyte using two immiscible solvents. It is a traditional sample preparation and become obsolete due to high consumption of organic solvents. The widely used solvents for extraction of

OPPs and other pesticide compounds using this technique are acetone, acetonitrile, ethyl acetate and n-hexane.

Mol and co-workers used ethyl acetate to extract six OPPs in vegetables and fruits (Mol *et al*., 2003). Acetone is commonly used to extract OPPs in various types of matrices such as pepper, tomato, honey and soil (Fenoll *et al*., 2007; Wang et al., 2008b; Pirard *et al*., 2007). This solvent has the capability to extract both polar and non-polar OPPs compounds. Despite the above solvents, methanol is also used to extract pesticide compounds in sediment sample using this technique (Hassan *et al*., 2010), while dichloromethane is use in extracting 24 OPPs in vegetables (Salvador *et al*., 2006). After the extraction using single solvent, the organic layer was added with two solvents to partition analytes and increase phase separation. Different ratios of the solvents (4:1, v/v) were used for extracting OPPs and other pesticide compounds (Pinho *et al*., 2010). Satisfactory recoveries were obtained for all pesticide compounds (74% -105%) with relative standard deviations (RSD) lower than 19% (Frenich *et al*., 2004).

Through this technique, further procedure such as clean-up or pre-concentration steps (e.g. vortexing, rotary evaporation, and purification) were applied to increase extraction efficiency. Although LLE gave satisfactory results in extracting OPPs and other pesticide compounds, it consumed large amounts of solvents, involve several steps, time consuming, labour intensive and difficult to be automated.

2.1.2 Solid phase extraction (SPE)

SPE is one of the most common techniques currently available for multiresidue pesticides analysis as an alternative method to overcome limitation observed in LLE. It is usually used for the purification of samples after the LLE steps. Many of the previous methods for pesticide determination in fresh fruit and vegetables use a combination of two or more SPE columns for clean-up. Normally, four steps are involved in this technique: conditioning (solvent is passed through the SPE cartridge), sample loading (sample passed through the cartridge), washing (Interferences selectively removed from the column) and elution (target analytes been removed by solvent) (Fig. 2.1).

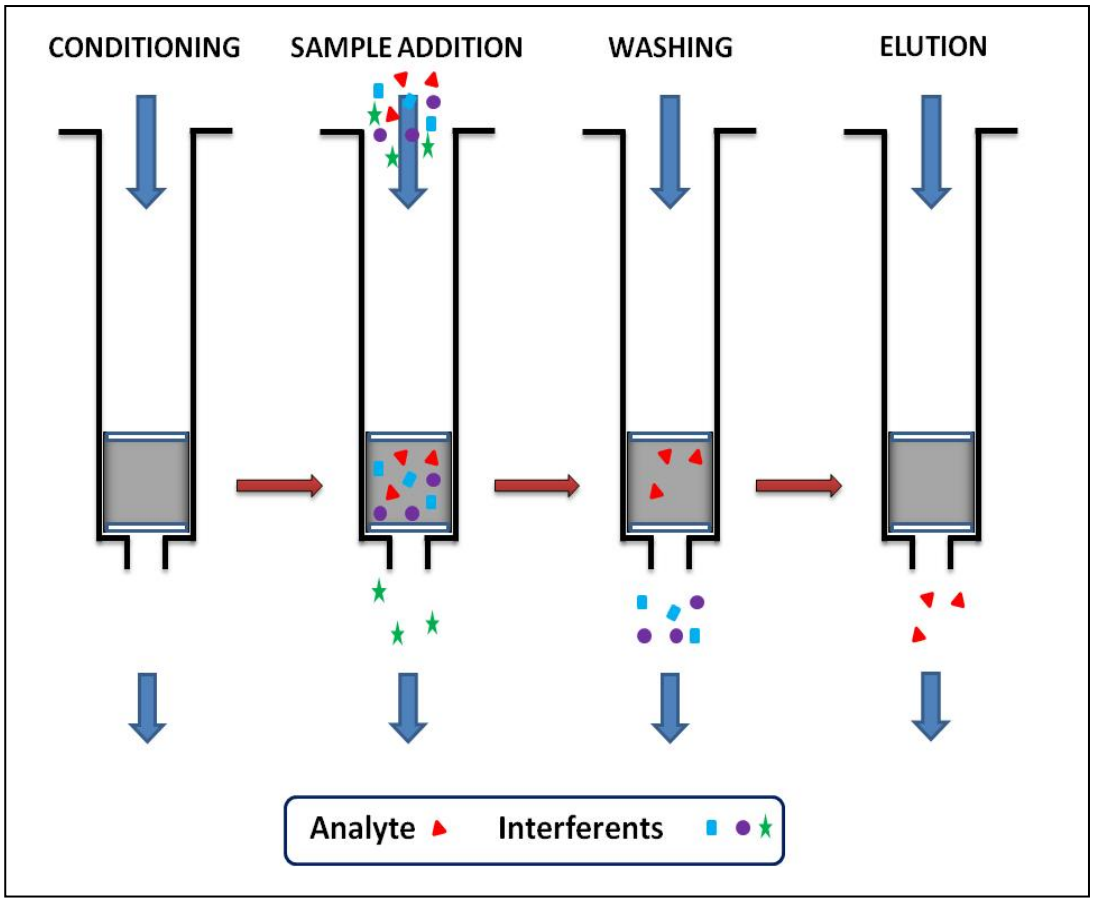


Fig. 2.1 Schematic of steps involved in SPE process (Lucci et al., 2012)

Up to date, various sorbents are available based on their base material and functional groups (e.g. polymeric resin-based sorbents and silica-based sorbents). Different types of sorbents were employed for the analysis of OPPs and others pesticides compounds in various agricultural samples, such as primary secondary amine (PSA), Envi-carb, polypyrolidone-divinylbenzene copolymer (Oasis HLB), activated carbon, anion-exchange sorbent (SAX) (Wang *et al*., 2009; Sharif *et al*., 2006; Yang *et al*., 2011, Xie *et al*., 2011). For determination of pesticides in water and juice, the sorbents used are octadecyl bonded silica (C18) (López-Blanco *et al*., 2006; Albero *et al*., 2005), meanwhile sorbent used in fish and baby food is aminopropyl (Chen *et al*., 2009; Hercegová *et al*., 2005).

Sorbent such as acidified activated carbon (AAC) is used to remove pigments in fresh vegetables (Wang *et al*., 2008a). However, this sorbent can cause absorption of OPPs and might resulted in lower recoveries. After clean-up using sorbent, further evaporation step under nitrogen stream is applied to the samples to pre-concentrate the pesticide analytes. However, lower recoveries for some of the pesticide compounds were found. Through it advantages, SPE grows continuously as a preferable technique to extract pesticides compounds.

2.1.3 Accelerated/assisted solvent extraction

Another sample preparation method that can be used to determine OPPs and other pesticide residues in environmental samples (e.g. sludge, soil and other wastes) is accelerated solvent extraction (ASE). The extraction process in ASE involved the use of organic solvents at high pressures and temperatures to increase solubility,

diffusion rate and mass transfer. ASE involved the use of extractor (e.g. ASE 200-Dionex) commonly equipped with 33-mL extraction cells (Fig. 2.2). The sample will be loaded into the cell together with desiccant (e.g. Extrelut 20) to reduce moisture and increased permeation. Then, the cell will be filled with solvents.

In the work of Wu et al. three solvents were compared for ASE systems such as acetonitrile, hexane-acetone (2:1, v/v) and cyclohexane-ethyl acetate (1:1, v/v) in determining OPPs and other pesticide residue in the foods of animal origin (Wu *et al*., 2011). Different temperature and pressure were used to heat the sample to determine the pesticide residues. In soil, sludge and other wastes the heats used were between 100°C to 140°C and the pressures were in the range of 1450 psi to 2000 psi (Popp *et al*., 1997; Conte *et al*., 1997). After the ASE procedure (30 min), the sample was evaporated to dryness under gentle nitrogen stream and transferred for clean-up. Common clean-up step used with this technique is automated gel permeation chromatography (GPC) (Frenich *et al*., 2006). This clean-up is applied to remove fat and others matrix interferences.

The advantages of ASE method is that it consumed small amounts of solvent and can be automated which is more environmental-friendly. It can be considered as an alternative technique that can replace the traditional methods such as Soxhlet-extraction and LLE. This method required 2 hours for the entire sample preparation and analysis. However, this method is widely used to determine OPPs and other pesticides in environmental samples compared to vegetables samples.

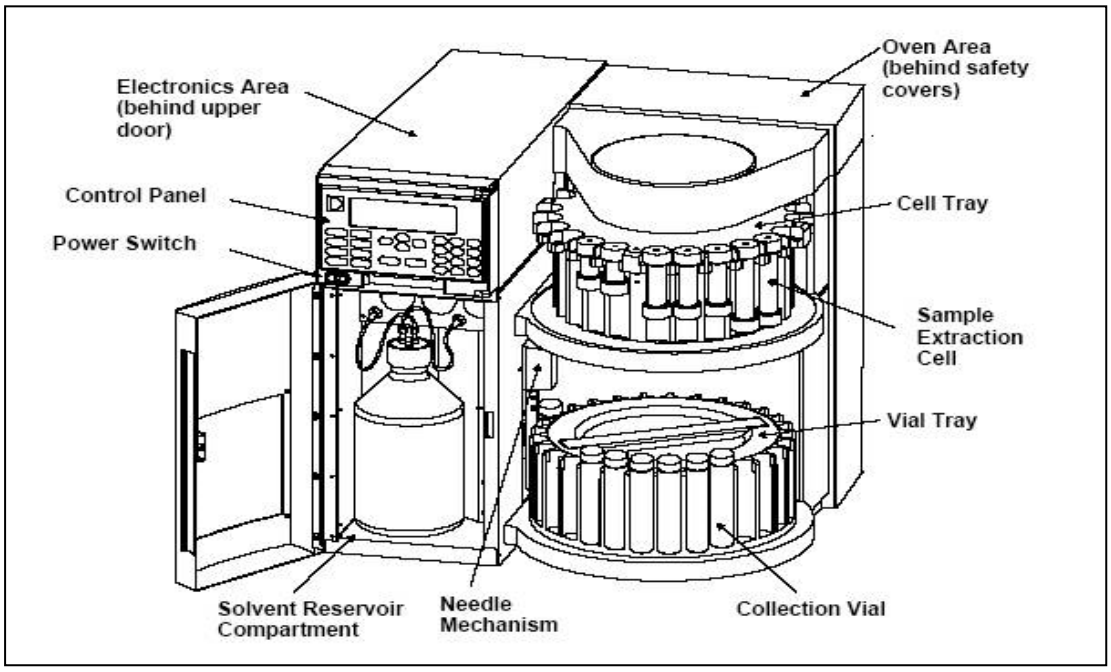


Fig. 2.2 Schematic of accelerated solvent extractor system (ASE) (Thermo Fisher Scientific, 2013)

2.1.4 Matrix solid phase dispersion

Matrix solid phase dispersion (MSPD) is a technique which requires simple devices such as glass mortar, pestle and chromatographic column. This technique involves blending the samples with a bonded solid support material, followed by sample purification or clean-up and elution using small volume of solvent (Fig. 2.3). It has been applied in variety of analysis such as veterinary drugs, herbicides, pesticides and other pollutants in fresh and processed foods (Barker, 2000).

Solid support materials were added into sample to achieve complete disruption and dispersal. For fruits and vegetables samples (orange, apple, tomato, carrot), octadecylsilyl (C_{18}) is used as the dispersant sorbent in determining OPPs

and other pesticide compounds (fungicide) and was blended together with the samples to form a homogenous condition (Torres *et al*., 1996; Navarro *et al*., 2002).

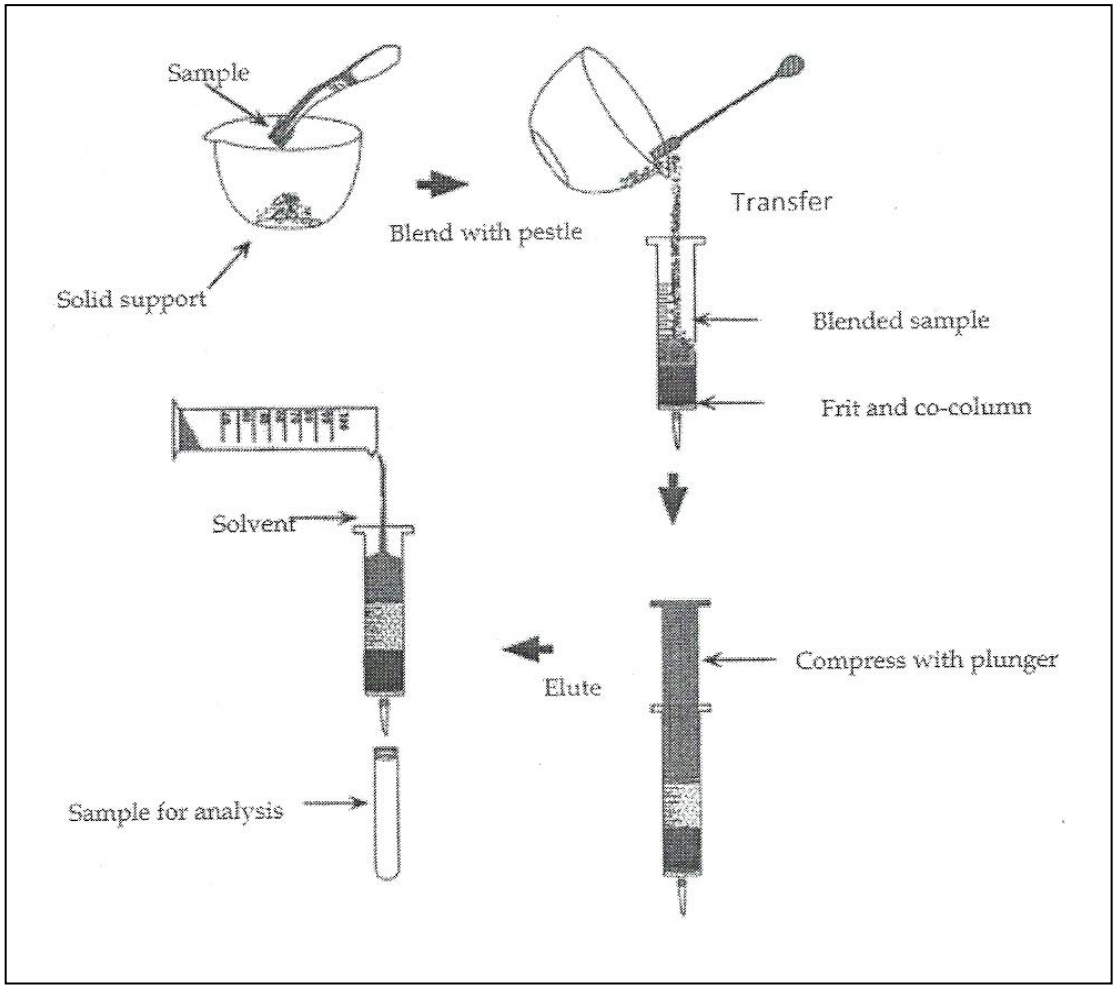


Fig. 2.3 Schematic of MSPD extraction procedure (Barker, 2000)

MSPD technique has also been applied in fruit juices. In contrast to solid and semi-solid samples, fruit juice and milk samples were analysed using this technique to determine pesticide residues and both used diatomaceous earth as dispersant sorbent (Radišić *et al*., 2009; Muccio *et al*., 1997). Milk sample is complex as it contains fatty materials and therefore further clean-up step was performed by using size-exclusion chromatography.

After the blending process completed, the blended samples were transferred to a column and compressed with a plunger. The column contains frits and co-column material such as florisil and silica. The last step in MSPD is elution using appropriate solvents. Different types of solvent used in this technique to extract OPPs and other pesticide compounds were dichloromethane, ethyl-acetate, *n*-hexane, petroleum ether, acetonitrile and ethanol. The volumes of solvent ranged between 5 mL to 10 mL. The advantages of MSPD are that it requires small amount of sample and solvents, non-time consuming and inexpensive. It is an alternative method to LLE or SPE. In addition, the used of solid-phase materials and unsuitable organic solvents can cause irreversible adsorption which resulted in lower extraction efficiency.

2.1.5 Dispersive solid phase extraction

A combination of single-phase extraction followed by partitioning and clean-up step is called a dispersive-solid phase extraction (d-SPE). It is rapid process due to a few analytical steps and inexpensive because of less solvent usage and laboratory glassware used in this method. This technique is commonly used to determine pesticide residues because of its ability to extract simultaneously, reducing interferences and easy to carry out in a minimal time.

The original d-SPE was first introduced in 2003 by Anastassiades et al. (Anastassiades *et al*., 2003). They have developed a method termed QuEChERS refers to Quick, Easy, Cheap, Effective, Rugged and Safe. The optimization parameter that give a significant effect to this technique have been evaluated such as sample size, type of extraction solvents, comparison of various salts,

extraction/partitioning step involved, pH effect and comparison of different SPE sorbents for dispersive clean-up steps. After the optimization, they found the highest extraction efficiency condition were 10 g of sample extracted with acetonitrile, followed by liquid-liquid partitioning formed by addition of anhydrous magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl). Further clean-up step was carried out using primary secondary amine (PSA) and $MgSO_4$ to remove interferences compounds such as pigments, sugars and organic acid (Anastassiades *et al*., 2003).

Up to date, this technique has gone through various modifications to improve recovery of pesticides in specific types of food. A total of 160 pesticide compounds were developed simultaneously using d-SPE in wines sample (Walorczyk *et al*., 2011). In this study, sample was added with acetonitrile and vortexing a few minutes before trisodium citrate dehydrate, anhydrous magnesium sulphate and sodium chloride were added to remove water and increase phase partitioning. After these three salts were added, the mixture was immediately shaked by hand and centrifuged a few minutes. Further clean-up step was done using $MgSO_4$, PSA and C_{18} to get a clear extract and consistent recovery readings. Simultaneous analysis of 44 types of pesticides was also been evaluated in raw bovine milk (Dagnac *et al*., 2009). Milk samples were diluted in a mixture of formic acid and acetonitrile followed by shakes for a few minutes and centrifugation. Further clean-up step was performed using a mixture containing $MgSO_4$, DSC_{18} and PSA.

More recently, the modification of this technique was successfully evaluated with amine modified graphene as a sorbent in determining 28 pesticide residues in oil crops samples (Guan *et al*., 2013). This sorbent was obtained from a synthesis

process and has higher ability to clean-up fatty acids and other interferences of oil crops. Samples were extracted using acetonitrile, added with NaCl followed by centrifugation step. The supernatant was transferred to a micro-centrifuge tube containing sorbent for clean-up process before analyzing by the instrument. A combination of d-SPE and others existing technique had also been applied in sample preparation for pesticide residues analysis. A method to determine seven (7) pesticide compounds was developed using d-SPE combined with dispersive liquid-liquid micro extraction (d-LLME) in grain samples (Wang *et al*., 2012). In this study, samples were extracted by a mixture of acetonitrile and formic acid followed by clean-up using PSA, C_{18} and graphitized carbon black (GCB). Further analysis was carried out by d-LLME to enhance sensitivity for the determination of trace level of pesticide residues.

Most of the studies discussed above have shown satisfactory result regarding the application of d-SPE in various food samples. However, this technique has it own disadvantages. The addition of anhydrous MgSO4 without immediate shaking can caused conglomeration in the sample which reduced the extraction efficiency. Moreover, the application of d-SPE can only be used when the selected sorbent removes the matrix interferences and not the target analytes. One type of sorbent could not eliminate all the interferences containing in the sample. Hence, the target analytes could also been absorbed by the sorbents consequently reducing recovery. Thus, it is very important to select the most suitable sorbents to absorb unwanted compounds effectively.

2.1.6 Solid phase micro-extraction

This technique was introduced by Pawliszyn and his co-workers in 1990 to fulfil the limitations of the SPE and LLE techniques (Kin and Huat, 2010). It consists of a coated fiber and syringe-like handling device that are used to isolate and concentrate analytes into a range of coating materials. SPME is known as a solvent free technique saved about 70% of sample preparation time (Chen *et al*., 2010).

Several fiber coatings are commercially available such as polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), polydimethylsiloxane (PDMS) and carbowax divinylbenzene (CW-DVB). PDMS has been used to extract OPPs in fruit and vegetables (Chai *et al*., 2009). PDMS was selected as the coating materials because of its ability to absorb polar to semi polar pesticides. PDMS-DVB is a bipolar fiber and has been use to extract OPPs in milk and fruit juice (Rodrigues *et al*., 2011; Cortés-Aguado *et al*., 2008). The application of PA fiber is reported in mango and honey sample (Filho *et al*., 2010; Campillo *et al*., 2006). The good ability of the PA fiber in extracting pesticide residue was previously reported by Tankiewicz *et al*. (2013). There are two modes of extraction in SPME: direct immersion SPME (DI-SPME) and headspace SPME (HS-SPME). A comparison between DI-SPME and HS-SPME modes were also reported (Filho *et al*., 2010). Several pesticide peaks were not detected with HS-SPME but were successfully absorbed when the DI-SPME was applied. For that reason, DI-SPME approach was more sensitive because the fiber was directly in-contact with the samples. HS-SPME mode could possibly save the fiber coating as it is not directly in-contact to the samples, but the increase of vapour pressure and formation of air

bubbles may result in degradation of the pesticides and decreasing the extraction efficiency.

Several methods for determining pesticide compounds in different matrices (mangoes, fruit juices, honey) have been reported using direct-immersion SPME (Cortés-Aguado *et al*., 2008; Campillo *et al*., 2006). Another extraction mode, head-space SPME was also evaluated in fruits and vegetables (Kin and Huat, 2010). The SPME with the circulation cooling technique (CC-SPME) have been introduced to determine five types of OPPs in tomato samples (Chai *et al*., 2008). This cooling technique was done by heating the sample while cooling the fiber coating. CC-SPME applied activated carbon fiber (ACF) and was compared to commercial fibers. Cooling the fiber resulted in better performance for HS-SPME techniques in terms of sensitivity, linearity and recovery. More details about DI-SPME will be discussed in Chapter 4 (section 4.1).

2.2 Identification and quantification of OPPs analysis.

A summary of the instrumental techniques reported for OPPs and other pesticides compounds is shown in Table 2.1. The most common method for determining OPPs is gas chromatography (GC) coupled with various types of detectors such as flame photometric detector (FPD), electron capture detector (ECD), nitrogen phosphorus detector (NPD) and mass spectrometry detector (MSD). Despite GCs, liquid chromatography (LC) and capillary electrophoresis (CE) have been employed for the determination of OPPs.

Table 2.1: Modern instrumental reported in the analysis of OPPs and other pesticides compounds (e.g. organochlorine, carbamates, herbicide)

No	Instrument	Detection	Name of pesticide compounds	Matrix	Analysis time, flow rate	LOD (µg L^{-1})	RSD (%), recovery (%)	Linearity range, R^2	Ref.
1)	GC	ECD	Malathion, cypermethrin, lambda-cyhalothrin	Soil	21.5 1.6	0.01 – 0.04	2.3 – 9.6 77.10 – 98.5	0.05 – 50 0.9993 – 0.9998	Wang *et al*., 2008b
2)	GC	ECD	Aldrin, bromopropylate, chlorothalonil, diclofop-methyl, dicofol,endosulfan, HCB, methoxychlor, tetradifon, buprofezin, dicloran, etaconazole, hexaconazole, imazalil, linuron, metolachlor, prochloraz, propiconazole, quizalofop-ethyl, tebuconazole, triadimefon, triadimenol, trifluralin, vinclozolin, chlorpyrifos**,** diazinon, dichlorvos, dimethoate, cyfluthrin, cypermethrin, fenvalerate.	Honey	63.75 NR	2 – 10	4.2 – 6.0 88 - 98	0.01 – 0.10 NR	Rissato *et al*., 2004

- NR – Not reported