July 2020

DEVELOPMENT OF HEADSPACE ANALYSIS OF LIVING AND POSTHARVEST FRESH PRODUCE USING SURFACE-ENHANCED RAMAN SPECTROSCOPY (SERS)

Xinyi Du

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A Dissertation Presented

by

XINYI DU

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 2020

Food Science

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Xinyi Du

Approved as to style and content by:

_____
Lili He, Chair

_____
Hang Xiao, Member

_____
Amanda Kinchla, Member

_____
Eric Decker, Department Head
Food Science

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Lili He, for her patience and constant motivation on my master study and life. Her high efficiency in work and multiple perspectives opinions for research stimulate me to move forward in food science study. I am grateful to be her student.

I would also like to thank my committee members Dr. Hang Xiao and Dr. Amanda Kinchla for their time and research recommendations. Finally, a special thanks to all my lab mates for their feedback, cooperation and of course friendship.

ABSTRACT

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MAY 2020

XINYI DU, B.A., NANJING NORMAL UNIVERSITY

M.A., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Lili He

The increasing market demand for fresh produce promotes a keen interest in developing a rapid, sensitive and reliable method for monitoring plant health and determining the shelf-life of postharvest produce. The objective of this study is to explore the capability of Surface-enhanced Raman spectroscopy (SERS) in these applications. SERS integrates Raman spectroscopy which measures molecular vibrations and nanotechnology which enhances the weak Raman signals. Herein, we developed two SERS methods based on a surface detection approach using nanoparticles solution and a headspace detection approach using gold nanoparticles (AuNPs) fibers, to detect biochemical changes during postharvest storage of arugula leaves. Compared with surface detection, the headspace detection revealed significant spectral changes during the storage, particularly in the shifts around 500, 950 and 1030 cm^{-1}. These changes analyzed using principal component analysis (PCA) to establish a prediction model for shelf-life determination. Through analyzing reference standard compounds, we identified the dimethyl disulfide (DMDS), 1-propanethiol and methanethiol (MT) were most likely to account for the signature spectra of headspace arugula at the late storage period due to the activities of spoilage bacteria. The headspace detection method was also applied to monitor the stress responses of living basil to abiotic stresses (pesticide/salinity). However, the volatile analysis of the basil plants response to abiotic stresses (pesticide/salinity) showed indistinctive

results. In conclusion, the headspace detection based on SERS provides a new strategy for quality monitoring of fresh produce in the food industry.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS iv

ABSTRACT v

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF ABBREVIATIONS xiii

CHAPTER

1. INTRODUCTION 1

1.1 Justification 1

1.1.1 Living plants stress response and plant phenotyping 1
1.1.2 Postharvest produce shelf-life and quality 2

1.2 Traditional detection technology 4

1.2.1 Traditional detection methods for stresses of preharvest produce 4
1.2.2 Traditional detection methods for shelf-life of postharvest produce 5

1.3 Headspace detection technology 6
1.4 Surface-enhanced Raman spectroscopy (SERS) 7
1.5 Goals and objectives of the study 8

2. DEVELOPMENT OF SURFACE DETECTION METHOD FOR REAL-TIME FRESHNESS OF ARUGULA WITH SERS 10

2.1 Introduction 10

2.1.1 Colloid-based substrates 10
2.1.2 The degradation reactions of fresh-cut arugula leaves during storage 11
2.1.3 Objectives of this study 11

2.2 Material and methods 11

2.2.1 Materials 11
2.2.2 Preparation of fresh-cut arugula samples for surface detection 12
2.2.3 Raman Instrument and data analysis 12
2.2.4 Standard plate count 13

2.3 Result and discussion 13

2.3.1 Characterization of arugula leaves SERS spectra with nanoparticles 13
2.3.2 Characterization of arugula leaves SERS spectra over time 14
2.3.3 Model design for monitoring the shelf-life of fresh-cut arugula samples over time 18

2.4 Conclusion 19

3. MONITORING THE SHELF-LIFE OF FRESH-CUT ARUGULA LEAVES BASED ON HEADSPACE ANALYSIS USING A SERS-ACTIVE FIBER 21

3.1 Introduction 21

3.1.1 Volatile metabolites in fresh-cut arugula 21
3.1.2 Solid surface-based substrates 22
3.1.3 Objectives of this study 23

3.2 Material and methods 23

3.2.1 Materials 23
3.2.2 Fabrication and characterization of gold nanoparticles coated fibers 24
3.2.3 Preparation of fresh-cut arugula samples for headspace detection 25
3.2.4 References compounds analysis 26
3.2.5 Raman Instrumentation and data analysis 26
3.2.6 Microbial analysis 27

3.3 Result and discussion 27

3.3.1 Characterization of the gold nanoparticle coated fiber 27

3.3.1.1 Optimization of incubation time 29

3.3.2 Characterization of arugula leaves SERS spectra over time 30
3.3.3 PCA analysis of the spectra over time and its application to predict unknown samples 33
3.3.4 In situ detection of arugula leaves within the original package 35
3.3.5 Chemical volatile compounds identification 36
3.3.6 Microbial analysis and isolates identification 41

3.4 Conclusion 42

4. DEVELOPMENT OF A HEADSPACE DETECTION METHOD FOR LIVING BASIL PLANTS STRESS RESPONSE 43

4.1 Introduction 43

4.1.1 Plant stress response and VOCs 43
4.1.2 Response of living basil to abiotic stress 44
4.1.3 Objectives of this study 45

4.2 Material and methods 45

4.2.1 Materials 45
4.2.2 Fabrication of gold nanoparticles coated fibers 45
4.2.3 Preparation of samples 45
4.2.4 Instrument and data analysis 46

4.3 Result and discussion 46

4.3.1 Characterization of the SERS spectra of living basil against pesticide 46
4.3.2 Characterization of the SERS spectra of living basil against high salinity 48

4.4 Conclusion 49

5. CONCLUSION 50

APPENDIX: SUPPLEMENTARY INFORMATION 51

BIBLIOGRAPHY 54

LIST OF TABLES

Table Page

1. Assignments of experimental SERS bands of postharvest arugula leaves under two degree of freshness: 7 days left on shelf-life and 0 days left on shelf-life 40

LIST OF FIGURES

Figure Page

1. Schematic illustration of the surface detection method.....12

2. Representative spectra of Arugula leaves, AgNPs background, AuNPs background, AgNPs on fresh arugula leaves and AuNPs on fresh arugula leaves.....14

3. (A) SERS spectra of arugula leaves under varying degrees of freshness using AgNPs and (B)SERS spectra of arugula leaves under varying degrees of freshness using AuNPs.....16

4. (A) arugula leaves picture exhibiting varying degrees of freshness and (B) Colony-forming units (CFU/g) among arugula leaves under varying degrees of freshness.....17

5. Schematic illustration of arugula samples structure.....18

6. SERS spectra of arugula leaves under varying degrees of freshness using AgNPs.....18

7. Principal component scores 3D Display model of the Surface Detection Method.....19

8. Schematic illustration of AuNPs coated fiber fabrication.....24

9. Schematic illustration of the headspace detection method. (A) arugula leaves incubated in glass bottles at 22±2°C (B) arugula leaves incubated in glass bottles at 4±2°C (C) arugula leaves incubated in original package at 4±2°C.....25

10. AuNPs coated fiber made by wire (ϕ203 μm) under (A) 50 μm SEM, (B) 10 μm SEM, (C) 2 μm SEM, (D) 50 nm SEM.....28

11. SERS spectra of DMS using AuNPs coated fiber over storage time.....29

12. SERS spectra of fresh arugula after 0.5h, 1h, 1.5h and 2h incubation using the AuNPs coated fiber and controls.....30

13. (A) SERS spectra of arugula leaves under varying degrees of freshness using AuNPs coated fiber and (B) Representative SERS spectra of arugula leaves in varying shelf-life using AuNPs coated fiber.....32

14. Comparison of Raman intensities of signature peaks and corresponding colony forming units (CFU/g) among arugula leaves under varying degrees of freshness over storage time.....33

15. Principal component scores 3D Display model of the headspace detection method.....33

16. (A) Arugula leaves purchased from Whole Foods Market Inc. that were in good appearance. (B) SERS spectra obtained from each respective sample 34

17. Comparison of SERS spectra of arugula leaves headspace obtained in glass containers and within the original package in varying shelf-life using AuNPs coated fiber 36

18. SERS spectra of relative volatile compounds: (a) arugula leaves have 7 days left on shelf-life, (b) arugula leaves have 1 day left on shelf life, (c) dimethyl disulfide, (d) methanethiol, (e) 1-propanethiol, (f) DMSO, (g) dimethyl trisulfide, (h) dimethyl sulfide, (i) S-MMTSO, (j) dipropyl trisulfide, (k) propyl disulfide, (l) allyl methyl sulfide, (m) methanesulfonic acid, (n) erucin, (o) allyl isothiocyanate, (p) diallyl disulfide, (q) ethylene, (r) allyl sulfide, (s) cis-3-Hexen-1-ol, (t) cis-3-Hexenyl butyrate and (u) limonene.....38

19. Representative SERS spectra of fresh-cut arugula have 7 days left on shelf-life and 1 day left on shelf-life with comparison to potential volatile compounds at 22±2°C.....39

20. Raman spectra of sterilized fresh arugula leaves, sterilized fresh arugula leaves inoculated with TSB solution, sterilized fresh arugula leaves inoculated with bacteria No.1 culture solution and sterilized fresh arugula leaves inoculated with bacteria No.2 culture solution 41

21. SERS spectra of living basil plants under stress of imidacloprid with control.....47

22. SERS spectra of living basil plants under stress of high salinity with control.....48

A 1. Principal component scores 3D Display model of AuNPs coated fibers for healthy living basil plants.....51

A 2. Principal component scores 3D Display model of AuNPs coated fibers for living basil plants under stress of imidacloprid with controls.....52

A 3. Principal component scores 3D Display model of AuNPs coated fibers for living basil plants under stress of high salinity with controls.....53

LIST OF ABBREVIATIONS

SERS - Surface-enhanced Raman spectroscopy

AuNPs - gold nanoparticles

AgNPs - silver nanoparticles

SNV - standard normal variate

PCA- principal component analysis

PLSR - partial least square regression

SAM - spectral angle mapper

DT - decision tree

GC-MS - Gas Chromatography-Mass Spectrometry

SPME - Solid-Phase Microextraction

GC-O - GC-Olfactometry

SIFT-MS- selected Ion Flow Tube-Mass Spectrometry

GC-FID - Chromatography-Flame Ionization Detector

PTR-MS - Proton Transfer Reaction-Mass Spectrometry

NMR - Nuclear Magnetic Resonance spectroscopy

LSPR - localized surface plasmon resonance

$HAuCl_4$- Hydrogen tetrachloroaurate(III) hydrate

ppb - part-per-billion

CFU/g - colony-forming units

DMS - dimethyl sulfide

DMDS - dimethyl disulfide

MT - methanethiol

DMSO- dimethyl sulfoxide

S-MMTSO - S-methyl methanethiosulfonate

DMTS - dimethyl trisulfide

C-S lyase - cysteine sulfoxide lyase

SAR - systemic acquired resistance

CHAPTER 1

INTRODUCTION

1.1 Justification

Due to the increasing health-conscious lifestyle, the market for fresh vegetables and fruits is expanding. In 2016, the fresh fruit and vegetable market was valued approximately 104.7 billion dollars(James, 2015). To maintain the high quality of that perishable produce in market, it is significantly essential to ensure both the plants health during growth and freshness before sale. Recently, there is limited technology for sensing environmental stresses, which are closely related to plant quality and yield. Hence, developing a timely approach for measuring noninvasive precise stress response is of great importance in modern agriculture. On the other hand, the “best by” labels are commonly used to indicate the imprecise shelf-life in the food industry. Accordingly, some fresh goods which are still safe for consumption could be discarded merely because they passed the expiration date. Therefore, developing a rapid detection for perishable produce quality and shelf-life is helpful to eliminate food waste as well as to promote food safety with fresh produce, thus bringing economic benefits.

1.1.1 Living plants stress response and plant phenotyping

When exposure to unfavorable environments, plants will make use of their genetically determined defense mechanisms to adjust to the surrounding environment. For living plants, these self-defense systems against stresses commonly exist by multiple metabolic and physiological pathways during growth. More specifically, abiotic stresses (includes drought, wounding, ultraviolet light, temperature, and other non-living environmental factors) or biotic (includes fungi, bacteria, insects, and other living factors) will affect the secondary metabolic pathways of preharvest plants by regulating activities of the biosynthesis-related enzyme, resulting in phytochemical accumulation or loss(Spadafora et al., 2016)(Reviews, 2003) or even

the structural adaption(Pieruschka & Schurr, 2019). For example, microbial infection, ultraviolet radiation, or chemical stressors could stimulate the synthesis of phenolic compounds (Formica-Oliveira et al., 2017)(Muhammad Siddiq, 2018). Therefore, selected stresses treatments are capable of enhancing the quality or yield of produce at the preharvest stage(Reviews, 2003). Furthermore, plants exposed to abiotic stresses have the stress resistance system of trying to decrease the accumulation of reactive oxygen species (ROS), which are highly reactive and harmful in vivo(Altangerel et al., 2017). Generally, crop yields and nutrient composition are influenced by plant stresses. Accordingly, promptly predicting the potential biotic or abiotic stresses to living plants is particularly beneficial to facilitate sustainable crop production management, improve the quality of products, and to reduce the use of pesticide(Mahlein, 2016). Plant phenotyping, which refers to quantitative assessment of plant traits in vivo, is commonly used in plant stress resistance study for noninvasive sensing metabolites or physiological responses and further understanding the plant interactions(Pieruschka & Schurr, 2019)(Singh et al., 2016). However, currently, there are still relatively few applications non-destructive field-based analytical technology of plant phenotyping adopted in agriculture.

1.1.2 Postharvest produce shelf-life and quality

The “fresh-cut” vegetable, also called “ready-to-eat” vegetable, is usually sold only after washing, slicing, chopping or shredding(De Corato, 2019). Compared with cooked vegetable, minimally-processed vegetable maintains a higher content of vitamin, mineral, fiber, and antioxidant. However, ready-to-eat produce has met the challenge of food safety, because they are generally consumed raw, often without proper decontamination practices(Ahmad et al., 2018). There have been a large number of foodborne outbreaks related to fresh produce(J. N. Farber et al., 2006). Additionally, the physiological processes of postharvest produce, like respiration, usually cause adverse effects on the quality(Nielsen et al., 2008). Therefore,

monitoring the microbiological and physiological parameters associated with freshness is significantly important to control the quality of fresh produce.

Arugula, also commonly known as “rocket,” “garden rocket” or “rucola” in British, Australian, South African, Irish and New Zealand English, is widely popular for its distinctive aroma and flavor. It is a rich source of beneficial nutrients, such as flavonols, phenols, vitamin C, and carotenoids(Toledo-Martín et al., 2017)(Bell et al., 2015)(Nunes et al., 2013). Furthermore, arugula also has a high concentration of glucosinolates (GSLs), which contribute to its high nutritional values, spicy flavor(Bell et al., 2015)(Inestroza-Lizardo et al., 2016), and bitter tastes(Bell & Wagstaff, 2014). The physical operation, like harvesting, may accelerate the pathways of producing GSLs. And when exposure to wounding, GSLs in leaves cells will be hydrolyzed to isothiocyanates (ITCs) under the catalysis of myrosinase(Bell & Wagstaff, 2014) ITCs, including erucin and sulforaphane, have the potential of anticarcinogen(Melchini et al., 2009), antibacterial, and antifungal(Bell & Wagstaff, 2014). However, all these phytochemical contents and related volatile organic compounds (VOCs) would change according to their surroundings(Bell et al., 2016). Some reports(Bell et al., 2017) demonstrate that GSLs, ITCs, and amino acids (AAs) would increase at the early storage stage. However, most of the researches showed that the phytochemical contents of fresh-cut arugula leaves decreases over storage time; for example, glucosinolates would significantly decline(Force et al., 2007). Generally, the major symptom of senescence during storage is yellowing, wilting, and the production of off-odors(Nielsen et al., 2008)(Koukounaras et al., 2007). Among these, yellowing has been proved caused by chlorophyll degradation(Siomos & Koukounaras, 2007). Besides, Spadafora et al. (Spadafora et al., 2016) verified that there is a negative correlation between ascorbic acid content and the storage time of packaged arugula salad. Furthermore, some reports(Spadafora et al., 2016)demonstrated that O_2 concentration inside the salad packages was negatively

correlated with storage time, while CO_2 concentration performed a positive correlation. Of particular interest, Siomos indicated that endogenous C_2H_4 had little effect on the quality decay of arugula(Siomos & Koukounaras, 2007), which was different from most of the fresh produce. In addition, both the total polyphenol content (TPC) and the total antioxidant activity (TAC) were relatively consistent over time(Gutiérrez & Rodríguez, 2017)(Char et al., 2012)(Char et al., 2017). Most of the references(Gutiérrez & Rodríguez, 2017)(Char et al., 2012)(Char et al., 2017) reported that the decline of sensory quality is slow and almost invisible for untrained labors. More specifically, there is no discrimination among fresh-cut arugula leaves over storage time, no matter in color measured by colorimeter, or sensory evaluated by professional staff(Gutiérrez & Rodríguez, 2017). In contrast, the escalating microbial populations was a significant variance in arugula shelf-life studies(Gutiérrez & Rodríguez, 2017). In conclusion, the senescence of postharvest arugula leaves is a complicated process, involving sensory change, phytochemical compounds transformation, and volatile organic compounds variation.

1.2 Traditional detection technology

1.2.1 Traditional detection methods for stresses of preharvest produce

Up till now, the practical detections of diseases or stresses on living plants still mainly rely on early visible symptoms(Lowe et al., 2017). Manual detection, which is mainly appropriate for identification of stress resistance in the middle to late stages, contributes little to management decision or early intervention(Mahlein, 2016). Besides, most of the physicochemical indexes are analyzed by complex indirect approaches. For example, when monitoring crop water stress, both soil water balance, and plant-based parameters have to be measured to get the final results. Most methods for studying biosynthetic routes are destructive analyses, which have high requirements of intensive labors, lab environment, and duration of the operation. Another problem for stress sensing is that the types and levels of stresses, as well

as concurrent physiological changes in vivo, will impact on plant phenotyping(Altangerel et al., 2017). Hence, the fresh produce industry has a keen interest in developing rapid, sensitive, and automated identification approaches to stress responses before any visible signs occur(Lowe et al., 2017). Compared with traditional analyses, noninvasive sensors are promising for field-sensing. RGB-imaging, spectral sensors, thermal sensors, and fluorescence imaging are typical optical sensors for plant phenotyping analysis(Mahlein, 2016). Specifically, chlorophyll fluorescence(Ač et al., 2015) and Near-IR spectroscopy reflectance spectroscopy(Altangerel et al., 2017)have been reported capable of stress sensing. Farber et al. (C. Farber & Kurouski, 2018) proposed a pathway of combining a hand-held Raman spectrometer with chemometric analyses to diagnose diseases of maize. In addition, Altangerel et al. (Altangerel et al., 2017) demonstrated that Raman microscopic could also be used to observe post-stress induction in Coleus plants, especially the change of the reactive oxygen-scavenging pigments concentration. Although emerging reports provide insight on various advanced sensors, remote sensing still meets the challenge of complex data analyses and statistical methods(Mahlein, 2016)(Singh et al., 2016). Despite that various advanced analysis methods, like partial least square regression (PLSR), spectral angle mapper (SAM), and decision tree (DT), etc. have been applicated for plant physiology timely identification(Maimaitiyiming et al., 2017)(Lowe et al., 2017), the combination of the advanced sensors with complicated analysis technology is demanding for practical agriculture. Breeders require a simple, convenient and robust system for phenotyping and stress response detection.

1.2.2 Traditional detection methods for shelf-life of postharvest produce

Standard shelf-life determinations of produce include sensory evaluations, physical testing, and microbial analyses. In practical experiment design based on fresh vegetables, flavor, color, texture, phenols content, antioxidant capacity, chlorophyll, and carotenoid content, as

well as microbiological analysis, were common indicators for deciding storage condition(Gutiérrez & Rodríguez, 2017)(Tsironi et al., 2017). However, the aerobic plate count for the total bacteria colony, which is the standard gold test for microbiological method, needs waiting time for original results of more than 24 hours(Pearson et al., 2018), and also requires for specialized laboratory setting and well-trained labors as well as physical testing. Hence, microbial analyses and physical properties tests are time-consuming and complex for industrial quality controls. Also, the assessments of sensory attributes are strikingly affected by subjective views, which is unreliable for practical monitoring. Particularly, the aroma of the intact vegetable can be too mild, resulting in the air from the storage package superimposed the rotten smells (Nielsen et al., 2008). To sum up, traditional detections cannot meet the requirement for rapid, simple, and sensitive monitoring of freshness in the modern industry.

1.3 Headspace detection technology

Volatile organic compounds (VOCs), which can vaporize at room temperature, naturally exist as fatty acids, aromatics, amino and sulfur-containing compounds in plants(Schulz & Dickschat, 2007)(Cozzolino et al., 2016). They are involved in various plants stress response and phytochemical metabolism in arugula as discussed above. Volatile compounds produced during multiple primary or secondary metabolism pathways(Pichersky et al., 2006)are capable of diffusing through the gas phase to provide signaling information. Based on this property, headspace detection technology has been widely used for detection and identification in food safety(Schnürer et al., 1999) or agricultural industries(Morath et al., 2012)(Rowan, 2011), environmental health(Korpi et al., 2009), and human health(Kai Zhang, 2014). For instance, headspace detection for specific fungi has been used in grains, fruit, and meat products for food safety(Morath et al., 2012). So far, a variety of headspace detection technology has been proposed. As one of the most popular approaches, Gas Chromatography-Mass Spectrometry

(GC-MS) is powerful in separation and detection sensitivity when combined with solid adsorbents, but it requires thermal pretreatment(Morath et al., 2012). Hence, Solid-Phase Microextraction (SPME) was introduced to this method to decrease sample preparation time as well as increase sensitivity(Zhang & Li, 2010). The combination of Dynamic Headspace GC-Olfactometry (GC-O)and GC-MS allowed broad-spectrum identification of flavor compounds(Engel et al., 2002).Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is capable of rapid detection in a moderate gas mixture. Additionally, it can be used for quantification in part-per-billion (ppb) levels(Senthilmohan et al., 2001). In addition, Proton Transfer Reaction-Mass Spectrometry (PTR-MS)(Ezra et al., 2004) and Nuclear Magnetic Resonance spectroscopy (NMR)(Booth et al., 2011)are also applicable for detection of VOCs or essential oils. Nonetheless, sniffing can be more sensitive than these technologies for some odors. As a substitution of human beings, the “Electronic nose” comprised by arrays of electronic chemical sensors is potential of detecting volatiles fingerprint in the food matrix(Morath et al., 2012). Consequently, the particular headspace identification approach depends on the intended objective.

1.4 Surface-enhanced Raman spectroscopy (SERS)

From the previous review, it is clear that the traditional detection approaches were unable to provide precise results in a short time. To increase the quality of perishable produce, surface-enhanced Raman scattering (SERS), which can give unique fingerprint spectral information about the target molecule could be taken into consideration. SERS is a rapid nondestructive ultra-sensitive spectroscopic technique that enhances the Raman signals utilizing electromagnetic enhancement and chemical enhancement. While the electromagnetic enhancement, induced by localized surface plasmon resonance (LSPR) on the surface of noble metal nanostructures(Willets, 2009), plays a dominant role in increasing Raman signals(Schatz et al., 2006), chemical enhancement, refers to the electronic interactions between the analyte

molecule and metal substrate, also provide an order of magnitude signal enhancement(Otto, 2005). For producing the enhanced electromagnetic field, the metal nanostructure substrates adopted is significantly essential. There are two kinds of commonly used SERS-active substrates: colloid-based substrates and solid surface-based substrates(Xie et al., 2017). The colloid-based substrates are produced by stabilizing colloidal nanoparticles in the solution-based system. They are inexpensive, convenient and suitable for large-scale production as well as laboratory setting. However, colloid-based substrates are difficult to control the target assembly and the location of analytes in a complex food matrix, thus resulting in low reproducibility. In contract, solid surface-based substrates, which refer to nanoparticles stabilized on solid substrates, have higher reproducibility and stable signals. SERS has been reported as rapid detection approaches of specific chemical constitutes, food chemical contaminants, foodborne pathogens, and other analytes in food matrices(Xie et al., 2017)(Pang et al., 2016)(Craig et al., 2013). Among various spectroscopic techniques based on electromagnetic enhancement, SERS is the most promising approach, due to the huge improvement in the detection limitation from ensembles of molecules to the single-molecule level(Boisselier & Astruc, 2009). Hence, SERS is possible to provide a unique insight into the shelf-life and stress response of fresh produce in the molecule level. However, few publications on the identification of food components have been reported. Therefore, developing SERS detection approach with proper substrates is promising in food industry and agriculture.

1.5 Goals and objectives of the study

We aim to develop an approach based on SERS to detect the real-time plant stress response. To achieve this goal, there are three objectives of this study.

Objective 1: Develop and optimize a surface detection method using colloid-based substrates to monitor the shelf-life of postharvest arugula leaves.

Objective 2: Develop and optimize a headspace detection method using solid surface-based substrates to monitor the shelf-life of postharvest arugula leaves.

Objective3: Apply the most effective SERS-based detection method to monitor living plants stress response.

CHAPTER 2

DEVELOPMENT OF SURFACE DETECTION METHOD FOR REAL-TIME FRESHNESS OF ARUGULA WITH SERS

2.1 Introduction

In chapter 1, several traditional detection methods of shelf-life and their drawbacks were discussed. However, Raman spectroscopy allowed simultaneous interrogation of various compounds in a food matrix. As our lab has successfully used commercial nanoparticle solutions to enhance SERS signals for investigating the pesticide behavior on tea leaves(Hou et al., 2016) and the penetration of pesticide in spinach leaves(T. Yang et al., 2016), we wanted to investigate the feasibility of monitoring the shelf-life of fresh-cut arugula by simply adopting colloid-based substrates on leaves surface, as well as comparing SERS with traditional approaches to figure out whether SERS is potentially advantageous over other methods in perceiving the quality decay of fresh-cut arugula.

2.1.1 Colloid-based substrates

Because our group has developed the detection approach of placing the nanoparticles on the leaves surface to monitor pesticide behavior using SERS(T. Yang et al., 2016)(Hou et al., 2016), we first evaluated the feasibility of adopting colloid-based substrates on the leaves surface to monitor shelf-life. The ability of signals enhancement of colloid-based substrates was largely related to the property of nanoparticles, such as shape, size, and surface morphology(Boisselier & Astruc, 2009). Recently, the commercial colloid-based substrates are mainly gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). Theoretically, the metal type of nanostructures has a decisive effect on the bulk plasma wavelength, which controls the spectral sensitivity(Lee & El-Sayed, 2006). For instance, AgNPs perform higher Raman signal-enhancement than similar size AuNPs, while AuNPs have better biocompatibility and

stability(Wang et al., 2013). Because the interaction between nanostructure and arugula leaves was unclear, we first investigated the spectra using both AuNPs and AgNPs.

2.1.2 The degradation reactions of fresh-cut arugula leaves during storage

In the previous review, we have already known that the proved degradation reactions of fresh-cut arugula over storage time, such as ascorbic acid content decline, and phytochemical compounds or related VOCs transformation. Furthermore, compared with sensory parameters, the microbial experiment is relatively reliable in the shelf-life study. Thus, the aerobic plate count, which was the gold standard test approach for total bacteria detection in food, was also adopted for comparative analysis in this study to indicate the degrees of the freshness of fresh-cut arugula. While color score and odors will not be further investigated, the images of representative arugula leaves of different degrees of freshness will still be attached to provide a better understanding of the senescence process.

2.1.3 Objectives of this study

The objectives of this study were to: (1) develop and optimize a surface detection method using SERS (2) characterize the signature SERS spectra from fresh arugula leaves to spoiled arugula leaves (3) build a shelf-life prediction model based on the surface detection method.

2.2 Material and methods

2.2.1 Materials

0.2 mg ml^{-1} 40nm citrate coated AgNPs solution, and 0.05 mg ml^{-1} citrate coated AuNPs solution were purchased from NANO PARTZ Inc. (Loveland CO). And tryptic soy agar was purchased from Fisher Scientific (Fair Lawn, NJ).

2.2.2 Preparation of fresh-cut arugula samples for surface detection

Fresh baby arugula leaves were purchased from the local Whole Foods Market (Waltham, MA). The arugula leaves were kept at -4℃ for storage. A volume of 2 µL of each of the commercial 0.2 mg ml^{-1} citrate coated AgNPs solution was then pipetted onto flat arugula leaves surface under room temperature, and air-dried in a fume hood for 30 min to form a "coffee ring" for measurement. The preparation method was demonstrated in Figure 1, Raman spectra were collected individually. Before drying, the pictures of leaves under varying degrees of freshness were also photographed.

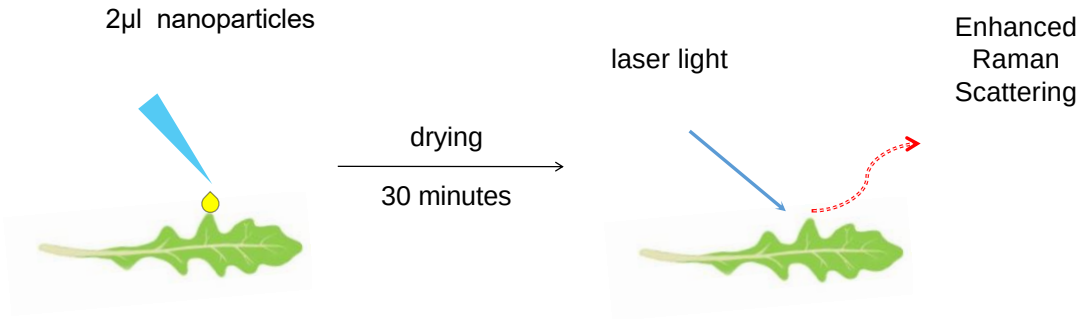


Figure 1: Schematic illustration of the surface detection method

2.2.3 Raman Instrument and data analysis

The "coffee ring" on fresh-cut arugula leaves surface was monitored under a DXR Raman microscope (Thermo Fisher Scientific, Madison, WI). The SERS laser setting was780 nm wavelength, 50 mm slit width for 2 seconds and a 20X confocal microscope objective. Each spectrum was scanned from 2000 to 400 $cm^{-1}$with 1mW laser power. The Raman instrument was controlled by OMNIC™ software (version 9.1). Thirty spectra were selected from each spot of nanoparticles on the surface of leaves and then averaged by OMNIC™ software, and then further analyzed by Thermo Scientific TQ Analyst (version 8.0). All Raman spectra were calculated from at least three replicates.

2.2.4 Standard plate count

20 g of tryptic soy agar was diluted in 500 ml of distilled water and then agitated while heating to boiling. The agar solution was sterilized (15 min at 121 °C) and subsequently poured into Petri dishes evenly in a molten state. Agar Plates were left to cool at room temperature until the liquid solidified. All plates were kept at 4 °C for storage and used for total plate count (TPC) of the fresh-cut arugula leaves every day.

5 g of arugula leaves were added to 50ml 0.9% sterilized salt water and placed in a stomacher to shake for 60 s (300 rpm) to create a 10% (w/v) digestive arugula dilution. 100 µl of the homogenized sample inoculum was diluted with 900 µl sterilized salt water to obtain serial dilution. 200 µl of each dilution was respectively added to agar Petri dishes. All these nutrient agar dished were incubated at 37±0.5 °C for approximately 24 hours. Quantification of viable colonies for each Petri dish was presented in the form of colony-forming units (CFU/g). Results from three replicates of each sample were averaged.

2.3 Result and discussion

2.3.1 Characterization of arugula leaves SERS spectra with nanoparticles

In order to control variables, the perfect fresh-cut arugula leaves samples without any operations were analyzed by SERS. The representative SERS data of pure arugula (Fig. 2A) showed very low intensity, which demonstrated that the background caused by arugula itself was negligible. Next, reference SERS spectra of AgNPs and AuNPs were recorded on the gold slides before it was applicated to the arugula leaves surface shown in Figure 2B and C. AgNPs and AuNPs were then pipetted onto arugula leaves under room temperature, and air-dried in a fume hood for 30 min. The SERS spectra of “coffee ring” were separately shown in Figure 2D and E. Both AuNPs and AgNPs produced similar SERS spectra on a control substrate with corresponding fresh arugula leaves surface.

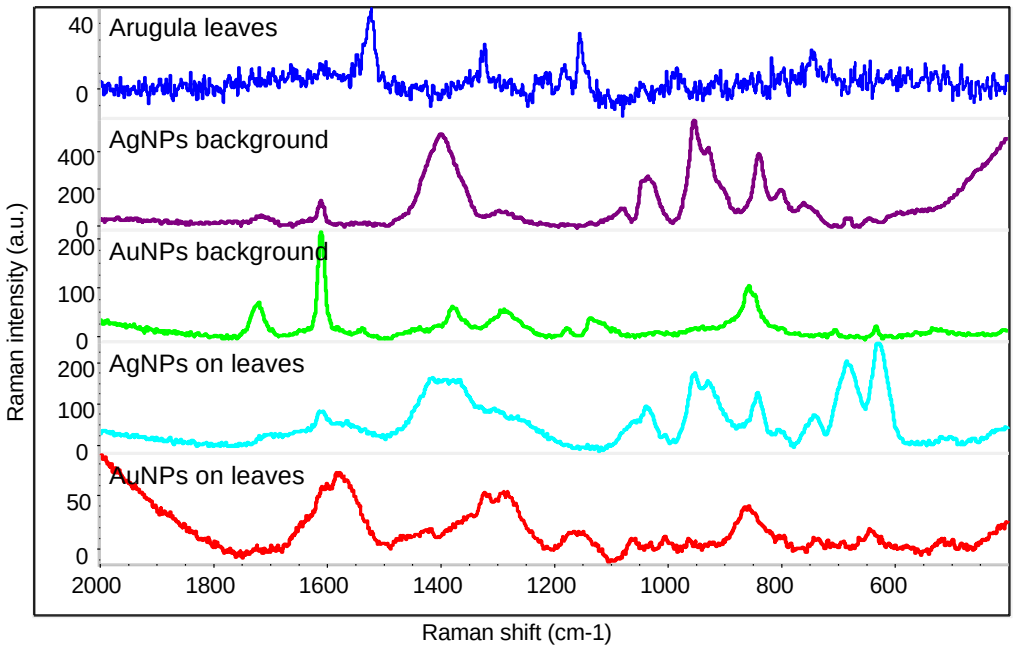


Figure 2: Representative spectra of Arugula leaves, AgNPs background, AuNPs background, AgNPs on fresh arugula leaves and AuNPs on fresh arugula leaves

2.3.2 Characterization of arugula leaves SERS spectra over time

The shelf-life of postharvest arugula leaves were reported to be 14 days at 4 °C(Nielsen et al., 2008). We collected 9 spectra from 3 leaves individually every day to figure out the preliminary variation regularity over time. Our Raman results are shown in Figure 3, under different degrees of freshness, the spectra of AgNPs on arugula leaves (Fig. 3A) observed obvious change in pattern, whereas the spectra of AuNPs on arugula leaves (Fig. 3B) had no significant change. Lee et al. (Lee & El-Sayed, 2006) measured the shift in the position of the plasmon bands and the band intensity to demonstrate that AgNPs are more sensitive to smaller amounts of the analyte, which might elucidate the reason why only the arugula leaves under AgNPs gave off diverse SERS pattern over time. Based on this result, we only utilized AgNPs as probes for the surface detection method in further experiments. However, the biggest challenge we met for the surface detection using AgNPs was that the full spectra of arugula leaves were remarkably inconsistent not only in the pattern but also in intensity. Hence, the 40nm citrate

coated commercial AgNPs solution needed to be well preserved and checked independently before every experiment to eliminate the external impact from nanoparticles. Furthermore, in order to increase the consistency of our results, a bunch of spectra need be taken into account when analyzing SERS results.

The representative picture of fresh-cut arugula in different degrees of freshness was present (Fig. 4A) with the colony growth curve during the storage (Fig. 4B). Based on these two traditional approaches, we concluded that the arugula leaves could stand for “0 days left on shelf-life”, in which condition leaves gave off a slightly unpleasant smell, transformed to yellow, and had been in exponential growth. And from the results shown in Figure 4, fresh-cut arugula after 4 days of storage was still could be acceptable for normal customers in both appearance and microbiological condition. In view of these identification rules, we build up our standard of shelf-life as a reference for further experimental analysis.

Meanwhile, in order to eliminate the individual difference of each spot on the same sample leaf, each leaf was divided into 6 parts according to the schematic illustration of arugula leaf structure (Fig. 4). And we made 3 “coffee ring” randomly in each division area, and collected 20 spectra from each “coffee ring” in those 6 divisions. Finally, there would be 360 spectra collected from every arugula leaf sample. For each day, SERS data from no less than 3 samples were fed into Thermo Scientific TQ Analyst (version 8.0) and used to provide a comparison among different degrees of freshness. As presented in Figure 5, the Raman spectra over storage time were complex and multiple. Moreover, the strong characteristic SERS patterns of AgNPs on arugula leaves based on the mass of data were similar to AgNPs background (Fig. 2B). Hence, it was hard to discriminate between fresh arugula group and unfresh arugula group simply based on SERS spectra. Future work needs include an investigation into the modeling approaches to help demonstrates the difference between fresh-cut arugula under varying degrees of freshness.

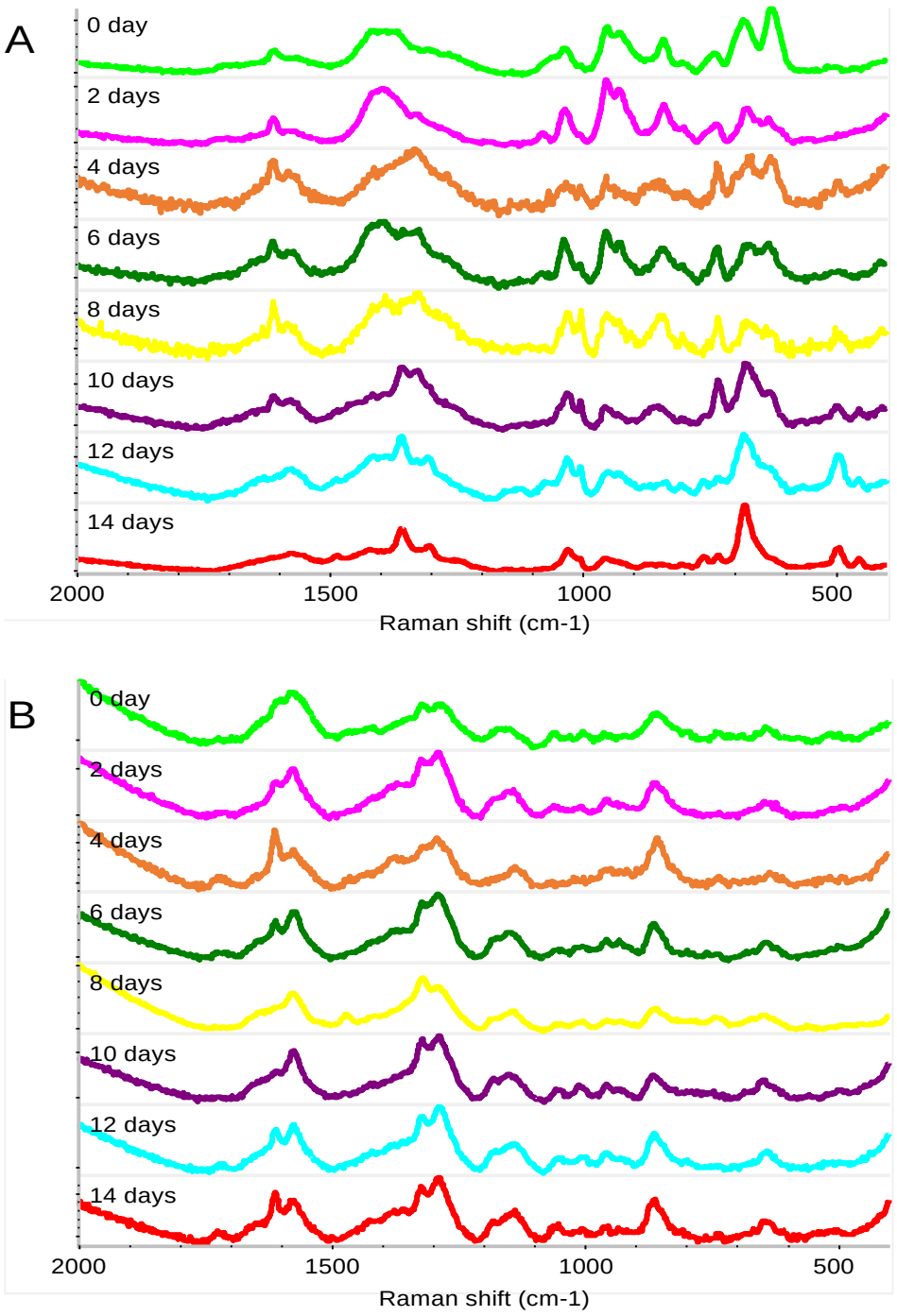
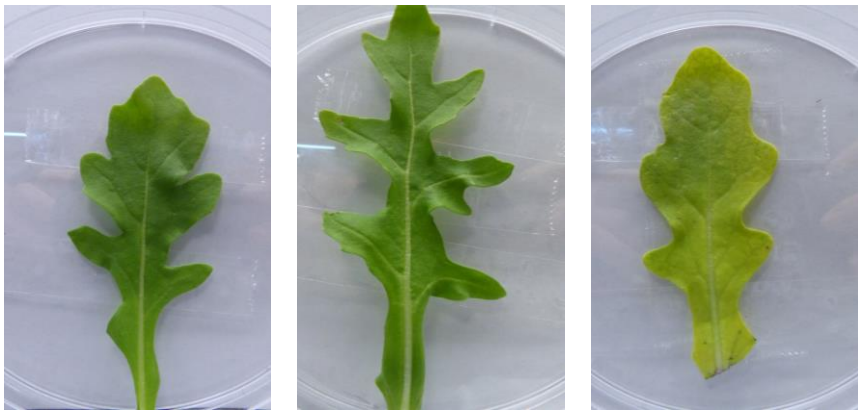


Figure 3: (A) SERS spectra of arugula leaves under varying degrees of freshness using AgNPs and (B)SERS spectra of arugula leaves under varying degrees of freshness using AuNPs

A



7 days left on shelf-life (Stored for 0 day)

3 days left on shelf-life (Stored for 4 day)

1 days left on shelf-life (Stored for 6 days)

B

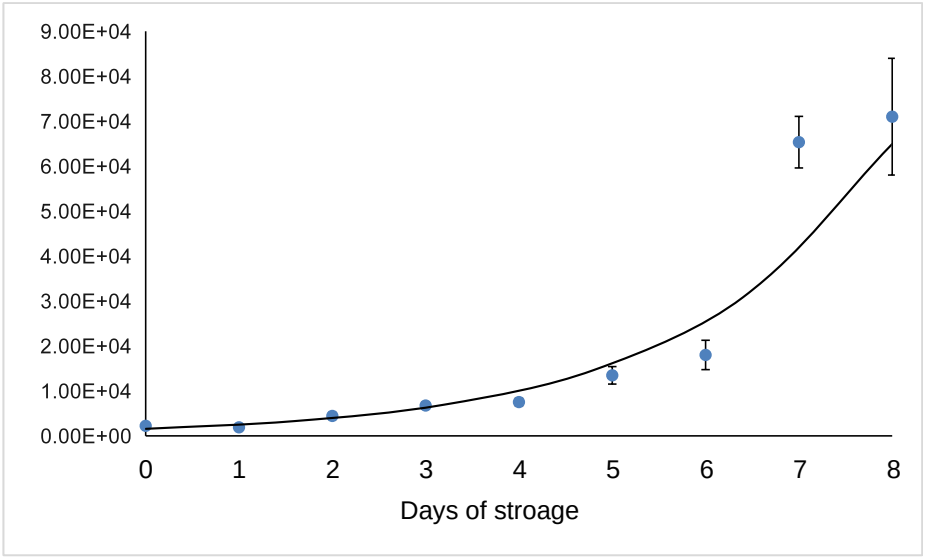


Figure 4: (A) arugula leaves picture exhibiting varying degrees of freshness and (B) Colony-forming units (CFU/g) among arugula leaves under varying degrees of freshness

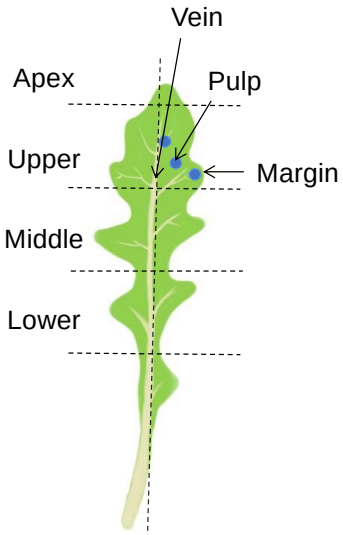


Figure 5: Schematic illustration of arugula samples structure

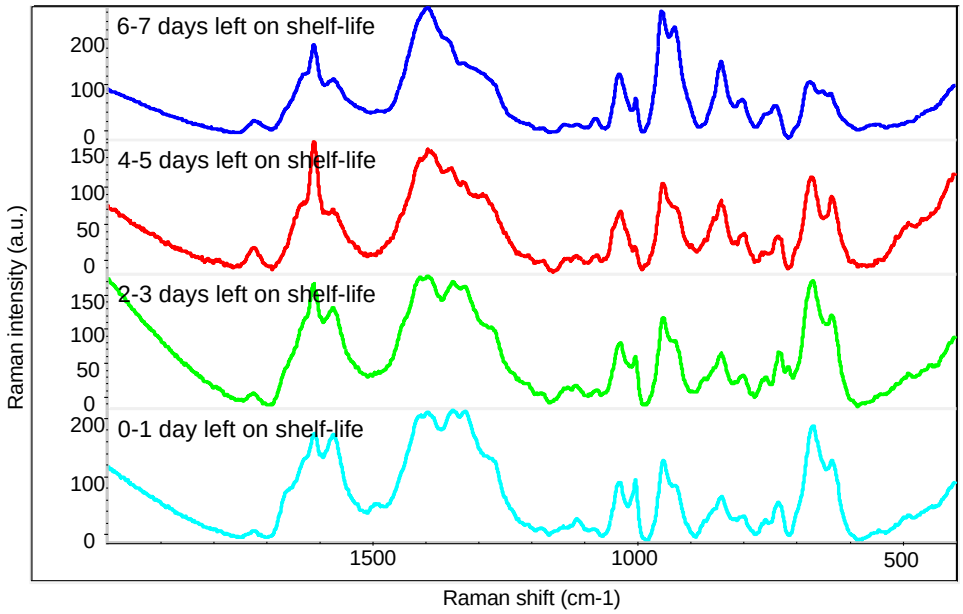


Figure 6: SERS spectra of arugula leaves under varying degrees of freshness using AgNPs

2.3.3 Model design for monitoring the shelf-life of fresh-cut arugula samples over time

In previous SERS spectra results (Fig. 6), we have already known that it is hard to summarize the variation with time based on visable SERS spectra pattern and peaks. Additionally, it is commonly accepted that the conventional zero-, first- and sec-order kinetics models were less flexible and insufficient to perform significant discrepancies in the shelf-life

study.(Amodio et al., 2015) To better perform the relevance between experimental data with estimated shelf-life of fresh produce, an innovative non-linear model is necessary.

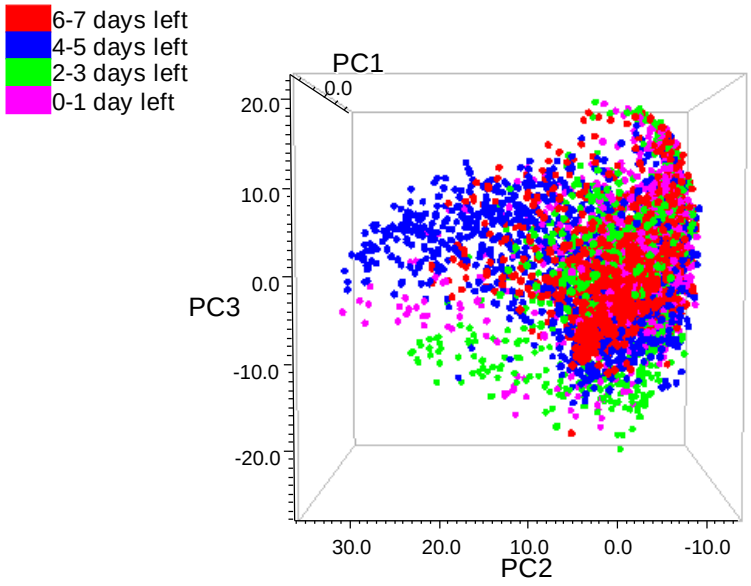


Figure 7: Principal component scores 3D Display model of the Surface Detection Method

Therefore, we analyzed the SERS data with principal component analysis (PCA). Generally, the PCA plot is able to show significant statistical discrimination of spectra from different data groups. However, in the PCA 3D model (Fig. 7), we found that the data points stood for different shelf-life groups were overlapped with each other, which agreed with the results showed in Figure 6. Therefore, both the Raman spectra and the PCA plots suggested that the surface detection method was unfeasible for shelf-life monitoring.

2.4 Conclusion

A shelf-life detection method, which simply adopting nanoparticles on the fresh-cut arugula leaves surface, based on SERS was developed in this study. Both AuNPs and AgNPs commercially used were tested for the optimization of this approach. A large amount of SERS spectra data were classified and analyzed for identifying the characteristic SERS signals change

of fresh-cut arugula leaves during storage. The visual appearance and microbial growth curve of fresh-cut arugula were added to set up reference standards of acceptance, as well as the association between storage time and exact shelf-life. However, we conclude that the surface method was unpractical for shelf-life monitoring. Hence, future work will mainly include developing a more effective way for shelf-life monitoring. In addition, the mechanism of arugula senescence would be analyzed to understand the relationship between fresh-cut arugula leaves shelf-life and SERS results.

CHAPTER 3

MONITORING THE SHELF-LIFE OF FRESH-CUT ARUGULA LEAVES BASED ON HEADSPACE ANALYSIS USING A SERS-ACTIVE FIBER

3.1 Introduction

In Chapter 2, we discussed the limitation and the possible reasons for the failure of the detection method based on fresh-cut arugula leaves surface. Besides, we also illustrate the merit and demerit of two types of SERS-active substrates: colloid-based substrates and solid surface-based substrates. Theoretically, developing a nanosensor could overcome the deficient of the surface detection method and improve the success rate. Chen(CHEN et al., 2018) developed an AuNPs coated fiber, which has been proved that could detect specific pesticide in apple juice, is potential for rapid volatile identification in the food matrix. Herein, we will detect the headspace above the arugula leaves using this solid surface-based substrate and establish a new prediction model.

3.1.1 Volatile metabolites in fresh-cut arugula

Although VOCs are mainly emitted by leaves, roots, flowers or fruits of living system(Rowan, 2011), as an aromatic species, postharvest arugula still has a high accumulation of volatiles in the packaging headspace. Olfactometry analyses proved that DMS and DMDS have comparatively potent odor intensity in fresh arugula(Nielsen et al., 2008). Besides dark-induced senescence, the off-odors of fresh-cut arugula would change during storage. Spadafora et al. (Spadafora et al., 2016) verified the parallel fall in specific VOCs contents and arugula quality, including phytochemical contents, armor score, and microbial growth over storage time. Among all the volatiles(Miyazawa et al., 2002)(Spadafora et al., 2016), 21 known compounds would decrease, whereas dimethyl sulfide (DMS), dimethyl sulfoxide (DMSO) and dimethyl disulfide (DMDS) showed a rising tendency. Although DMSO is almost odorless, DMS and DMDS have

distinctive odor with comparatively low thresholds. Additionally, DMS and DMDS can be the end-products from the breakdown of sulfur-containing compounds, like sulfurous glucosinolates in arugula(Nielsen et al., 2008). And DMS can be reduced from DMSO by microorganisms, which is similar to nitrate reduction(Glindemann et al., 2006). Because microbial growth will induce putrid smell, sour taste, and rot smell(Muhammad Siddiq, 2018), flavor and odor of fresh-cut arugula are compromised by days of storage. Hence, headspace detection could also be considered as an effective pathway of microbial spoilage test(Kai Zhang, 2014).

3.1.2 Solid surface-based substrates

Although the colloid-based substrate is the most straightforward application of nanoparticles in SERS, it shows low reproducibility and relatively unstable signals. Because the solution system was easy to be affected by PH or charges of the food matrix, additionally, using colloid-based substrates is difficult to control the location of the analyte molecules. In contrast, solid surface-based substrates exhibit higher reproducibility because they can target the location of analytes(Xie et al., 2017). There are various designs of nanoparticles with high-sensitivity has been reported. For instance, a leaning nano-pillar substrate fabricated on silicon (Si) was proved could be used for multiplex SERS detection of VOCs(Wong et al., 2014). The approach using a specific SiO_2@Au@Ag core-shell–shell NPs was also capable of rapid detection(Lu et al., 2014). Sun et al. (Sun et al., 2016) developed an aptamer-modified Ag nanorod capable of label-free trace detection of specific pollutants. However, Yang et al. (Y. Yang et al., 2012) reported an Ag nanoneedles technology, and she also explained that nanoneedles could provide the strongest SERS enhancement around the apex theoretically because of the sharp structures. Based on the reference, a nanoparticles coated fiber with high reproducibility and stability was developed by our group(Chen, 2018). The nanoparticles coating of this substrate will interact with the VOCs, and then provide valuable information about the

shelf-life. Based on the SERS-sensitive fiber fabrication technology, we investigated the feasibility of predicting shelf-life by headspace detection.

3.1.3 Objectives of this study

The objectives of this study were to: (1) develop and optimize a headspace detection method of fresh-cut arugula shelf-life based on SERS (2) characterize the signature SERS spectra from fresh arugula leaves to spoiled arugula leaves (3) establish and assess the shelf-life prediction model based on the headspace detection method (4) analysis the SERS assignment of VOCs of arugula leaves during storage.

3.2 Material and methods

3.2.1 Materials

Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4$) (99.999%), ethylene (≥99.95%), flow controlling valve for 600 mL and 1000 mL pressure tins, propyl disulfide (≥97%), dimethyl disulfide (>99.9%), dipropyl trisulfide (>97%), dimethyl trisulfide (DMTS) (≥98%), cis-3-Hexenyl butyrate (≥98%), 1-Propanethiol (99%), dimethyl sulfoxide (≥99.95%), dimethyl sulfide (≥99%), S-methyl methanethiosulfonate (S-MMTSO) (≥98%), methanesulfonic acid (≥99%), allyl isothiocyanate, allyl sulfide (97%), allyl methyl sulfide (98%), diallyl disulfide (≥98%) were procured from Sigma Aldrich (St. Louis, Mo., USA). Methanethiol (2000 μg/mL in Toluene) was purchased from AccuStandard. Erucin (98%) was purchased from Cayman Chemical. Hydrochloric acid (34%-37.5%), ethanol (100%), methanol (99.9%), cis-3-Hexen-1-ol (98%), (R)-(+)-Limonene (97%), tryptic soy broth were purchased from Fisher Scientific (Fair Lawn, NJ., USA). The stainless-steel wire (SUS304, ϕ203 μm) was purchased from the Small Parts, Inc. A stock solution of hydrogen tetrachloroaurate hydrate was prepared in distilled water at 1% and further diluted by distilled water. Fresh baby arugula leaves were purchased from the local Whole Foods Market (Waltham, MA). The arugula leaves were kept at -4°C for storage.

3.2.2 Fabrication and characterization 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 stainless wire. The stainless-steel wire (3 cm) was washed with distilled water and ethanol in an ultrasonic bath for 5 min respectively. The etched fiber was washed again with distilled water and methanol in the ultrasonic bath for 5 min respectively, then dried with blowing. The etched fiber was then immersed into $HAuCl_4$ solution (0.05%, w/w) to introduce gold to its porous surface shown in Figure 1. The coating reaction is the replacement reaction between iron and gold: $Fe + [AuCl_4]^- = Fe^{3+} + Au + 4Cl^-$. (M. Tréguer-Delapierre, J. Majimel, 2008) AuNPs coated fibers were kept in cool and dry places without direct sunlight until ready to use.

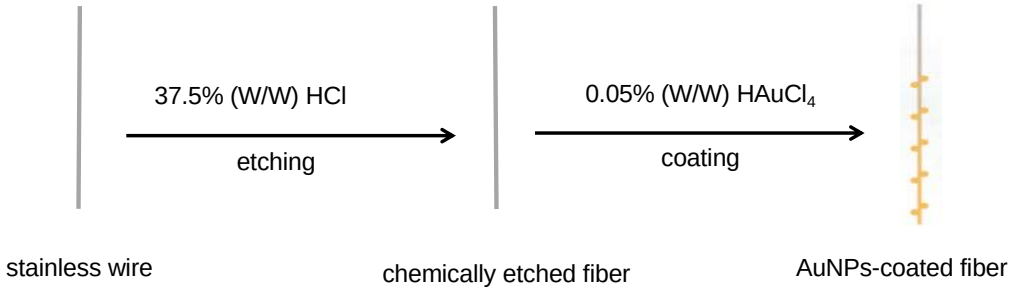


Figure 8: Schematic illustration of AuNPs coated fiber fabrication

The prepared fibers were analyzed using a Magellan 400 SEM (5.0 kV; FEI Company, Hillsboro, OR). The stability of the fibers was determined by periodically inserting the prepared fibers into the vial with a certain amount of DMS for two hours reaction and then recording SERS signals.

3.2.3 Preparation of fresh-cut arugula samples for headspace detection

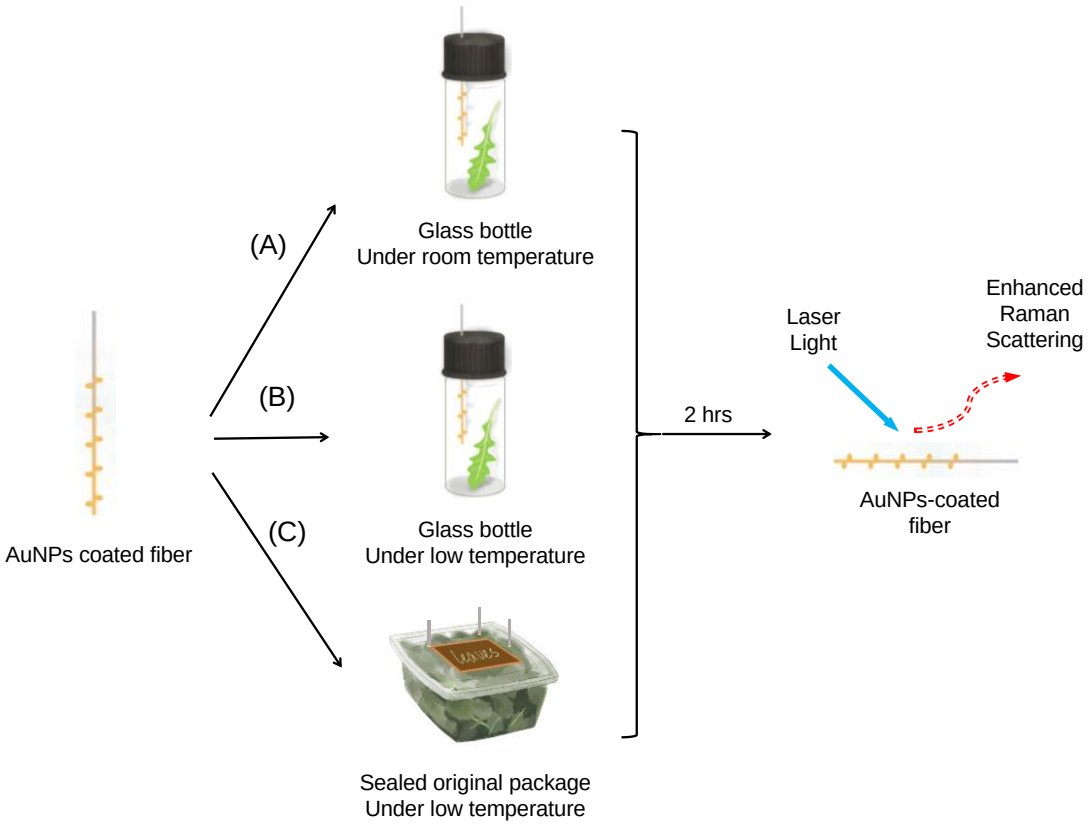


Figure 9: Schematic illustration of the headspace detection method. (A) arugula leaves incubated in glass bottles at 22±2°C (B) arugula leaves incubated in glass bottles at 4±2°C (C) arugula leaves incubated in original package at 4±2°C

To ensure the stable reactions between the AuNPs coated fibers and volatile compounds from arugula leaves, we utilized glass screw-thread sample vials with PTFE/Silicone septa and open-top polypropylene closure as a sealed bottle. Around 2 grams of fresh-cut arugula leaves were transferred from the original package to the bottles. And then the AuNPs coated fiber was inserted through the PTFE/silicone septum and exposes to the headspace above the leaves shown in Figure 9A and 9B. In addition, we also inserted AuNPs coated fibers through some small pores into the unopened packaging filled with fresh-cut arugula (Fig. 9C). While the experimental setting of Figure 9A was under room temperature, we operated in the

refrigerator in Figure 9B and 9C. After reacted for two hours, SERS spectra were collected from fibers.

3.2.4 References compounds analysis

AuNPs coated fibers and a moderate amount of volatile compounds were sealed in screw thread glass vials under room temperature. After incubated for two hours, SERS spectra were collected from fibers. Among all the chemicals, ethylene was commonly considered as the marker of ripeness and senescence in most of the fresh produce. The existence of cis-3-Hexenyl butyrate, cis-3-Hexen-1-ol, (R)-(+)-Limonene in arugula were supported by the united use of Solid Phase Microextraction (SPME), Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography-Flame Ionization Detector (GC-FID), GC-Olfactometry (GC-O)(Miyazawa et al., 2002)(Jirovetz et al., 2002). S-MMTSO, methanesulfonic acid, allyl isothiocyanate, DMSO, erucin, DMS, DMDS, MT and DMTS were hydrolysate from under the catalysis of cysteine sulfoxide lyase (C-S lyase), which could be activated by the harvesting and chopping in fresh-cut arugula(Chin & Lindsay, 1994)(Tulio et al., 2002)(Bennett et al., 2002). Besides, propyl disulfide, dipropyl trisulfide, allyl sulfide, allyl methyl sulfide and diallyl disulfide were common VOCs produced by fresh produce.

3.2.5 Raman Instrumentation and data analysis

Fibers were taken out after incubation and placed under a DXR Raman microscope (Thermo Fisher Scientific, Madison, WI) for measurement. Refer to 2.2.3 for SERS laser setting. However, each spectrum was scanned from 3410 to 400cm^{-1} with 3mW laser power. Thirty spectra were selected from each fiber by OMNIC™ software (version 9.1), and then further imported to Thermo Scientific TQ Analyst (version 8.0) for classification. The standard normal variate (SNV) calculation region was set from 400 cm^{-1} to 3410 cm^{-1}. Meanwhile, the region for

PCA 3D display is 450 - 1800 cm^{-1}. All Raman spectra were calculated from at least three replicates.

3.2.6 Microbial analysis

5 grams of relatively old arugula leaves were mixed with 50 ml 0.9% sterilized salt water, and then mechanically homogenizing for 60 seconds (300 rpm) in the stomacher. 100 µl of the homogenized sample inoculum was diluted with 900 µl sterilized salt water to obtain serial dilution. 200 µl of each dilution was respectively adopted to TSA plates. After incubated at 37±0.5 °C for approximately 24 hours, only agar plates that contained 30 to 300 colonies were adopted for later isolation. Different isolates would be selected, according to color, size, shape and surface texture, to be separately grown in 10 mL of tryptic soy broth (TSB). After incubated at 37±0.5 °C for more than 8 hours, pure cultures of selected isolates were obtained for inoculation in the study. The fresh arugula samples would undergo UV radiation treatment for 15min to eliminate microorganisms without damaging the fresh produce. 500µL of selected microbial cultures were mixed with 2 g sterilized fresh leaves respectively. And then the AuNPs coated fibers were inserted to incubate in the headspace above samples for 2 hours to collect data prior to analysis.

3.3 Result and discussion

3.3.1 Characterization of the gold nanoparticle coated fiber

The surface morphologies of AuNPs coated fiber was shown in Figure 10. Under SEM, the nanoparticles distributed evenly and densely on the stainless-steel fiber surface. In Figure 10D, the size of the gold nanoparticles was around 190 nm.

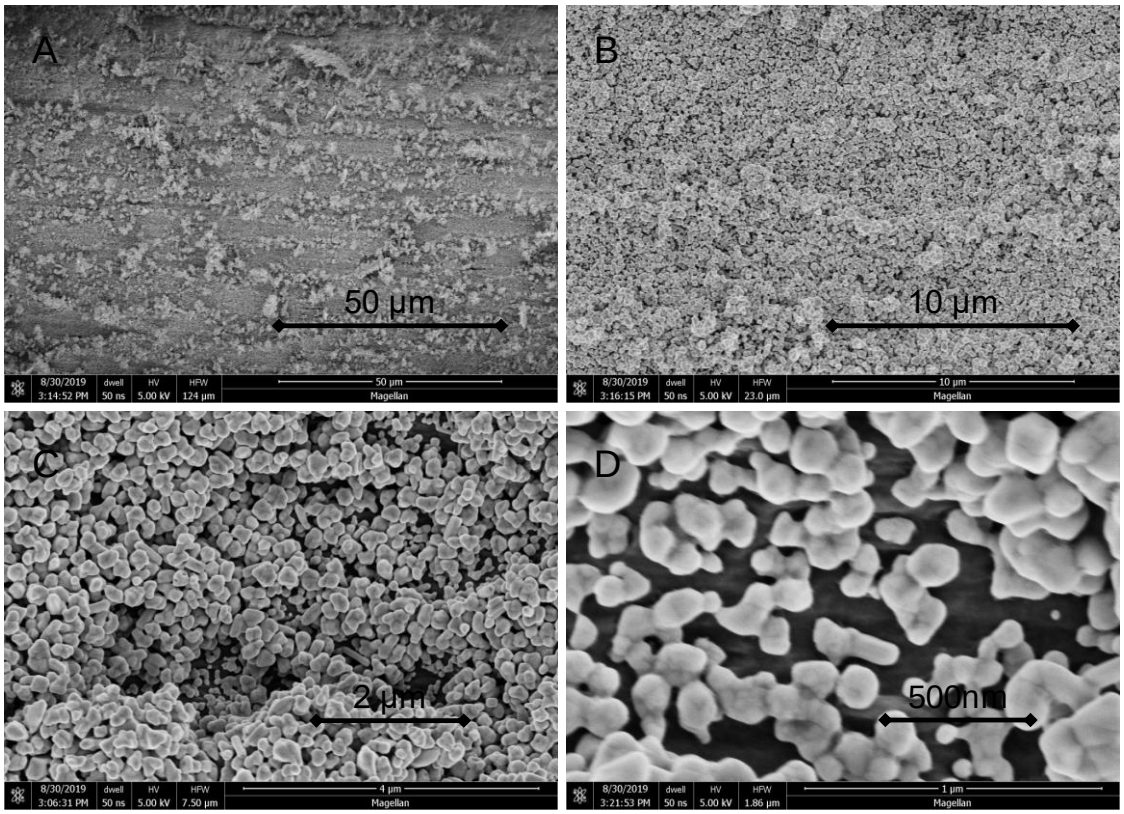


Figure 10: AuNPs coated fiber made by wire (ϕ203 µm) under (A) 50 µm SEM, (B) 10 µm SEM, (C) 2 µm SEM, (D) 50 nm SEM

The stability of the prepared fiber was determined using DMS as a volatile indicator. As shown in figure 11, DMS produces peaks at 500, 677, 725, 950, 985, 1035, 1225, 1325, 1420, 2910 and 2990 cm-1 Raman shift. The peak pattern and intensity were relatively consistent and stable in at least 15 days. On day 18, the average Raman intensity would significantly decline (Fig. 11). In addition, the peaks became relatively smooth. Hence, the conservatively estimated shelf-life of fiber is around 15 days.

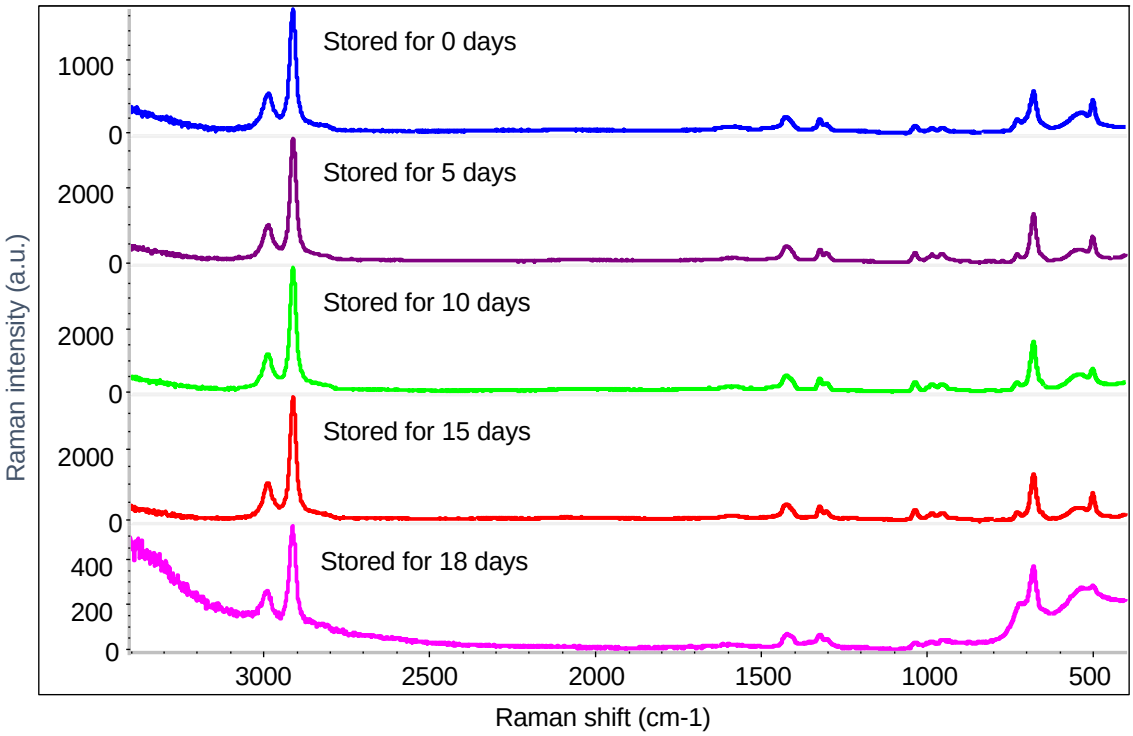


Figure 11: SERS spectra of DMS using AuNPs coated fiber over storage time

3.3.1.1 Optimization of incubation time

The prepared fiber was inserted into the vial with fresh leaves for 0 (control), 0.5, 1, 1.5 and 2 hours. The control spectrum of the fiber shows strong signals over the range of 400 cm^{-1} to 800 cm^{-1}. However, after reacted in the headspace, the pattern of fiber in this region was covered by volatile signals, which meant the fiber signals were negligible. As little as 0.5 hours, significant peaks were observed at 677, 1030, 1325, 1420, 2910 and 2990cm^{-1} Raman shift. The intensity of these peaks was gradually increased and at 2 hours, these peaks were sharp and clear. Therefore, we chose 2 hours as the optimum incubation time for the following studies.

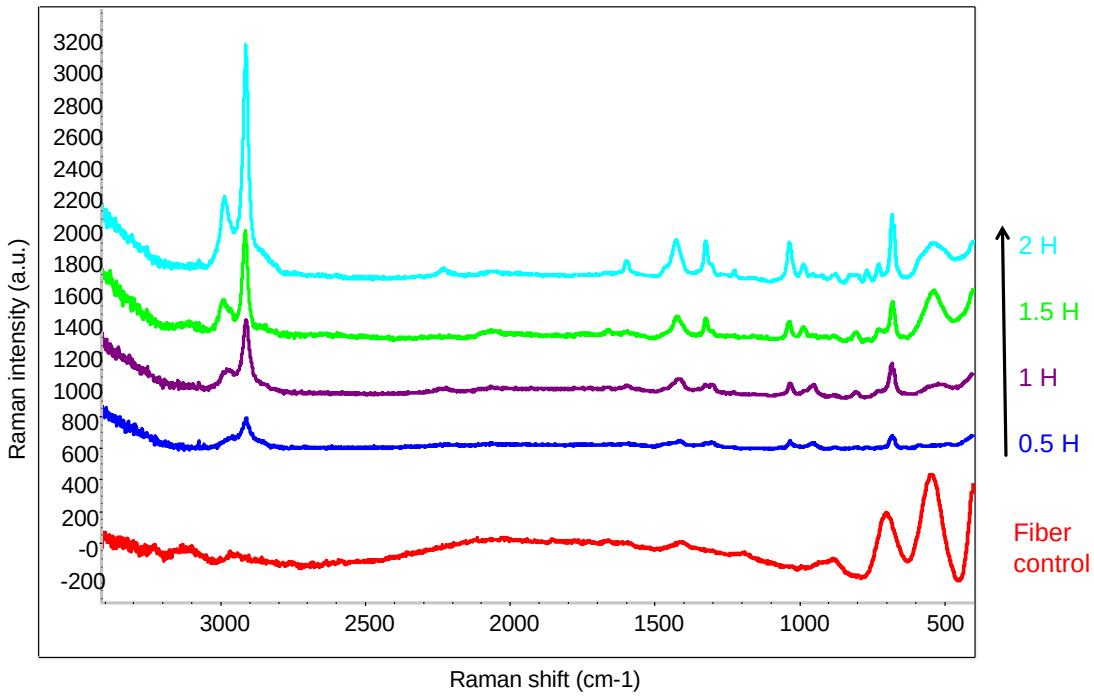


Figure 12: SERS spectra of fresh arugula after 0.5h, 1h, 1.5h and 2h incubation using the AuNPs coated fiber and controls

3.3.2 Characterization of arugula leaves SERS spectra over time

The SERS spectra of arugula leaves stored at different days were presented in Figure 13A. The most significant difference over time obtained from AuNPs coated fibers were mainly in 925-1050 cm^{-1} and 485-515 cm^{-1}. Meanwhile, there was a visible decrease of Raman intensity at 1600 cm^{-1} and 1225 cm^{-1}, which were tiny in Raman intensity, thus they would not be marked as indicators.

In the last chapter, we have already figured out the association between storage time and exact shelf-life referred to the visual appearance and microbial growth curve of fresh-cut arugula. Based on this standard, we compare the results that still have 7 days left on shelf-life, 5 days left on shelf-life, 3 days left on shelf-life with those only have 1 day left on shelf-life in Figure 13B. Basically, there were three significant differences. When only 1 day left, two sharp and strong characteristic peaks were emitted at 500 and 950cm^{-1}. According to reference, the

peak at 500 cm^{-1} is attributed to S-S stretching(López-Tobar et al., 2013)(Noh et al., 2007). It could be considered as the indicator of fresh decay in this study. Another noticeable difference was that the peak presented at 1030 cm^{-1} decrease sharply. Furthermore, the headspace detection methods based on SERS can not only provide the signature pattern but also be analyzed for quantitative representation.

The quantitative analysis based on the Raman intensity of peaks at 500, 950 and 1030 cm^{-1}, as well as the microbial population among varying shelf-life groups, were presented in Figure 14. Because the growth potential of the total number of the bacterial colony is very useful to determine safety threats to the ready-to-eat salad(Sant'Ana et al., 2012), it provided strong evidence that the Raman intensity of 3 signature peaks could be considered as the markers of fresh and quality decay of arugula. Therefore, the headspace detection method using AuNPs coated fiber was potential to monitor the senescence of fresh-cut arugula leaves.

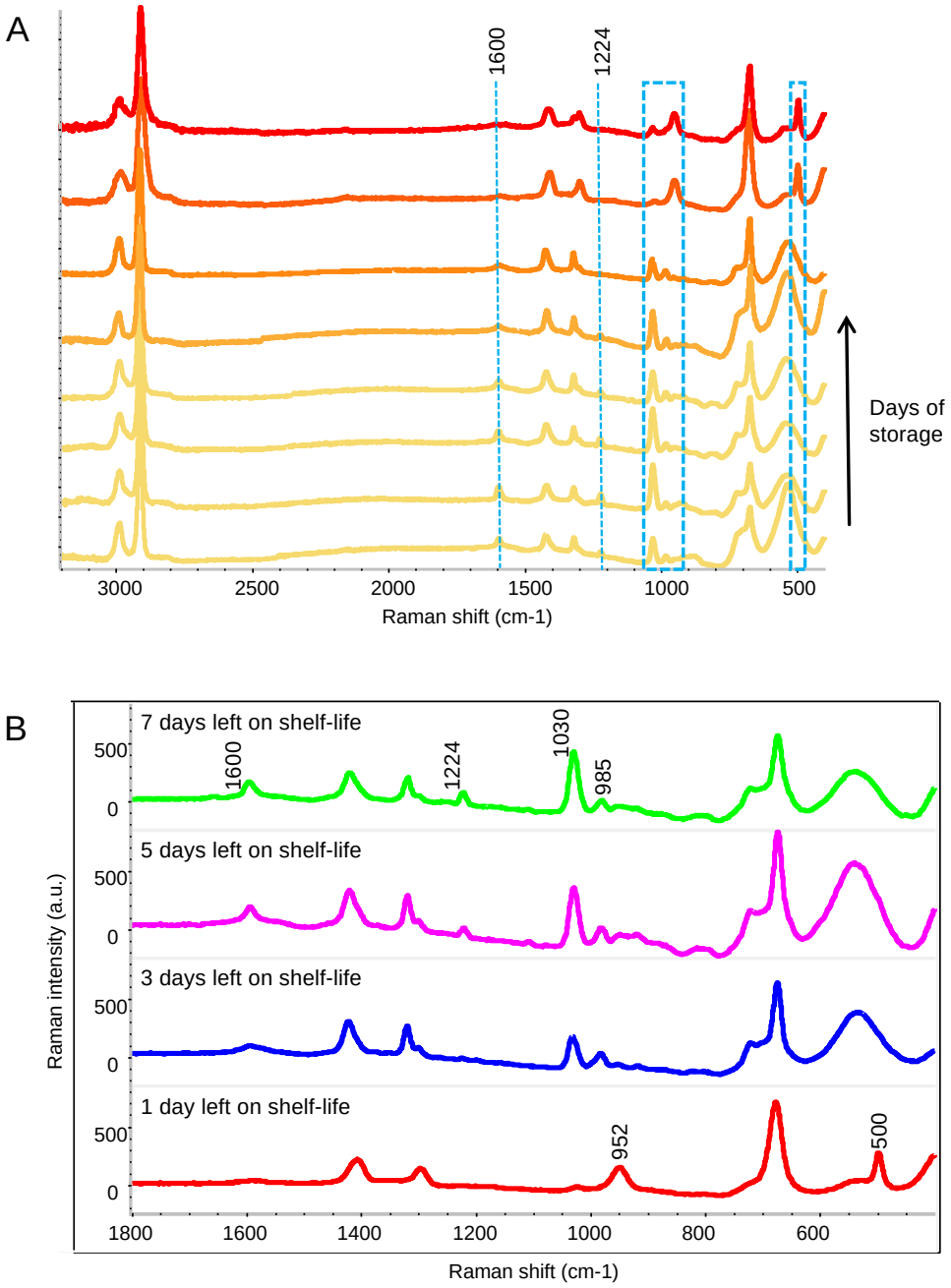


Figure 13: (A) SERS spectra of arugula leaves under varying degrees of freshness using AuNPs coated fiber and (B) Representative SERS spectra of arugula leaves in varying shelf-life using AuNPs coated fiber

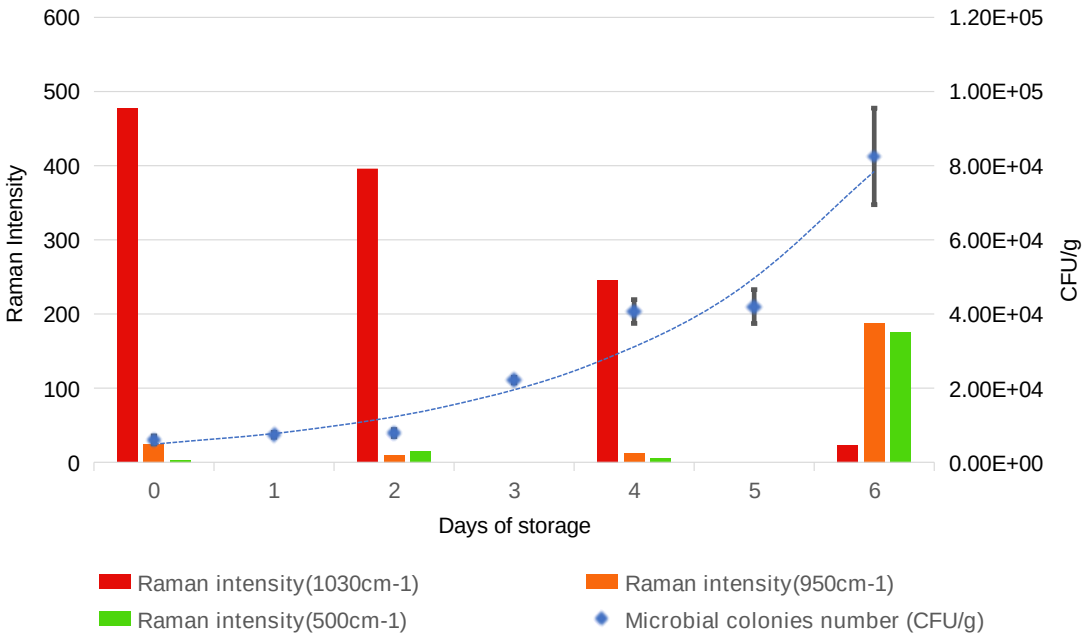


Figure 14: Comparison of Raman intensities of signature peaks and corresponding colony forming units (CFU/g) among arugula leaves under varying degrees of freshness over storage time

3.3.3 PCA analysis of the spectra over time and its application to predict unknown samples

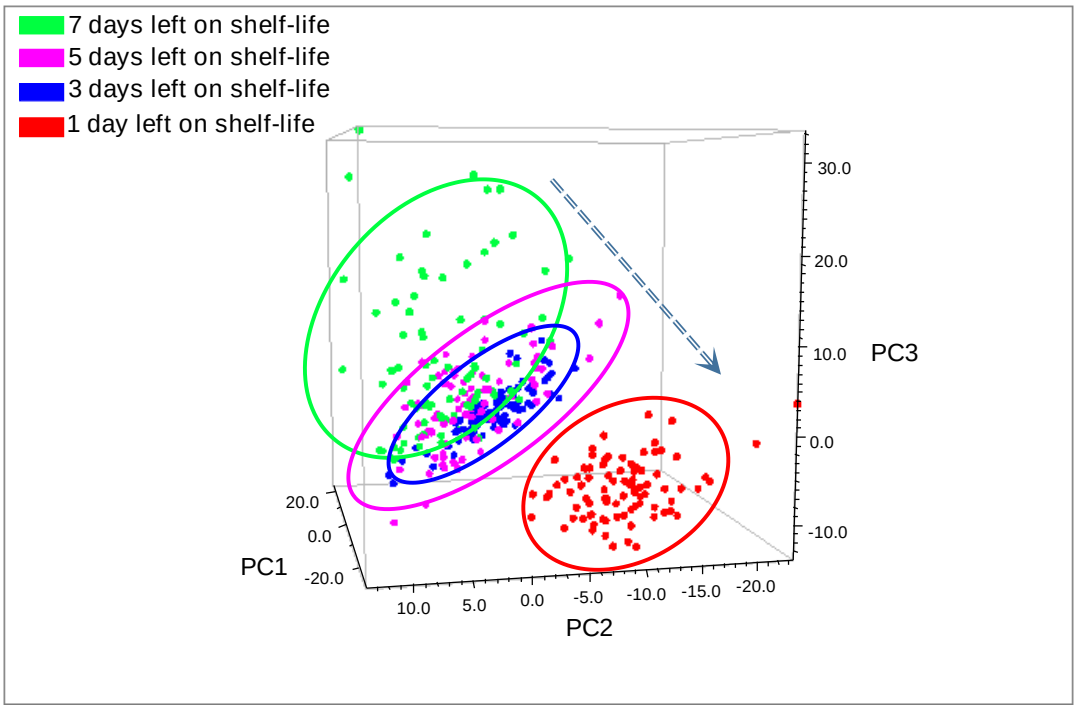


Figure 15: Principal component scores 3D Display model of the headspace detection method

Based on our previous discussion in 2.3.3, we analyzed the results in varying shelf-life with PCA. The PCA 3D model (Fig. 15) showed clear discrimination among 7days left on shelf-life, 5 days left on shelf-life, 3 days left on shelf-life and 1 day left on shelf-life, which indicated the significant statistical difference of different groups. Therefore, both the SERS spectra pattern and the PCA model had the potential to discriminate between fresh arugula and relatively unfresh arugula. As the headspace detection method using AuNPs coated fibers based on SERS was proved to be feasible for monitoring the arugula shelf-life, the database of SERS results of postharvest arugula leaves could be put into further application, such as predicting the exact shelf-life.

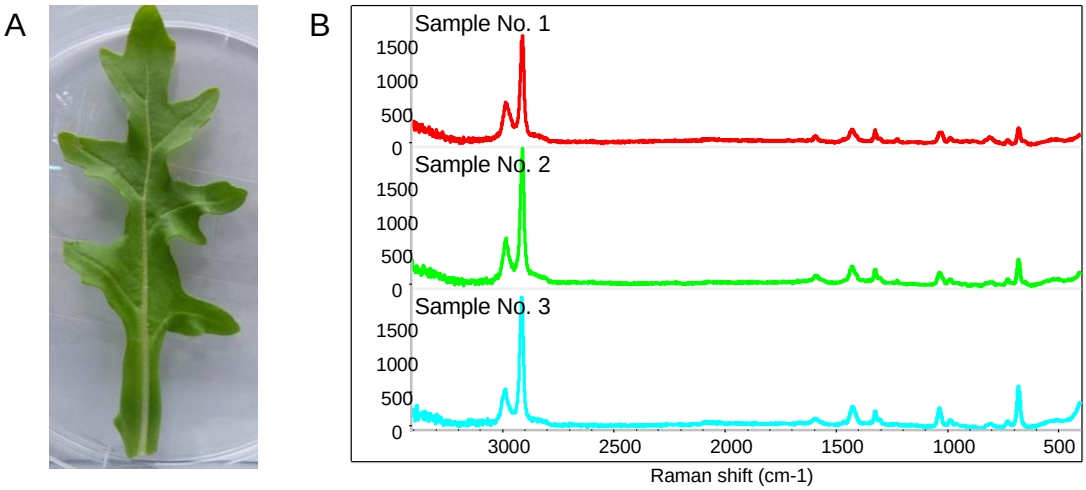


Figure 16: (A) Arugula leaves purchased from Whole Foods Market Inc. that were in good appearance. (B) SERS spectra obtained from each respective sample

To test the accuracy of the PCA model to predict the unknown sample, we randomly purchased a box of arugula leaves from a local grocery store and stored it for a week in the fridge. On day 8, we opened the box and these leaves were still with good quality as judged by the naked eyes from a regular consumer point of view. The spectra of the leaves were collected and analyzed using the PCA model, all three leaves were classified into the “3 days left on shelf-life”. After 3 days, most of the leaves in the box showed visible yellowing tendency as an

indication of low quality from a regular consumer perspective. This test demonstrates the potential usefulness of this method to predict the shelf life of fresh-cut produce.

3.3.4 In situ detection of arugula leaves within the original package

We designed comparing the fiber signals in glass incubators under normal temperature as well as under low temperature to the signals within the original package under low temperature to investigate whether the glass bottles were the suitable incubators for the volatile reaction. The comparison results presented in Figure 17 demonstrated that representative SERS spectra collected under different reaction conditions gave off nearly consistent signature peaks. Especially, for “7 days left on shelf-life”, “5 days left on shelf-life” and “3 days left on shelf-life” group, the Raman intensity of signature peaks at 677, 1325, and 1420 cm^{-1} were in the same level. However, when only 1 day left on shelf-life, the fibers incubated under room temperature gave off sharper peak at both the 500 and 950 cm^{-1}, which were essential indicators of fresh decay. Therefore, we could access the conclusion that generally the incubation setting of fibers, including temperature and container type, would not affect the headspace detection results. However, the results obtained in glass vials under room temperature were more sensitive for shelf-life analysis at the late storage stage.

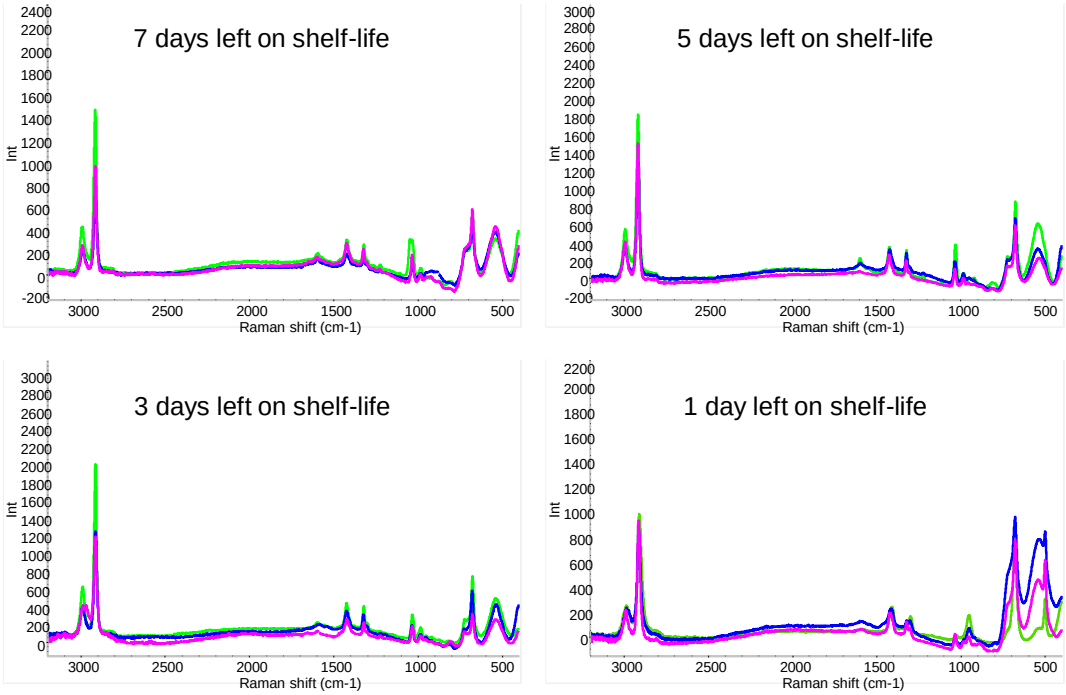


Figure 17: Comparison of SERS spectra of arugula leaves headspace obtained in glass containers and within the original package in varying shelf-life using AuNPs coated fiber

3.3.5 Chemical volatile compounds identification

To further investigate and assess the respective attributions of these peaks, we tested a set of reference compounds that are known to be produced from leafy greens at different stages. The representative spectra of related volatile compounds were arranged in terms of similarity (Fig. 18).

We successfully identified 3 chemical compounds that might contribute to SERS spectra of arugula senescence by the peak assignments presented in Figure 19. They are DMDS, MT and 1-propanethiol. DMDS and MT are original flavor components of fresh-cut arugula. However, with the catalysis action of microorganisms, sulfur-containing phytochemical compounds were proposed to be decomposed to DMDS, 1-propanethiol and MT(Miyazawa et al.,

2002)(Spadafora et al., 2016)(Korpi et al., 2009). Particularly, there would be a potent rise of DMDS as well as MT in related microbiological study based on arugula(Nielsen et al., 2008). Therefore, the SERS pattern of only 1 day left leaves could attribute to the dramatic increase of DMDS, 1-propanethiol or MT, and the dominant place of microbial spoilage at the late storage stage.

However, there was no chemical profile can completely match the spectra of fresh arugula that still have 7 days left on shelf-life. Therefore, the reason seems to be that various VOCs might collectively and synergistically contribute to the fresh arugula signature peaks.

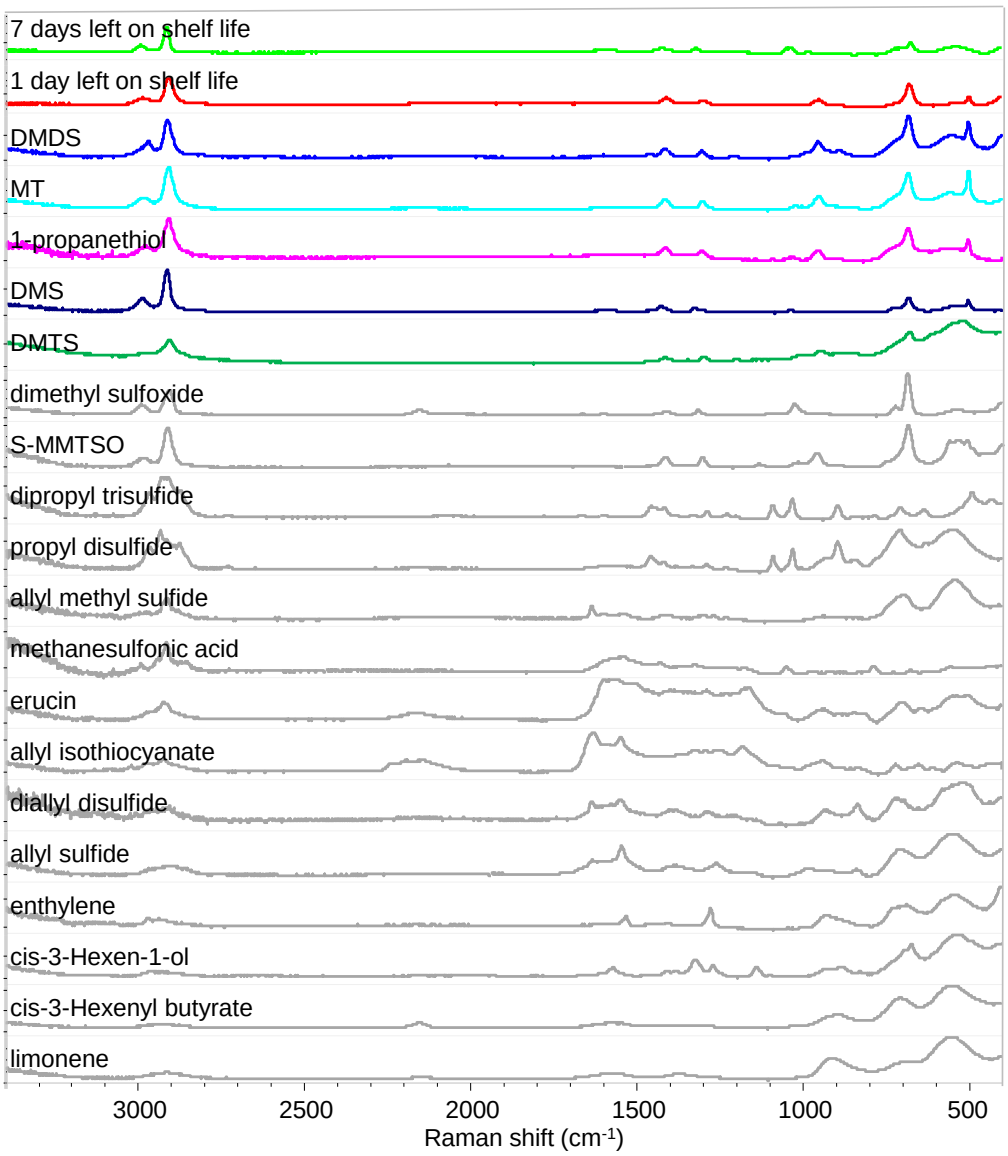


Figure 18: SERS spectra of relative volatile compounds: (a) arugula leaves have 7 days left on shelf-life, (b) arugula leaves have 1 day left on shelf life, (c) dimethyl disulfide, (d) methanethiol, (e) 1-propanethiol, (f) DMSO, (g) dimethyl trisulfide, (h) dimethyl sulfide, (i) S-MMTSO, (j) dipropyl trisulfide, (k) propyl disulfide, (l) allyl methyl sulfide, (m) methanesulfonic acid, (n) erucin, (o) allyl isothiocyanate, (p) diallyl disulfide, (q) ethylene, (r) allyl sulfide, (s) cis-3-Hexen-1-ol, (t) cis-3-Hexenyl butyrate and (u) limonene

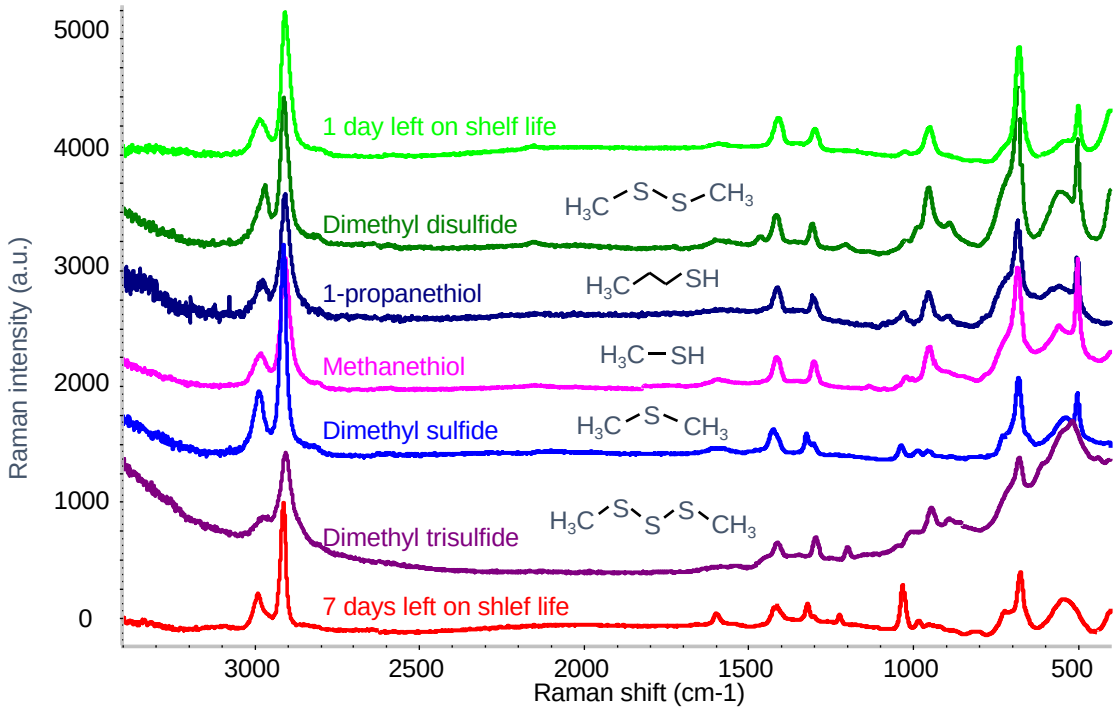


Figure 19: Representative SERS spectra of fresh-cut arugula have 7 days left on shelf-life and 1 day left on shelf-life with comparison to potential volatile compounds at 22±2°C

The SERS bands assignments referred to references were summarized in Table 1 to figure out the causes of signature peaks in the Raman pattern. However, the information provided by different literature could be complicated and even contradictory. Besides, specific peaks in different shelf-life groups had a slight difference in Raman shift, for example, fresh arugula leaves usually present a peak at 1420 cm^{-1}, while 1 day left on shelf-life leaves would present at 1410cm^{-1}. It was unclear whether these shifts were indicators of new volatile compounds or had to do with the influence of external factors. To sum up, the SERS bands assignments cannot elucidate the inherent Raman signals of arugula.

Table 1: Assignments of experimental SERS bands of postharvest arugula leaves under two degree of freshness: 7 days left on shelf-life and 0 days left on shelf-life

7 days left on shelf life	1 day left on shelf life	assignment	references
2990	2990	CH_3 stretching, C-H stretching	(Hope et al., 2001; Lim et al., 2006)
2910	2910	CH3 degenerated deformation	(Lim et al., 2006)
1600	/	S-ring, C=C vibration	(Yu et al., 2013)
1420	1410	CH3 rocking, CH3 degenerated deformation	(Hernández et al., 2011; Lim et al., 2006)
1325	/	CH_3 rocking, CH3 symmetric deformation	(Lim et al., 2006; SI et al., 2015)
/	1300	C-H bending	(SI et al., 2015)
1225	/	C-H bending	(SI et al., 2015)
1030	/	CH_3 rocking	(Lim et al., 2006)
985	/	C-S bending, C-H bending, CH_3 rocking	(Hope et al., 2001; Lim et al., 2006; López-Tobar et al., 2013; SI et al., 2015)
/	950	CH_3 rocking	(Hope et al., 2001)
677	677	C-S stretching	(Hernández et al., 2011; Kelly et al., 2018; Li et al., 2007; Lim et al., 2006; López-Tobar et al., 2013; Noh et al., 2007; Ohno et al., 1993; SI et al., 2015)
/	500	S-S stretching	(López-Tobar et al., 2013; Noh et al., 2007)

3.3.6 Microbial analysis and isolates identification

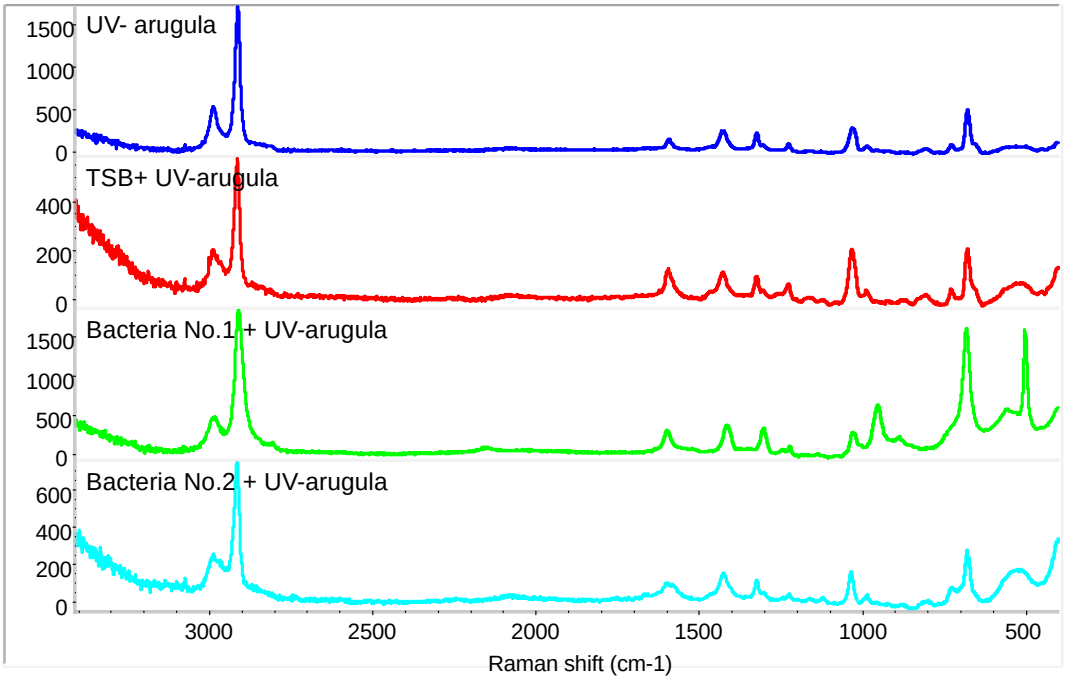


Figure 20: Raman spectra of sterilized fresh arugula leaves, sterilized fresh arugula leaves inoculated with TSB solution, sterilized fresh arugula leaves inoculated with bacteria No.1 culture solution and sterilized fresh arugula leaves inoculated with bacteria No.2 culture solution

We utilized inoculation with microorganisms to verify the microbial metabolism using the headspace detection method. After inoculated with the bacteria No.1 incubation solution, we found that two sharp and strong characteristic peaks were present at 500 and 950 cm^{-1} in sterilized fresh arugula spectra, while the peak at 1030 cm^{-1}declined (Fig. 20). More specifically, the sterilized fresh-cut arugula inoculated with target bacteria gave off similar signals as leaves had 1 day left on shelf-life. However, data from both the sterilized fresh arugula leaves inoculated with TSB solution and sterilized fresh arugula leaves inoculated with bacteria No.2 incubation solution was similar to the inherent fresh arugula spectra (Fig. 20). Hence, the bacteria, which was most likely to be Pseudomonadaceae&Xanthamonadaceae supported by Nielsen(Nielsen et al., 2008), could account for the off-odor emitted during senescence. Moreover, Chen(Chen, 2018) reported that spoiled meat also had peaks at 1410, 1300 and 950

cm^{-1} Raman shift. Since we had discussed that DMDS, 1-propanethiol and MT produced by microbial metabolism could account for the signals from unfresh leaves in 3.3.5, the principle of our headspace detection was due to the fiber reaction with DMDS, 1-propanethiol or MT. As numerous study had utilized volatiles analyses to identify and separate microorganisms(Schnürer et al., 1999)(Korpi et al., 2009), the future study could be characterizing microbial profiles using SERS.

3.4 Conclusion

The AuNPs coated fibers were applicable to monitor the volatiles signals from fresh-cut arugula leaves using SERS. Both the SERS spectra and PCA model showed clear discrimination between arugula leaves of different freshness degrees. Peak profile analysis demonstrates the characteristic peaks of dimethyl disulfide, 1-propanethiol and methanethiol that could be released by microbial metabolism are indicative of shelf-life decrease. Moreover, SERS spectra obtained from microbial inoculation arugula verified this explanation. In conclusion, the headspace detection method using AuNPs coated fiber is rapid and sensitive, and has the practical potential for shelf-life monitoring. Hence, future work will mainly focus on making use of this approach for shelf-life detection of various food.

CHAPTER 4

DEVELOPMENT OF A HEADSPACE DETECTION METHOD FOR LIVING BASIL PLANTS STRESS RESPONSE

4.1 Introduction

In Chapter 1, we explained the common remote sensing system compositions for plants phenotyping are typically a stress sensor in conjunction with advanced data analysis methods. Because we sketched a headspace detection method using an innovative AuNPs coated fiber based on SERS to monitor the fresh decay of fresh-cut arugula leaves in chapter 3. And this method has been proved that was capable of monitoring the differentiation among arugula leaves on different shelf-life, as well as predicting the real-time shelf-life. Hence, the AuNPs coated fiber could seem as an innovative stress sensor without the extra requirement for advanced analysis technology to sense the phenotyping and stress.

4.1.1 Plant stress response and VOCs

As discussed in chapter 1, exposure to abiotic or biotic stresses will result in a change in phytochemical content as well as VOCs. Living plants will release specific volatile compounds, such as ethylene, methyl salicylate, and jasmonate as defensive signals to modulate levels of systemic acquired resistance (SAR) against disease and predators(Rowan, 2011)(Heil & Ton, 2010)(Dicke et al., 2009). Isoprene was the secondary metabolite emitted to alleviate oxidative stress(Rowan, 2011). Hence, the volatiles produced in the internal and external communication of living is helpful to reveal plant responses to varied environmental stressors. For instance, herbivore-damaged leaves have the self-defensive mechanism of releasing specific VOCs to attract arthropod predators and parasitoids (Tang et al., 2012). Generally, most of the preharvest stress factors may increase the abundance of VOCs(Holopainen & Gershenzon, 2010)(Bell et al., 2016). Nevertheless, there is limited reference about the effect of abiotic

stresses on VOCs produced by living plants. Thus, the headspace detection method using AuNPs coated fiber is promising to examine post-stress induction and to sense the phenotyping.

4.1.2 Response of living basil to abiotic stress

As a herb species, basil is abundant in various VOCs, which are implicated in the defense systems against environmental stresses(Muhammad Siddiq, 2018).To preliminarily study the response of basil against abiotic stresses based on our headspace detection method, basil plants were subjected to selected abiotic stresses treatments, soil salinity as well as pesticide individually in this study. According to reference, although basil is considered to be a moderate saline-alkali tolerant plant(Scagel et al., 2019), salinity was demonstrated that could significantly decrease the plant biomass, stem length, leaves thickness and chlorophylls, with the enhancement of lipid peroxidation(Bekhradi et al., 2015). Because high salinity can cause osmotic imbalance as well as accumulation of Na+ or Cl- to toxic levels, uptake and assimilation of essential nutrients and photosynthetic activity will inevitably be inhibited in the diminished plant tissue(Scagel et al., 2019)(Attia et al., 2011). Further, salinity may trigger secondary-induced oxidative stress due to the production of reactive oxygen species (ROS)(Attia et al., 2011). In view of multiple interactions triggered by a single factor(Holopainen & Gershenzon, 2010), only the experiments held under realistic environmental setting will be able to study the natural occurrence of VOCs. Consequently, the basil plants are planned to be exposed to stress factors in the open-field. Compared with physical parameters, such as light, temperature, salinity, etc., the effect of artificial chemical applications like pesticides on the emission of VOCs in basil is unclear.

4.1.3 Objectives of this study

The objectives of this study were to: (1) characterize the SERS spectra of living basil plants under the high salinity stress (2) characterize the SERS spectra of living basil plants under the application of imidacloprid

4.2 Material and methods

4.2.1 Materials

Imidacloprid and sodium chloride (≥99.95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 100ppm imidacloprid stock solution was prepared in acetonitrile and diluted to needed concentration with distilled water. 200mM sodium chloride solution was prepared in distilled water to make saline water.

4.2.2 Fabrication of gold nanoparticles coated fibers

Refer to 3.2.2 for the AuNPs coated fibers fabrication and storage.

4.2.3 Preparation of samples

Sweet basil plants purchased from Big Y(Amherst, MA) were cultivated on well-drained soils under long days accessing to natural light(Taylor, 2008). The temperature was kept between 20 to 27°Cinindoor. For the control group, each plant was supplied with 20ml distilled water every day, while the high salinity group was adopted 20ml 200mM saline water. Meanwhile, 20ml 40ppm imidacloprid solution was singly adopted to basil at the beginning and supplied with the same amount of distilled water as the control group in the following days. To be noted, the basil plants were covered by transparent plastic buckets which were suspended with enough space to keep the normal air exchange. And there are several slight pores on the buckets' wall for inserting fibers to monitor the volatiles in the headspace inside. The reaction duration was 2 hours.

4.2.4 Instrument and data analysis

Refer to 3.2.3 for the experimental SERS setting and data analysis.

4.3 Result and discussion

4.3.1 Characterization of the SERS spectra of living basil against pesticide

To investigate the feasibility of sensing the basil phenotyping based on our headspace detection approach. We first compare the plants after a single application of pesticides with controlled healthy plants. Imidacloprid, as a representative systematic pesticide, was applied to the soil with maximum allowed usage at the beginning. The SERS spectra observe in the following ten days after imidacloprid application were presented in Figure 21. After two hours of exposure, Raman cannot sense the stress immediately. However, when exposed to pesticides for one day, basil gave off the volatile signals significantly different from the control. There were three robust peaks at 1596, 1225 and 1030cm^{-1}. Nevertheless, the volatile emission was transitory, and the SERS spectra completely recovered in three days. In the long run, we found that basil exposed to imidacloprid tended to have lower Raman intensity, especially after 10 days (Fig. 21). As systemic insecticide are usually absorbed by roots and then translocated to the rest of the plant via the xylem tissue, the mechanism of nutrient delivery in vivo lead to lagging phenotyping. Hence, after the application, we could not observe the stress in Raman spectra immediately. Basil showed early visible symptoms against stress after exposed for seven days. While healthy plants were growing vigorously, the growth tendency of pesticide group declined obviously. It demonstrated that AuNPs coated fibers only had the potential to sense the influence of imidacloprid at an early point in time after adoption. However, it was unfeasible to discriminate the differentiation of SERS pattern before visible phenotyping.

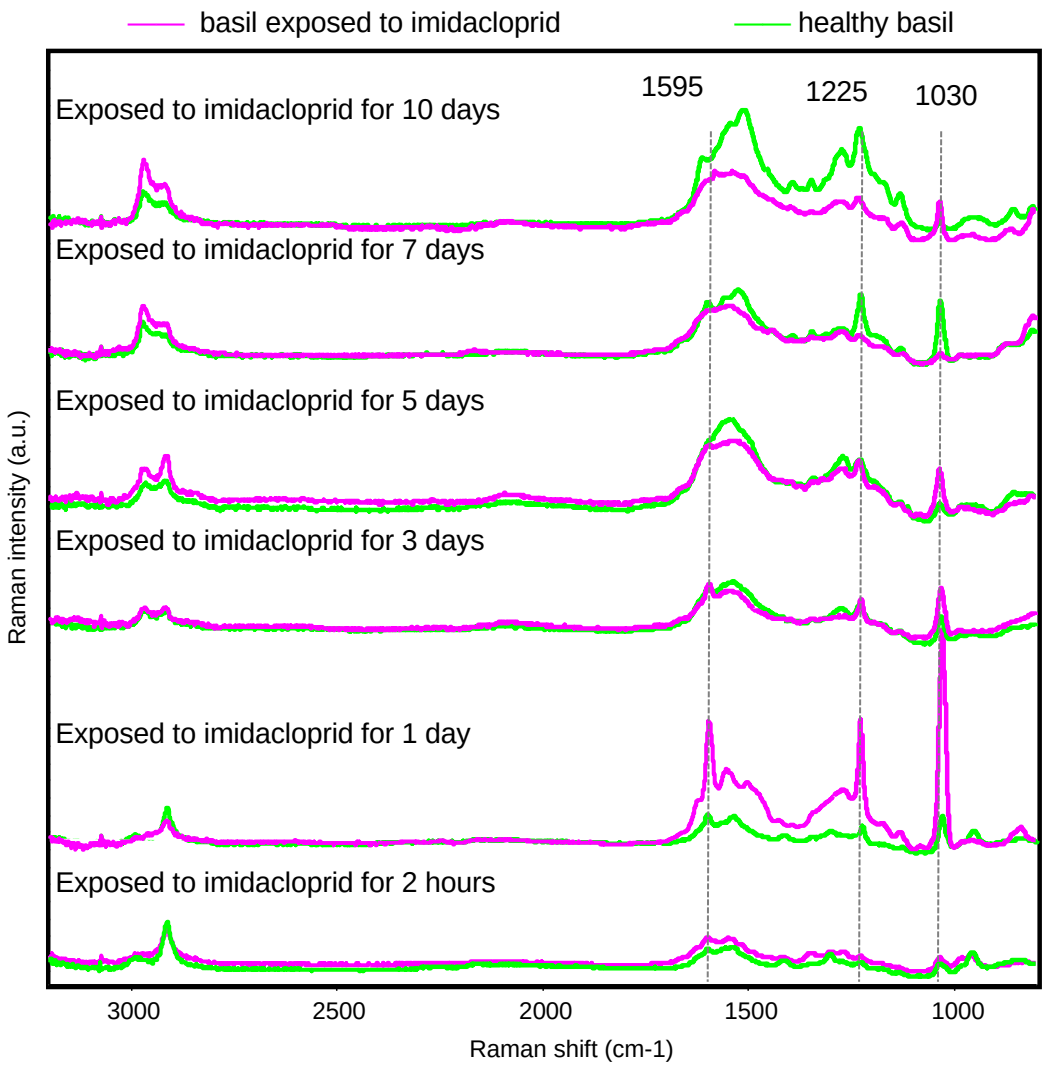


Figure 21: SERS spectra of living basil plants under stress of imidacloprid with control

4.3.2 Characterization of the SERS spectra of living basil against high salinity

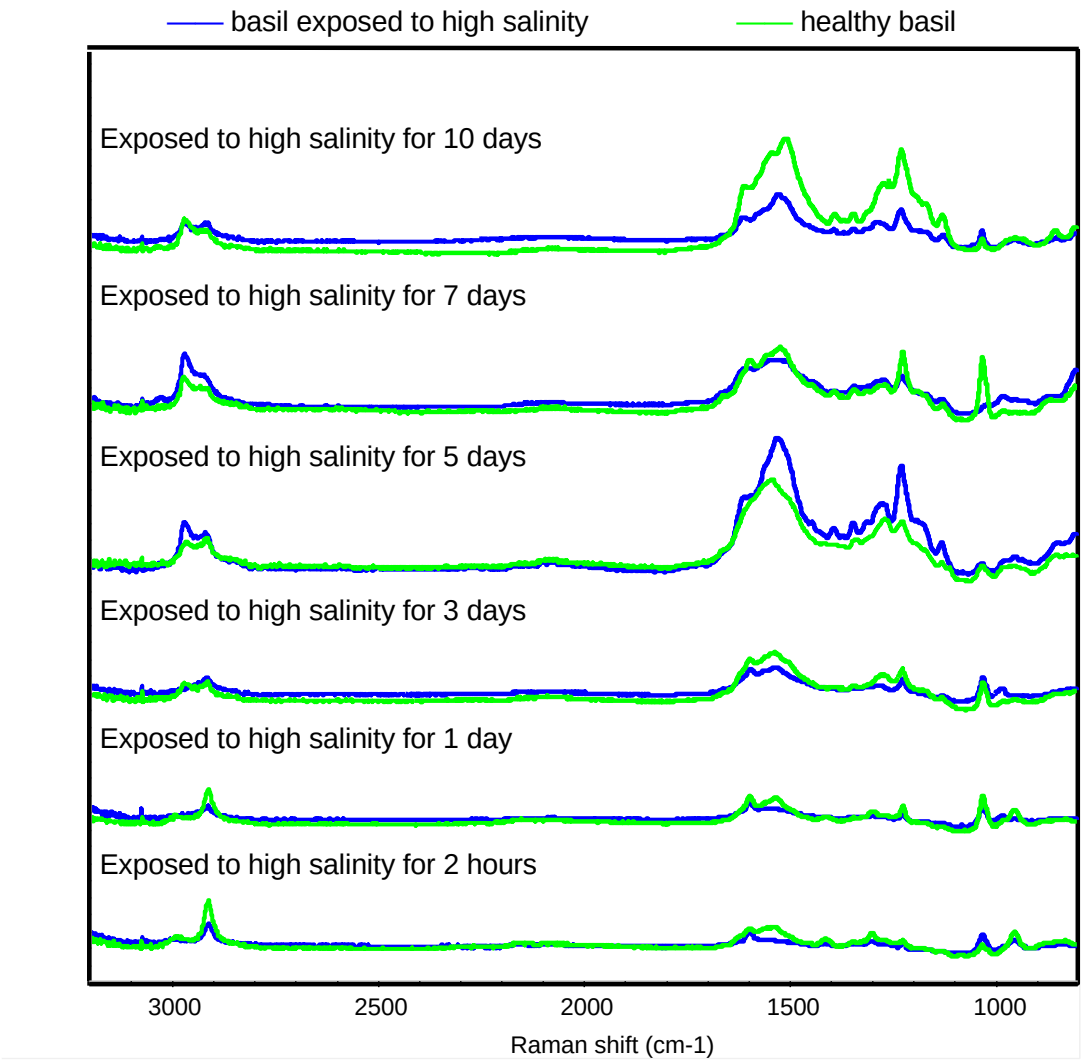


Figure 22: SERS spectra of living basil plants under stress of high salinity with control

The influence of high salinity on volatile metabolites produced by basil was also tested in the study by comparing with the healthy controlled plants. In Figure 22, we found that there was no significant difference between the SERS spectra of two groups. In the long run, we found that basil exposed to high salinity tended to have lower Raman intensity, especially after 10 days

(Fig. 22), which was similar to the pesticide group. However, when exposed to salty water for five days, basil plants had already shown a sign of wilting. And after ten days, basil samples had obvious senescence appearance. According to reference(Holopainen & Gershenzon, 2010)(Attia et al., 2011), high salinity was considered to be beneficial to enhance the VOCs emission. It could attribute to the fact that volatile metabolites profiles are multiple and highly method-dependent(Rowan, 2011). Because there was no single headspace detection approach capable of providing comprehensive volatile profiles, various volatile analyses need to be synergistically utilized. Consequently, it is unfeasible for the headspace detection method using AuNPs coated fibers with SERS to sense the early visible phenotyping.

4.4 Conclusion

We investigate the feasibility of using the headspace detection methods with AuNPs coated fibers to sense the basil plants response to abiotic stresses in this study. The influence of imidacloprid could be detected at early time points, however, the differentiation in SERS spectra was less potent than visible phenotyping. In addition, SERS was unable to discriminate basil plants under high salinity before visible phenotyping occurs. Nevertheless, after ten days of exposure, both groups emitted less phytochemical VOCs. In conclusion, these two abiotic stresses could be assessed with other methods. In further study, we could assess other parameters using the AuNPs coated fibers as well as increase the sensitivity.

CHAPTER 5

CONCLUSION

A rapid, sensitive and reliable headspace approach for monitoring the plant health and determining the shelf-life of the postharvest producer has been developed. The method utilized the AuNPs coated fibers as SERS-active substrates to detect the volatile composition released by fresh-cut arugula and living basil plants in this study. Compared with traditional nanoparticles solution, the fibers were more reproducible and stabler in Raman signals, as well as more sensitive to leafy greens senescence. As both of the SERS signature spectra peaks and PCA 3D display model could discriminate the arugula leaves close to shelf-life, the headspace analysis offered higher effectiveness than microbial analysis and visual inspection. Moreover, the method was capable of predicting the remaining shelf life. To be noted, the nanosensor was not only feasible in experimental incubators, but also suitable for integrating into the package. Furthermore, it was discovered that the DMDS and MT emitted by microbial metabolism were the major causes of the typical signals from unfresh arugula. In addition, the headspace analysis also secening some slight negative response of basils to abiotic stresses (pesticide/salinity).

Further development of this method will be focused on applying fibers to predict shelf-life of various fresh produce. Moreover, continued study for plant stress response study based on the headspace approach is promising.

APPENDIX

SUPPLEMENTARY INFORMATION

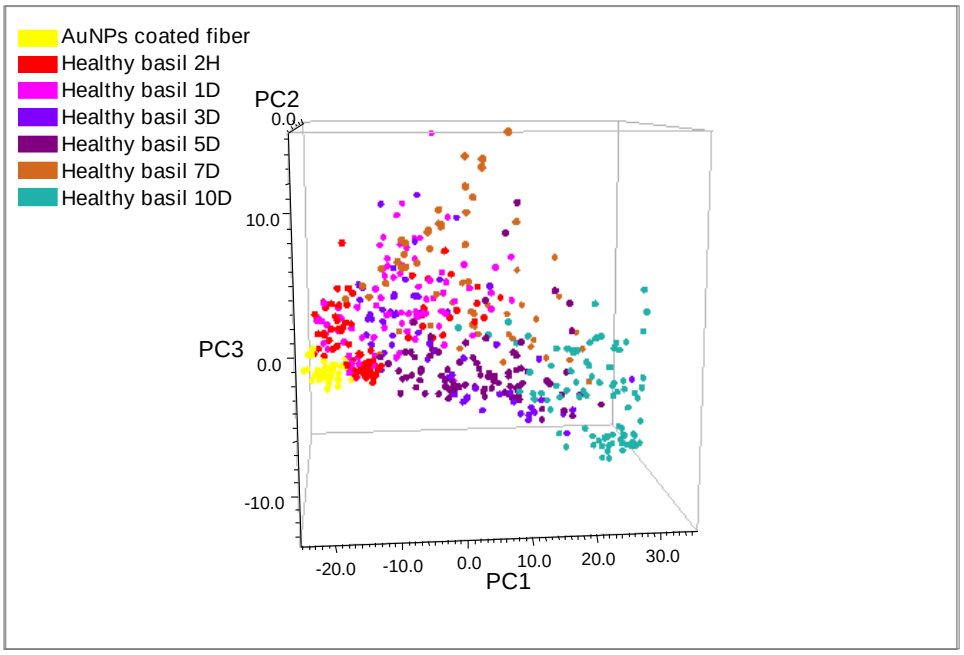


Figure A 1: Principal component scores 3D Display model of AuNPs coated fibers for healthy living basil plants

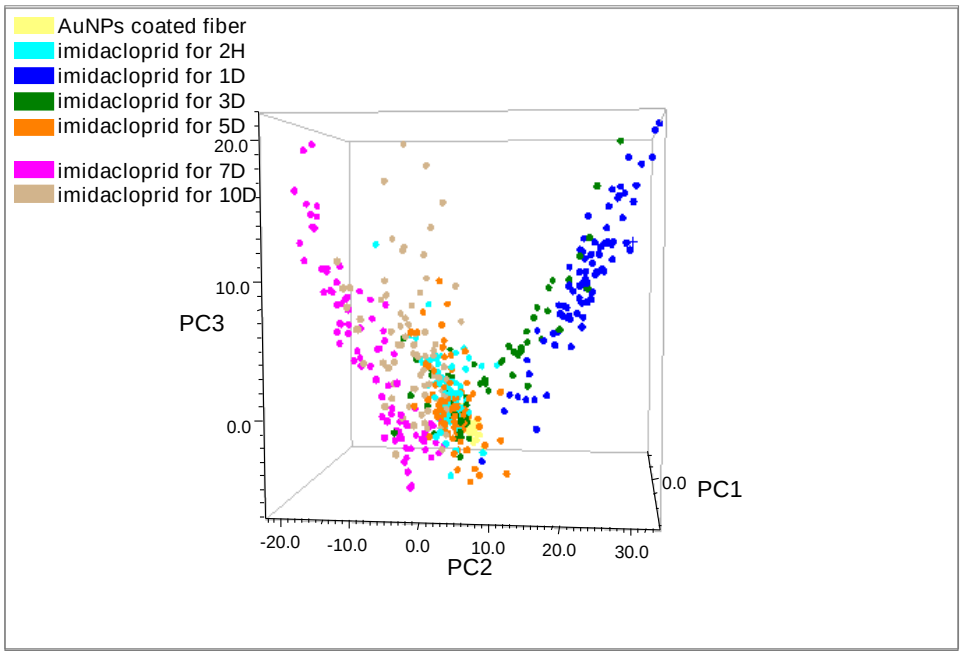


Figure A 2: Principal component scores 3D Display model of AuNPs coated fibers for living basil plants under stress of imidacloprid with controls

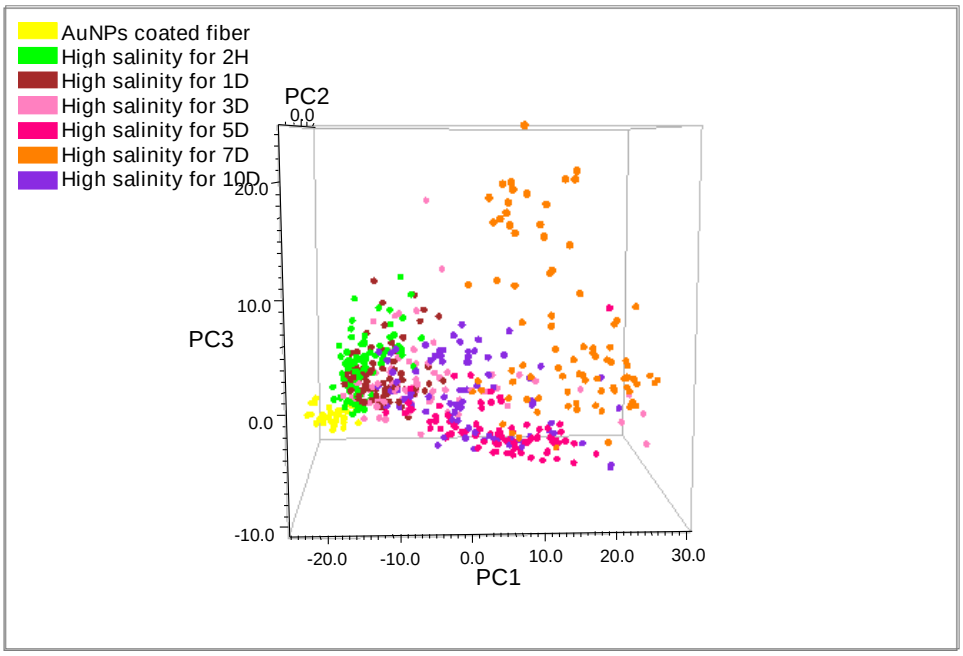


Figure A 3: Principal component scores 3D Display model of AuNPs coated fibers for living basil plants under stress of high salinity with controls

BIBLIOGRAPHY

Ač, A., Malenovský, Z., Olejníčková, J., Gallé, A., Rascher, U., & Mohammed, G. (2015). Meta-analysis assessing potential of steady-state chlorophyll fluorescence for remote sensing detection of plant water, temperature and nitrogen stress. *Remote Sensing of Environment*, *168*, 420–436. https://doi.org/10.1016/j.rse.2015.07.022

Ahmad, S., Ahmad, M., Maqbool, M., Dar, B. N., Greiner, R., & Roohinejad, S. (2018). Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. *Food Control*, *85*, 235–244. https://doi.org/10.1016/j.foodcont.2017.10.006

Altangerel, N., Ariunbold, G. O., Gorman, C., Alkahtani, M. H., Borrego, E. J., Bohlmeyer, D., Hemmer, P., Kolomiets, M. V., Yuan, J. S., & Scully, M. O. (2017). In vivo diagnostics of early abiotic plant stress response via Raman spectroscopy. *Proceedings of the National Academy of Sciences*, *114*(13), 3393–3396. https://doi.org/10.1073/pnas.1701328114

Amodio, M. L., Derossi, A., Mastrandrea, L., & Colelli, G. (2015). A study of the estimated shelf life of fresh rocket using a non-linear model. *JOURNAL OF FOOD ENGINEERING*, *150*, 19–28. https://doi.org/10.1016/j.jfoodeng.2014.10.030

Attia, H., Ouhibi, C., Ellili, A., Msilini, N., Bouzaïen, G., Karray, N., & Lachaâl, M. (2011). Analysis of salinity effects on basil leaf surface area, photosynthetic activity, and growth. *Acta Physiologiae Plantarum*, *33*(3), 823–833. https://doi.org/10.1007/s11738-010-0607-6

Bekhradi, F., Delshad, M., Marín, A., Luna, M. C., Garrido, Y., Kashi, A., Babalar, M., & Gil, M. I. (2015). Effects of salt stress on physiological and postharvest quality characteristics of different Iranian genotypes of basil. *Horticulture Environment and Biotechnology*, *56*(6), 777–785. https://doi.org/10.1007/s13580-015-1095-9

Bell, L., Nadia, H., Oluwadamilola, O., Methven, L., & Wagstaff, C. (2017). Changes in rocket salad phytochemicals within the commercial supply chain : Glucosinolates , isothiocyanates , amino acids and bacterial load increase significantly after processing. *Food Chemistry*, *221*, 521–534. https://doi.org/10.1016/j.foodchem.2016.11.154

Bell, L., Oruna-Concha, M. J., & Wagstaff, C. (2015). Identification and quantification of glucosinolate and flavonol compounds in rocket salad (Eruca sativa, Eruca vesicaria and Diplotaxis tenuifolia) by LC-MS: Highlighting the potential for improving nutritional value of rocket crops. *Food Chemistry*, *172*, 852–861. https://doi.org/10.1016/j.foodchem.2014.09.116

Bell, L., Spadafora, N. D., Müller, C. T., Wagstaff, C., & Rogers, H. J. (2016). Use of TD-GC-TOF-MS to assess volatile composition during post-harvest storage in seven accessions of rocket salad (Eruca sativa). *Food Chemistry*, *194*, 626–636. https://doi.org/10.1016/j.foodchem.2015.08.043

Bell, L., & Wagstaff, C. (2014). Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (eruca sativa and diplotaxis tenuifolia). *Journal of Agricultural and Food Chemistry*, *62*(20), 4481–4492. https://doi.org/10.1021/jf501096x

Bennett, R. N., Mellon, F. A., Botting, N. P., Eagles, J., Rosa, E. A. S., & Williamson, G. (2002). Identification of the major glucosinolate (4-mercaptobutyl glucosinolate) in leaves of Eruca sativa L. (salad rocket). *Phytochemistry*, *61*(1), 25–30. https://doi.org/10.1016/S0031-9422(02)00203-0

Boisselier, E., & Astruc, D. (2009). Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. *Chemical Society Reviews*, *38*(6), 1759–1782. https://doi.org/10.1039/b806051g

Booth, E., Strobel, G., Knighton, B., Sears, J., Geary, B., & Avci, R. (2011). A rapid column technique for trapping and collecting of volatile fungal hydrocarbons and hydrocarbon derivatives. *Biotechnology Letters*, *33*(10), 1963–1972. https://doi.org/10.1007/s10529-011-0660-2

Char, C., Silveira, A. C., Inestroza-Lizardo, C., Hinojosa, A., Machuca, A., & Escalona, V. H. (2012). Effect of noble gas-enriched atmospheres on the overall quality of ready-to-eat arugula salads. *Postharvest Biology and Technology*, *73*, 50–55. https://doi.org/10.1016/j.postharvbio.2012.05.010

Char, C., Yoplac, I., & Escalona, V. H. (2017). Microbiological and Functional Quality of Ready-to-Eat Arugula as Treated by Combinations of UV-C and Nonconventional Modified Atmospheres. *Journal of Food Processing and Preservation*, *41*(3), e12978. https://doi.org/10.1111/jfpp.12978

Chen, H. (2018). Development and Application of A SERS Needle for One-step Multi-phase Analysis. *Masters Theses*, *713*.

CHEN, H., WANG, C., ZHANG, Z., & HE, L. (2018). Combining Headspace Solid-Phase Microextraction and Surface-Enhanced Raman Spectroscopy To Detect the Pesticide Fonofos in Apple Juice. *Journal of Food Protection*, *81*(7), 1087–1092. https://doi.org/10.4315/0362-028X.JFP-17-505

Chin, H. W., & Lindsay, R. C. (1994). Mechanisms of Formation of Volatile Sulfur Compounds following the Action of Cysteine Sulfoxide Lyases. *Journal of Agricultural and Food Chemistry*, *42*(7), 1529–1536. https://doi.org/10.1021/jf00043a026

Cozzolino, R., Pace, B., Cefola, M., Martignetti, A., Stocchero, M., Fratianni, F., Nazzaro, F., & De Giulio, B. (2016). Assessment of volatile profile as potential marker of chilling injury of basil leaves during postharvest storage. *Food Chemistry*, *213*, 361–368. https://doi.org/10.1016/j.foodchem.2016.06.109

Craig, A. P., Franca, A. S., & Irudayaraj, J. (2013). Surface-Enhanced Raman Spectroscopy Applied to Food Safety. *Annual Review of Food Science and Technology*, *4*(1), 369–380. https://doi.org/10.1146/annurev-food-022811-101227

De Corato, U. (2019). Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: A comprehensive critical review from the traditional technologies into the most promising advancements. *Critical Reviews in Food Science and Nutrition*, *0*(0), 1–36. https://doi.org/10.1080/10408398.2018.1553025

Dicke, M., Van Loon, J. J. A., & Soler, R. (2009). Chemical complexity of volatiles from plants induced by multiple attack. *Nature Chemical Biology*, *5*(5), 317–324. https://doi.org/10.1038/nchembio.169

Engel, E., Baty, C., Le Corre, D., Souchon, I., & Martin, N. (2002). Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *Journal of Agricultural and Food Chemistry*, *50*(22), 6459–6467. https://doi.org/10.1021/jf025579u

Ezra, D., Jasper, J., Rogers, T., Knighton, B., Grimsrud, E., & Strobel, G. (2004). Proton transfer reaction-mass spectrometry as a technique to measure volatile emissions of Muscodor albus. *Plant Science*, *166*(6), 1471–1477. https://doi.org/10.1016/j.plantsci.2004.01.022

Farber, C., & Kurouski, D. (2018). Detection and Identification of Plant Pathogens on Maize Kernels with a Hand-Held Raman Spectrometer. *Analytical Chemistry*, *90*(5), 3009–3012. https://doi.org/10.1021/acs.analchem.8b00222

Farber, J. N., Harris, L. J., Parish, M. E., Beuchat, L. R., Suslow, T. V., Gorney, J. R., Garrett, E. H., & Busta, F. F. (2006). Microbiological Safety of Controlled and Modified Atmosphere Packaging of Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety*, *2*(s1), 142–160. https://doi.org/10.1111/j.1541-4337.2003.tb00032.x

Force, L. E., Hare, T. J. O., Wong, L. S., & Irving, D. E. (2007). *Impact of cold storage on glucosinolate levels in seed-sprouts of broccoli , rocket , white radish and kohl-rabi*. *44*, 175–178. https://doi.org/10.1016/j.postharvbio.2006.11.014

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Aguayo, E., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2017). A Functional Smoothie from Carrots with Induced Enhanced Phenolic Content. *Food and Bioprocess Technology*, *10*(3), 491–502. https://doi.org/10.1007/s11947-016-1829-4

Glindemann, D., Novak, J., & Witherspoon, J. (2006). Dimethyl sulfoxide (DMSO) waste residues and municipal waste water odor by dimethyl sulfide (DMS): The north-east WPCP plant of Philadelphia. *Environmental Science and Technology*, *40*(1), 202–207. https://doi.org/10.1021/es051312a

Gutiérrez, D. R., & Rodríguez, S. C. (2017). *Combined Effect of UV-C and Modified Atmosphere Packaging for Keeping Antioxidant Compounds and Extend to Shelf-Life of Fresh-Cut Rocket Leaves*. *6*, 62–68.

Heil, M., & Ton, J. (2010). *Progress in Botany, Vol. 71*. *71*(September). https://doi.org/10.1007/978-3-642-02167-1

Hernández, B., Pflüger, F., Adenier, A., Kruglik, S. G., & Ghomi, M. (2011). Side chain flexibility and protonation states of sulfur atom containing amino acids. *Physical Chemistry Chemical Physics*, *13*(38), 17284–17294. https://doi.org/10.1039/c1cp21054h

Holopainen, J. K., & Gershenzon, J. (2010). Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, *15*(3), 176–184. https://doi.org/10.1016/j.tplants.2010.01.006

Hope, G. A., Watling, K., & Woods, R. (2001). A SERS spectroelectrochemical investigation of the interaction of isopropyl, isobutyl and isoamyl xanthates with silver. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *178*(1–3), 157–166. https://doi.org/10.1016/S0927-7757(00)00692-0

Hou, R., Zhang, Z., Pang, S., Yang, T., Clark, J. M., & He, L. (2016). Alteration of the Nonsystemic Behavior of the Pesticide Ferbam on Tea Leaves by Engineered Gold Nanoparticles. *Environmental Science and Technology*, *50*(12), 6216–6223. https://doi.org/10.1021/acs.est.6b01336

Inestroza-Lizardo, C., Silveira, A. C., & Escalona, V. H. (2016). Metabolic activity, microbial growth and sensory quality of arugula leaves (Eruca vesicaria Mill.) stored under non-conventional modified atmosphere packaging. *Scientia Horticulturae*, *209*, 79–85. https://doi.org/10.1016/j.scienta.2016.06.007

James, S. (2015). Grand View Research. *Curcumin Market Analysis By Application (Pharmaceutical, Food, Cosmetics) And Segment Forecasts To 2022*, *5699*. http://www.grandviewresearch.com/industry-analysis/turmeric-extract-curcumin-market

Jirovetz, L., Smith, D., & Buchbauer, G. (2002). Aroma compound analysis of Eruca sativa (Brassicaceae) SPME headspace leaf samples using GC, GC-MS, and olfactometry. *Journal of Agricultural and Food Chemistry*, *50*(16), 4643–4646. https://doi.org/10.1021/jf020129n

Kai Zhang, L. Z. (2014). Volatile Organic Compounds as Novel Markers for the Detection of Bacterial Infections. *Clinical Microbiology: Open Access*, *03*(03). https://doi.org/10.4172/2327-5073.1000151

Kelly, J., Patrick, R., Patrick, S., & Bell, S. E. J. (2018). Surface-Enhanced Raman Spectroscopy for the Detection of a Metabolic Product in the Headspace Above Live Bacterial Cultures. *Angewandte Chemie - International Edition*, *57*(48), 15686–15690. https://doi.org/10.1002/anie.201808185

Korpi, A., Järnberg, J., & Pasanen, A. L. (2009). Microbial volatile organic compounds. *Critical Reviews in Toxicology*, *39*(2), 139–193. https://doi.org/10.1080/10408440802291497

Koukounaras, A., Siomos, A. S., & Sfakiotakis, E. (2007). Postharvest CO2 and ethylene production and quality of rocket (Eruca sativa Mill.) leaves as affected by leaf age and storage temperature. *Postharvest Biology and Technology*, *46*(2), 167–173. https://doi.org/10.1016/j.postharvbio.2007.04.007

Lee, K. S., & El-Sayed, M. A. (2006). Gold and silver nanoparticles in sensing and imaging: Sensitivity of plasmon response to size, shape, and metal composition. *Journal of Physical Chemistry B*, *110*(39), 19220–19225. https://doi.org/10.1021/jp062536y

Li, S., Wu, D., Xu, X., & Gu, R. (2007). Theoretical and experimental studies on the adsorption behavior of thiophenol on gold nanoparticles. *Journal of Raman Spectroscopy*, *38*(11), 1436–1443. https://doi.org/10.1002/jrs.1791

Lim, J. K., Kim, I. H., Kim, K. H., Shin, K. S., Kang, W., Choo, J., & Joo, S. W. (2006). Adsorption of dimethyl sulfide and methanethiolate on Ag and Au surfaces: Surface-enhanced Raman scattering and density functional theory calculation study. *Chemical Physics*, *330*(1–2), 245–252. https://doi.org/10.1016/j.chemphys.2006.08.020

López-Tobar, E., Hernández, B., Ghomi, M., & Sanchez-Cortes, S. (2013). Stability of the disulfide bond in cystine adsorbed on silver and gold nanoparticles as evidenced by SERS data. *Journal of Physical Chemistry C*, *117*(3), 1531–1537. https://doi.org/10.1021/jp3112606

Lowe, A., Harrison, N., & French, A. P. (2017). Hyperspectral image analysis techniques for the detection and classification of the early onset of plant disease and stress. *Plant Methods*, *13*(1), 1–12. https://doi.org/10.1186/s13007-017-0233-z

Lu, Y., Yao, G., Sun, K., & Huang, Q. (2014). β-Cyclodextrin coated SiO2@Au@Ag core-shell nanoparticles for SERS detection of PCBs. *Physical Chemistry Chemical Physics*, *17*(33), 21149–21157. https://doi.org/10.1039/c4cp04904g

M. Tréguer-Delapierre, J. Majimel, S. M. et al. (2008). Synthesis of non-spherical gold nanoparticles. *Gold Bull*, *41*(2), 195–207.

Mahlein, A.-K. (2016). Plant Disease Detection by Imaging Sensors - Parallels and Specific Demands for Precision Agriculture and Plant Phenotyping. *Plant Disease*, *100*(2), 1–11. https://doi.org/10.1007/s13398-014-0173-7.2

Maimaitiyiming, M., Ghulam, A., Bozzolo, A., Wilkins, J. L., & Kwasniewski, M. T. (2017). Early detection of plant physiological responses to different levels of water stress using reflectance spectroscopy. *Remote Sensing*, *9*(7), 1–23. https://doi.org/10.3390/rs9070745

Melchini, A., Costa, C., Traka, M., Miceli, N., Mithen, R., De Pasquale, R., & Trovato, A. (2009). Erucin, a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells. *Food and Chemical Toxicology*, *47*(7), 1430–1436. https://doi.org/10.1016/j.fct.2009.03.024

Miyazawa, M., Maehara, T., & Kurose, K. (2002). Composition of the essential oil from the leaves of Eruca sativa. *Flavour and Fragrance Journal*, *17*(3), 187–190. https://doi.org/10.1002/ffj.1079

Morath, S. U., Hung, R., & Bennett, J. W. (2012). Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. *Fungal Biology Reviews*, *26*(2–3), 73–83. https://doi.org/10.1016/j.fbr.2012.07.001

Muhammad Siddiq, M. A. U. (Ed.). (2018). *Handbook of Vegetables and Vegetable Processing*. https://doi.org/10.1002/9781119098935

Nielsen, T., Bergström, B., & Borch, E. (2008). The origin of off-odours in packaged rucola (Eruca sativa). *Food Chemistry*, *110*(1), 96–105. https://doi.org/10.1016/j.foodchem.2008.01.063

Noh, J., Jang, S., Lee, D., Shin, S., Ko, Y. J., Ito, E., & Joo, S. W. (2007). Abnormal adsorption behavior of dimethyl disulfide on gold surfaces. *Current Applied Physics*, *7*(6), 605–610. https://doi.org/10.1016/j.cap.2006.12.007

Nunes, T. P., Martins, C. G., Faria, A. F., B??scola, V., de Oliveira Souza, K. L., Mercadante, A. Z., Cordenunsi, B. R., & Landgraf, M. (2013). Changes in total ascorbic acid and carotenoids in minimally processed irradiated Arugula (Eruca sativa Mill) stored under refrigeration. *Radiation Physics and Chemistry*, *90*, 125–130. https://doi.org/10.1016/j.radphyschem.2013.03.044

Ohno, N., Uehara, J., & Aramaki, K. (1993). A SERS Study on Adsorption of Dibenzyl Disulfide, Sulfide, and Sulfoxide on an Iron-Deposited Silver Electrode in a Hydrochloric Acid Solution. *Journal of the Electrochemical Society*, *140*(9), 2512–2519. https://doi.org/10.1149/1.2220853

Otto, A. (2005). The “chemical” (electronic) contribution to surface-enhanced Raman scattering. *Journal of Raman Spectroscopy*, *36*(6–7), 497–509. https://doi.org/10.1002/jrs.1355

Pang, S., Yang, T., & He, L. (2016). Review of surface enhanced Raman spectroscopic (SERS) detection of synthetic chemical pesticides. *TrAC - Trends in Analytical Chemistry*, *85*, 73–82. https://doi.org/10.1016/j.trac.2016.06.017

Pearson, B., Mills, A., Tucker, M., Gao, S., McLandsborough, L., & He, L. (2018). Rationalizing and advancing the 3-MPBA SERS sandwich assay for rapid detection of bacteria in environmental and food matrices. *Food Microbiology*, *72*, 89–97. https://doi.org/10.1016/j.fm.2017.11.007

Pichersky, E., Noel, J. P., Dudareva, N., & Arbor, A. (2006). Biosynthesis of Plant Volatiles : Nature ’ s Diversity and Ingenuity PV Biosynthetic Pathways Branch Off from Primary Metabolism. *Science*, *311*(5762), 808–811. https://doi.org/doi: 10.1126/science.1118510

Pieruschka, R., & Schurr, U. (2019). Plant Phenotyping: Past, Present, and Future. *Plant Phenomics*, *2019*, 1–6. https://doi.org/10.34133/2019/7507131

Reviews, C. (2003). The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. *JOURNAL OF FOOD SCIENCE*, *68*(5), 1560–1565.

Rowan, D. D. (2011). Volatile metabolites. *Metabolites*, *1*(1), 41–63. https://doi.org/10.3390/metabo1010041

Sant’Ana, A. S., Barbosa, M. S., Destro, M. T., Landgraf, M., & Franco, B. D. G. M. (2012). Growth potential of Salmonella spp. and Listeria monocytogenes in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. *International Journal of Food Microbiology*, *157*(1), 52–58. https://doi.org/10.1016/j.ijfoodmicro.2012.04.011

Scagel, C. F., Lee, J., & Mitchell, J. N. (2019). Salinity from NaCl changes the nutrient and polyphenolic composition of basil leaves. *Industrial Crops and Products*, *127*(July 2018), 119–128. https://doi.org/10.1016/j.indcrop.2018.10.048

Schatz, G. C., Young, M. A., & Duyne, R. P. Van. (2006). Electromagnetic Mechanism of SERS. *Physics*, *46*, 19–46.

Schnürer, J., Olsson, J., & Börjesson, T. (1999). Fungal volatiles as indicators of food and feeds spoilage. *Fungal Genetics and Biology*, *27*(2–3), 209–217. https://doi.org/10.1006/fgbi.1999.1139

Schulz, S., & Dickschat, J. S. (2007). Bacterial volatiles: The smell of small organisms. *Natural Product Reports*, *24*(4), 814–842. https://doi.org/10.1039/b507392h

Senthilmohan, S. T., McEwan, M. J., Wilson, P. F., Milligan, D. B., & Freeman, C. G. (2001). Real time analysis of breath volatiles using SIFT-MS in cigarette smoking. *Redox Report*, *6*(3), 185–187. https://doi.org/10.1179/135100001101536166

SI, M., LI, L., & Chuanyun, Z. (2015). Identification and Characterization of Volatile Organic Compounds of Fresh Plant Using Headspace Combined with Surface-Enhanced Raman Scattering. *Journal of Food Processing & Technology*, *6*(12). https://doi.org/10.4172/2157-7110.1000520

Singh, A., Ganapathysubramanian, B., Singh, A. K., & Sarkar, S. (2016). Machine Learning for High-Throughput Stress Phenotyping in Plants. *Trends in Plant Science*, *21*(2), 110–124. https://doi.org/10.1016/j.tplants.2015.10.015

Siomos, A. S., & Koukounaras, A. (2007). *Quality and Postharvest Physiology of Rocket Leaves*.

Spadafora, N. D., Amaro, A. L., Pereira, M. J., Müller, C. T., Pintado, M., & Rogers, H. J. (2016). Multi-trait analysis of post-harvest storage in rocket salad (Diplotaxis tenuifolia) links sensorial, volatile and nutritional data. *Food Chemistry*, *211*, 114–123. https://doi.org/10.1016/j.foodchem.2016.04.107

Sun, K., Huang, Q., Meng, G., & Lu, Y. (2016). Highly Sensitive and Selective Surface-Enhanced Raman Using DNA Aptamer-Modi fi ed Ag-Nanorod Arrays. *ACS Appl. Mater. Interfaces*, *8*(8), 5723–5728. https://doi.org/10.1021/acsami.5b12866

Tang, R., Zhang, J. P., & Zhang, Z. N. (2012). Electrophysiological and Behavioral Responses of Male Fall Webworm Moths (Hyphantria cunea) to Herbivory-Induced Mulberry (Morus alba) Leaf Volatiles. *PLoS ONE*, *7*(11), 1–7. https://doi.org/10.1371/journal.pone.0049256

Taylor, P. (2008). Ocimum sp . (Basil): Botany , Cultivation , Pharmaceutical Properties , and Biotechnology Access details : Access Details : [subscription number 772810551]. *Journal of Herbs, Spices & Medicinal Plants*, *6475*(May). https://doi.org/10.1300/J044v13n03

Toledo-Martín, E. M., Font, R., Obregón-Cano, S., De Haro-Bailón, A., Villatoro-Pulido, M., & Del Río-Celestino, M. (2017). Rapid and cost-effective quantification of glucosinolates and total phenolic content in rocket leaves by visible/near-infrared spectroscopy. *Molecules*, *22*(5). https://doi.org/10.3390/molecules22050851

Tsironi, T., Dermesonlouoglou, E., Giannoglou, M., Gogou, E., Katsaros, G., & Taoukis, P. (2017). *International Journal of Food Microbiology Shelf-life prediction models for ready-to-eat fresh cut salads : Testing in real cold chain*. *240*, 131–140. https://doi.org/10.1016/j.ijfoodmicro.2016.09.032

Tulio, A. Z., Yamanaka, H., Ueda, Y., & Imahori, Y. (2002). Formation of methanethiol and dimethyl disulfide in crushed tissues of broccoli florets and their inhibition by freeze-thawing. *Journal of Agricultural and Food Chemistry*, *50*(6), 1502–1507. https://doi.org/10.1021/jf010673g

Wang, Y., Yan, B., & Chen, L. (2013). SERS Tags: Novel optical nanoprobes for bioanalysis. *Chemical Reviews*, *113*(3), 1391–1428. https://doi.org/10.1021/cr300120g

Willets, K. A. (2009). Surface-enhanced Raman scattering (SERS) for probing internal cellular structure and dynamics. *Analytical and Bioanalytical Chemistry*, *394*(1), 85–94. https://doi.org/10.1007/s00216-009-2682-3

Wong, C. L., Dinish, U. S., Schmidt, M. S., & Olivo, M. (2014). Non-labeling multiplex surface enhanced Raman scattering (SERS) detection of volatile organic compounds (VOCs). *Analytica Chimica Acta*, *844*, 54–60. https://doi.org/10.1016/j.aca.2014.06.043

Xie, X., Pu, H., & Sun, D.-W. (2017). Recent advances in nanofabrication techniques for SERS substrates and their applications in food safety analysis. *Critical Reviews in Food Science and Nutrition*, *0*(0), 1–14. https://doi.org/10.1080/10408398.2017.1341866

Yang, T., Zhang, Z., Zhao, B., Hou, R., Kinchla, A., Clark, J. M., & He, L. (2016). Real-Time and in Situ Monitoring of Pesticide Penetration in Edible Leaves by Surface-Enhanced Raman Scattering Mapping. *Analytical Chemistry*, *88*(10), 5243–5250. https://doi.org/10.1021/acs.analchem.6b00320

Yang, Y., Li, Z., Yamaguchi, K., Tanemura, M., Huang, Z., & Jiang, D. (2012). Nanoscale Controlled fabrication of silver nanoneedles array for SERS and their application in rapid detection of narcotics. *Nanoscale*, *4*, 2663–2669. https://doi.org/10.1039/c2nr12110g

Yu, T. H., Ho, C. H., Wu, C. Y., Chien, C. H., Lin, C. H., & Lee, S. (2013). Metal-organic frameworks: A novel SERS substrate. *Journal of Raman Spectroscopy*, *44*(11), 1506–1511. https://doi.org/10.1002/jrs.4378

Zhang, Z., & Li, G. (2010). A review of advances and new developments in the analysis of biological volatile organic compounds. *Microchemical Journal*, *95*(2), 127–139. https://doi.org/10.1016/j.microc.2009.12.017