2018

Development and Application of A SERS Needle for One-step Multi-phase Analysis

Haoxin Chen

DEVELOPMENT AND APPLICATION OF A SERS NEEDLE FOR ONE-STEP MULTI-PHASE ANALYSIS

A Thesis Presented

By

HAOXIN CHEN

Submitted to the Graduate School of the

University of Massachusetts Amherst in partial fulfillment

of the requirements for the degree of

MASTER OF SCIENCE

September 2018

Food Science

DEVELOPMENT AND APPLICATION OF A SERS NEEDLE FOR ONE-STEP MULTI-PHASE ANALYSIS

A Thesis Presented

By

HAOXIN CHEN

Approved as to style and content by:

_____

Lili He, Chair

_____

Eric Decker, Member

_____

Matthew Moore, Member

_____

Eric Decker, Department Head

Department of Food Science

ACKNOWLEDGEMENTS

I would like to express my gratitude toward my advisor, Dr. Lili He, for her tremendous support, insightful guidance and warm encouragement on my master study and life. Her intelligence, full motivation on science, high working efficiency, and kind help impressed and motivate me a lot. Being her student is the luckiest thing I've ever made. I would also like to thank Dr. Eric Decker and Dr. Matthew Moore for being my committee members. A big thank to all my lab mates for their help and discussion on my projects, as well as making our lab a big family. Last but not least, a special thank to lovely UMass badminton team for making my life in UMass. Go UMass Badminton!

This work was funded by the China Scholarship Council (201606150089).

ABSTRACT

DEVELOPMENT AND APPLICATION OF A SERS NEEDLE FOR ONE-STEP MULTI-PHASE ANALYSIS

SEPTEMBER 2018

HAOXIN CHEN, B.S., SOUTH CHINA UNIVERSITY OF TECHNOLOGY,

RUTGERS UNIVERSITY

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Lili He

Surface-enhanced Raman spectroscopy (SERS) is an emerging and sensitive technique in food analysis providing advantages of rapid detection, simple sample preparation and on-site detection capability over GC and LC methods. Most SERS applications focus on detecting trace amount of analyte in liquid as an alternative approach to HPLC. Herein, we invented an innovative SERS-active needle which is composed with an injection needle and a gold-nanoparticles coated fiber inside the injection needle. The gold nanoparticles-coated fiber was fabricated by reducing gold (III) on a chemically etched stainless wire. The SERS needle can be used to insert into the headspace and liquid sample for simultaneous multiphase sample detection, or a soft tissue like a tomato fruit to detect the analyte inside of the tissue with minimum invasion. Using this needle, we can detect as low as 5 ppb of fonofos in the headspace of water and apple juice samples, compared with the dip method, which cannot detect lower than 10 ppb in water and 50 ppb in apple juice. The SERS needle was also applied in real time pesticide translocation study to monitor internalized thiabendazole in tomato fruit after

root uptake. The SERS needle detected thiabendazole inside tomato fruits 30 days after the pesticide exposure in a hydroponic planting environment. Moreover, realizing the advantage of detecting volatile components in the headspace of food sample, we applied the SERS needle in a ground beef spoilage study to detect the spoilage biomarkers in the headspace of the raw beef. As a result, the SERS needle detected volatile spoilage compounds produced by bacteria *Lactobacillus*. Overall, this invention opens a new field of SERS strategy for broad analytical applications.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATION

SERS- surface-enhanced Raman spectroscopy

GC- gas chromatography

LC- liquid chromatography

HPLC- high performance liquid chromatography

MS- mass spectrometry

VOC- volatile organic compound

SPME- solid phase microextraction

SEM- scanning electron microscopy

LOD- limit of detection

LOQ- limit of quantification

TSA- trypticase soy agar

PCA- principal component analysis

CHAPTER 1

INTRODUCTION

1.1 Justification

Microbial and chemical contamination in food are two major food safety and quality concerns. Detection of these two contaminations in food is critically important to monitor the quality and safety of food products and prevent the contaminated products distributed further in the food chain. A variety of detection methods have been developed for these two classes of contaminations.

1.1.1 Chemical contamination in food

The sources of chemical contaminants in food is various, including pesticide residue, environmental contaminants, food processing contaminants, and unapproved food additives and adulterants. Specifically, pesticide and insecticide are widely used on agricultural produce and soil to control pest, and the amount of pesticides used in the United States is 7.0 kg/ha annually[1]. Pesticides can bioaccumulate and transfer from soil to plant and animal via food chain, and ultimately persist in food products like milk, juice, and meat [2]. The consumption of pesticide residue in food has potential harm on human health, such as carcinogenic, teratogenic, mutagenic, immunotoxic, immunopathological and neuropathic effects [3]. In addition to direct application on crops and plants, the systemic fungicides are more widely applied in growing soil or hydroponic environment thanks to their ability to translocate in plant via xylem and thus, they are more effective in controlling pests and plant diseases than non-systemic fungicides [4]. However, it posts another threat that the fungicides can be absorbed by plant and ultimately present inside the fruit that cannot be simply washed away [5].

1.1.2 Microbial contamination in food

Foodborne bacterial contamination would cause illness and sometimes death, so the fatal pathogens have zero tolerance in food like *E. coli* O157:H7 and *Salmonella* [6]. In addition to pathogenic contamination, spoilage bacterial contaminations occur more often in food and cause deterioration and economic loss. Although the spoilage bacteria may not be harmful, their growth and metabolism result in change of texture and production of off-odor to food like poultry, vegetables and fruits [7–9]. Since the spoilage could happen in a short time, a rapid and sensitive analytical method is in need to ensure food safety and quality and predict shelf life.

1.2 Gold standard methods for chemical residue detection in food

For chemical contamination, gas chromatography (GC) and liquid chromatography (LC) with mass spectrometry (MS) detection are recognized as the "gold standard" methods because of their high separation ability, selectivity, sensitivity and identification capabilities [10,11]. GC is a strong tool to analyze components from vapor-phase mixtures, and a vapor-phase extraction is employed to partition analytes between a non-volatile liquid or solid phase and the vapor phase above the liquid or solid [12]. In this scene, less components in the headspace are expected to be transferred into GC and analyzed than the complex liquid or solid mixture. In pesticide detection, the choice of GC or LC depends on the properties of the pesticides. For examples, some thermally labile and/or high boiling-point pesticides are more amenable for GC. Organochlorine and organophosphorus pesticides have been better analyzed by GC-MS and do not show sufficient LC-MS response owing to their low polarity and good thermal stability and volatility [10,13–16].

1.2.1 Classic sample preparation methods of GC and LC

Sample preparation is the key for both GC and LC methods. Solvent extraction followed by solid phase extraction is the most widely used technique in LC sample preparation, while solid samples are required to be homogenized before extraction, and matching solvent polarity and analyte solubility is also essential for the method [17]. The method is overall laborious, time-consuming, expensive and unsuitable to evaporating analytes. The solvent-free sample preparation in GC methods can be classified into three categories: gas phase extraction, membrane extraction and sorbent extraction. Gas phase extraction method involves static headspace sampling and dynamic headspace (purge and trap) sampling. Static headspace is mainly used in the high-ppb to percent concentration ranges because of its limited sensitivity. In dynamic headspace sampling, carrier gas passes through a liquid sample, and the volatile analytes on a sorbent is trapped and desorbed onto a GC. The sensitivity is higher to detect ppb to ppt of volatile organic compounds in aqueous matrices [12]. However, these headspace methods are limited to volatile compounds only, while many contaminants including pesticides are non-volatile or little volatile, which transfers the focus onto polarity of analytes. In membrane extraction, analytes are extracted by the membrane material or by a stripping phase. It is effective in trapping volatile compounds, while unsuitable for more polar compounds due to the lack of specific membrane. The membrane also shows slow response to the change of concentration [18,19]. Hence, they are not effective methods to determine pesticides. Solid phase extraction belongs to sorbent extraction which is to use an adsorbent material to extract analytes.

Among all these methods, solid phase microextraction (SPME) is gaining more and more popular as a solvent-free method in GC and LC sample preparation.

1.2.2 SPME preparation method

In SPME, a fused silica or other appropriate materials made fiber is coated with sorbent, and then the coated fiber is used to capture and concentrate analytes from a static or dynamic headspace of a liquid or solid sample [12,18]. It allows rapid mass transfer during extraction and desorption, meanwhile addressing the plugging problem of solid phase extraction. It also offers the benefit based upon selectivity since only volatile and semi-volatile organic compounds that can be released into the headspace and trapped by the fiber [14]. Utilizing this advantage, SPME-headspace has been greatly employed on food and flavor analysis to determine the components in the volatile fraction. A main complaint about SPME-headspace is on the extraction reproducibility in method validation[12]. When determining pesticides in sample matrix using SPME-headspace, researchers have worked on the optimization of extraction conditions and development of a clear protocol to improve the extraction rate, efficiency and sensitivity. The relation between multiple parameters and the extraction rates were studied with attention to detail. Agitating the sample like constantly stirring the liquid sample can speed up the extraction because stirring creates a continuously new and high surface water-gas interface [10,18]. Heating accelerates the release of analytes from matrix, while it is sometimes unsuitable for analytes that are unstable at high temperature [18]. The pH of the sample could have impact on the extraction efficiency, while it is not a controlling parameter for neutral pesticides. For slightly acidic or basic compounds, extraction rate will be promoted if these compounds are kept in dissociated forms [10]. Ionic strength can be enhanced by

adding NaCl or Na_2SO_4, which results in a less solubility of the organic compounds and improved partition coefficient [19]. Optimization of these parameters helps to enhance the evaporation of volatile analyte and improve the sensitivity and efficiency in following SERS analysis.

1.3 Chemical detection of microbial contamination

In microbial spoilage detection, microbiological analysis and/or sensory evaluation are widely applied, while they have drawbacks. Microbiological methods are too lengthy for industrial controls and cannot trouble shoot the spoilage not from microbial origins. Sensory evaluation has strong reliance on trained panels and is very costly and unattractive to food industry. On the other hand, chemical analysis has been recognized to diagnose spoilage and assign shelf life. In particular, GC method has been applied in detecting volatile organic compounds (VOC) from microbial spoilage, since microbial VOCs have often been related with their use as markers of microbial growth [20]. Couple sulfur-containing compounds producing bacteria have been reported to be mainly responsible for VOCs production and spoilage like *Pseudomonas* and *Lactobacillus,* and VOCs identified include dimethyl sulfide, acetone and methyl ethyl ketone from ground beef specifically[8,20–24]. While H_2S indicates the growth of an atypical flora including *Enterobacteriaceae* since *Pseudomonas* do not product it [24]. Acetoin and diacetyl have been suggested to indicate the microbial quality of pork [25]. Trimethylamine is the most tested and discussed compound responsible for the "fishy" odor of spoiling seafoods [26]. What is more, microbial VOCs are also utilized in clinical specimens and taxonomic studies to detect and classify bacteria with specific metabolism behaviors [7,27–29].

1.4 Surface-enhanced Raman spectroscopy (SERS)

Surface-enhanced Raman spectroscopy (SERS) is a vibrational spectroscopy method for rapid detection. Raman spectroscopy can reveal structural characteristics of the molecules based on molecular vibrations by hitting the targeted molecules with a laser, and the surface-enhanced effect is achieved by a metallic nano-substrate, such as gold or silver nanoparticles (Figure 1) [30,31]. The enhancement factors can be up to 10^{10} to 10^{11} times, so the technique can even detect single molecules and satisfy identification purpose [32]. There are two enhancement mechanisms have been proposed: electromagnetic and chemical effects. In both, the analytes must be absorbed on a SERS active substrate and irradiated by laser. The electromagnetic enhancement factor arises from enhanced optical fields because of excitation of electromagnetic resonances in the metallic structures. Chemical enhancement results from a metal electron-mediated resonance Raman effect via a charge transfer intermediate state called "active sites" [33]. SERS technique has been rapidly developed as a sensitive analytical tool for the detection of chemical and microbial contaminants in agricultural, food, and environmental samples [34–37]. In particular, SERS has been explored for the detection of a variety of pesticides from simple to complex matrices [36,38–40]. SERS technique has the advantages of rapid detection, little or no sample preparation and high levels of sensitivity, and it is becoming a promising method for pesticide detection considering its sensitivity, reproducibility and portability.

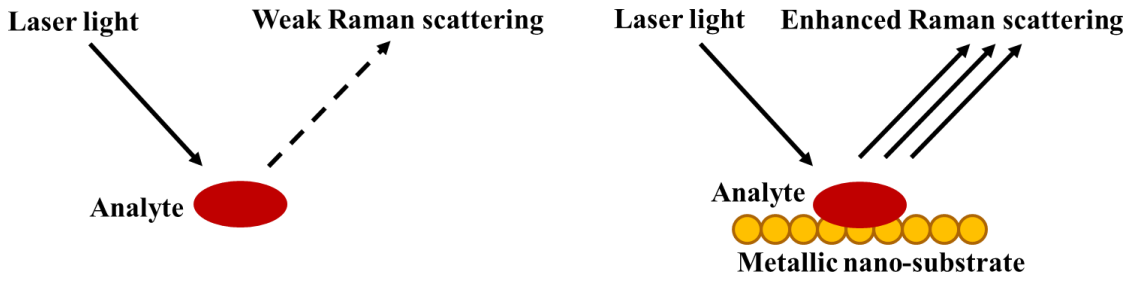

Figure 1. Illustration of SERS mechanism

1.4.1 Limitations of current SERS substrates

Most SERS applications focus on detecting in liquid matrices. The sample preparation often involves mixing the liquid sample with nanoparticles and then drying on a solid surface to form a "coffee ring" for measurement [36]. In other sample preparations, a liquid sample is dropped on a solid substrate and allowed to dry or the solid substrate is dipped into the liquid sample and then taken out and dried. Inclusion of the drying process reduces the interference from volatile solvents and concentrates the target molecules on the surface of the nanostructure. However, when the target compound is also volatile, the drying process results in loss of the target compound. In volatile organic compound detection, some SERS researchers introduce a gas chamber to the SERS instrument, and compounds in the vapor phase are detected when passing through the gas chamber [29,41]. However, the use of a gas chamber and pump does not offer an advantage over GC.

1.5 Goals and objectives of the study

To extend the SERS application for VOC detection, we aim to develop a new strategy that is based on the modification of a stainless-steel fiber with gold-nanoparticles. The resulting gold-nanoparticles coated fiber combining a real injection needle forms a SERS needle will be tested to perform SPME in both liquid and

headspace in one-step. In addition, the SERS needle will be evaluated for probing internal pesticides in a soft tissue like a tomato fruit with minimum invasion.

To complete this goal, there are four objectives of this study. Upon competition of these four objectives, we expect to open a new field of SERS strategy for broad analytical applications.

Objective 1: Fabricate and optimize a gold-nanoparticles coated SERS needle

Objective 2: Apply the SERS needle to detect pesticide in liquid and headspace from food matrix.

Objective 3: Apply the SERS needle to detect internalized pesticide and monitor pesticide translocation in tomato plant.

Objective 4: Apply the SERS needle to detect microbial VOCs during meat spoilage.

CHAPTER 2

DEVELOPMENT OF GOLD-NANOPARTICLES COATED FIBER FOR SPME HEADSPACE DETECTION OF PESTICIDE WITH SERS

2.1 Introduction

In chapter 1, several conventional SERS substrates and their drawbacks on volatile compound detection were discussed. With the rapid and sensitive detection capabilities of the Raman microscopy, we wanted to make improvement on SERS substrate for volatile pesticide detection using solid phase microextraction (SPME) approach.

2.1.1 The development of a SERS substrate for SPME approach

SPME is firstly developed in GC analysis that a solid substrate, mostly a fiber, is coated with sorbent and used to extract the volatile analytes in the headspace of sample. Unlike GC analysis, the choice of sorbents is based upon the affinity between different pesticides and analytes to improve extraction rate, many pesticides have good interaction with metal nanoparticles to generate enhanced SERS signals. Therefore, the SPME approach can be applied in SERS detection and the fiber can be made into SERS substrate by coating it with gold or silver nanoparticles. Some SERS researchers fabricated silver or gold nanoparticles coated fiber probe or needle to detect chemicals[42–45]. Different fabrication methods were adopted like laser ablation, annealing, sputtering, simple immersion into nanoparticles solution, and chemical reaction layer by layer[43–48]. The laser ablation, annealing and sputtering offer the advantage that the metal nanoparticle size is precisely controlled, while they are usually expensive. The chemical reaction layer by layer to grow nanoparticles is very time-consuming that requires dozens

of reactions. The simple immersion of the probe into nanoparticles solution does not provide strong binding between nanoparticles and the fiber probe, and nanoparticles can easily fall off during the detection of samples. In order to develop a simple and rapid way to fabricate a gold-nanoparticles coated fiber, a chemical etching and coating method was adopted in this study[49]. Then the fiber is put inside a injection needle and can penetrate samples.

2.1.2 One-step multi-phase sample preparation

In order to capture and detect volatile chemicals, some SERS researchers introduced gas chamber, pump, or thermoelectric cooler to preconcentrate the volatile analytes[29,41,50]. However, the involvement of these instruments adds more cost and labor intension to the experiment. To better analyze the volatile chemicals, we combined headspace and SPME to extract evaporating analytes. In a sealed bottle, the SERS needle inserts through the PTFE/silicone septum and exposes to the liquid sample and the headspace above the sample solution shown in Figure 2. Headspace approach has a large benefit based upon selectivity since only volatile and semi-volatile compounds can evaporate into the headspace and captured by the SERS needle[14]. Hence, the employment of headspace method eliminates the interference from other chemicals and results in better sensitivity. Based on it, we combine the headspace approach and dip approach to measure multiple phases in on-step. The headspace approach and simple dip approach to detect VOCs were compared and evaluated in this study.

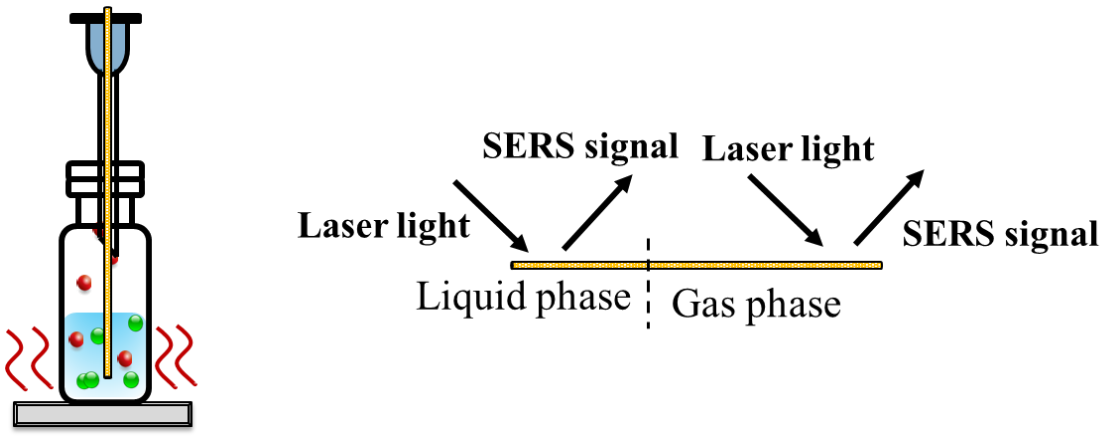

Figure 2. Scheme of one-step multi-phase sample detection

2.1.3 Objectives of this study

The objectives of this study were to (1) fabricate a gold-nanoparticles coated needle for SERS analysis (2) characterize the SERS needle substrate and (3) compare and evaluate headspace and dip methods in detecting volatile chemicals.

2.2 Materials and methods

2.2.1 Materials

Analytical grade standard of fonofos (>99.9%), 2, 4, 6 trimethylpyridine (>99.9%), allyl methyl sulfide (>99.9%), diphenyl sulfide (>99.9%), hydrogen tetrachloroaurate hydrate (99.999%), and sodium chloride (>99.5%) were procured from Sigma Aldrich (St. Louis, Mo., USA). Hydrochloric acid (34%-37.5%), Acetonitrile (99.9%), ethanol (100%) and methanol (99.9%) were purchased from Fisher Scientific (Fair Lawn, NJ., USA). The stainless-steel wire (SUS304, φ140 μm) was purchased from the Small Parts, Inc. Stock solution of fonofos was prepared in acetonitrile as at 100 ppm and further diluted by distilled water. Stock solutions of trimethylpyridine, allyl methyl sulfide, and diphenyl sulfide were diluted by distilled water.

2.2.2 Preparation of gold nanoparticles-coated fibers.

An acid etching reaction was used to increase the roughness and the surface area which can strengthen the binding between the gold-nanoparticles coating and the porous stainless wire [49]. The stainless-steel wire (5 cm) was washed with methanol, ethanol and distilled water in an ultrasonic bath for 10 min respectively. The etched fiber was washed again with methanol and distilled water in the ultrasonic bath for 5 min respectively, then dried at 60℃. The etched fiber was then immersed into $HAuCl_4$ solution (0.05%, w/w) to introduce gold to its porous surface as demonstrated in Figure 3. The coating reaction is the replacement reaction between iron and gold:

$$Fe + [AuCl_4]^- = Fe^{3+} + Au + 4Cl^-$$

The surface morphologies of unetched fiber, acid-etched fiber and gold-nanoparticles coated fiber were characterized under microscopes and SEM.

2.2.3 Headspace SPME and dip SPME

After fabrication, the fiber was put inside an injection needle and used to penetrate the PTFE/silicon septum of a sealed vial. In headspace detection, the SERS needle was inserted into the headspace overlying the working solution, and the solution was heated at 75℃. In dip detection, the SERS needle was immersed into the working solution without heating. The extraction time was 30 minutes for each. When extraction step was finished, the fiber was removed from the needle and immobilized on a slide for SERS measurement. The fabrication and extraction methods are demonstrated in Figure 3.

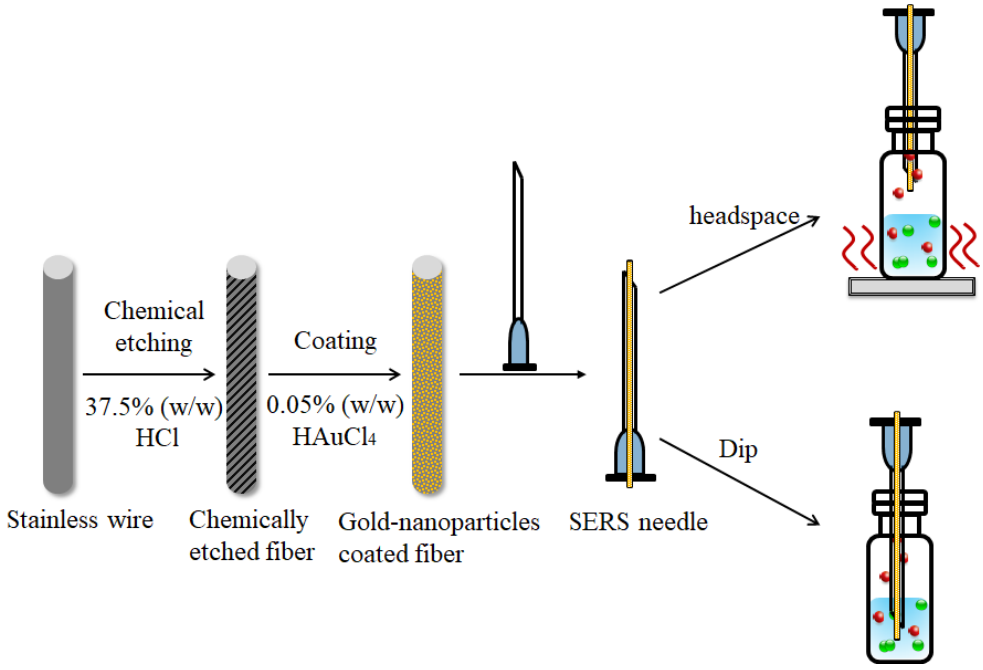

Figure 3. Schematic illustration of fiber fabrication and headspace-dip method. The stainless wire was etched by HCl and then coated with gold nanoparticles by HAuCl4. In headspace detection, the SERS needle was inserted above the solution. In dip detection, the fiber was dipped into the solution.

2.2.4 Instruments and data analysis

The surface morphology of unetched fiber, etched fiber and gold-nanoparticles coated fiber were characterized by FEI Magellan 400 scanning electron microscope (SEM, Hillsboro, OR) with the voltage of 5.0 kV.

A DXR Raman microscope (Thermo Fisher Scientific, Madison, Wis., U.S.A.) with a 780 nm laser and a 50X confocal microscope objective (0.8 mm spot diameter and 2 cm^{-1} spectral resolution) was used in this study. Each spectrum was scanned from 2000 to 800 cm^{-1} with 5 mW laser power and a 50 mm slit width for 2 seconds integration time. OMNIC™ software version 9.1 was used to control the Raman instrument. Fifteen scans were selected from each fiber and then averaged by the software.

The Raman spectra were analyzed using Thermo Scientific TQ Analyst 8.0 software. All Raman intensities were calculated from at least three replicates and standard deviations were recorded.

2.3 Result and discussion

2.3.1 Characterization of fiber substrate and fonofos SERS spectra

Figure 4 showed the surface morphologies of unetched fiber, acid-etched fiber and gold-nanoparticles coated fiber. The unetched fiber has a smooth and polished surface, as shown in Figure 4(A) through 4(D). After acid etching, the etched fiber has a rough surface as shown through Figure 4(E) through 4(H). The etching effect was controlled through these parameters: reaction temperature, HCl concentration, and reaction time. The parameters were optimized to maximize the roughness while avoiding the fragility. We found out 30 minutes reaction time with 37.5% HCl at room temperature reach the ideal condition for coating. Longer reaction time (i.e., 45 min) and/or higher reaction temperature (i.e., 45-60℃) attributed to fiber's fragility that is not liable for coating. The increased surface area provides more area for gold-nanoparticles to grow and a stronger binding between the gold-nanoparticles and the fiber. After replacement reaction, the coated fiber showed optically golden color which indicates the successful coating of gold in Figure 4(I) and 4(J). Under SEM, the nanoparticles were at around 100 nm and evenly and densely distributed in Figure 4(K) and 4(L). This fabrication method is a simple and rapid way to coat nanoparticles onto a stainless-steel fiber and offer great advantage compared with other fabrication methods such as laser ablation, annealing and chemical reaction layer by layer.

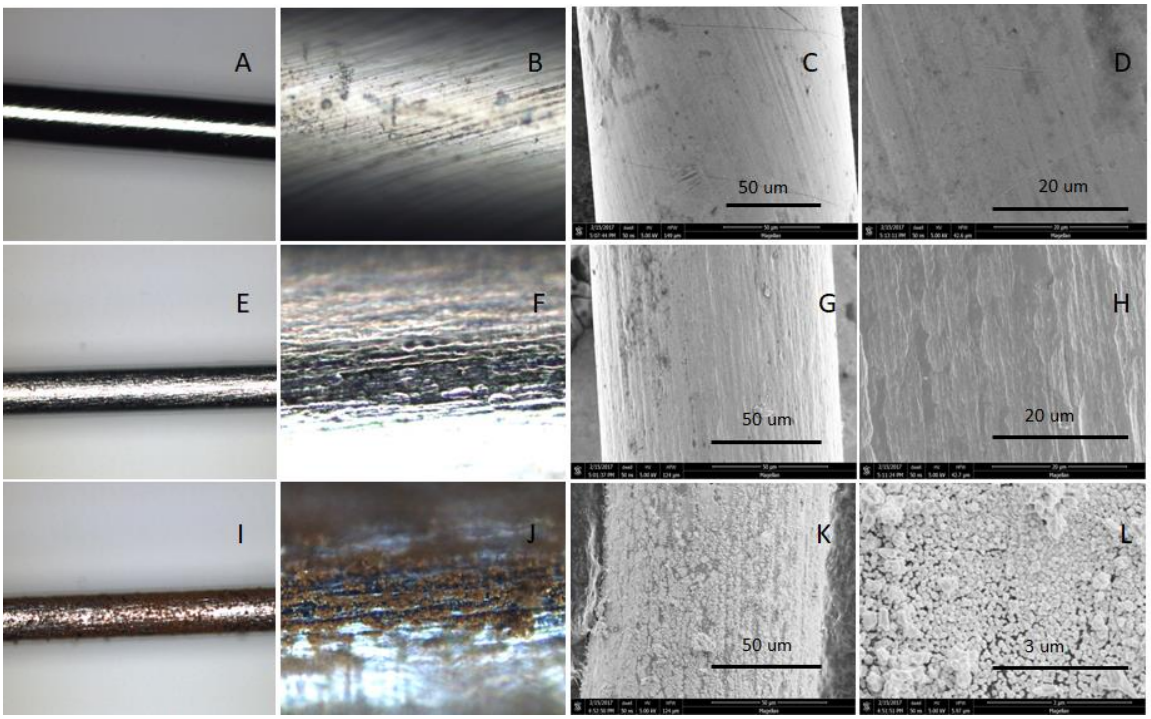

Figure 4. Unetched fiber under 10X objective (A), 50X objective (B), 50-um SEM (C), and 20-um SEM (D); etched fiber under 10X objective (E), 50X objective (F), 50-um SEM (G), and 20-um SEM (H); and coated fiber under 10X objective (I), 50X objective (J), 50-um SEM (K), and 3-um SEM (L).

After the fiber was fabricated, its SERS-active capability and extraction efficiency were tested in 1 ppm fonofos water solution with dip and headspace methods. In the headspace-SPME approach, 20% NaCl solution was added to the sample because the addition of salt usually increases the ionic strengths and decreases the solubility of organic analytes in the aqueous phase [51]. From Figure 5(A), the fiber has minimal background noise between 800 to 2000 cm^{-1} Raman shift, providing no interference to pesticide signals. In dip and headspace tests, the four most obvious peaks of fonofos on 1001, 1024, 1081 and 1576 cm^{-1} Raman shift were observed and characterized in Figure 5(B) and 5(C). The peak at 1576 cm^{-1} is attributed to ν(C=C) phenyl stretch which is used

for quantitative analysis later. The peaks at 1081, 1024 and 1001 cm^{-1} are respectively attributed to ν(S–C phenyl)+δ(C–H)phenyl, δ(C–H)phenyl + ν(S–C phenyl), and δ(CCC)phenyl [31]. Moreover, headspace method generates higher intensity of signals and minimal interference compared to dip method, indicating the advantage and feasibility of headspace approach for fonofos detection.

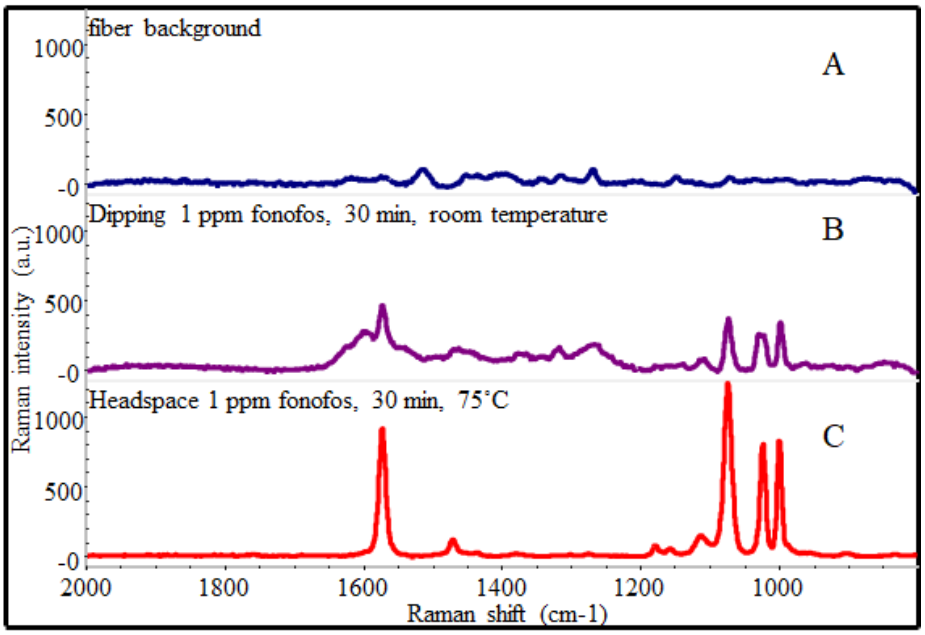

Figure 5. SERS spectra of (A) fiber background, (B) 1 ppm of fonofos detection with dip-SPME, (C) 1 ppm of fonofos detection with headspace-SPME.

In addition, more volatile chemicals were tested using the SERS needle with headspace and dip method. 2, 4, 6 trimethylpyridine, allyl methyl sulfide and diphenyl sulfide are the characterized spoilage biomarkers from ground meat, which consist off-odor from the spoiled meat[3,9]. Using the SERS needle, these chemicals can be detected from both liquid phase and gas phase. In dip detection, 10% of each working solution was used in Figure 6 (A), and 1% of each working solution was tested with headspace in Figure 6 (B). Each volatile chemical produced characteristic spectra, while headspace method generated stronger Raman signals than dip method even though lower

concentrations of working solutions were used. It clearly demonstrated that headspace method is more effective in detecting volatile compounds as compounds evaporate to the gas phase and are captured by the SERS needle. Moreover, the benefits of using the SERS needle to detect multi-phases sample is revealed.

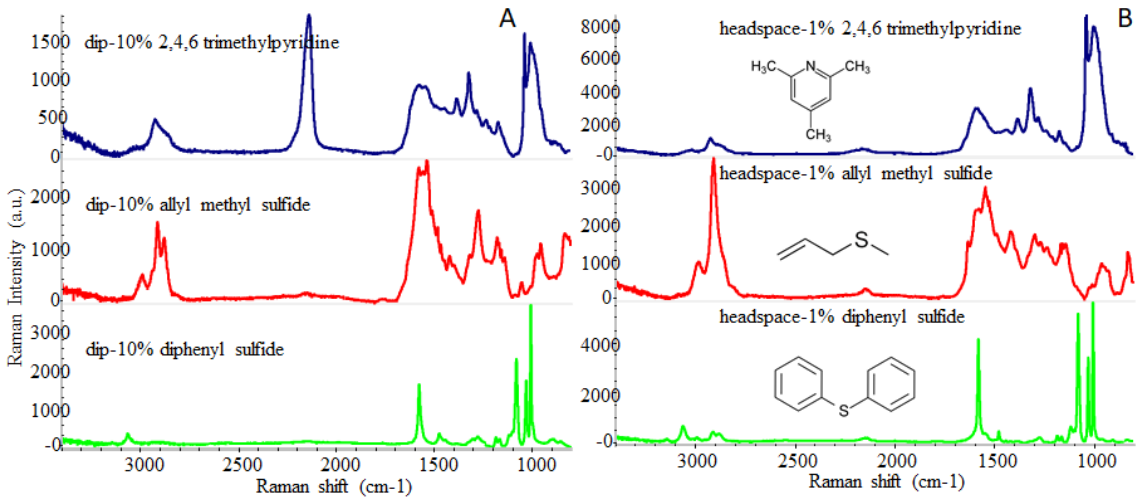

Figure 6. SERS spectra of (A) 10% 2, 4, 6 trimethylpyridine, allyl methyl sulfide and diphenyl sulfide with dip-SPME and (B) 1% 2, 4, 6 trimethylpyridine, allyl methyl sulfide and diphenyl sulfide with headspace-SPME.

2.4 Conclusion

A rapid and easy way to fabricate a gold-nanoparticles coated fiber was developed in this study. Using acid etching and chemical replacement reaction can produce an evenly and densely distributed gold-nanoparticles coating, as well as strengthened binding between the coating and the fiber. The fiber has minimal background noise in a certain range of Raman shift that provides no interference to analyte's signals. The VOCs fonofos, 2, 4, 6 trimethylpyridine, allyl methyl sulfide, and diphenyl sulfide were tested using the SERS needle with both headspace and dip approach. The headspace-SPME approach had better sensitivity and effectiveness in detecting volatile compounds from

solution than dip-SPME in this study. The SERS needle coupled with headspace SPME overcomes the difficulty in detecting volatile compounds in SERS research, and states the benefit of one-step multi-phase sample detection. It provides a convenient, simple and rapid way to fabricate a SERS substrate, capture and detect volatile compound from a solution. Future study will focus on detecting the volatile pesticide from food matrices and expanding the application of the SERS needle in more chemicals detection.

CHAPTER 3

QUANTIFICATION AND DETECTION OF PESTICIDE FONOFOS IN WATER AND APPLE JUICE WITH SERS NEEDLE

3.1 Introduction

In Chapter 2, we discovered the advantages and feasibility of the SERS needle and headspace approach for fonofos detection. Herein, we will expand the detection of fonofos to food matrix (i.e. apple juice) and establish its quantification curve in water solution.

3.1.1 Fonofos detection

Fonofos, or O-ethyl S-phenyl ethylphosphonodithiolate, is selected as a model for detection using this method because of its volatility (i.e., boiling point is 130°C at 0.1mm Hg). It is one of the organophosphate soil insecticides that can control pests such as corn rootworms. According to Environmental Protection Agency regulatory document, the oral exposure of to fonofos can induce acethylcholinesterase inhibition and cause acute toxicity. The chronic reference dose for fonofos is 0.002 mg/kg/day, the health reference level is 10 ppb, and the minimum reporting level is 0.5 ppb [51].

GC method is the current golden method to analyze pesticide fonofos, while the more rapid and easy-manipulating Raman method could be a potential alternative approach to the GC method. Recent SERS researchers proposed some innovative methods to detect fonofos from food matrices, such as dried silver and gold nanoparticles film, metal-doped sol gel filled capillary, and self-assembly silver nanoparticle mirror[31,52,53]. Although they reached sensitive limit of detection (LOD), none of them tested the gaseous phase of the pesticide fonofos as fonofos easily evaporates.

3.1.2 Objectives of this study

The objectives of this study were to (1) quantify fonofos in water solution (2) and detect fonofos in spiked apple juice with the SERS needle and headspace SPME approach.

3.2 Materials and method

3.2.1 Materials

The Langres® apple juice was purchased from local Stop & Shop supermarket (Amherst, MA., USA). Other chemicals refer to Section 2.2.1.

3.2.2 Detection of pesticides fonofos using headspace-SPME and dip-SPME methods

Each test pesticide stock solution of 100 mg/L (ppm) was prepared with acetonitrile and further diluted to needed concentrations (0.5 ppm to 0.005 ppm) with distilled water or apple juice prior to use. 5 mL of working solution were mixed with 3 mL of 20% sodium chloride solution in a 16-mL vial with a sealed PTEF/silicone septa top. The addition of 20% NaCl solution can increase the ionic strengths and, thus, decreases the solubility of organic analytes in the aqueous phase in headspace-SPME detection[54]. In the headspace-SPME method, the fiber was inserted through the PTFE/silicon septum into the headspace above the working solution to extract the volatile compounds. The extraction condition was 75℃ for 30 min. After extraction, the fiber was fixed on a slide for SERS measurement. In dip-SPME detection, working solution remains the same while the fiber dipped into the working solution without salt for 30 min at room temperature. The fiber was then air-dried and measured using Raman microscopy.

3.2.3 Data collection

The peak at 1571 cm^{-1} Raman shift of fonofos was chosen for quantification analysis due to its good consistency and least interference with the gold-nanoparticle background and apple juice signals.

3.3 Result and discussion

3.3.1 Quantification of fonofos in water sample

To investigate the sensitivity and quantification quantitative reliability of the method, we applied the headspace-SPME-SERS to detect fonofos of various concentrations (0.005 ppm to 0.5 ppm) in water as shown Figure 7 (A). The lowest detectable concentration was 5 ppb (0.005 ppm). Current SERS studies in detecting fonofos report higher detectable concentration at 10 ppm, and their limit of detection ranges from 0.1 ppm to 1 ppm[31,35,55]. In comparison, our method offers a huge improvement on sensitivity due to the use of the headspace method for capturing volatile fonofos. We then selected the peak intensity at 1576 cm^{-1} for quantitative analysis, and the linear range was obtained from 0.025 ppm to 0.5 ppm as shown in Figure 7 (B). Fonofos concentration and Raman intensity present a nice linear relation with coefficient of determination (R^2) as 0.9883. The Limit of Detection (LOD) value was calculated to be 0.0052 ppm according to the equation of 3.3 σ/S, where σ is the standard deviation of the blank, and S is the slope of the calibration curve. The LOD value is confirmed by the detection of 0.005 ppm (5 ppb) fonofos in Figure 7(A). The theoretical Limit of Quantification (LOQ) value can be extended to 0.015 ppm according to the equation of 10 σ/S [56]. However, the error bars revealed that the method had large variations that needs to be further reduced. The variation may come from varied sizes and

aggregations of the gold-nanoparticles on the fiber which may be improved by using a stainless wire fiber with a higher quality and purity and further optimizing the coating reaction conditions.

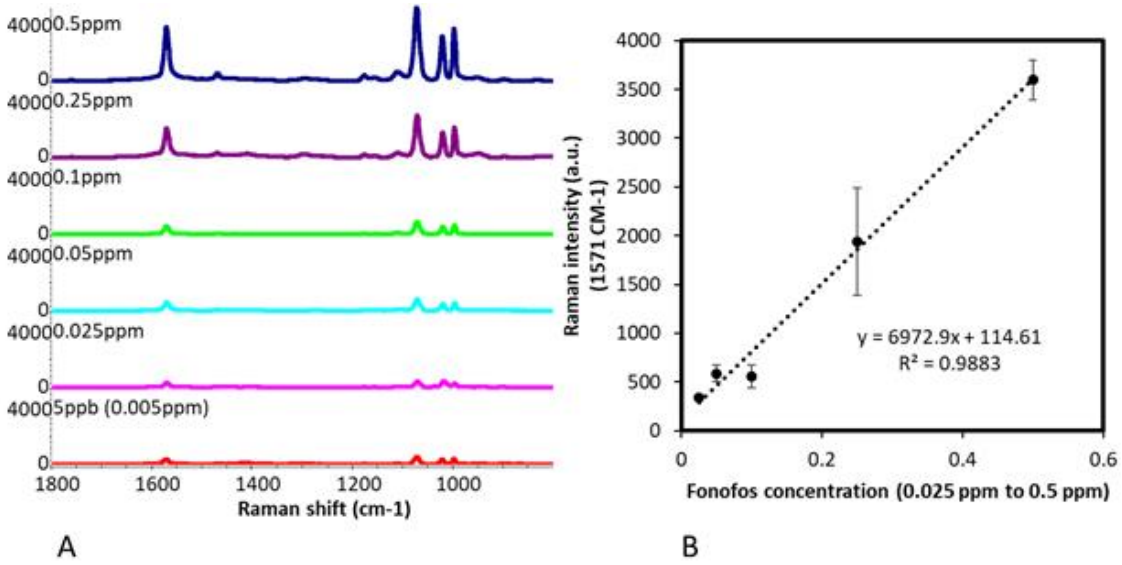

Figure 7. (A) SERS spectra of fonofos detection in water sample with headspace method. (B) Raman intensity of the peak at 1567 cm^{-1} versus the sample concentration corresponding from 0.025 to 0.5 ppm.

3.3.2 Headspace vs dip of fonofos detection in apple juice sample

To further illustrate the advantage of headspace method to detect a volatile pesticide in a real matrix, we applied the headspace method and compared with the dip method to detect fonofos in apple juice. Even though apple juice creates an acidic system, fonofos is stable at low pH according to Hazardous Substances Data Bank (HSDB), so the pH would not affect the extraction. As seen in Figure 8(A), the dip-SPME- SERS method detected 50 ppb fonofos spiked in apple juice and cannot detect lower concentration at 10 ppb because it was affected by the inferencing compounds from apple juice. On the other hand, headspace-SPME- SERS detected 5 ppb fonofos spiked in apple juice (Figure 8(B)). These data demonstrate that the headspace method is more sensitive

and effective than the dip method when detecting the volatile pesticide fonofos in complex matrices. In the headspace method, only volatile compounds occupy the space and have the chance to bind to the fiber. While in the dip detection, other components from the sample matrix may bind to the fiber and cause interference. The lowest detectable concentration at 5 ppb in a food sample is comparable to the nano-liquid chromatography and the common GC method in complex samples detection, which are 5.3 ppb and 30 ppb, respectively[32,57].

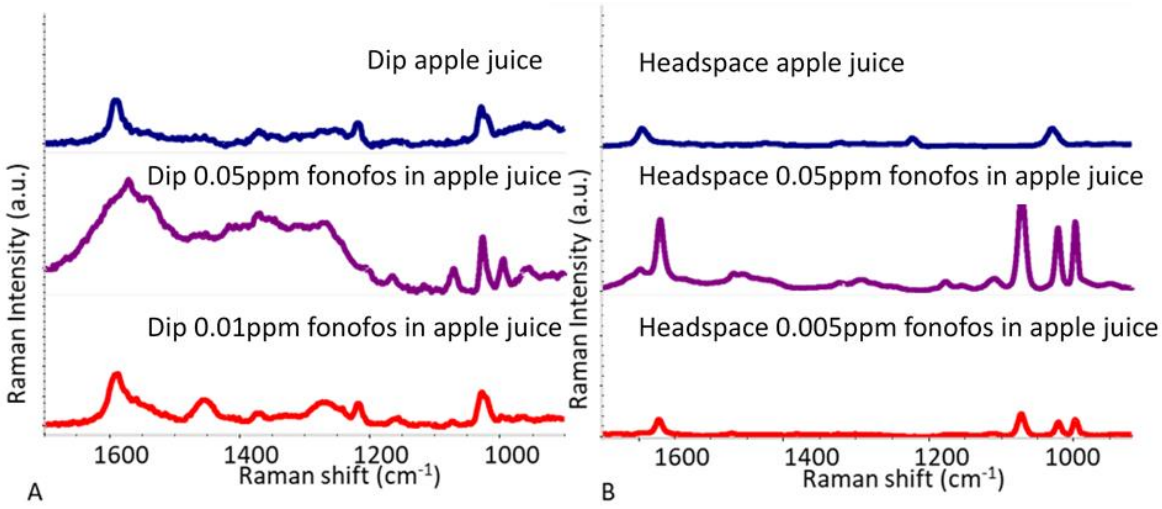

Figure 8. (A) SERS spectra of fonofos detection in apple juice sample with dip method. (B) Raman spectra of fonofos detection in apple juice sample with headspace method.

3.4 Conclusion

This study demonstrated the SERS needle ability to quantitative and detect volatile pesticide fonofos in water and apple juice. A quantification curve was obtained in the range from 0.025 ppm to 0.5 ppm with coefficient of determination as 0.9883. The LOD in water and apple juice samples was 5 ppb, which offers a huge improvement on sensitivity in SERS detection of fonofos due to the use of headspace method other than dip method. On the other hand, dip method can only reach 10 ppb fonofos in water and

50 ppb fonofos in apple juice while signal interference from apple juice sample was observed. The SERS needle coupled with headspace is an alternative method to detecting VOCs, offering the advantages of minimum sample preparation, rapid detection and satisfying sensitivity compared to current GC/LC methods in food industry. Future studies will focus on the minimization of the signal variation and on testing in a variety of target compounds and matrices. Overall, we successfully developed an innovative and simple approach to detect the volatile pesticide fonofos in a complex matrix (i.e., apple juice) by combining SERS with headspace and SPME. The approach has the advantages of simple sample preparation and rapid detection compared with SPME-GC, as well as improved sensitivity in detecting vaporizable and volatile compounds compared with traditional SERS using SPME-dip or gas chamber.

CHAPTER 4

REAL-TIME MONITORING OF PESTICIDE THIABENDAZOLE TRANSLOCATION IN TOMATO PLANT BY SERS NEEDLE

4.1 Introduction

In Chapter 2 and 3, the SERS needle has shown its capability to determine pesticide from food matrix in multiple phases. In addition, it has the advantage to detect internal pesticides from fruits and causing minimal invasion compared to other detection methods.

4.1.1 Pesticide translocation in plants

Pesticides are widely used in agricultural product during growth. To control pests and diseases, systemic fungicides are greatly applied to plants over protectant fungicides because of their ability to translocate through the cuticle and across leaves [4]. After applied to roots, systemic fungicides are taken up and translocated intact to stems and foliage via the xylem tissue [58]. Little amount of certain fungicides was reported to translocate downward. After uptake and translocation, some researches also reported and suggested a complexing or binding of fungicides to plant constituents [4]. Thiabendazole is one of the systemic fungicides that widely and commercially used to control postharvest citrus fruit decay [59]. Understanding the translocation of fungicides in plants is important to control plant disease and internalized fungicides residue.

Conventionally, chromatographic techniques are employed to detect pesticide translocation and internalized pesticides of plant and fruit [5,16]. To study dynamic uptake and translocation, researchers labelled pesticides with radiocarbon and trace the molecular weight during analysis [4]. Unfortunately, these methods are very time-

consuming and require complex sample preparation. On the other hand, SERS not only provides the advantage of rapid and *in situ* detection, but is also able to locate pesticide distribution on plant tissues using nano-particles [40,60].

4.1.2 Objectives of this study

The objectives of this study were to (1) characterize internalized pesticide thiabendazole in tomato fruit using the SERS needle and (2) in situ and real time monitor pesticide translocation from plant roots to tomato fruit using the SERS needle.

4.2 Materials and method

4.2.1 Materials

Thiabendazole (systemic fungicide: 2-(4-thiazolyl)-1H-benzimidazole, >99%, analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1000 ppm thiabendazole stock solution was prepared in methanol and diluted to needed concentration with Hoagland solution or distilled water. Hoagland modified basal salt mixture was purchased from PhytoTechnology Laboratories (Lenexa, KS, USA). Hoagland stock solution was prepared with dissolving 1.62 grams of Hoagland modified basal salt mixture per liter ultrapure water. Tomato seeds was purchased from W. Atlee Burpee & Co. (Warminster, PA, USA). Vermiculite potting media were provided by Greenhouse Centre at University of Massachusetts (Amherst, MA, USA).

4.2.2 Plant culture

Tomato seeds were firstly planted in soil system, and pesticide translocation was performed in hydroponic system. To begin, each seed was placed in one small plastic pots filled with vermiculite potting media in greenhouse for 30 days (temperature of 25℃, relative humidity between 50% to 60%, and a 16-h photoperiod with light intensity

of 200 umol photons $m^{-2}s^{-2}$). After that, plants were transferred to 4 L of 100% Hoagland solution in a container. The solution was covered with aluminum foil paper to block light. The plants roots were grown in the solution and other parts were outside and exposed to light. After 7 days, each plant was transferred to a light-blocking 250-mL bottle containing 100 mL of 50% Hoagland solution and 200 ppm thiabendazole. 50% Hoagland solution was replenished to each vial every day to maintain 100 mL volume.

4.2.3 Characterization of the SERS signals of internalized pesticide in tomato fruit

Characterization of thiabendazole signals was obtained by immersing the SERS needle in 100 ppm thiabendazole solution. For characterization of SERS signals of internalized thiabendazole in tomato fruit, harvested fruits from tomato plants were inserted with 100 ppm thiabendazole. After 2 days of translocation, a SERS needle was inserted into different parts of the tomato fruit to capture pesticide. After 30 minutes extraction, the needle was pulled out and fixed on a slide for Raman measurement (Figure 9). The organic tomato fruits without pesticide were examined as well as negative control. For pesticide translocation study, a SERS needle was inserted into tomato fruits after 0, 10, 20, and 30 days after pesticide application in the culture solution. Raman spectra were collected respectively.

Figure 9. Schematic illustration of injecting and detecting pesticide thiabendazole in different locations in tomato fruit.

4.3 Result and discussion

4.3.1 Characterization of thiabendazole spectra

To characterize thiabendazole Raman spectra, the SERS needle immersed in 100 ppm thiabendazole solution and measured with Raman. The characteristic Raman peaks at 1012 and 1275 cm^{-1} were attributed to pesticide thiabendazole, and they can be observed from tomato fruits injected with pesticide (Figure 10). The peak at 1012 cm^{-1} of thiabendazole was selected for examination in the following studies. There were some other peaks from immersion of thiabendazole solution coming from the SERS needle that may attributes to solvent. The tomato fruit itself has various background peaks due to the abundant organic components that can bind to the SERS needle, such as the pigment lycopene. What is more, after injected thiabendazole pesticide at one spot, the extraction and measurement were happened at other spots after 48 hours, and the SERS needle can detect pesticide signals, which indicate that the pesticide could spread and migrate inside the tomato fruit. The SERS needle also accurately controls the measurement point and depth, providing the benefits of *in situ* monitoring.

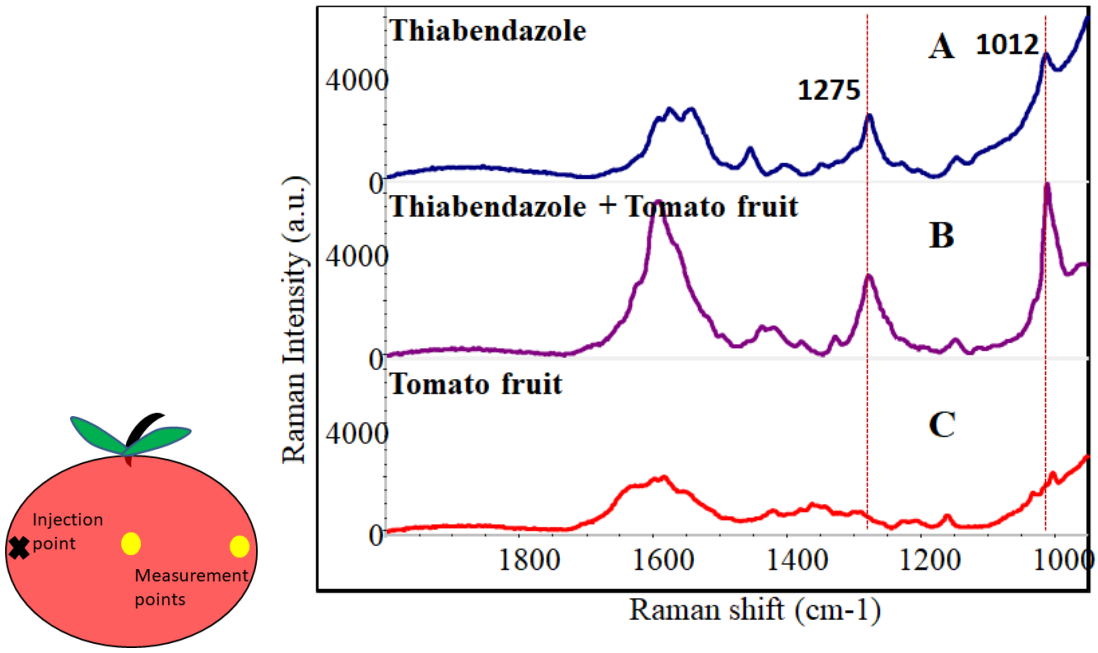

Figure 10. Raman spectra of (A) immersing 100 ppm thiabendazole solution with the SERS needle, (B) harvested organic tomato fruit injected with 100 ppm thiabendazole solution, (C) harvested organic tomato fruit with no pesticide injected.

4.3.2 Real time monitoring of the translocation of thiabendazole in tomato fruits

Systemic fungicide can be absorbed and transported to stems, foliage, flower, and fruit. We monitor thiabendazole translocation to tomato fruits in a hydroponic system. Tomato plants were removed and exposed to 200 ppm thiabendazole and Hoagland solution after they grown fruits. The measurement was conducted before the exposure, and on the 10^{th}, 20^{th}, and 30^{th} day during the exposure. To detect the internalized pesticide in tomato fruit, a SERS needle was inserted in the fruit to capture the pesticide, and then removed and measured with Raman spectroscopy. We successfully detected the signals from pesticide thiabendazole on the 30th day from tomato fruit by looking at the previously characterized Raman peaks at 1012 and 1275 cm^{-1} (Figure 11(A)). We analyzed the spectra with principal component analysis (PCA). The PCA plot showed clear discrimination among day 0, day 20, day 30 and the positive control, which

demonstrated the significant statistical difference of their spectra. What is more, the spectra of day 10 overlapped with day 0 and day 20 and cannot be separated out, which showed the spectra of day 10 is a transit from day 0 and day 20. The result confirmed that pesticide thiabendazole can be translocated in tomato plant and ultimately showed up in tomato fruit after 30 days.

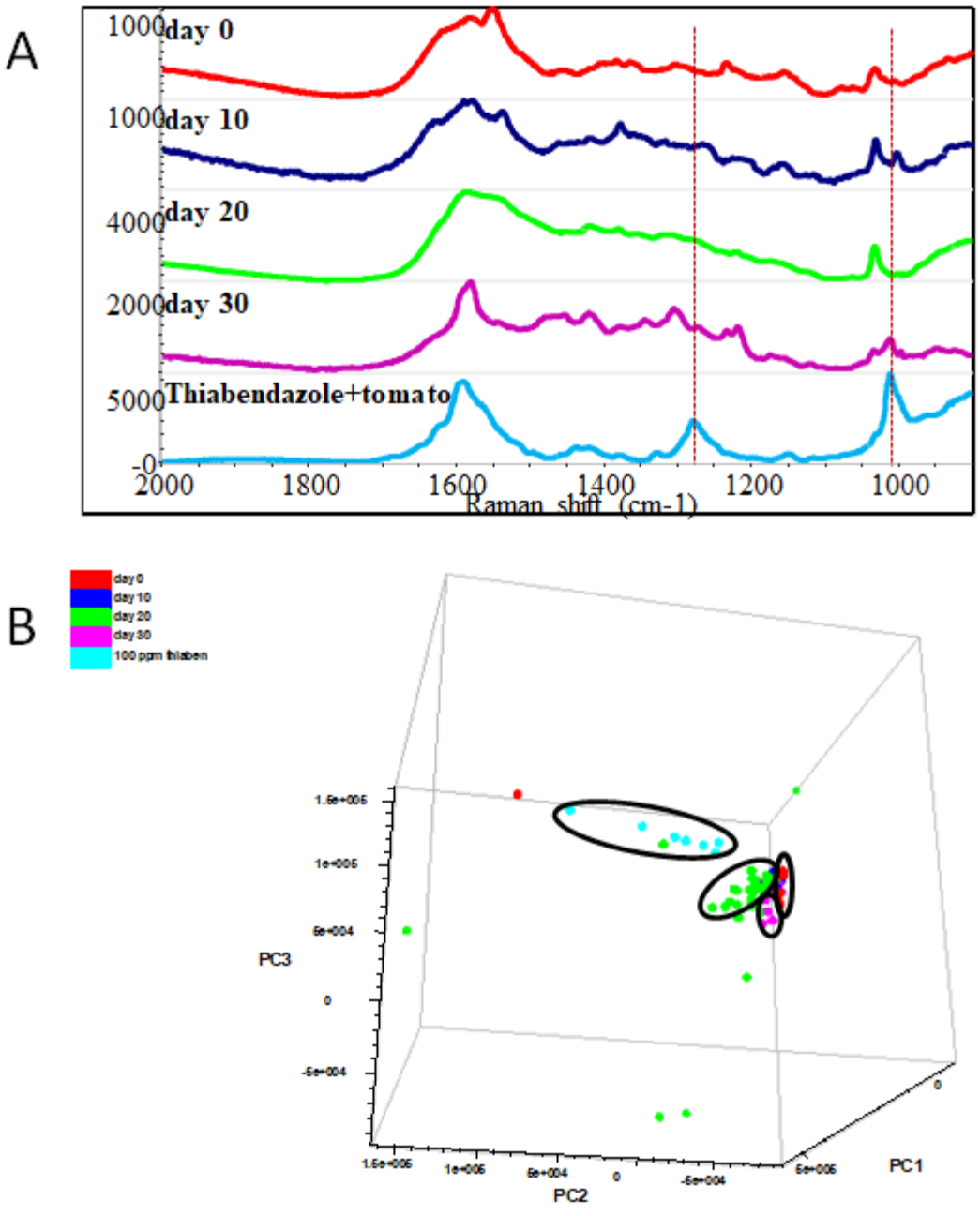

Figure 11. (A)Raman spectra of on tomato fruits after pesticide thiabendazole exposure and positive control at harvested tomato fruit inserted with thiabendazole. (B) a PCA plot of the spectral data of Figure 11(A).

4.4 Conclusion

This work demonstrated the application of SERS needle in real time monitor of pesticide translocation in plant. Using the SERS needle, the pesticide thiabendazole was characterized solely and inside tomato fruit with Raman. Thiabendazole has characteristic Raman peaks at 1012 and 1275 cm^{-1}, and the peak at 1012 cm^{-1} was selected for analysis due to its good consistency. Tomato fruit was injected with pesticide and the SERS needle detected it at different points, indicating that the pesticide can spread and migrate inside the fruit. Moreover, the SERS needle also monitored that thiabendazole translocation to tomato fruit after 30 days exposure. The SERS needle provides the benefits of simple and easy extraction preparation and *in situ* monitor of pesticide translocation compared to LC method. In future work, the result should be validated using LC method.

CHAPTER 5

HEADSPACE SOLID PHASE MICROEXTRACTION FOR THE DETECTION OF VOLATILE COMPOUNDS IN THE SPOILAGE OF RAW GROUND BEEF USING SERS NEEDLE

5.1 Introduction

5.1.1 Ground meat spoilage and volatiles

Odor, flavor and taste are related to meat quality and have significant impact on customers' acceptance. Unlike foodborne illness, food spoilage relates to freshness and shelf life. Along the aging time of raw meat, the color, texture, and odor of the raw meat change due to the oxidation and microbial metabolism, and spoilage occurs[61]. Moreover, ground meat degrades more quickly than a whole muscle meat. It is because ground meat has greater surface area exposed to air, higher microbial contamination risk during grinding processing, and the larger loss of intracellular reductant as well as more oxidant and enzyme released to oxidize myoglobin and cause browning[62].

The volatile profile of ground meat from lipid oxidation and microbial metabolism were well studied using GC-MS[8,9,62]. It is reported that the microbes that responsible for sulfury-associated spoilage were *pseudomonads* and related gram-negative organisms. Several volatile compounds are highlighted for spoilage indicators[9]. Currently, GC method and sensory analysis are employed to evaluate meat spoilage volatiles, while running GC takes loads of time and cannot provide a real-time monitor. Sensory panel training is more time and cost consuming. What is more, if microbial analysis is in demand, a traditional microbial plate count takes much more time for analysis. Hence, a rapid and real-time analytical method for meat spoilage is in need.

5.1.2 Objective of this study

In Chapter 2, several spoilage indicators were used to test the sensitivity and effectiveness of the SERS needle and received good outcomes. Herein, we applied the SERS needle to monitor spoilage from ground beef using headspace-SPME technique. We also use GC method and conventional plate count to validate the SERS result and identify the source of spoilage volatiles.

5.2 Materials and method

5.2.1 Preparation of ground meat

Fresh ground beef (20% fat and 80% lean), ground chicken and ground pork were purchased from local Stop & Shop (Amherst, MA). The meat was stored at -70℃ until analysis. Changes in the volatile profile of the meat samples were measured after 0-48 hours at room temperature.

5.2.2 Microbial analysis and selecting isolates

The aerobic plate count and selection of isolates were obtained by stomaching a 1:10 dilution of 10 grams of fresh or spoiled ground beef in 0.9% sterilized salt water. Serial dilutions of the stomached ground beef mixture were made in 0.1% peptone broth. 0.1 mL solution of each dilution was spread on TSA plates and the petri dishes were incubated at 25℃ for 48 hours. For each determination, 2 samples were analyzed in duplicate and results averaged.

To select isolates, petri dishes containing 30 to 300 distinctly separated colonies were used. Isolates were chosen according to their appearance, color, edge, size, shape and surface texture. At least 20 isolates from all dilutions were selected and grown on TSA tubes at 25℃ for 24 hours to obtain pure cultures. Two selected isolates were

analyzed using the scheme for initial classification of isolates from ground beef (Figure 12)[8].

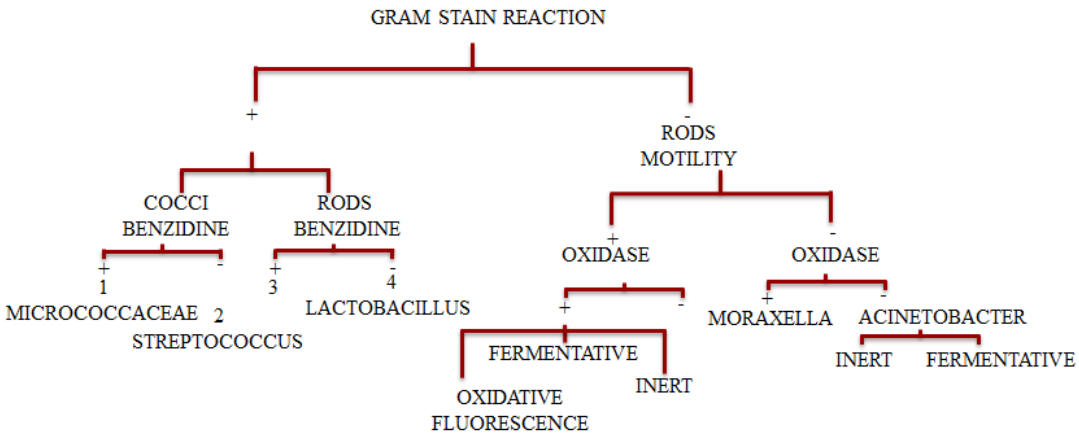

Figure 12. Scheme for initial classification of isolates from ground beef

5.2.3 Inoculation of sterile ground beef

Sterility of the ground beef was performed by freeze-thawing for multiple times to suppress gram-negative bacteria[63]. 6 isolates were selected to inoculate to sterile ground beef and considered the most representative of their group. All inoculations were conducted in an air flow hood. 1 mL of culture suspension was added to a small portion of ground beef and mixed. Inoculated beef was incubated under 25℃ for 24 hours prior to analysis.

5.2.4 SERS analysis

During spoilage measurement, 5 grams of ground meat was put in a 20-mL vial and the vial is sealed with parafilm. A SERS fiber was inserted to the headspace of vials to capture the volatile molecules. In the real-time monitor, the SERS fiber was measured every 2-hour until the observed spoilage (i.e., 54 hours). Another organic solvent extraction method was employed as well for comparison. 5 grams of spoiled or fresh

ground meat was mixed with 10 mL acetonitrile, then the mixture was vortaged for 1 minute and centrifuged at 2000 rpm for 10 minutes. 5 mL of upper liquid was collected for headspace detection for 30 minutes at 75℃, with addition of salt.

5.2.5 GC-SPME-headspace analysis

An optimized GC-SPME method was developed by other researchers to detect volatile profile of spoiled ground beef [62]. The 50/30 um DVB/CAR/PDMS coated SPME fiber was used for capturing the volatile compounds in the headspace of ground beef. For headspace-SPME, 1 gram of ground beef was placed in an 8-mL glass vial and covered with a PTFE/silicone septum. The analysis was performed at room temperature. A Shimadzu 2014 GC coupled with an auto-sampler was used in this experiment. The split ratio of injection port was 1:10. The carrier gas was at a flow rate of 1 mL/min. The injector port temperature for the fibers was 230℃, and the interface temperature was 250℃. The oven temperature was maintained at 40℃ for 5 minutes, programmed at 5℃/min to 200℃ and maintained at 200℃ during following analysis.

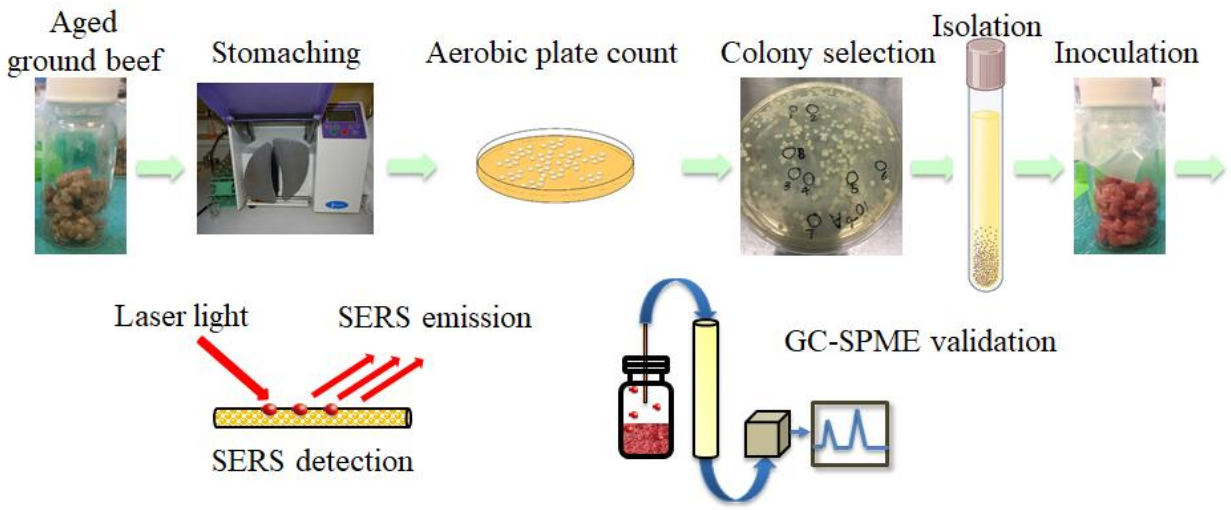

Figure 13. Schematic illustration of microbial analysis following by SERS analysis and GC-SPME-headspace validation. The aged beef was stomached to extract microorganisms, and the proper dilutions were used for aerobic plate count. The representative colonies were selected from each qualified plate and isolated to pure culture. The selected isolates were inoculated to fresh sterile beef and incubated. After aging, the spoiled ground beef was analyzed by SERS-SPME-headspace and GC-SPME-headspace.

5.3 Result and discussion

Preliminarily, ground beef, ground chicken and ground pork were all used to spoilage study using SERS needle, and only ground beef showed sufficient signals in the range of 900-2000 cm^{-1} Raman shift (Figure 14). Moreover, an organic solvent extraction method was employed to compare the extraction effectiveness demonstrated in Figure 15. After 24 to 48 hours of temperature abuse, the deleterious spoiled odor was developed in all ground meat, while direct headspace-SERS method showed consistent and obvious signals from ground beef instead of ground chicken and ground pork. In contrast, although the organic solvent acetonitrile brought the deleterious odor out from the sentimental meat after centrifugation, the extraction method did not bring up consistent signals. Since the spectra patterns from fresh meat and spoiled meat were different, the

organic solvent could extract the meat flavor as well and interfere the spoiled compounds' signals. Therefore, the organic solvent extraction method did not work well. The ground beef with 20% fat and 80% lean was chosen for later microbial analysis due to its consistent signals.

To monitor the real-time spoilage and reach the lowest detectable level, the fresh ground beef experienced temperature abuse and was detected by the SERS needle every 2-hour until completed spoilage. In Figure 16(A), all spectra showed common peaks at 1410, 1300 and 950 cm^{-1} Raman shift, and the intensity increased along with the aging time and reach the maximum at 18 hours, and then decreased after. Then all the spectra were analyzed using PCA in Figure 16(B), and the result suggested the same trend. Normally, the concentration of the volatile compounds should increase during the aging time, while the intensity reached the peak at 18 hours in this experiment. It could because when using the same fiber testing the volatile spoilage compounds, one fiber reached maximum combination with analytes and could not bind more molecules. It could also because the microbial and chemical reactions went to the different stage and produced different volatile profile, so the ones causing signals at 1410, 1300 and 950 cm^{-1} Raman shift decreased. Therefore, it is important to identify the source of spoilage. Since the spoilage occurs at temperature abuse during a short period of time, the primary concern is microbial spoilage. A microbial analysis was followed up.

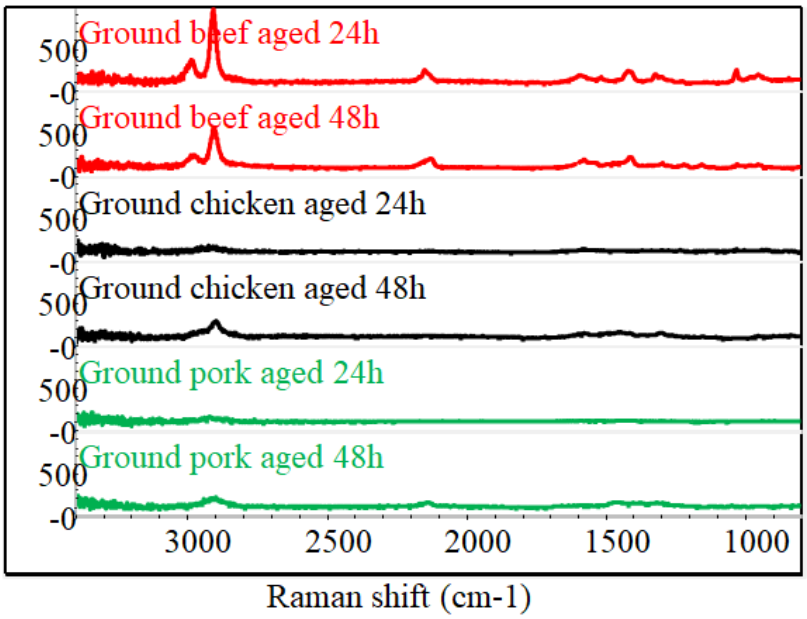

Figure 14. SERS spectra of aged ground beef, ground chicken and ground pork after 24 h and 48 h using direct headspace-SERS.

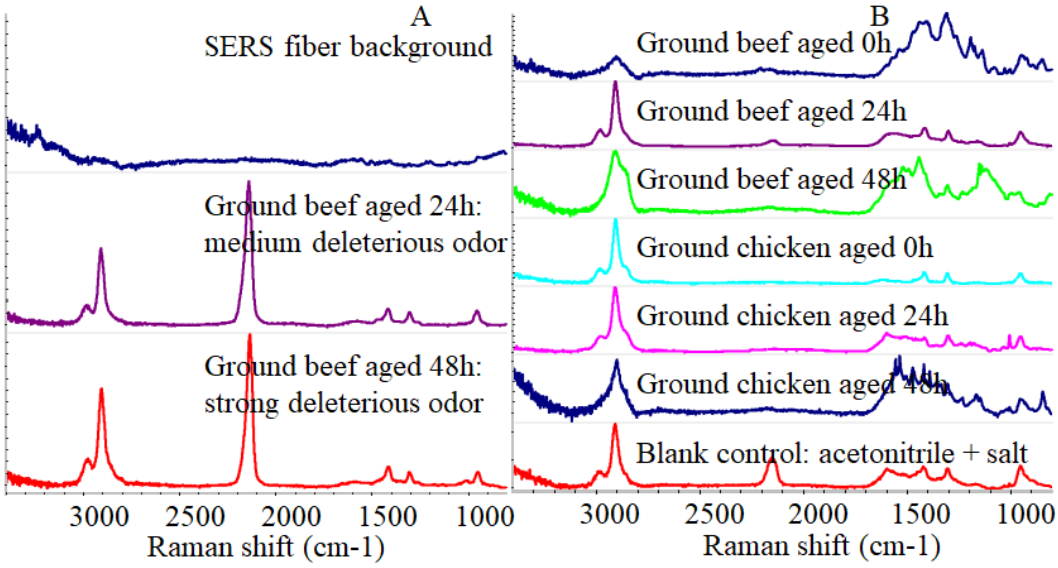

Figure 15. (A) SERS spectra of aged ground beef after 0h, 24h, and 48h using direct headspace-SERS, (B) SERS spectra of aged ground beef and ground chicken after 0h, 24h and 48h and blank control using organic solvent extraction method.

After 48 hours of temperature abuse, the ground beef was stomached, and the microorganisms were plated on TSA plates. 22 colonies were isolated based on the appearance such as color, shape, size, edge, and texture. Six isolates (labelled bacteria 1-6) were considered the most representative ones from the plates and inoculated to fresh ground beef to grow. The sterilization of ground beef was performed by freeze-thawing for multiple cycles which can greatly suppress the gram-negative microbes that are believed to be dominant in odor-producing spoilage microorganisms [8]. After 24-hour incubation, ground beef inoculated with bacteria 1 produced the most similar signals compared to naturally spoiled ground beef (Figure 17(A)). Other groups produced reduced or minimal signals compared with group 1. Hence, we proposed that bacteria 1 is mainly responsible to produce the volatile compounds that cause the same Raman signals from naturally spoiled ground beef. We continue conducted gram staining test, morphology observation under microscope and oxidase test to further confirm bacteria 1 as *Lactobacillus* using Figure 12. Another study reported that *Lactobacillus* species produce tyramine and hydrogen sulfide in beef [22]. The Raman signals from the spoiled ground beef partially match allyl methyl sulfide in Figure 17(B), suggesting that the detected compounds could contain similar structure to this molecule. Nevertheless, more solid validation should be made in the future study. GC-SPME-headspace method was used to differentiate the difference between SERS detected samples and non-detected samples, while the result did not suggest any difference among samples. The most critical issue of this study is the inconsistency of the signals because it is hard to control the source of spoilage. As mentioned in the introduction, ground meat has greater risk of contamination and higher chance of microbial and chemical oxidation, it is hard to

maintain the same situation and starting microbiota of different purchased ground beef in this study. Moreover, future study should meet the consumer acceptance by improving the sensitivity of determining spoilage. Although researchers use sensitive techniques like electronic nose and GC to detect meat spoilage, it is reported that human nose is the most sensitive tool. Exceeding its sensitivity is one of the challenges.

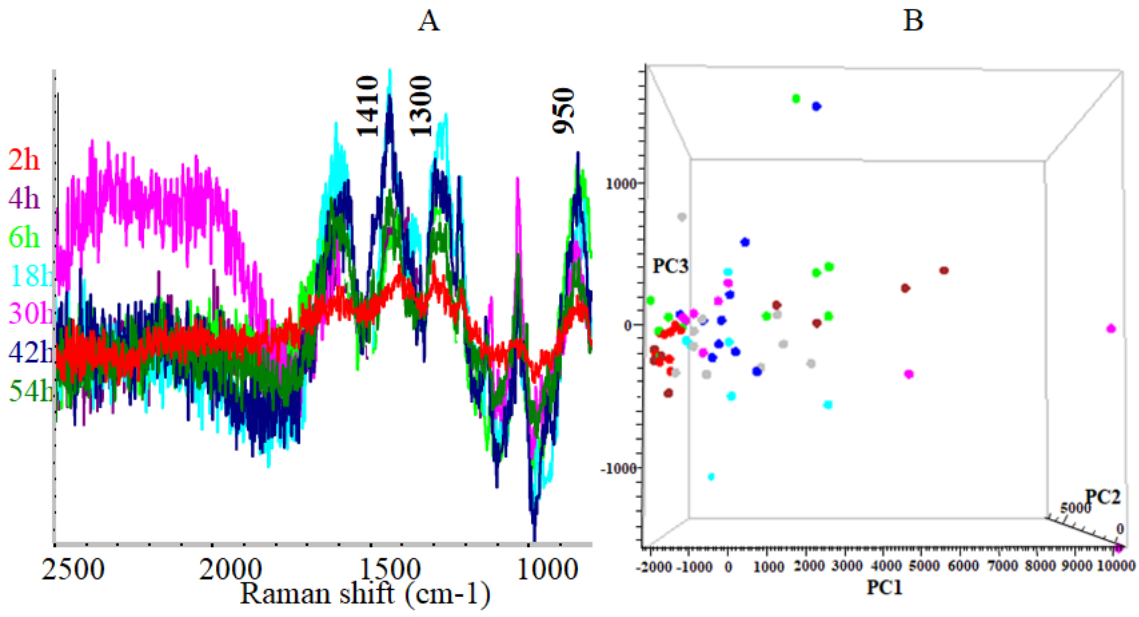

Figure 16. (A)SERS spectra and (B)PCA plot of real-time monitor of ground beef spoilage from 2 h to 54 h.

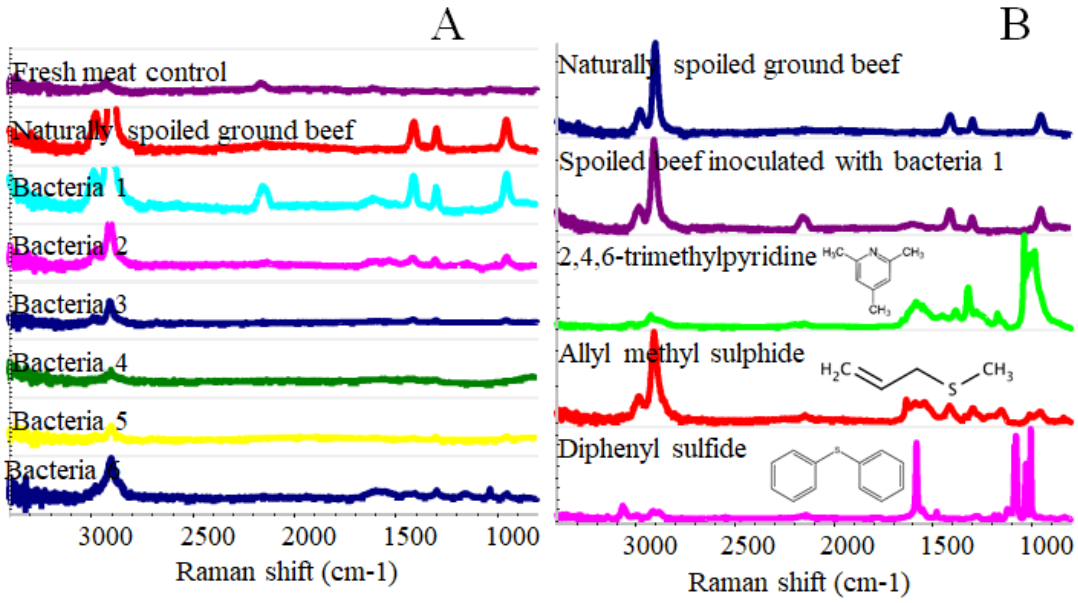

Figure 17. (A) SERS spectra of fresh ground beef, naturally spoiled ground beef, and spoiled ground beef inoculated with bacteria 1 to 6. (B) SERS spectra of naturally spoiled ground beef, bacteria 1 inoculated spoiled ground beef, and spoilage biomarkers detection: 2, 4, 6 trimethylpyridine, allyl methyl sulfide and diphenyl sulfide.

5.4 Conclusion

In this study, we used headspace-SERS to detect volatile spoilage compounds from ground beef, since microbial VOCs serve as the indicator for meat spoilage. Preliminarily, spoiled ground beef showed sufficient signals other than ground chicken and ground pork, and the direct headspace approach behaved better than organic solvent extraction. Therefore, ground beef (20% fat and 80% lean) and direct headspace approach were chosen for following study. A real-time monitor of spoilage was performed within 54 hours when the SERS needle was measured every 2h. The spoilage spectra were recorded, and the signals reached maximum at 18 hours. It can because the SERS needle reached maximum combination, and/or the spoilage went to a different stage with different products that did not cause the same SERS signals. The initial microorganisms

from spoiled ground beef generating the SERS signals was identified to be *Lactobacillus*. The spoilage signal from this study was similar to a spoilage biomarker: allyl methyl sulfide, suggesting that a similar structural molecule may present. Future study is needed to validate the results, identify the chemical compounds and improve the sensitivity.

CHAPTER 6

CONCLUSION

This work invented a gold-nanoparticles coated SERS needle for one-step multi-phase sample detection. The SERS fiber is capable for liquid and gas phase sample detection simultaneously using dip and headspace approaches. Moreover, the headspace approach offered better sensitivity and effectiveness than dip method when testing VOCs because the volatile compounds evaporate into headspace. With the SERS needle, we overcame the difficulty in detecting volatile compounds from gas phase in SERS research. Other than volatile pesticides detection, the SERS needle also works well in dipping into solution and testing non-volatile pesticides. To our best knowledge, it is the first study to combine SERS and headspace in detecting vapor fraction of samples which also provides a simple and easy way for multi-phase analysis. What is more, the SERS needle showed its advantage to penetrate and insert into soften bio-sample like tomato fruit to detect the internal analytes with controlled depth, minimum invasion and sample pre-treatment. By inserting into tomato fruits, the SERS needle detected internalized pesticide thiabendazole and real-time monitored pesticide translocation in tomato plants *in situ*. The SERS needle further exhibited its benefits in microbial VOCs detection and real-time monitor in ground beef spoilage study. Overall, the SERS needle coupled with headspace is an alternative method to detect VOCs, offering the advantages of minimum sample preparation, rapid detection and satisfying sensitivity compared to current GC/LC methods in food industry.

In future work, the SERS needle can work with a variety of food samples with multiple phases, such as tea, wine, and cheese that their flavor fraction is important for

food quality. What is more, since microbial VOCs are utilized a lot in clinical specimens and taxonomic studies, the SERS needle can be incorporated in more studies like breath analysis.

REFERENCE

(1) Chauhan, R. S.; Singhal, L. Harmful Effects of Pesticides and Their Control Through Cowpathy. *Int. J. Cow Sci.* **2006**, *2* (1), 61–70.

(2) LeDoux, M. Analytical Methods Applied to the Determination of Pesticide Residues in Foods of Animal Origin. A Review of the Past Two Decades. *Journal of Chromatography A*. **2011**, *1218* (8), 1021-1036.

(3) Solomon, G. M.; Huddle, A. M. Low Levels of Persistent Organic Pollutants Raise Concerns for Future Generations. *Journal of Epidemiology and Community Health*. **2002**, *56* (11), 826-827.

(4) Erwin, D. C. Systemic Fungicides : Disease Control , Translocation , and Mode of Action. *Annu. Rev. Phytopathol.* **1973**, *11* (1), 389–422.

(5) Ben-Aziz, A.; Aharonson, N. Dynamics of Uptake, Translocation, and Disappearance of Thiabendazole and Methyl-2-Benzimidazolecarbamate in Pepper and Tomato Plants. *Pestic. Biochem. Physiol.* **1974**, *4* (2), 120-126.

(6) Hedberg, C. W.; Bender, J. B.; Sampedro, F.; Wells, S. J. Potential Impacts of a Zero Tolerance Policy for Salmonella on Raw Meat and Poultry. **2014**.

(7) Schnürer, J.; Olsson, J.; Börjesson, T. Fungal Volatiles as Indicators of Food and Feeds Spoilage. *Fungal Genet. Biol.* **1999**, *27* (2–3), 209–217.

(8) STUTZ, H. K.; LEVIN, R. E. Bacteria and Volatile Compounds Associated with Ground Beef Spoilage. *J. Food Sci.* **1991**, *56* (5), 1147–1153.

(9) Lovestead, T. M.; Bruno, T. J. Detection of Poultry Spoilage Markers from Headspace Analysis with Cryoadsorption on a Short Alumina PLOT Column. *Food Chem.* **2010**, *121* (4), 1274–1282.

(10) Beltran, J.; Lopez, F. J.; Hernandez, F. Solid-Phase Microextraction in Pesticide Residue Analysis. *J Chromatogr.A* **2000**, *885* (1–2), 389–404.

(11) Sharma, D.; Nagpal, A.; Pakade, Y. B.; Katnoria, J. K. Analytical Methods for Estimation of Organophosphorus Pesticide Residues in Fruits and Vegetables: A Review. *Talanta* **2010**, *82* (4), 1077–1089.

(12) Snow, N. H.; Slack, G. C. Head-Space Analysis in Modern Gas Chromatography. *TrAC - Trends Anal. Chem.* **2002**, *21* (9–10), 608–617.

(13) Famiglini, G.; Palma, P.; Pierini, E.; Trufelli, H.; Cappiello, A. Organochlorine Pesticides by LC-MS. *Anal. Chem.* **2008**, *80* (9), 3445-3449.

(14) Doong, R. a; Liao, P. L. Determination of Organochlorine Pesticides and Their Metabolites in Soil Samples Using Headspace Solid-Phase Microextraction. *J. Chromatogr. A* **2001**, *918* (1), 177-188.

(15) Ng, W. F.; Teo, M. J. K.; Lakso, H.-Å. Determination of Organophosphorus Pesticides in Soil by Headspace Solid-Phase Microextraction. *Fresenius. J. Anal. Chem.* **1999**, *363* (7), 673-679.

(16) Gonzalez, M.; Miglioranza, K. S. B.; Aizpún De Moreno, J. E.; Moreno, V. J. Occurrence and Distribution of Organochlorine Pesticides (OCPS) in Tomato (Lycopersicon Esculentum) Crops from Organic Production. *J. Agric. Food Chem.* **2003**, *51* (5), 1353-1359.

(17) Lambropoulou, D. A.; Albanis, T. A. Methods of Sample Preparation for Determination of Pesticide Residues in Food Matrices by Chromatography-Mass Spectrometry-Based Techniques: A Review. *Analytical and Bioanalytical Chemistry*. **2007**, *389* (6), 1663-1683.

(18) Pawliszyn, J. NEW DIRECTIONS IN SAMPLE PREPARATION FOR ANALYSIS OF ORGANIC-COMPOUNDS. *TrAC-Trends Anal. Chem.* **1995**, *14* (3), 113–122.

(19) Prosen, H.; Zupančič-Kralj, L. Solid-Phase Microextraction. *TrAC - Trends Anal. Chem.* **1999**, *18* (4), 272–282.

(20) Schöller, C.; Molin, S.; Wilkins, K. Volatile Metabolites from Some Gram-Negative Bacteria. *Chemosphere* **1997**, *35* (7), 1487-1495.

(21) Filipiak, W.; Sponring, A.; Baur, M. M.; Filipiak, A.; Ager, C.; Wiesenhofer, H.; Nagl, M.; Troppmair, J.; Amann, A. Molecular Analysis of Volatile Metabolites Released Specifically by Staphylococcus Aureus and Pseudomonas Aeruginosa. *BMC Microbiol.* **2012**, *12* (1), 113.

(22) Edwards, R. A.; Dainty, R. H.; Hibbard, C. M.; Ramantanis, S. V. Amines in Fresh Beef of Normal PH and the Role of Bacteria in Changes in Concentration Observed during Storage in Vacuum Packs at Chill Temperatures. *J. Appl. Bacteriol.* **1987**, *63* (5), 427-434.

(23) Leisner, J. J.; Greer, G. G.; Stiles, M. E. Control of Beef Spoilage by a Sulfide-Producing Lactobacillus Sake Strain with Bacteriocinogenic Leuconostoc Gelidum UAL187 during Anaerobic Storage at 2°C. *Appl. Environ. Microbiol.* **1996**, *62* (7), 2610-2614.

(24) Dainty, R. H. Chemical/Biochemical Detection of Spoilage. *Int. J. Food Microbiol.* **1996**, *33* (1), 19-33.

(25) Ordóñez, J. A.; de Pablo, B.; de Castro, B. P.; Sanz, B.; Asensio, M. A. Selected Chemical and Microbiological Changes in Refrigerated Pork Stored in Carbon Dioxide and Oxygen Enriched Atmospheres. *J. Agric. Food Chem.* **1991**, *39* (4), 668-672.

(26) Boskou, G.; Debevere, J. Reduction of Trimethylamine Oxide by Shewanella Spp. under Modified Atmospheres in Vitro. *Food Microbiol.* **1997**, *14* (6), 543-553.

(27) Ndip, R. N.; MacKay, W. G.; Farthing, M. J. G.; Weaver, L. T. Culturing Helicobacter Pylori from Clinical Specimens: Review of Microbiologic Methods. *Journal of Pediatric Gastroenterology and Nutrition*. **2003**, *36* (5), 616-622.

(28) Sohrabi, M.; Zhang, L.; Zhang, K.; Ahmetagic, A.; Wei, M. Q. Volatile Organic Compounds as Novel Markers for the Detection of Bacterial Infections. *Clin. Microbiol: open access.* **2014**.

(29) Wong, C. L.; Dinish, U. S.; Schmidt, M. S.; Olivo, M. Non-Labeling Multiplex Surface Enhanced Raman Scattering (SERS) Detection of Volatile Organic Compounds (VOCs). *Anal. Chim. Acta* **2014**, *844*, 54–60.

(30) Haynes, C. L.; Mcfarland, A. D.; Duyne, R. P. Van. RAMAN SPECTROSCOPY. *Anal. Chem.* **2005**, 338A-346A.

(31) Vongsvivut, J.; Robertson, G.; Mcnaughton, D. Surface-Enhanced Raman Spectroscopic Analysis of Fonofos Pesticide Adsorbed on Silver and Gold Nanoparticles. *J. Raman Spectrosc.* **2010**, *41* (10), 1137–1148.

(32) Buonasera, K.; D'Orazio, G.; Fanali, S.; Dugo, P.; Mondello, L. Separation of Organophosphorus Pesticides by Using Nano-Liquid Chromatography. *J. Chromatogr. A* **2009**, *1216* (18), 3970–3976.

(33) Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I. Single Molecule Detection Using Surface-Enhanced Raman Scattering (SERS). *Phys. Rev. Letters.* **1997**, *78* (9), 1667.

(34) Lutz Alder, Kerstin Greulich, Gunther Kempe, and B. V. Residue Analysis of 500 High Priority Pesticides: Better by GC-MS or LC-MS/MS? *Mass Spectrom. Rev.* **2006**, *25*, 838–865.

(35) Shende, C.; Gift, A.; Inscore, F.; Maksymiuk, P.; Farquharson, S. Inspection of Pesticide Residues on Food by Surface-Enhanced Raman Spectroscopy. *Monit. Food Safety, Agric. Plant Heal.* **2004**, *5271*, 28–35.

(36) Zheng, J.; He, L. Surface-Enhanced Raman Spectroscopy for the Chemical Analysis of Food. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 317–328.

(37) Lin, M.; He, L.; Awika, J.; Yang, L.; Ledoux, D. R.; Li, H.; Mustapha, A. Detection of Melamine in Gluten, Chicken Feed, and Processed Foods Using Surface Enhanced Raman Spectroscopy and HPLC. *J. Food Sci.* **2008**, *73* (8), T129-T134.

(38) Pang, Shintaro, Theodore P. Labuza, and L. H. Development of a Single Aptamer-Based Surface Enhanced Raman Scattering Method for Rapid Detection of Multiple Pesticides. *Analyst* **2014**, *139* (8), 1895–1901.

(39) Yang, T.; Doherty, J.; Zhao, B.; Kinchla, A. J.; Clark, J. M.; He, L. Effectiveness of Commercial and Homemade Washing Agents in Removing Pesticide Residues on and in Apples. *J. Agric. Food Chem.* **2017**, *65* (44), 9744-9752.

(40) Yang, T.; Zhao, B.; Kinchla, A. J.; Clark, J. M.; He, L. Investigation of Pesticide Penetration and Persistence on Harvested and Live Basil Leaves Using Surface-Enhanced Raman Scattering Mapping. *J. Agric. Food Chem.* **2017**, *65* (17), 3541–3550.

(41) Wong, C. L.; Dinish, U. S.; Buddharaju, K. D.; Schmidt, M. S.; Olivo, M. Surface-Enhanced Raman Scattering (SERS)-Based Volatile Organic Compounds (VOCs) Detection Using Plasmonic Bimetallic Nanogap Substrate. *Appl. Phys. A Mater. Sci. Process.* **2014**, *117* (2), 687–692.

(42) Dong, J.; Tao, Q.; Guo, M.; Yan, T.; Qian, W. Glucose-Responsive Multifunctional Acupuncture Needle: A Universal SERS Detection Strategy of Small Biomolecules in Vivo. *Anal. Methods* **2012**, *4* (11), 3879-3883.

(43) Dong, J.; Chen, Q.; Rong, C.; Li, D.; Rao, Y. Minimally Invasive Surface-Enhanced Raman Scattering Detection with Depth Profiles Based on a Surface-Enhanced Raman Scattering-Active Acupuncture Needle. *Anal. Chem.* **2011**, *83* (16), 6191-6195.

(44) Yuen, C.; Liu, Q. Towards in Vivo Intradermal Surface Enhanced Raman Scattering (SERS) Measurements: Silver Coated Microneedle Based SERS Probe. *J. Biophotonics* **2014**, *7* (9), 683-689.

(45) Liu, C.; Zhang, X.; Li, L.; Cui, J.; Shi, Y.; Wang, L.; Zhan, J. Silver Nanoparticle Aggregates on Metal Fibers for Solid Phase Microextraction–surface Enhanced Raman Spectroscopy Detection of Polycyclic Aromatic Hydrocarbons. *Analyst* **2015**, *140* (13), 4668–4675.

(46) Lan, X.; Han, Y.; Wei, T.; Zhang, Y.; Jiang, L.; Tsai, H.-L.; Xiao, H. Surface-Enhanced Raman-Scattering Fiber Probe Fabricated by Femtosecond Laser. *Opt. Lett.* **2009**, *34* (15), 2285–2287.

(47) Li, B.; Shi, Y. E.; Cui, J.; Liu, Z.; Zhang, X.; Zhan, J. Au-Coated ZnO Nanorods on Stainless Steel Fiber for Self-Cleaning Solid Phase Microextraction-Surface Enhanced Raman Spectroscopy. *Anal. Chim. Acta* **2016**, *923*, 66–73.

(48) Liu, C.; Wang, S.; Chen, G.; Xu, S.; Jia, Q.; Zhou, J.; Xu, W. A Surface-Enhanced Raman Scattering (SERS)-Active Optical Fiber Sensor Based on a Three-Dimensional Sensing Layer. *Sens. Bio-Sensing Res.* **2014**, *1*, 8–14.

(49) Yang, Y.; Li, Y.; Liu, H.; Wang, X.; Du, X. Electrodeposition of Gold Nanoparticles onto an Etched Stainless Steel Wire Followed by a Self-Assembled Monolayer of Octanedithiol as a Fiber Coating for Selective Solid-Phase Microextraction. *J. Chromatogr. A* **2014**, *1372*, 25–33.

(50) Mosier-Boss, P. .; Lieberman, S. . Detection of Volatile Organic Compounds Using Surface Enhanced Raman Spectroscopy Substrates Mounted on a Thermoelectric Cooler. *Anal. Chim. Acta* **2003**, *488* (1), 15-23.

(51) U.S. Environmental Protection Agency (EPA). EPA Report 815-R-08-012. Chapter 9 : Fonofos. In Regulatory Determinations Support Document for Selected Contaminants from the Second Drinking Water Contaminant Candidate List (CCL 2); **2008**; 1–26.

(52) Shende, C. S.; Inscore, F.; Gift, A.; Maksymiuk, P.; Farquharson, S. Analysis of Pesticides on or in Fruit by Surface-Enhanced Raman Spectroscopy. *Nondestruct. Sens. food safety, Qual. Nat. Resour.* **2004**, *5587*, 170–177.

(53) Qu, Y.; Tan, C.; Zhang, Z.; He, L. A Facile Solvent Mediated Self-Assembly Silver Nanoparticle Mirror Substrate for Quantitatively Improved Surface Enhanced Raman Scattering. *Analyst* **2017**, *142* (21), 4075–4082.

(54) Song, W.; Zhang, Y.; Guo, M.; Wang, T.; Yang, Y.; Wang, X.; Du, X. Rapid Electrochemical Preparation of Porous Sponge-like Zinc–zinc Oxide Coating Deposited on an Etched Stainless Steel Fiber for Selective Determination of UV Filters in Environmental Water Samples. *Anal. Methods* **2015**, *7* (16), 6619–6628.

(55) Lee, Y.-H.; Farquharson, S. SERS Sample Vials Based on Sot-Gel Process for Trace Pesticide Analysis. *Photonic Detect. Interv. Technol. Safe Food.* **2001**, *4206*, 140–147.

(56) Ich. ICH Topic Q2 (R1) Validation of Analytical Procedures : Text and Methodology. *Int. Conf. Harmon.* **2005**, *1994* (November 1996), 17.

(57) Li, H.-P.; Li, G.-C.; Jen, J.-F. Fast Multi-Residue Screening for 84 Pesticides in Tea by Gas Chromatography with Dual-Tower Auto-Sampler, Dual-Column and Dual Detectors.

(58) Sicbaldi, F.; Sacchi, G. A.; Trevisan, M.; Del Re, A. A. M. Root Uptake and Xylem Translocation of Pesticides from Different Chemical Classes. *Pestic. Sci.* **1997**.

(59) Jankowska, M.; Kaczynski, P.; Hrynko, I.; Lozowicka, B. Dissipation of Six Fungicides in Greenhouse-Grown Tomatoes with Processing and Health Risk. *Environ. Sci. Pollut. Res.* **2016**.

(60) Yang, T.; Zhang, Z.; Zhao, B.; Hou, R.; Kinchla, A.; Clark, J. M.; He, L. Real-Time and in Situ Monitoring of Pesticide Penetration in Edible Leaves by Surface-Enhanced Raman Scattering Mapping. *Anal. Chem.* **2016**.

(61) Mottram, D. S. Flavour Formation in Meat and Meat Products : A Review. *Food Chem.* **1998**, *62* (4), 415–424.

(62) Perez, R. A.; Rojo, M. D.; Gonzalez, G.; Lorenzo, C. De. Solid-Phase Microextraction for the Determination of Volatile Compounds in the Spoilage of Raw Ground Beef. *J AOAC Int* **2008**, *91* (6), 1409–1415.

(63) Walker, V. K.; Palmer, G. R.; Voordouw, G. Freeze-Thaw Tolerance and Clues to the Winter Survival of a Soil Community. *Appl. Environ. Microbiol.* **2006**, *72* (3), 1784–1792.