EFFECT OF PRODUCTION SYSTEM ON TOMATO FRUIT QUALITY AND LEAF METABOLITE PROFILE CHANGES IN RESPONSE TO BACTERICERA COCKERELLI-CANDIDATUS LIBERIBACTER SOLANACEARUM INSECT-PATHOGEN COMPLEX

A Dissertation

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ABSTRACT

Tomato (*Solanum lycopersicum*) is one of the most widely consumed horticultural crops in the world, and an important source of multiple health-promoting compounds. Fresh and processed tomatoes have huge demand globally, and the fruit qualities will influence consumers' preferences and market value. Consequently, to improve the yield, quality and health-promoting compounds, controlled production systems such as high-tunnel (HT) and disease resistant genotypes have been studied. This dissertation mainly focuses on two main objectives: the first is to evaluate the effect of production systems on tomato qualities associated with health-promoting compounds. The second is to elucidate the distinct profiles of susceptible and resistant tomato genotypes against *Bactericera cockerelli*, tomato potato psyllid (TPP), vectoring or not the phloem-limited bacterial pathogen *Candidatus* Liberibacter solanacearum (Lso).

The first two studies, determined the effect of production systems (net-house (NH) and open-field (OF)) on volatile profiles using optimized headspace solid phase microextraction (HS-SPME) conditions (2g fresh weight (FW), a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber at 60 °C for 45 min) for extraction. About 40 volatile metabolites were identified using gas chromatography coupled with a mass spectrometer (GC-MS) and relatively quantified in four tomato varieties grown in north Texas (Amarillo) and eight tomato varieties grown in south Texas (Weslaco). The levels of flavor-associated β-damascenone, geranylacetone, and d-limonene were significantly affected by production systems.

In the third, fourth, and fifth studies, the eight different varieties of tomatoes, including three Texas A&M University (TAMU) and five commercial varieties were grown in Weslaco to examine the *in vitro* bile acids binding capacities and the levels of health-promoting compounds, including ascorbic acid, carotenoids, phenolics, indole amines, as well as quality-associated enzymes polyphenol oxidase (PPO) and peroxidase (POD). Effect of production systems was evaluated by ultrahigh performance liquid chromatography (UPLC) coupled to quadrupole time-of-flight high-resolution mass spectrometry (QTOF-HRMS) combined chemometric analysis to understand the differential effect based on genetics.

Lastly, the changes of metabolic profiles of insect-susceptible (cv CastleMart) and -resistant (RIL LA3952) genotypes were evaluated by HS-SPME/GC-MS and UHPLC/APCI-QTOF-HRMS. Furthermore, multivariate analysis using partial least squares-discriminant analysis (PLS-DA) distinguished the resistant tomato genotype response to TPP carrying or not the pathogen by confirming response volatile compounds.

DEDICATION

I dedicate this dissertation to my father (Jae Han Lee) and mother (Hyun Sook Bae) for their unconditional love, support, and patience.

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NOMENCLATURE

SPME	Solid phase microextraction
GC	Gas chromatography
MS	Mass spectrum
MS/MS	Tandem mass spectrometry
HT	High-tunnel
NH	Net-house
OF	Open-field
HPLC	High-performance liquid chromatography
UHPLC	Ultrahigh performance liquid chromatography
APCI	Atmospheric pressure chemical ionization
QTOF	Quadrupole time-of-flight
HRMS	High-resolution mass spectrometry
ESI	Electrospray ionization
DAD	Diode array detector
PDA	Photodiode array
TSS	Total soluble solids
TA	Total acidity
CA	Cholic acid
CDCA	Chenodeoxycholic acid
DCA	Deoxycholic acid

GCDCA	Glycochenodeoxycholic acid
GCA	Glycocholic acid
GDCA	Glycodeoxycholic acid
AA	Ascorbic acid
BHT	Butylated hydroxytoluene
PCA	Principal component analysis
PLS-DA	Partial least squares-discriminant analysis
VIP	Variable importance on projection
ANOVA	Analysis of variance
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
FCR	Folin–Ciocalteu reagent
TP	Total phenolics
FW	Fresh weigh
DW	Dry weight
PPO	Polyphenol oxidase
POD	Peroxidase
EDTA	Ethylenediaminetetraacetic acid
PVPP	Polyvinylpolypyrrolidone
BCA	Bicinchoninic acid
K_m	Michaelis–Menten constant
V_{max}	Velocity

TPP	Tomato potato psyllid
Lso	*Candidatus* Liberibacter solanacearum
RIL	Recombinant inbred lines

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to a family of Solanaceae and originated from South America.[1] It is one of the most consumed horticultural crops in the world and an important source of essential nutrients.[2] In 2017, the production of fresh tomato was 10.9 million tons in the United States, which is the fourth largest producing country after China, India, and Turkey.[3] Moreover, tomato is the seventh most produced crops after maize, soybeans, wheat, sugar beet, sugar cane, and potatoes in the United States.[3] Tomato contains several natural antioxidants such as vitamin C, carotenoids, indoleamines, and phenolics.[4-7] Furthermore, epidemiological studies have been demonstrated that the consumption of tomato may reduce the risk of chronical and degenerative diseases based on their health beneficial effects such as antioxidant activity, anti-inflammation, and neurotransmitter properties.[8-10] Based on potential benefits and meet the dietary guidelines, the United States Department of Agriculture's (USDA's) MyPlate recommends the consumption of ½ cup (90 g) of fresh tomatoes on the 2,000-calorie daily diet.[11]

The flavor, color, texture, and appearance are considered as the important criteria for consumer preference.[12] However, tomato breeding programs have mainly focused on improving productivity over the past 50 years.[4] The development has been acquired at the expense of flavor quality-related constituents such as aroma-active volatile compounds.[5] Consequently, consumers have expressed dissatisfaction with fresh tomato due to the lack

of flavor and texture in commercial tomatoes.[13-14] In addition, various factors such as genotypes, environmental effects, management practices, and postharvest treatments showed influence on the chemical composition in association with fruit quality.[15-18] For example, a wide range of tomato genetic variations is allowed to develop genotypes with the selected properties such as increased nutritional components or disease resistance.[19] Recently, the usage of high tunnels has spread in the United States based on the beneficial effects, such as increasing the yield and extended production seasons.[20-22]

In addition, tomato-based products accounted for 75% of total tomato consumption.[23] The essential fruit quality attributes such as color, nutritional constituents, and viscosity are changed during tomato processing, mainly, due to the quality-related enzymes, polyphenol oxidase (PPO) and peroxidase (POD).[24] Therefore, understanding the effect on enzyme activity is essential to avoid quality deterioration during the processing and storage of foods.[25] However, little information is available in relation to effect of genotype, production system, and their interaction on the tomato quality in Texas. Therefore, the main goal of the proposed research work is to evaluate the effects of different production systems and/or genotypes on the studied components involved in fruit quality or disease resistance. This scientific information would be useful for breeders to enhance the fruit quality and to meet the needs of consumers.

Objectives

1. To determine the production systems influence on volatile biomarkers in tomato in North Texas.

2. To investigate the metabolomic studies of volatiles from tomatoes grown in net-house and open-field conditions in South Texas.
3. To assess the effects of variety and production systems on quality of tomato fruits and in vitro bile acid binding capacity.
4. To estimate the genotype and production system effects on melatonin, serotonin, phenolics, and antioxidant activities of tomatoes.
5. To determine the effect of production system and inhibitory potential of aroma volatiles on polyphenol oxidase and peroxidase activities of tomatoes.
6. To determine the metabolic changes of susceptible and resistant tomato in response to infestation with tomato/potato psyllid vectoring or not *Candidatus* Liberibacter Solanacearum.

CHAPTER II

LITERATURE REVIEW

Tomato (*Solanum lycopersicum*) is one of the main ingredients in modern diets worldwide and consumed in forms of fresh and processed products.[249] Tomato breeding programs have been aimed to increase the yield with enhanced amounts of nutritional constituents. [4] In that context, breeders develop tomato genotypes based on specifically targeted traits such as disease resistance and/or chemical compositional profiles.[26-27] In addition to fundamental potentials, environmental conditions such as high-tunnel systems have been applied for fruit qualities. [28-29] Therefore, understanding the genotype, production system, and their interaction on tomato quality according to consumers' preference is essential for breeder and industry.

Bioactive compounds in tomato

Ascorbic acid

Ascorbic acid is a water-soluble antioxidant in plants, and humans are not able to synthesize endogenous ascorbate and acquire it from dietary sources such as tomato.[30-32] It has been reported important roles of ascorbic acid in the detoxification of reactive oxygen species and in photoprotection in plants.[33] The short-lived monodehydroascorbate (MDHA) radical is produced by ascorbic acid oxidation and able to be recycled by monodehydroascorbate reductase.[34] Dehydroascorbate (DHA) can be generated from the disproportionation of MDHA and also able to be recycled into ascorbic acid by

dehydroascorbate reductase (DHAR) while using glutathione (GSH) as a reductant before undergoing irreversible hydrolysis to 2,3-diketogulonic acid.[35] The levels of ascorbic acid in tomatoes were influenced by various factors such as genotype, climatic conditions, cultural practices, and maturity.[36] For example, In cherry tomato, the levels of ascorbic acid were gradually increased during the ripening in fruit grown in the greenhouse.[37]

Carotenoids

Tomato is a rich source of carotenoids, and lycopene is an important plant pigment synthesized during fruit ripening, and its contents are around 85-90% of the total carotenoids in red ripe tomatoes.[38-40] It is noteworthy that tomato and its products account for more than 85% of lycopene consumption in a human diet.[41] Tomatoes contain different carotenoids and their isomers such as lycopene, β-carotene, lutein, phytoene, phytofluene, γ-carotene, and ζ-carotene.[42] Previous studies showed that lycopene was found dominantly among carotenoids in human serum, liver, testes, and prostate.[43-45] The cis-isomers of lycopene have been reported as more bioavailable than the trans lycopene, and more than 50% of cis-isomers of lycopene were detected in human serum and tissues.[44, 46-47] Tomatoes contain mainly lycopene, a carotenoid with a high oxygen-radical scavenging and quenching capacity. Additionally, epidemiological studies have been suggested that intake of carotenoids may reduce risks of certain types of cancers and degenerative diseases.[8-9] California-grown processing tomatoes were reported to contain 84 to 173mg/kg of trans-lycopene. Previous researchers demonstrated that levels of lycopene were influenced by genotype, environment, growing location and harvesting season.[42]

However, most of the intricate inter-related biological processes including different production systems leading to volatile emission still remain unclear.

Phenolics

Polyphenols in tomatoes consist of mainly phenolic acids and flavonoids. Phenolic compounds are considered important secondary metabolites in plants produced through the phenylpropanoid pathway. This group of compounds has shown antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory activities.[48] Flavonoids can contribute to decreased risk of cardiovascular diseases and cancer.[49-50] In addition, free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways have been reported in these metabolites.[51] It has been demonstrated that the variation of polyphenols in tomato fruit may be caused by genetic and environmental effects.[52] Red tomato contains flavonoids such as naringenin (45%), followed by quercetin (39%), myricetin (10%) and kaempferol (5%).[53-56] Specifically, chlorogenic acid and its related compounds are considered as the main phenolic compounds in tomato fruits. Chlorogenic acid was demonstrated to have potential health benefits based on their potent antioxidant activity as well as hepatoprotective, hypoglycemic and antiviral activities.[57] These acids may also be responsible for their astringent taste.[58-59] Ferulic, caffeic and chlorogenic acids were extracted in tomato fruit, and p-coumaric acid was detected in a tomato skin extract. Sinapic acid of green tomato fruits was also reported.[59-60] Those types of phenolic compounds seem to significantly contribute to antioxidant activity.[61] In addition to

genotype, cultural practices also influence levels of phenolics and phenolic acids in tomatoes.[62]

Serotonin, melatonin, and plant hormones

Melatonin (N-acetyl-5-methoxytryptamine) and serotonin (5-hydroxytryptamine) are indoleamines and were identified as neurotransmitters in vertebrates.[63] Both melatonin and serotonin were discovered in plants several years after their discovery in mammals, and their presence was confirmed in almost all plant families.[7] These components also play important roles in plant growth and development, including functions in energy acquisition, seasonal cycles, modulation of reproductive development, control of root and shoot organogenesis, maintenance of plant tissues, delay of senescence, and responses to biotic and abiotic stresses.[64] Melatonin contents ranged from 1.4 to 142.5 ng/g fresh weight (FW) were analyzed in three organs, including leaf, stem, and root of tomato plants grown under different environmental conditions.[65] Tomato fruits showed relatively high melatonin concentrations such as 14.77 ng/g FW or 249.98 ng/g dry weight (DW) in *Solanum lycopersicum* L. cv. Optima and 23.87 ± 2.02 ng/g FW in *Lycopersicon esculentum* cv. Bonda.[66-67] Serotonin content was also reported as 6.4 μg/g FW.[68] Moreover, endogenous melatonin has also been shown to play an important role in plant physiology that may be related to auxin metabolism. Tryptophan is known as the common precursor for melatonin, serotonin and indole-3-acetic acid (IAA) in higher plants.[7] Consequently, there are potential roles of melatonin and serotonin in acting as regulators of plant developmental responses.[69] It was reported that alterations of the endogenous

concentration of melatonin and inhibitors of the transport of serotonin and melatonin auxin-induced root and cytokinin-induced shoot organogenesis were inhibited.[70]

Flavor-associated volatile compounds

In recent years, consumers use flavor, as one of the most important criteria along with color, texture, and appearance while purchasing vegetables and fruits.[12] The flavor of fresh tomato is mainly characterized by the complex interaction among sugar, acids, and volatile compounds. Major total soluble solids are glucose and fructose. Citric acid is a major constituent of organic acids followed by malic acid in red tomato.[71] Approximately, 400 volatile compounds have been reported in tomato fruit. However, only 15-20 volatile compounds seemed to have major impacts on human perception of tomato aroma and flavor based on orthonasally measured odor thresholds.[72-75] These aroma volatiles are derived from various precursors including fatty acid (linoleic acid and linolenic acid), essential amino acid (leucine, isoleucine, and phenylalanine), and carotenoids (lycopene, ß-carotene, and ζ-carotene).[76-79] Tomato volatiles are mainly formed either during ripening (isobutylthiazole, 3-methylnitrobutane, geranyl acetone, and ß-ionone) or during maceration by cutting or eating (C_6 compounds in the lipid oxidation pathway).[80-81] Various factors, such as ripening, genotypes, environmental effects, management practices, and postharvest treatments influence tomato volatile composition.[15-18] Potential roles of aroma volatile compounds are not only restricted to their flavor attributes but also expanded to human health benefits.[82-85] In tomato fruit, several aroma-active volatiles such as (E)-2-Hexenal (green, leafy) and hexanal (fresh, cut grass) have been reported to

provide antimicrobial properties against microorganisms.[86-87] In addition, linalool (citrus, floral) showed anti-inflammatory effects and its precursor, geraniol (floral), is also considered as a potent therapeutic agent of neurological disease.[83, 88-90] Therefore, further investigation into multiple roles of tomato aroma-active volatiles on flavor, health benefits and anti-microbial properties and their synergistic effects are critical.[82]

Influence of production systems on tomato quality

Tomato is consumed daily as plant food and it is considered as one of the major ingredients in the Mediterranean diets and generally planted in mid to late May with harvest beginning in late July to early August.[91] Consumers consider fresh tomato fruit qualities based on flavor, color, shape, firmness, and nutritional value.[37] In terms of production, tomato is one of the three largest crops along with sweet corn and snap beans in the United States. Therefore, growers have been using greenhouses for cultivating high-value crops in order to meet consumer needs of tomato with good quality year-round.[92-94] Even though this approach has been successful compared with the field, the cost of heating during the winter is expensive. Consequently, the unheated greenhouse system, such as high tunnel, has been adopted for supplying tomato to local markets throughout the United States rapidly. [20-22] High tunnel facilitates watering uniformly and protects plants from rainfall, wind, snow, insects, foliar disease, and disorders like cracking.[91, 95] Additionally, high tunnel systems can provide an extension of cultivating season up to two months as well as the increase of marketable fruit yields of tomato, strawberry, and blueberry.[96-100] Production systems have not only increased productivity, but also fruit qualities were positively

affected. Generally, lower vitamin C levels were reported in greenhouse-grown tomatoes than those grown outside due to the lower light intensity.[101] Furthermore, approximately two times higher soluble phenolics, including rutin and chlorogenic acid, were found in cherry tomatoes under high light than plants grown under lower light in the greenhouse.[102] Moreover, influence of different colors of net materials have been reported to have alteration on the levels of bioactive compounds, and volatile compounds in tomatoes and coriander leave based on spectral quality of photo-selective nets.[103-105]

Fruit quality-related enzymes Polyphenol oxidase (PPO) and peroxidase (POD)

The quality of plant-based foods such as nutritional components and appearance is important for influencing consumers to purchase vegetables and fruit.[12] Furthermore, fresh-like quality attributes of processed food such as color, texture, and flavor are demanded by customer's preferences.[106] However, the deterioration of these attributes can be mainly caused by biochemical and enzymatic reactions, and this may lead to the reduction of product shelf-life.[107] The major quality-related enzymes, involved in enzymatic browning, are polyphenol oxidase (PPO) and peroxidase (POD) are found in plastids, whereas antioxidant compounds such as phenols are mainly located in the vacuoles in plant cells.[108]

Polyphenol oxidase (PPO) is a copper-containing enzyme and participates in synthesis of an o-diphenol from monophenol substrates such as catechol, 4-methylcatechol, pyrogallol, and phenol by monooxygenase, and the subsequent oxidation of o-diphenols to o-quinones is catalyzed by diphenolase.[109-110] Continuously, non-enzymatic secondary reactions occur

and these compounds are converted to melanins, brown complex polymers.[111] Peroxidase (POD) is also widely present in fruits and vegetables and catalyzes the oxidation of a wide variety of substrates such as guaiacol and pyrogallol in the presence of hydrogen peroxide as an antioxidative enzyme.[112] POD also plays roles related to the deterioration food quality such as color and flavor as well as the many functions, such as control of elongation and defense mechanisms.[113-114] The damage of sub-cellular compartmentalization due to various reasons, including wounding, senescence, pathogen attack, processing, and storage, allows the contact between these enzymes and phenolic substrates in the vacuole to initiate the enzymatic browning reaction.[115] Consequently, the deterioration of nutritional and sensory qualities according to these enzyme reactions may lead to a significant economic impact on producers and the food processing industry by decreased consumer acceptability.[116] Therefore, the inactivation of POD and PPO enzymes is a key prerequisite indicator to assess food quality in fruits and vegetables. [117] Understanding kinetic properties of PPO and POD enzymes is essential to easily predict the enzyme activity linked to decolorization. There are several parameters that can significantly influence accurate measurement of the reaction, depending on the enzyme source.[118] For example, the extraction buffer containing supplements such as polyvinylpolypyrrolidone (PVPP) may effectively remove polyphenols while extracting enzymes.[109] Furthermore, these enzyme activities are depending on the pH which influences the binding of substrates and catalytic activity.[111] The temperature also affects enzyme activities by the enzyme denaturation based on the solubility of oxygen.[119-120] It has been reported that the change of tomato color may be linked to browning and lycopene degradation based on PPO

activity.[24] In addition, processing techniques such as high-pressure and temperature treatment have been adapted to produce tomato products such as a puree, and PPO and POD activities are considerably affected according to the parameters.[121-122] For instance, the 25% decreased POD activity was found at 350 MPa and 20 °C, whereas 10% reduced PPO activity at 200 MPa and 20 °C.[122]

Development of tomato genotypes with disease resistance

The food security is a global concern according to the population growth in the world, and plant pathogens are one of the critical reasons causing the loss of crops.[26] The tomato potato psyllid (TPP), *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae), was first identified infesting plants of the Solanaceae, including potatoes in New Zealand in 2006.[123] The damages such as yellowing leaves and stunted growth by feeding of TPP occur on tomatoes and potatoes.[124-125] Substantial losses caused by TPP in potato and tomato crops were found in eastern Mexico and the central United States, and tomato losses up to 80% was reported in western North America in 2001.[126] Furthermore, *B. cockerelli* is a vector for the phloem-limited bacterium '*Candidatus* Liberibacter solanacearum' (Lso) which is associated with potato zebra chip (ZC) diseases resulting in serious economic losses in the potato industry.[127] Several haplotypes of Lso are identified in distinct geographical regions, including New Zealand (hapA), North and Central America (hapA and hapB), northern Europe (hapC), and southern Europe and southern Mediterranean region (hapD and hapE).[128] Different psyllid vectors are infected with Lso haplotypes and harbor them to host plants, for example, hapA and hapB can be transmitted

in solanaceous crops by tomato potato psyllid, whereas haplotype C, carried by *Trioza apicalis,* is linked to carrot disease *B. cockerelli*.[128-129] The distinctive response of tomato host plants against pathogens have been investigated using genetic approaches.[130-133] Several plant pathways linked to plant-defensive metabolites such as hormones, phenolics, and volatiles are regulated by the pathogen infection.[130, 134-136] Furthermore, vector-borne bacterial pathogens are considered to interact with both vector and host plant by regulating the fitness of its vector insect and/or modulating the gene expression in the host plants.[131, 133] To reduce crop losses, pesticides have been applied to control vectors and reduce pathogen infection.[137-138] However, the different and decreased efficiency of insecticides were observed among tomato cultivars.[139-140] In that context, the tomato breeding programs have focused on characterizing disease resistance in *Solanum habrochaites* and developing recombinant inbred lines (RILs) against TPP and/or Lso.[26]

CHAPTER III

PRODUCTION SYSTEM INFLUENCES VOLATILE BIOMARKERS IN TOMATO*

Introduction

Tomato (*Lycopersicum esculentum*) is one of the most widely consumed horticultural crops in the world and an important source of essential nutrients.[141] In 2015, the tomato market, including fresh and processed products, accounted for more than 2.6 billion dollars in the United States.[142] The most important criteria for consumer preference include flavor, color, texture, and appearance.[12] Generally, the flavor of a fresh tomato is influenced by the complex interactions of sugars, acids, and volatile compounds. In red tomatoes, glucose and fructose are the major soluble solids and citric acid is the major organic acid, followed by malic acid.[71] Unfortunately, until now, tomato-breeding programs around the globe have mainly focused on improving the productivity, disease resistance, and firmness of tomatoes at the expense of flavor and texture. One main reason for this is the genetic complexity of flavor and the lack of a simple assay that can predict consumer-preferred values of the factors that contribute to flavor.[76] Consequently, important alleles related to aromatic volatiles have been lost and consumers have been disappointed with the lack of flavor in commercial tomatoes.[143]

At present, around 400 volatile compounds have been reported in the tomato fruit.[144-145] However, based on the threshold levels of odor detection, only 15–20 volatile compounds are considered to have a strong effect on the human perception of tomato aroma and flavor.[73, 144] During ripening, tomatoes produce 2-isobutylthiazole, 3-methylnitrobutane, geranylacetone, and ß-ionone. Tomatoes also produce C_6 compounds in the lipid oxidation pathway during maceration.[80] Several research groups have used genetics and metabolomics approaches to understand the characteristics of aroma-associated volatile compounds of tomato fruits and improve flavor quality. For instance, Klee and Tieman have focused on elucidating the chemistry of consumer flavor preferences, examining the mechanism of flavor deterioration in tomato fruits, and delineating a molecular roadmap for flavor enhancement.[76, 144] Similarly, Bauchet et al. reported the pathways and distinct gene-metabolite regulation involved in fruit acidity and phenylpropanoid-derived volatiles in tomato.[146] Moreover, Tikunov et al. investigated tomato volatile profiles by using metabolomic strategies with GC-MS datasets and discriminated metabolite variation among different tomato genotypes.[147-148]

Previous studies have demonstrated that the sample preparation and analytical methods for analysis of volatile compounds have a substantial influence on tomato volatile profiles. In particular, the headspace solid phase microextraction (HS-SPME) method has been widely tested, using fibers coated with different types of polymeric stationary phases that extract the target analytes from a complex sample matrix by absorption. For example, Rambla et al., investigated the effect of four commonly used sample processing methods

on volatile levels by HS-SPME using polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers and demonstrated that each sample processing method produced characteristic volatile profiles.[149] Similarly, other studies focused on the extraction efficiency by comparing various fibers and found that divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber had a higher extraction efficiency than PDMS/DVB fiber.[150-151] However, from these studies, the effects of sample processing methods on volatile patterns have not been clearly understood, and this is a prerequisite for accurate quantification of volatiles.

Accumulating evidence suggests that several factors influence tomato volatiles, such as the ripening stage, genotype, environmental effects, management practices, and postharvest treatments [15-16], but little information is available about the effect of the production system on specific chemical markers. Among different production systems, unheated greenhouse systems, such as the high-tunnel, have increasingly been adopted to supply local markets throughout the United States.[21] The main advantages of high-tunnel cultivation are uniform watering, protecting plants from rainfall, wind, snow, insects, and foliar disease.[95] The properties of the materials used to construct the high tunnels, such as the color or photo-selective nets, may influence the level of health-promoting compounds, including volatiles, in the fruits.[103] However, at present, very little information is available about the exact effect of high-tunnel production systems on tomato volatiles. In addition, there is no comparative study on the effect of high-tunnel versus open-field production systems on tomato flavor constituents.

The main objective of this study was to optimize the extraction and quantification conditions using HS-SPME coupled with GC-MS to determine the volatile composition of tomatoes as well as to measure the influence of the production system on the specific molecular marker, using a metabolomics approach.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were analytical grades. All 21 authentic volatile standards (see Table 1) were procured from Sigma-Aldrich (Sigma-Aldrich Chemical Co, St. Louis, MO, USA).

Production systems

All samples were obtained from the Texas A&M AgriLife Research and Extension farm located in Bushland, Texas, United States (35° 11' 25.89" N 102° 3' 50.08" W). Three tomato cultivars were developed at the Vegetable and Fruit Improvement Center of Texas A&M University by Dr. Kevin Crosby (TAM Hot-Ty, TAM exp 1, TAM Exp 2), and a commercial variety (USAT 0121) (Supplementary Fig. S1). These four tomato varieties were grown in the high-tunnel system (96 feet long, 30 feet wide and 12 feet tall) and in the open field. The high-tunnel metal frames were covered with a fiberglass-impregnated tarp to allow sunshine in and keep most of the weather out. The plants were fertigated using drip irrigation. The experimental design was a completely randomized design and

each treatment contained five fruits with four replications. At sampling time, whole tomato fruits were obtained after removing the calyx and stem scar. Fruits were harvested in October 2016.

Sample preparation and basic quality measurement

The five fruits from each replication were cut into pieces, mixed together, and quickly blended for 30 sec. For volatile analysis, 2 g of each sample was placed into a 20-mL GC-MS vial containing saturated $CaCl_2$ (2 mL) and 2-octanone (10 µL, 0.025% in ethanol, v/v) as an internal standard and stored at -20°C until analysis. The total soluble solids content (TSS) of tomato was determined at 25°C using a hand refractometer (American Optical Corp., South Bridge, MA, USA). For each sample, 5 g of sample was mixed with 45 mL of nanopure water and total acidity (TA) was measured by titrating with 0.1 M NaOH up to pH 8.1 through a DL 22 Food and beverage analyzer (Mettler Toledo, Columbus, OH, USA). All samples were measured in four replicates and the results were averaged.

Optimization of HS-SPME conditions

Fresh Roma tomatoes (*Solanum lycopersicum*) were purchased from a local supermarket (HEB, College Station, TX, USA) for optimizing extraction conditions using HS-SPME. Tomato fruits were washed with deionized water and sliced into six pieces. Then, samples were blended for 30 seconds to facilitate the release of volatile compounds

by solid diffusion. Tomato puree samples (2 g) were put into 20-mL glass headspace vials with 2 mL of saturated $CaCl_2$ solution in nanopure water and kept frozen at -20°C until analysis.

Selection of fibers. To compare the extraction efficiency of fiber types on the measurement of volatile compounds in tomatoes, five types of coated fibers, polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and polyacrylate (PA) were used to compare the areas of selected peaks of hexenal, 6-methyl-5-heptene-2-one, hexen-1-ol, linalool, geranyl acetone, and *ß*–ionone. Samples (2 g) with 2 mL of saturated $CaCl_2$ solution in the 20-mL headspace vial were used to evaluate the fibers by extracting at 60°C for 20 min.

Sample weight. To determine optimal sample weight for the quantification of volatiles, we placed different amounts (0.5, 1, 2, 4, and 8 g) of tomato samples into 20-mL GC-MS glass vials and added equivalent amounts of saturated $CaCl_2$. Then extraction was carried out using DVB/CAR/PDMS fibers at 60°C for 20 min.

Extraction time and temperature. Two grams of sample and DVB/CAR/PDMS fibers were used to test the effect of temperature and time on the extraction. To identify the best conditions, the GC-MS analysis was performed for various extraction times (15, 30, 45, and 60 min) at different temperatures (40, 60, and 80°C).

GC-MS analysis

Electron impact (EI) analysis. The GC-MS analysis was performed using a Thermo Finnigan GC–MS (Thermo Fisher Scientific, Inc., San Jose, CA, USA) equipped with an electron ionization source with a Dual-Stage Quadrupole (DSQ II) mass spectrometer (Thermo Scientific, Austin, TX, USA). The separation was achieved with a Zebron ZB-Waxplus column coated with 100% polyethylene glycol of 30 m × 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Inc. Torrance, CA). Helium was used as the carrier gas at a constant flow rate of 1 mL/min in splitless mode. For optimizing HS-SPME extraction condition, the initial oven temperature was maintained at 50°C for 2°C min and then increased to 225°C at a rate of 4°C/min and the temperature of the column was maintained for 8 min. To determine the effect of production system on the volatile compounds from four tomato varieties, the optimized method consisted of an initial oven temperature of 40°C, held for 1 min, then increased to 90°C at a rate of 10°C/min, and increased to 175°C at a rate of 3°C/min. Finally, it was increased to 230°C at a rate of 35°C/min and held for 2 min at the final temperature, with a total run time of 38 min. Electron impact (EI) data from *m/z* 40 to 450 were acquired at a scanning speed of 11.5 scans per sec and with an ionization voltage of 70 eV. The ion source temperature and mass transfer line temperature were maintained at 280°C. The data were recorded and processed using Xcalibur software (v. 2.0.7., Thermo-Fisher Scientific, San Jose, CA, USA).

Positive-ion chemical ionization (PCI): Positive-ion chemical ionization was also performed to confirm the volatile compounds. The chromatographic separation conditions used were the same as those used for EI mode, except the ion source temperature and mass transfer line temperature were maintained at 180 and 250°C, respectively. Methane was used as the ionization source with a flow rate of 1.3 mL/min.[152] The mass spectral data of the separated compounds were acquired in CI mode.

Samples were vortexed for one min and sonicated for 30 min at room temperature, and then loaded onto a TriPlus autosampler (Austin, TX, USA). The volatile compounds were extracted by HS-SPME with a 50/30 µm CAR/PDMS/DVB fiber. The incubation and extraction times were 2 and 45 min, respectively, at 60°C under continuous agitation. Desorption was carried out in the injector at 225°C for 2 min and fiber conditioning was carried out for 7 min. Forty-one volatile constituents were identified by comparing retention time, Kovat's index (KI), and mass spectra with those of reported compounds in tomato fruits and the NIST library. KI values were calculated by the retention time of a mixture of *n*-alkane standards (C_{10}–C_{24}) analyzed under the same conditions as the samples.[153] Among these, 21 volatiles were confirmed by matching the retention times and mass spectra patterns to their authentic standards. Furthermore, 19 volatiles were confirmed by positive chemical ionization (PCI) mode. The levels of volatiles were expressed relative to 2-octanone, as per published protocols.[154]

Statistical analysis

The univariate statistical analysis was performed using SPSS (v. 23, BM SPSS Statistics, IBM Corp., Chicago, IL, USA). The multiple mean comparisons (*P*-value < 0.05) were carried out using one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test. Significant differences between production systems were assessed with Student's *t*-test (*P*-value < 0.05). The multivariate analysis was performed by exporting GC-MS data in Excel format to MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/).

Results

Optimization of HS-SPME parameters

The present study examined the effect of different fibers on the extraction efficacy of volatiles of tomato. We choose six volatile compounds (hexanal, 6-methyl-5-heptene-2-one, hexen-1-ol, linalool, geranyl acetone, and *β*-ionone) for detailed analysis, based on their prevalence. In addition, these compounds represent a broad range of retention times and different volatilities, which helps us to understand the factors affecting extraction efficiencies using headspace analysis. In comparing the extraction of these six compounds, the lowest amounts of volatiles were extracted with PDMS fiber, except *β*-ionone, and significantly higher ($P < 0.05$) amounts of volatile compounds were extracted with 50/30 µm DVB/CAR/PDMS fiber amongst studied fibers (Fig. 1A). Therefore, the 50/30 µm DVB/CAR/PDMS fiber was selected for the extraction of volatile compounds from

tomatoes for further experiments to determine the influence of production system on volatile metabolites.

Subsequently, we tested the effect of different amounts of sample (by weight) on the extraction of volatiles from tomato (with the equivalent volume of saturated $CaCl_2$) to increase the partition coefficient of the analytes between the gas phase and the sample. Fig. 1B shows the efficiency of extraction as determined by the analysis of the six compounds from tomato samples. In these tests, the 2-g samples produced the maximum amounts of volatiles. For instance, linalool, geranyl acetone, and β-ionone were significantly higher in 2-g samples, whereas the lower molecular weight compounds hexanal, 3-methyl-5-heptene-2-one, and hexan-1-ol were significantly higher in 4, 1, and 0.5-g samples, respectively.

Finally, the effect of the extraction temperature and time was also assessed using DVB/CAR/PDMS fiber and 2-g samples. Fig. 1C–E depict the efficiency of the extraction time and temperature on the tomato volatile compounds at 40, 60, and 80°C, respectively. The highest peak area for low molecular weight volatiles was observed for samples extracted at 40°C compared to 60°C and 80°C. However, the extraction efficacy of the high molecular weight volatiles increased with increasing extraction time and temperature. Based on the extraction efficacy of a maximum number of volatile compounds, 60°C was chosen for further experiments. Interestingly, we found that comparable amounts of volatiles were extracted at 45 min and 60 min at 60°C (P <0.05) (Fig. 1D). Based on

statistical significance and a shorter run time, we chose 45 min as the optimal condition for further experiments.

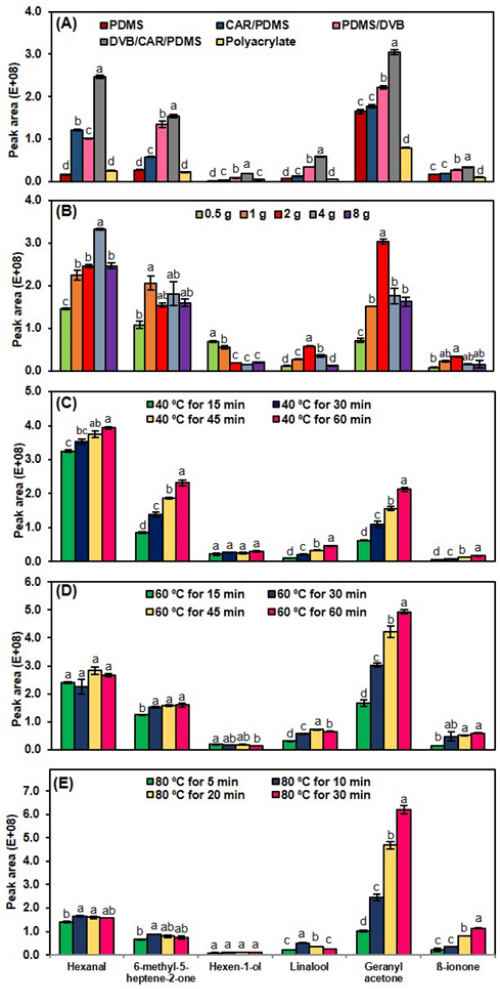

Figure 1. Optimization (A) The effects of coated fibers on the peak areas of the representative volatile compounds of tomato. (B) The effects of sample weights 0.5–8 g on the peak areas of the representative volatile compounds of tomato. Among studied sample weights, the 2-g sample showed extraction of the maximum amount of volatiles. The effects of extraction temperatures and times on the peak areas of the representative volatile compounds of tomatoes. (C) The extractions of tomato volatiles at 40°C for 15, 30, 45, and 60 min. (D) at 60°C for 15, 30, 45, and 60 min. (E) at 80°C for 10, 15, 20, and 30 min. Among these, extraction of volatile at 60°C for 45 min was found to be optimized condition (Desorption for 2 min at 225°C).

Chemical composition of the tomato volatiles

The optimized method described above was used to analyze volatiles from high-tunnel and open-field grown tomatoes of four varieties (Supplementary Fig. S2). In total, 41 volatile compounds were identified from all the tomato varieties using authentic standards, mass spectra, and KI values (Table 1). The identification of each metabolite was also performed based on spectral similarity with mass spectral libraries (Wiley registry 8e, Replib, and Mainlib) by considering Metabolomics Standard Initiative (MSI) levels proposed by Chemical Analysis Working Group [155] (Table 1 and Supplementary Table S1 and S2). The identified volatiles were classified as alcohols, aldehydes, fatty acids, furans, ketones, and sulfur- and nitrogen-containing compounds. These compounds were quantified and expressed as 2-octanone equivalents (Table 1 and 2). Alcohols, aldehydes, and ketones were the major classes in all studied tomato varieties.

The alcohol 1-hexanol (green, resin and, flowery odor) was the primary contributor to the total alcohol content. In tomato, cis-3-hexen-1-ol contributes green odor and linalool contributes citrus, fruity and sweet odor.[156] Notably, our results indicated that three varieties (TAM Hot-Ty, TAM EXP-1, and TAM EXP-2) in two different production systems had eugenol (alcohol) contents more than 29 ng/g. Whereas, the commercial variety USAT-012 had the least amount (2 ng/g) of eugenol (Table 1). Among aldehydes, hexanal (green, grassy odor) and trans-2-hexenal (green odor) were found in comparatively higher levels than geranial and trans-2-octenal (green, grassy odor).

Similarly, among all identified ketones, geranyl acetone (sweet, floral odor) and 6-methyl-5-hepten-2-one (sweet, fruity odor) were the main components.

Furthermore, we conducted an analysis of variance to examine the overall effect of genotype and production system on the tomato volatiles (Table 2). Our results showed that the average levels of alcohols and fatty acids in all four varieties were significantly higher in the tomatoes grown in the high-tunnel system. Conversely, the levels of the total aldehydes, furans, ketones, and nitrogen compounds from all four varieties were higher in the open-field tomatoes. However, the hydrocarbons and sulfur groups were not affected by the production system. The univariate analysis showed that decanal levels were significantly higher in the open-field tomatoes for all four varieties. Moreover, TAM Exp 1 and USAT 0121 grown in the open field showed significantly higher levels of the furan derivative 2-pentyl furan compared with tomatoes grown in the high-tunnel system. The amino acid-derived volatile 1-nitro-3-methylbutane showed the highest levels for all varieties grown in the open field. The levels of neral (lemon odor), geranial (citrus odor), β-ionone (fruity, floral odor), and farnesyl acetone (ethereal floral odor) exhibited higher levels in all open-field tomatoes. In particular, 2-phenylethanone (floral odor) exhibited the highest levels in high-tunnel tomatoes for all varieties studied. Furthermore, tomatoes grown in the high-tunnel system had the highest levels of β-damascenone (fruity odor) while open-field grown tomatoes had the highest levels geranylacetone (sweet, floral odor) of all four varieties.

Table 1. Identified volatile compounds and their concentrations (ng/g) in four tomato varieties grown in the high-tunnel and open-field conditions.

RT	Compounds	KI[a]	ID[b]	Mass library similarity	MSI[c] level	TAM Hot-Ty		TAM Exp 1		TAM Exp 2		USAT 0121	
						High-tunnel	Open-field	High-tunnel	Open-field	High-tunnel	Open-field	High-tunnel	Open-field
4.38	1-Penten-3-one*	1013	MS, KI, ST	850	1	11.6 ± 2.2	11.2 ± 1.3	7.7 ± 1.2b	13.1 ± 1.2a	11.2 ± 2.1	11.4 ± 1.4	6.5 ± 0.3b	9.3 ± 1.1a
5.01	Hexanal	1076	MS, KI, ST	930	1	162.0 ± 13.6b	345.5± 29.1a	248.3 ± 39.5b	358.8± 24.9a	303.1 ± 50.3	220.7 ± 30.9	93.3 ± 10.2a	59.3 ± 7.5b
6.90	Trans-2-hexenal	1207	MS, KI, PCI	900	2	70.3 ± 5.2b	145.4± 15.7a	82.7 ± 10.3b	193.7± 19.4a	105.2 ± 16.1	131.0 ± 17.0	88.0 ± 7.0	75.6 ± 12.4
7.07	2-Pentyl furan	1217	MS, KI, ST, PCI	924	1	13.5 ± 0.51	21.3 ± 0.8	16.3 ± 1.1b	26.8 ± 1.8a	18.8 ± 2.4	21.4 ± 0.7	10.9 ± 0.7b	14.3 ±1.0a
7.78	*p*-Cymene	1258	MS, KI	893	2	27.8 ± 12.6	3.3 ± 0.2	9.1 ± 1.6a	3.5 ± 0.4b	4.7 ± 1.1	3.0 ± 0.1	2.7 ± 0.1	3.3 ± 0.3
8.75	Trans-2-heptenal*	1308	MS, KI, ST, PCI	844	1	12.0 ± 0.7	11.5 ± 0.8	12.8 ± 1.8	15.2 ± 1.3	18.0 ± 2.6a	11.2 ± 1.3b	8.4 ± 0.7	6.1 ± 1.0
8.87	1-Nitro-3-methylbutane*	1314	MS, KI	837	2	2.2 ± 0.5b	10.1 ± 1.4a	3.0 ± 0.3b	9.7 ± 1.3a	10.1 ± 1.8	12.3 ± 1.6	4.1 ± 0.8b	7.6 ± 1.1a
8.99	6-Methyl-5-hepten-2-one*	1320	MS, KI, ST, PCI	957	1	397.5 ± 9.4	369.3±25.1	355.2 ± 25.0	372.5± 36.2	478.2 ± 76.9	385.6 ± 38.8	275.2 ± 17.8b	394.1 ± 48.5a
9.33	1-Hexanol	1336	MS, KI, ST	930	1	174.1 ± 62.6	62.9 ± 13.6	267.3 ± 50.8a	94.0 ± 30.9b	55.8 ± 8.6	60.2 ± 9.2	44.5 ± 4.0b	82.3 ± 8.9a
10.04	Cis-3-hexen-1-ol*	1367	MS, KI, ST, PCI	924	1	32.0 ± 6.4a	14.3 ± 1.6b	43.0 ± 6.1	28.1 ± 6.4	23.7 ± 2.9	16.2 ± 2.3	33.5 ± 2.7	38.8 ± 5.0
10.22	Nonanal	1374	MS, KI	950	2	16.0 ± 0.6	15.8 ± 1.0	16.0 ± 0.0	18.1 ± 1.3	16.4 ± 1.9	16.7 ± 1.0	14.0 ± 0.7	15.0 ± 1.4
10.57	2-Isobutylthiazole*	1389	MS, KI, ST, PCI	913	1	50.5 ± 15.6	37.2 ± 4.3	40.2 ± 6.5	62.4 ± 9.0	27.4 ± 3.1b	56.5 ± 8.6a	29.5 ± 3.4	31.5 ± 5.8
11.07	Trans-2-octenal	1421	MS, KI, PCI	873	2	71.4 ± 2.8	76.9 ± 2.5	77.2 ± 6.7	94.5 ± 7.8	105.6 ± 15.7	80.3 ± 6.5	49.6 ± 1.7	51.8 ± 3.3
11.61	1-Octen-3-ol	1442	MS, KI	972	2	9.0 ± 1.8	7.6 ± 0.5	6.7 ± 0.5	8.2 ± 0.8	8.0 ± 0.8	7.2 ± 0.6	5.8 ± 0.5	5.7 ± 0.4
11.96	*β*-thujone	1455	MS, KI, PCI	813	2	12.1 ± 1.4b	17.8 ± 1.2a	14.5 ± 1.0b	21.1 ± 1.4a	17.2 ± 0.8	15.4 ± 1.5	10.6 ± 0.2	11.4 ± 1.2
12.71	(E,E)-2,4-heptadienal	1482	MS, KI	869	2	10.4 ± 0.5	12.1 ± 1.1	13.7 ± 1.3	13.1 ± 0.8	14.5 ± 2.1	11.3 ± 1.0	12.6 ± 0.5a	9.4 ± 0.3b
12.86	Decanal	1487	MS, KI	952	2	2.0 ± 0.3b	4.2 ± 0.8a	2.5 ± 0.2b	5.9 ± 0.8a	2.0 ± 0.1b	4.5 ± 0.4a	1.8 ± 0.2b	3.8 ± 0.5a
13.48	Benzaldehyde*	1508	MS, KI, ST, PCI	904	1	23.7 ± 1.0b	37.7 ± 3.0a	31.7 ± 3.7	36.9 ± 2.9	36.5 ± 4.1	38.3 ± 4.9	40.5 ± 2.3	33.2 ± 3.1
13.89	2-Nonenal	1521	MS, KI	958	2	16.9 ± 1.1b	29.0 ± 1.7a	12.5 ± 2.3b	32.4 ± 5.4a	21.2 ± 2.4	30.4 ± 4.4	11.1 ± 0.5b	21.1 ± 1.2a
14.01	Cis-4-decenal*	1524	MS, KI	845	2	2.4 ± 0.5b	5.9 ± 1.0a	5.2 ± 1.1	6.6 ± 0.7	3.6 ± 0.5b	6.1 ± 0.7a	7.8 ± 0.3	6.8 ± 1.3
14.31	Linalool	1534	MS, KI, ST	910	1	26.6 ± 0.5	27.7 ± 4.0	25.3 ± 0.6	24.3 ± 2.9	34.2 ± 5.0	27.3 ± 3.5	29.4 ± 1.4a	15.7 ± 1.6b
14.66	1-Octanol	1544	MS, KI	942	2	23.0 ± 4.9a	7.7 ± 0.5b	56.6 ± 12.8a	9.8 ± 1.6b	12.4 ± 1.5	8.8 ± 1.2	12.0 ± 0.6	13.1 ± 2.4
15.35	(E,Z)-2,6-nonadienal	1565	MS, KI	900	2	1.4 ± 0.1b	1.9 ± 0.2a	2.0 ± 0.2	2.1 ± 0.3	1.4 ± 0.1b	2.0 ± 0.2a	1.5 ± 0.2	1.9 ± 0.1
16.39	*β*-Cyclocitral	1593	MS, KI, PCI	885	2	21.6 ± 1.0b	33.1 ± 1.7a	32.0 ± 4.1	36.4 ± 3.6	31.6 ± 3.5	36.0 ± 4.8	18.5 ± 0.6	25.5 ± 3.7
16.95	Benzeneacetaldehyde	1663	MS, KI	900	2	2.7 ± 0.2b	4.2 ± 0.2a	7.6 ± 4.3	4.4 ± 0.4	4.1 ± 0.3	4.0 ± 0.4	4.9 ± 0.3	4.8 ± 0.6
17.12	4-Methylbenzaldehyde	1668	MS, ST, PCI	922	1	7.2 ± 0.5	8.3 ± 0.8	9.3 ± 1.0	9.4 ± 1.0	8.0 ± 1.2	8.0 ± 1.0	6.5 ± 0.9	8.2 ± 1.3
17.23	1-phenylethanone	1671	MS, ST	889	1	3.4 ± 0.1	2.8 ± 0.3	5.2 ± 0.5a	2.5 ± 0.3b	4.9 ± 0.6a	2.2 ± 0.2b	3.2 ± 0.2a	1.9 ± 0.2b
18.29	Neral	1699	MS, ST, PCI	969	1	25.5 ± 2.4b	47.3 ± 5.5a	28.6 ± 2.6b	58.9 ± 5.1a	37.4 ± 7.0	48.5 ± 6.0	18.3 ± 0.5b	32.7 ± 2.3a
18.91	(E,E)-2,4-Nonadienal	1715	MS, KI	864	2	13.5 ± 0.7	18.5 ± 2.4	16.8 ± 1.7	17.5 ± 1.4	19.6 ± 3.8	14.8 ± 1.8	14.8 ± 0.5a	9.1 ± 0.8b
19.01	4-methoxy-6-methyl phenol	1718	MS	842	2	20.0 ± 4.8	28.3 ± 4.2	19.8 ± 2.3b	43.0 ± 8.1a	30.0 ± 4.9	37.9 ± 7.3	18.0 ± 2.9	24.1 ± 4.5
19.90	Geranial	1740	MS, KI, ST, PCI	940	1	68.2 ± 6.1b	101.0 ± 9.6a	78.4 ± 7.6b	122.2 ± 9.8a	95.4 ± 18.7	97.4 ± 10.8	42.0 ± 1.5b	60.0 ± 4.3a
20.95	(E,Z)-2,4-decadienal	1764	MS, KI, PCI	894	2	7.9 ± 0.6	14.3 ± 1.5a	9.3 ± 1.4b	19.3 ± 2.0a	11.1 ± 2.1	13.2 ± 1.3	4.4 ± 0.2	5.5 ± 0.6
22.39	(E,E)-2,4-decadienal	1796	MS, KI, PCI	882	2	34.1 ± 3.0b	53.7 ± 5.6a	41.2 ± 6.5b	68.6 ± 8.2a	45.1 ± 7.4	54.5 ± 6.4	15.4 ± 0.9	16.4 ± 1.4
22.74	*β*-damascenone*	1875	MS, KI, ST, PCI	896	1	65.1 ± 3.2a	42.3 ± 8.3 b	82.3 ± 9.9a	31.8 ± 4.1b	85.4 ± 15.3a	34.0 ± 6.1b	35.7 ± 2.1a	12.1 ± 2.0b
23.72	Hexanoic acid	1897	MS, KI	982	2	21.3 ± 5.0	21.1 ± 1.1	16.4 ± 1.1a	0.7 ± 0.1b	19.8 ± 2.0	17.7 ± 1.9	7.2 ± 0.6	11.1 ± 2.5
23.92	Geranyl acetone	1902	MS, ST, PCI	983	1	228.4 ± 23.2b	629.8± 70.4a	432.5 ± 51.4b	691.6± 81.0a	278.4 ± 48.7b	611.7 ± 89.2a	140.1 ± 6.0b	262.5 ± 23.2a
26.49	*β*-ionone	1955	MS, KI, ST, PCI	911	1	25.2 ± 1.1b	41.3 ± 2.2a	31.8 ± 3.1b	46.9 ± 5.6a	36.0 ± 5.8	40.4 ± 6.2	17.7 ± 0.6	24.1 ± 3.7
26.82	Benzothiazole	1961	MS, ST, PCI	904	1	11.3 ± 0.6	12.8 ± 1.0	14.5 ± 1.8	11.3 ± 1.1	14.7 ± 2.6	11.1 ± 1.4	11.0 ± 0.7a	8.9 ± 0.7b
30.58	Octanoic acid	2126	MS, KI	967	2	8.1 ± 0.9	10.1 ± 1.2	20.1 ± 4.1	13.5 ± 2.2	20.3 ± 3.0a	9.3 ± 1.5b	4.7 ± 0.4a	3.2 ± 0.2b
33.58	Eugenol	2179	MS, KI, ST	927	1	23.2 ± 0.9b	34.0 ± 3.0a	35.8 ± 3.3	33.0 ± 3.5	50.3 ± 6.4	43.8 ± 6.2	2.3 ± 0.5	1.0 ± 0.2
36.56	Farnesyl acetone	2394	MS, ST	951	1	22.4 ± 2.7b	72.1 ± 8.4a	60.0 ± 11.7	78.5 ± 11.3	47.7 ± 5.9b	73.0 ± 9.6a	16.7 ± 1.1b	28.6 ± 4.0a

*Volatiles positively correlated with consumer preferences [73, 144].

[a] KI: Kovat's index, relative to *n*-alkanes (C_8-C_{24}) on the ZB-Wax Plus capillary column.

[b] ID: Identification methods, MS: Mass spectra; KI values that agreed with the data reported in previous studies or the database (http://www.nist.gov).

ST: Standard comparison, compounds identified using authentic standards; PCI indicates compounds identified using positive chemical ionization mode.

[c] MSI level: Metabolomics Standards Initiative level [155].

Different letters in the same row indicated significant differences between production systems at 95%.

Table 2. Effect of genotype and production system on the tomato volatile compounds.

	Alcohols	Aldehydes	Fatty Acids	Furans	Hydrocarbons	Ketones	Nitrogen compounds	Sulfur compounds
Genotype								
TAM Hot-Ty	228.8ab	525.3a	29.9a	17.4a	30.5a	961.1a	5.8b	55.9a
TAM Exp 1	325.3a	642.9a	25.1a	20.4a	24.1ab	1105.7a	6.2b	64.1a
TAM Exp 2	181.9b	621.5a	33.6a	20.1a	20.2ab	1049.9a	11.2a	54.8a
USAT 0121	148.3b	390.4b	13.0b	12.6b	14.0b	613.6b	4.7b	40.4a
Significance	**	***	***	***	*	***	***	ns
Production system								
High tunnel	265.9a	469.1b	29.3a	14.9b	24.7a	791.3b	4.5b	49.7a
Open field	176.2b	621.0a	21.5b	20.4a	19.7a	1073.9a	9.4a	57.9a
Significance	**	**	*	***	ns	***	***	ns

Unit = concentration (ng/g of fresh tomato sample, equivalent of 2-octanone) and mean values with different letters indicated significant difference (ns: no significance, * < 0.05, ** < 0.01, and *** < 0.001).

Multivariate analysis and potential volatile marker

One-way analysis of variance (ANOVA) was performed to understand the statistical significance of the observed differences in volatile metabolites of tomato varieties grown in high-tunnel and open-field systems. In addition, multivariate analysis of GC-MS data was performed to determine the variance and discriminant features between the production systems in each variety using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). In PCA analysis, three components explained 80%, 73.7%, 73.3%, and 80.8% of variances in the TAM Hot-Ty, TAM Exp 1, TAM Exp 2, and USAT 0121 varieties, respectively (Fig. 2A–D). The score plots between component 1 and component 2 of four PLS-DA models are shown in Fig. 2E–H. Two clusters of PLS-DA models defined production systems (high-tunnel and open-field) in each variety using four biological replicates. The R^2 and Q^2 values were calculated by the "Leave one out" cross-validation method to evaluate the goodness of fit and prediction ability of four PLS-DA models (Supplementary Table S3).

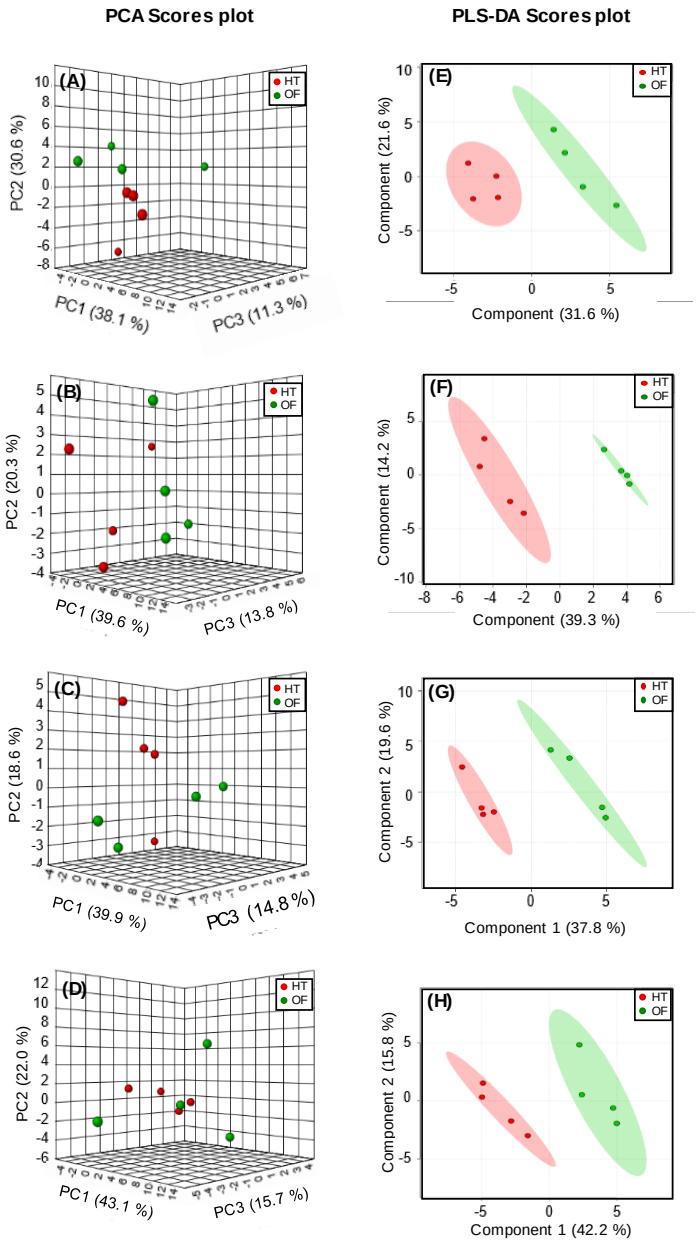

Figure 2. Multivariate analysis: (A–D) Principal component analysis (PCA) score plots corresponding to a model aimed at the discrimination between production system (high-tunnel, HT) and open-field, OF), influencing tomato volatile profiles. (E–H) Partial least squares discriminant analysis (PLS-DA) score plots from each tomato variety grown in the different production systems. The colored ellipses indicate 95% confidence intervals for each class. (A) and (E) TAM Hot-Ty, (B) and (F) TAM Exp1, (C) and (G) TAM Exp 2, and (D) and (H) USAT 0121.

Furthermore, the variable importance on projection (VIP) score plots were derived from the PLS-DA models. The compounds responsible for clustering in four varieties were identified based on their VIP scores exceeding 1.0 (Fig. 3 and Supplementary Table S4).[157] The VIP score plots showed that the four compounds, 4-methoxy-6-methyl phenol, 1-phenylethanone, β-damascenone, and geranylacetone, were common in the four varieties. In the univariate analysis, β-damascenone and geranylacetone were significantly different between the two production systems ($P < 0.05$). Therefore, based on the multivariate and univariate analysis, these two compounds could be considered as potential volatile biomarkers to distinguish high-tunnel and open-field grown tomatoes.

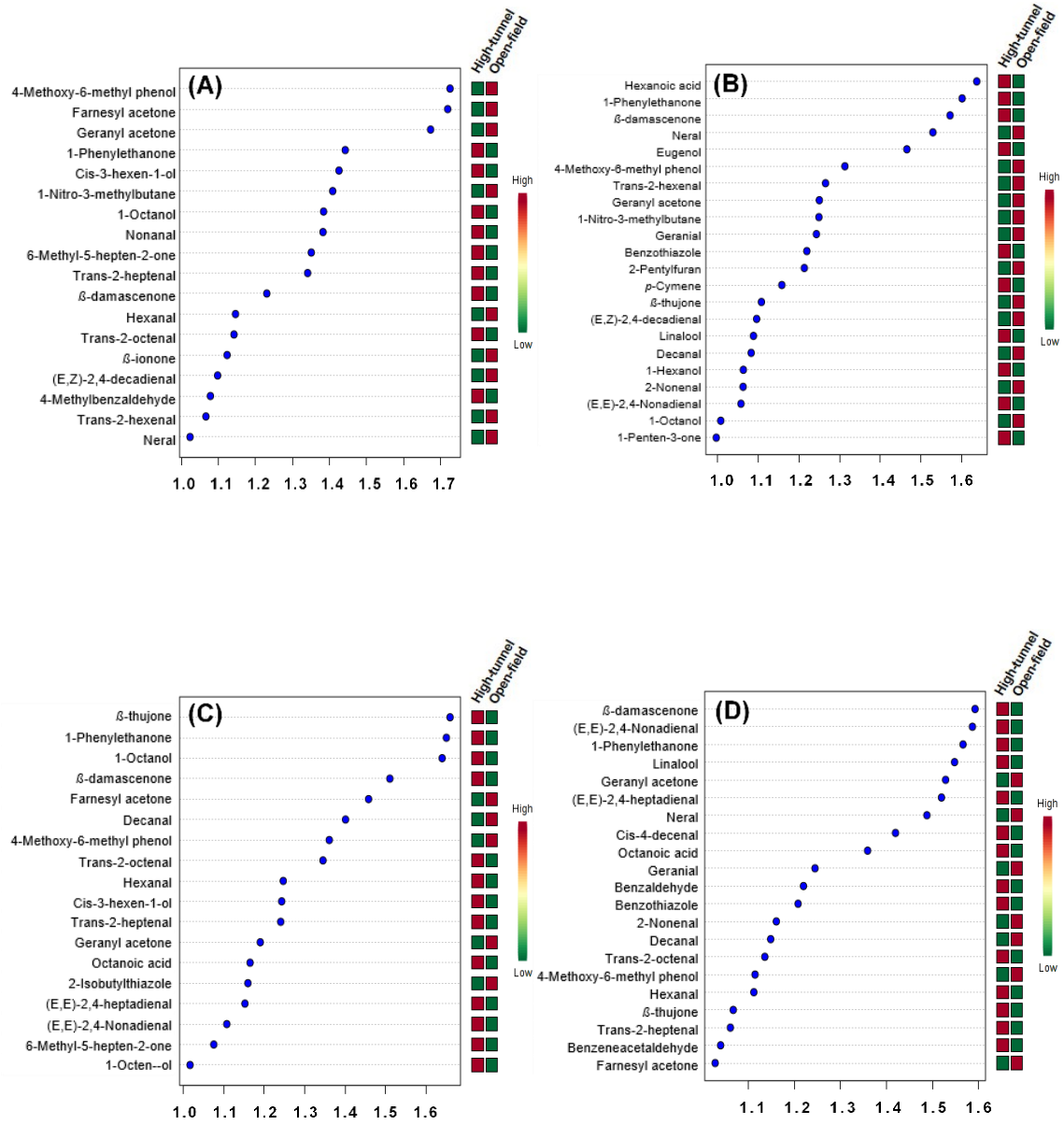

Figure 3. The discriminating metabolite features based on variable importance on projection (VIP) scores ≥1.0 from partial least squares discriminant analysis (PLS-DA) of each tomato variety. (A) TAM Hot-Ty, (B) TAM Exp1, (C) TAM Exp 2, and (D) USAT 0121. Red and green on the right indicate relatively high and low concentrations of metabolites from high-tunnel and open-field grown tomatoes.

Discussion

Flavor has a considerable effect on consumer preferences for tomatoes. As a result, a number of plant breeding and genetic engineering studies have aimed to enhance the flavor of tomato fruits. In addition, researchers have investigated the influence of processing and post-harvest handling on tomato volatiles.[149, 158] However, consumer-preferred tomato flavors are difficult to attain in many cases, due to the complex interaction between genetics and the production system.[159] Furthermore, quantification of flavor-linked metabolites is also difficult due to their complex chemical nature and low concentrations.[143] Therefore, there is a critical need to develop efficient quantification methods for flavor-linked volatiles. Moreover, an optimized quantification method to identify flavor-linked volatiles can be used for establishing the relationship between genetics, production system, and specific metabolites. Consequently, this information will provide essential clues for improving tomato flavor.

GC-MS is a valuable technique routinely used for aroma characterization.[156, 160-161] Previous studies reported that sampling procedures affect the release of volatile metabolites from tomato fruits. Therefore, in these studies, whole and halved fruit, paste, frozen powder, and filtered juice were used to analyze the volatile profiles of tomato fruits.[12, 149, 162] For instance, Tikunov et al. reported the use of blended tomato fruit to identify key glycoconjugated volatiles by fusion approaches using GC-MS and LC-MS.[148] Therefore, selecting proper sample preparation and extraction techniques is crucial for the analysis of tomato volatiles, due to their low concentrations and the complex

physicochemical properties of tomato samples. The process of sample preparation and the technique used for analysis have a significant effect on the observed profiles of tomato volatiles.[149-151] In the present study, blended tomato samples were used for the optimization of the method. Previous studies also demonstrated that blended samples were optimal for extraction of volatiles from strawberry and *Monstera deliciosa* fruits.[163-164]

The headspace solid phase microextraction (HS-SPME) technique was initially introduced by Arthur and Pawliszyn (1989), and it has been widely used in combination with GC-MS, mainly due to its many advantageous features such as solvent-free sample preparation, robustness, high sensitivity, and reproducibility.[165-166] A previous study showed that the HS-SPME method allowed extraction of a wider range of compounds than headspace-trap (HS) and Tenax adsorption-thermal desorption (TD) methods.[149] HS-SPME involves many steps, making it important to optimize the extraction conditions to achieve the greatest efficiency. The fibers used, extraction temperature, and time seem to play a major role in extraction efficiency. Different fibers have different polarities and retention capabilities, depending on their types of coating. A previous report indicated that the majority of volatile metabolites can be extracted at lower extraction temperatures, whereas higher temperatures facilitate the release of higher concentrations of semi-volatile compounds from the matrix.[167] Additionally, the extraction time influences the distribution of compounds between the sample matrix, the headspace phase, and the fiber coatings; therefore, extraction time will significantly affect the HS-SPME results.[150-151] The present study aimed to optimize the extraction method using blended samples. Taken

together, the extraction of two-gram blended tomato samples at 60°C for 45 min with DVB/CAR/PDMS fiber was found to show the maximum amounts of volatiles.

We also aimed to assess the volatile profiles of different tomato varieties grown in two different production systems. The 9 volatiles out of 16 major aroma- and flavor-determining volatiles of tomato were common in all studied varieties, such as 1-penten-3-one, hexanal, trans-2-hexenal, trans-2-heptenal, 6-methyl-5-hepten-2-one, cis-3-hexen-1-ol, 2-isobutulthiazole, ß-damascenone, and ß-ionone.[73] Among these compounds, the levels of ß-damascenone were significantly influenced by production system and were higher in the high-tunnel tomatoes than in the open-field grown tomatoes. We used four tomato varieties with similar maturity indices (total soluble solids content (TSS)/total acidity (TA)) to determine the effect of the production system on tomato volatile compounds. The TSS and TA contents are important for the tomato flavor, along with aroma-active volatile compounds. However, we did not find significant differences between production systems for TSS and TA (Supplementary Table S5).

In recent years, metabolic markers have been identified that can serve as indicators or predictors of disease outbreak frequency, developmental stage, food sensory evaluation, and crop yield.[168] Usually, in metabolomics studies, chromatographic techniques are coupled with chemometric methods such as PCA and PLS-DA to understand the patterns in the data and to identify molecular markers. PCA is a mathematical algorithm that reduces multidimensional data and provides a graphical interpretation of the data in which similar samples cluster close together and dissimilar

samples fall further apart. PLS-DA is a supervised method for classification and discriminant analysis which can be used to describe the relationships among the measured variables.[169]

The PCA, PLS-DA, and variable importance of projection (VIP) analyses were performed to identify metabolite markers for tomatoes grown in the two production systems (Fig. 2 and 3). The PCA and PLS-DA analysis of tomato volatiles showed that high-tunnel and open-field production systems have a considerable impact on tomato volatile profiles in each of the four varieties (Fig. 3A–D). Furthermore, using chemometric studies, prominent volatile compounds were ranked from VIP plot based on their importance in discriminating production systems. To assess the statistical significance of class discrimination in the PLS-DA model, a permutation test was performed. In addition, a one-way ANOVA (analysis of variance) with Tukey's Honestly Significant Difference (Tukey's HSD) test was performed on the metabolomics data, to assess which metabolites were mainly involved in each of the various groups. The threshold of significance was set at $p < 0.05$. In summary, findings of multivariate and univariate analyses confirmed that *β*-damascenone and geranyl acetone may be considered potential volatile markers for high-tunnel and open-field grown tomatoes. *β*-Damascenone (sweet, apple odor) has a considerable role in the flavor of tomato due to its extremely low odor threshold, 0.002 ppb compared to 60 ppb for geranylacetone.[73, 170] Based on our observation, the significantly increased levels of *β*-damascenone under the high-tunnel system may have an important role in the flavor of the fresh tomato. In addition, several potent health-

promoting properties have been reported for *β*-damascenone, including UV protective potential.[171] Similarly, geranylacetone is a well-known antimicrobial agent and has a potential role in the treatment of Alzheimer's disease.[172-173] We believe that the present study will help to select the proper production system to produce aroma-rich tomatoes or to select tomato varieties well-suited for a particular production system.

CHAPTER IV

METABOLOMIC STUDIES OF VOLATILES FROM TOMATOES GROWN IN NET-HOUSE AND OPEN-FIELD CONDITIONS*

Introduction

Tomatoes (*Solanum lycopersicum*) are an important source of health-promoting compounds such as carotenoids, ascorbic acid, phenolic acids, and flavonoids.[4-6] Moreover, it is a valuable horticultural crop; for example, in 2016, the United States produced about 14 million tons of fresh market and processing tomatoes, with a value of more than 2.6 billion dollars.[174] The chemical composition of the tomato determines its color and the interactions of sugars, acids, and aromatic volatile compounds define its flavor.[175-176] So far, over 400 volatile compounds have been identified in the tomato fruit. Generally, the major tomato volatiles are derived from amino acids, fatty acids, terpenoids, and carotenoid pathways.[176] However, according to the concentrations and odor thresholds, only 16 aroma-active volatiles are found to contribute to tomato flavor.[177-178]

Most breeding programs have focused on the selection of fruit size, sugar and acid levels, but have not examined volatile profiles.[4] Consequently, such breeding programs

could be unintentionally contributing to the deterioration of flavor in modern tomato varieties. In addition, the growing environment, seasons, and locations have

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different influences on tomato volatiles.[28-29] Moreover, the demand for locally grown food has increased; to meet this claim, protected agricultural systems such as high tunnels are used to extend the growing season as well as enhance crop quality and yield profitably and effectively.[179] However, only a few comparative studies have examined the effects of production system on tomato volatiles.[180]

The study of tomato volatiles requires accurate methods for quantitation and identification of these molecules. Gas chromatography with mass spectrometry (GC-MS) along with headspace solid phase microextraction (HS-SPME) has been used as a method for the efficient investigation of the complex volatile components.[181-182] These chromatographic and spectroscopic data have been analyzed by metabolomics approaches, which allow identification of potential metabolite marker(s) based on their characteristic profiles of volatile metabolites by genotype and growing conditions.[6, 183-184] This metabolomic information is essential for plant breeding, along with traditional molecular markers for crop improvement and adaptation of suitable growing practices.[185] However, except for these few reports, little is known about the individual or collective influence of these factors on the volatiles of fresh tomato fruits.

In the present study, the volatile profiles of three local Texas A&M University (TAMU) and five commercial tomato varieties grown in net-house and open-field conditions were analyzed by HS-SPME/GC-MS methods. In addition, the individual and collective influences of genotype and the two production systems were further studied using univariate and multivariate chemometric approaches. This scientific information

may be useful to recommend tomato varieties and cultivation practices to improve or preserve the desirable flavor components of tomato fruit.

Materials and methods

Chemicals and reagents

Analytical grades of ethanol and calcium chloride as well as twenty two authentic volatile standards, including 1-penten-3-one, hexanal, d-limonene, 2-pentylfuran, (E)-2-heptenal, 6-methyl-5-hepten-2-one, 1-hexanol, (Z)-3-hexen-1-ol, 2-isobutylthiazole, benzaldehyde, linalool, 4-methylbenzaldehyde, neral, geranial, methyl salicylate, β-damascenone, geranylacetone, β-ionone, benzothiazole, pseudoionone, eugenol, and farnesylacetone, were procured from Sigma-Aldrich (St. Louis, MO, USA).

Plant materials and production systems

Eight tomato cultivars including Texas A&M F_1 hybrids (TAM Hot-Ty, T3, and L501-55) and commercial varieties (SV8579TE, Shourouq, Seri, Mykonos, and DRP-8551) were grown in net-house and open-field production systems. The fruits of TAM Hot-Ty, T3, and L501-55, Shourouq, Mykonos were Beefsteak round type; SV8579TE, Seri, and DRP-8551 were Roma oval type fruits (Supplementary Fig. S3). The net-house type high-tunnel structure consisted of a 2000-ft^2 (185-m^2) hoop house structure completely covered with 50-mesh insect screen (20–29% shade). Five-week-old seedlings were transplanted on February 29th, 2016 at the Texas A&M AgriLife Research and

Extension Center, Weslaco, TX (26° 09' 19.1" N 97° 57' 42.9" W). The production system evaluation was set in plots side by side to reduce soil and field variation. Three replications per cultivar for each production system were evaluated in a complete randomized design (8 plants/plot at 1.5-ft (0.46-m) spacing between plants and 5-ft (1.5 m) between rows, with an average of 5,808 plants/acre). Irrigation, fertilization, and pesticide applications were consistent between production systems. Soludrip Tomatoes Stage fertilizer (Vital Fertilizers, Mission, TX) was applied according to the manufacturer's recommendations. Weather stations were installed to monitor temperature, relative humidity, wind speed, and solar radiation. The average daily temperature was similar between production systems (~82°F). However, the average temperature in the open field was more variable, showing higher and lower peaks as compared to protected structures where the temperature was more stable. Relative humidity average values were also similar between the two production systems (72–75%). As a result of the insect screen, the average solar radiation and wind speed was ~5.6 MJ/day and ~4 mph lower inside the net-house as compared to the open-field conditions.

Sample preparation for volatile analysis

Fresh tomato fruits were sliced into six pieces and blended for 30 sec and the samples (2 g) were put into a 20 mL glass vial. Saturated $CaCl_2$ (2 mL) and 10 µL of 2-octanone (0.025%, v/v) in ethanol were also added into the GC-MS vial and stored at –

20℃ until analysis. Tomato samples consisted of two replications and three subsamples taken from each replication.

HS-SPME/GC-MS analysis

Tomato samples in GC-MS vials were vortexed for one min and sonicated for 30 min at room temperature, and then loaded onto a TriPlus autosampler (Austin, TX, USA). The volatile compounds from fresh tomatoes were extracted by HS-SPME with a 50/30 µm CAR/PDMS/DVB fiber (Sigma-Aldrich, St. Louis, MO). The incubation and extraction times using SPME fibers were 2 and 45 min, respectively, at 60 ℃ with continuous agitation (5 sec/min). As soon as the SPME fiber was desorbed by being inserted into the GC injector at 225 ℃ for 2 min, fiber conditioning was followed for 7 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min with a splitless mode. The Thermo Finnegan gas chromatograph (Thermo Fisher Scientific, Inc., San Jose, CA, USA) coupled with a Dual-Stage Quadrupole (DSQ II) mass spectrometer (Thermo Scientific, Austin, TX, USA) was used for volatile analysis from eight tomato varieties. Chromatographic separation was carried out on Zebron ZB-Wax column coated with 100% polyethylene glycol of 30 m × 0.25 mm i.d., 0.25 µm film thickness (Phenomenex, Inc. Torrance, CA). The oven temperature was started at 40℃ and held for 1 min, then increased to 90 ℃ at a rate of 10 ℃/min, increased to 175 ℃ at a rate of 3 ℃/min. Finally, it was ramped up to 230 ℃ at a rate of 35 ℃/min and held for 2 min with a total run time of 38 min. Electron impact (EI) mass data from *m/z* 40 to 450 were acquired at a scanning

speed of 11.5 scans per sec and with an ionization voltage of 70 eV. The ion source temperature was maintained at 280 ℃. In addition, positive-ion chemical ionization was performed with methane as a reagent gas (reagent gas flow: 1 mL/min). The ion source temperature and mass transfer line temperature were maintained at 180 and 250 ℃, respectively. The data were recorded and processed using Xcalibur software (v. 2.0.7., Thermo-Fisher Scientific, San Jose, CA, USA).

Identification and quantification of tomato volatile compounds

Identification of volatile compounds was achieved by comparison of their mass spectra, Kovats indices (KI), and retention times to authentic standards. The KI values were calculated by the retention time of *n*-alkane standards (C_{10}–C_{24}) analyzed under the same conditions as the samples. Each mass spectrum was also compared in Wiley 8 and NIST 05 mass spectral library. Relative quantification of volatiles in tomato was performed by direct comparison of 2-octanone as an internal standard according to a previous study [175].

Univariate and multivariate analysis

The significance of variation in the levels of 40 different volatiles across the 8 varieties grown in the net-house and open-field was analyzed by univariate statistics using SPSS software (v. 23, BM SPSS Statistics, IBM Corp., Chicago, IL, USA). Significant differences between production systems were assessed with a Student's *t*-test (*P*-value <

0.05). The multiple mean comparison (*P*-value ≤ 0.05) analysis was carried out by using a one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test. Further, multivariate analysis was performed by exporting GC-MS data in Excel format to online software, MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/). Both unsupervised and supervised multivariate analysis were performed to evaluate metabolite patterns and correlations.

Results and discussion

Effects of genotype and production system, and their interaction on volatile profiles of fresh tomato fruits

The volatile profiles of tomatoes from eight varieties grown in the net-house and open-field conditions were obtained by headspace solid phase microextraction coupled with gas chromatography and mass spectrometer (HS-SPME/GC-MS). A total of 40 different volatile compounds were identified and quantified and these are summarized in Table 3. Identified volatiles were categorized into eight groups: alcohols, aldehydes, esters, fatty acids, furans, hydrocarbons, ketones, and sulfur compounds (Table 4). The results clearly indicated that the volatile profiles of tomatoes were quite different for various genotypes and production systems. Among identified compounds, benzothiazole and 2-isobutylthiazole were found to be sulfur-containing volatiles. The production system significantly affected the volatile components, including alcohols, aldehydes, hydrocarbons, ketones, and sulfur compounds. All studied tomato varieties grown under

the net-house system had higher levels of aldehydes, ketones, and sulfur compounds. The volatile ester, fatty acids, and furan derivatives did not show significant differences between production systems. The genotype also significantly influenced compounds from certain volatile classes such as aldehydes, esters, fatty acids, and ketones ($P < 0.001$), and hydrocarbons ($P < 0.05$). We found no statistical difference in alcohols, furans, and sulfur compounds among genotypes. The volatiles from the furan ($P < 0.001$), fatty acids ($P < 0.01$), esters, and ketones chemical classes ($P < 0.05$) were significantly influenced by genotype-by-production system interactions. However, genotype-by-production systems interaction had no statistically significant influence on the volatile compounds from alcohols, aldehydes, hydrocarbons, and sulfur-containing volatiles.

We found that these factors had a significant influence on the profiles of tomato volatiles in the different groups and only ketones were significantly influenced by all parameters. Similarly, the previous study also found that genotype and cultivation systems variously affected the tomato volatiles.[186] Therefore, identifying genotypes with low environmental variability might be effectual for a breeding program to improve targeted aroma qualities.

Table 3. Identified volatile compounds and their concentrations (ng/g) in eight tomato varieties grown in the net-house (NH) and open-field (OF) conditions.

RT	Identified compounds	KI[a]	ID[b]	TAM Hot-Ty		T3		L501-55		SV8579TE		Shourouq		Seri		Mykonos		DRP-8551	
				NH	OF	NH	OF	NH	OF	NH	OF	NH	OF	NH	OF	NH	OF	NH	OF
4.38	1-Penten-3-one	1013	MS, KI, ST	5.7 ± 0.7b	8.7 ± 0.9a	5.8 ± 0.3b	11.3 ± 1.3a	6.7 ± 0.5	8.1 ± 0.8	6.1 ± 0.5b	23.0 ± 2.6a	9.5 ± 0.5	48.5 ± 19.5	10.7 ± 1.4	48.0 ± 24.5	4.0 ± 0.4b	16.6 ± 2.2a	8.8 ± 0.4	10.6 ± 2.1
5.04	Hexanal	1076	MS, KI, ST	236.3 ± 15.0	154.2 ± 32.3	216.1 ± 22.3	144.1 ± 24.9	253.3 ± 27.8	187.5 ± 19.2	109.5 ± 5.4	95.1 ± 3.6	102.8 ± 11.2	100.2 ± 18.2	83.6 ± 10.4	107.3 ± 10.1	71.8 ± 13.1	72.9 ± 8.3	117.1 ± 6.8	123.5 ± 16.4
6.65	d-Limonene	1199	MS, KI, ST	2.2 ± 0.7	26.8 ± 11.5	2.0 ± 0.4b	33.6 ± 5.2a	1.6 ± 0.20.2	9.7 ± 3.5	1.4 ± 0.4b	27.7 ± 4.2a	2.4 ± 0.6b	38.8 ± 12.8a	1.2 ± 0.2	20.7 ± 12.1	0.5 ± 0.1b	26.5 ± 4.8a	1.6 ± 0.5	16.9 ± 8.2
6.89	Trans-2-hexenal	1206	MS, KI, PCI	100.3 ± 13.9	71.6 ± 15.8	104.9 ± 7.3	72.2 ± 12.8	128.9 ± 8.6	113.9 ± 15.8	64.1 ± 9.0a	31.4 ± 8.1b	35.0 ± 9.7	61.4 ± 20.1	34.0 ± 3.3	37.8 ± 13.5	24.8 ± 2.3	48.2 ± 14.3	68.1 ± 5.2	69.7 ± 14.5
7.05	2-Pentyl furan	1216	MS, KI, ST, PCI	17.3 ± 1.6a	7.8 ± 0.8b	14.8 ± 1.5	12.1 ± 0.2	15.5 ± 1.6	13.4 ± 1.4	10.5 ± 1.2b	15.5 ± 0.9a	12.8 ± 1.9	14.5 ± 0.8	12.3 ± 1.4	15.5 ± 1.9	8.3 ± 1.1b	12.7 ± 1.5a	13.2 ± 1.0	16.4 ± 2.3
7.75	*p*-Cymene	1256	MS, KI	3.5 ± 0.5	3.8 ± 0.8	4.9 ± 0.2a	3.9 ± 0.3b	4.4 ± 0.5	5.3 ± 0.5	2.4 ± 0.1	2.6 ± 0.4	2.0 ± 0.3	1.9 ± 0.2	3.4 ± 0.2a	2.2 ± 0.3b	1.7 ± 0.2	2.6 ± 0.3	3.0 ± 0.3	2.4 ± 0.3
8.74	Trans-2-heptenal	1308	MS, KI, ST, PCI	30.9 ± 1.7a	15.1 ± 1.9b	22.3 ± 2.6	15.7 ± 1.9	22.8 ± 1.9a	16.8 ± 1.3b	21.7 ± 1.5	20.7 ± 1.3	18.6 ± 1.7	16.7 ± 2.0	21.7 ± 2.5	19.7 ± 1.8	16.7 ± 1.9	16.4 ± 1.3	26.4 ± 1.7	28.3 ± 4.0
8.98	6-Methyl-5-hepten-2-one	1319	MS, KI, ST, PCI	722.4 ± 11.4	723.6 ± 31.0	722.9 ± 36.7a	519.4 ± 49.0b	720.4 ± 38.1	637.5 ± 29.5	767.3 ± 15.2a	685.1 ± 16.8b	708.5 ± 16.7a	422.0 ± 92.1b	847.5 ± 27.5	866.0 ± 53.6	487.1 ± 21.8	447.6 ± 10.9	776.6 ± 12.4	647.5 ± 80.4
9.31	1-Hexanol	1335	MS, KI, ST	20.0 ± 4.9	13.8 ± 4.0	13.7 ± 5.9	9.2 ± 3.1	16.4 ± 8.1	21.3 ± 8.3	5.8 ± 0.5b	24.6 ± 3.3a	7.1 ± 1.4	9.2 ± 2.5	4.9 ± 1.3	11.1 ± 3.7	5.3 ± 0.8	9.5 ± 1.8	6.4 ± 1.5	8.1 ± 2.2
10.00	Cis-3-hexen-1-ol	1365	MS, KI, ST, PCI	18.7 ± 2.4	23.9 ± 2.9	14.1 ± 5.9	8.4 ± 5.5	7.5 ± 2.1	19.6 ± 7.6	6.3 ± 0.8b	47.1 ± 8.3a	9.2 ± 2.3	15.1 ± 4.5	4.6 ± 1.5	13.6 ± 5.6	6.8 ± 1.3b	13.5 ± 2.3a	6.3 ± 2.1	10.1 ± 3.7
10.20	Nonanal	1374	MS, KI	17.3 ± 1.5a	12.7 ± 0.6b	14.9 ± 16a	9.0 ± 0.4b	15.1 ± 1.5	13.9 ± 1.3	20.9 ± 1.2	17.8 ± 1.1	14.7 ± 2.0	11.0 ± 1.7	18.3 ± 1.0	19.1 ± 0.8	10.1 ± 1.2	11.0 ± 1.3	24.7 ± 1.5	19.1 ± 2.8
10.54	2-Isobutylthiazole	1388	MS, KI, ST, PCI	20.8 ± 1.6	16.43.6	22.5 ± 2.3a	14.0 ± 1.1b	21.4 ± 2.7	17.4 ± 1.5	18.3 ± 1.3	17.1 ± 1.9	29.3 ± 2.3a	15.3 ± 4.2b	16.0 ± 1.3	12.3 ± 1.5	22.7 ± 3.6	16.0 ± 2.9	26.0 ± 1.1a	17.2 ± 2.1b
11.05	Trans-2-octenal	1420	MS, KI, PCI	56.1 ± 3.5a	31.3 ± 2.2b	46.7 ± 3.4a	29.1 ± 3.4b	45.7 ± 5.2	36.1 ± 2.6	49.2 ± 2.5	55.6 ± 3.5	48.7 ± 3.3	38.8 ± 4.8	63.9 ± 6.7	65.5 ± 5.9	41.0 ± 5.0	46.6 ± 4.2	59.2 ± 3.3	59.8 ± 7.7
11.58	1-Octen-3-ol	1441	MS, KI	7.3 ± 0.4a	5.8 ± 0.3b	8.0 ± 0.5a	4.3 ± 0.3b	7.2 ± 0.8a	4.9 ± 0.3b	6.6 ± 0.2	6.6 ± 0.2	7.0 ± 0.6	5.6 ± 0.7	7.8 ± 0.6	8.8 ± 0.5	5.3 ± 0.6	5.0 ± 0.4	8.1 ± 0.3	7.8 ± 1.0
11.94	*β*-thujone	1454	MS, KI, PCI	29.6 ± 1.6	34.6 ± 8.4	25.9 ± 4.6	17.0 ± 1.4	23.7 ± 1.2	20.8 ± 1.0	26.4 ± 1.8	25.3 ± 0.9	28.0 ± 1.0	23.4 ± 3.9	40.5 ± 7.1	51.0 ± 9.7	21.3 ± 1.8	21.7 ± 1.6	31.6 ± 1.4	30.6 ± 4.0
12.67	(E,E)-2,4-heptadienal	1480	MS, KI	13.9 ± 0.7	18.8 ± 2.3	17.0 ± 0.8a	10.5 ± 0.8b	18.1 ± 1.8	13.8 ± 0.8	28.8 ± 0.5	27.5 ± 1.0	31.5 ± 1.8	23.7 ± 3.4	43.4 ± 3.5	44.1 ± 6.3	23.7 ± 2.9	24.8 ± 0.8	37.2 ± 1.4	28.6 ± 3.5
12.84	Decanal	1486	MS, KI	5.8 ± 0.3	6.4 ± 1.4	3.9 ± 0.5	3.3 ± 0.2	4.9 ± 0.3	5.6 ± 1.0	6.6 ± 0.7	7.3 ± 0.9	8.8 ± 1.0	8.4 ± 1.6	7.3 ± 1.7	9.2 ± 1.5	4.8 ± 0.8	5.5 ± 0.6	6.7 ± 0.3	8.1 ± 1.2
13.46	Benzaldehyde	1507	MS, KI, ST, PCI	14.2 ± 1.2b	17.8 ± 0.9a	20.3 ± 1.7a	9.4 ± 0.7b	23.7 ± 5.0	17.1 ± 1.6	20.0 ± 0.8	23.9 ± 2.0	20.3 ± 0.7	18.2 ± 1.9	39.9 ± 2.6b	49.1 ± 2.3a	15.1 ± 1.9	16.5 ± 1.2	22.0 ± 0.7	21.8 ± 2.2
13.86	2-Nonenal	1520	MS, KI	12.8 ± 0.8a	7.2 ± 0.9b	11.3 ± 1.0	8.7 ± 0.7	9.6 ± 1.4	8.6 ± 0.7	11.3 ± 0.5	10.5 ± 0.4	9.5 ± 1.0	6.6 ± 1.2	12.3 ± 1.0	12.2 ± 1.0	6.5 ± 0.8	6.6 ± 0.7	16.0 ± 0.7	14.0 ± 2.2
14.27	Linalool	1532	MS, KI, ST	12.6 ± 0.3b	17.6 ± 0.6a	15.2 ± 1.3	21.1 ± 2.6	16.4 ± 0.8b	21.9 ± 1.5a	16.7 ± 0.6	16.7 ± 0.5	14.6 ± 0.9	15.9 ± 1.1	29.3 ± 1.0	29.0 ± 3.1	14.4 ± 0.5	17.4 ± 1.9	21.9 ± 1.0	22.6 ± 3.6
14.64	1-Octanol	1544	MS, KI	5.5 ± 0.3	4.8 ± 0.2	6.4 ± 0.3a	4.4 ± 0.2b	5.2 ± 0.7	4.9 ± 0.2	6.5 ± 0.2b	8.7 ± 0.8a	6.6 ± 0.4a	5.1 ± 0.5b	7.4 ± 0.4	8.9 ± 0.7	4.6 ± 0.4	4.8 ± 0.2	7.0 ± 0.4	5.8 ± 0.8
16.36	*β*-Cyclocitral	1593	MS, KI, PCI	18.9 ± 0.6	20.9 ± 2.5	26.0 ± 1.4a	20.5 ± 0.7b	23.8 ± 1.4	24.4 ± 1.0	11.3 ± 2.1b	18.2 ± 1.0a	24.4 ± 1.2	21.6 ± 3.1	18.5 ± 1.2	20.2 ± 1.1	19.7 ± 1.0	23.5 ± 1.5	17.7 ± 0.6	19.6 ± 2.5
16.94	Benzeneacetaldehyde	1663	MS, KI	0.7 ± 0.2b	3.5 ± 0.9a	3. ± 0.4	2.1 ± 0.3	1.7 ± 0.6	2.0 ± 0.3	0.6 ± 0.2	0.9 ± 0.2	1.8 ± 0.3	1.8 ± 0.5	2.0 ± 0.2b	3.3 ± 0.4a	1.2 ± 0.3	1.4 ± 0.3	1.1 ± 0.2	1.1 ± 0.3
17.11	4-Methylbenzaldehyde	1667	MS, KI, ST, PCI	31.4 ± 1.2a	25.0 ± 1.3b	29.7 ± 2.1	28.1 ± 1.4	32.1 ± 1.0	30.9 ± 1.1	36.2 ± 1.0	37.2 ± 1.2	33.3 ± 1.7	32.3 ± 3.0	32.3 ± 2.5	33.8 ± 2.8	34.0 ± 1.4	38.3 ± 1.4	44.5 ± 1.9	38.6 ± 5.1
18.25	Neral	1698	MS, KI, ST, PCI	57.5 ± 2.5	38.3 ± 6.7	30.1 ± 7.0	33.9 ± 5.8	55.7 ± 3.8	40.0 ± 6.4	61.5 ± 2.8	56.4 ± 2.3	60.2 ± 1.8a	35.4 ± 8.8b	69.7 ± 8.1	65.3 ± 5.0	53.7 ± 3.3	48.2 ± 3.2	76.4 ± 3.1	56.6 ± 7.6
18.91	(E,E)-2,4-Nonadienal	1715	MS, KI	15.9 ± 1.5	14.5 ± 0.8	18.4 ± 2.6	14.3 ± 1.0	18.9 ± 2.2	15.9 ± 1.1	17.9 ± 1.0	21.0 ± 0.8	21.8 ± 2.1	18.6 ± 2.8	29.0 ± 3.5	33.5 ± 2.6	17.8 ± 2.5	21.2 ± 2.6	30.4 ± 1.8	30.7 ± 4.2
19.02	4-methoxy-6-methyl phenol	1718	MS, KI	7.5 ± 1.3	8.9 ± 1.3	7.9 ± 1.0	5.7 ± 1.3	6.7 ± 2.1	6.0 ± 1.3	8.6 ± 1.4	8.2 ± 1.0	12.8 ± 2.8	10.8 ± 2.3	9.7 ± 1.9	9.6 ± 1.8	8.3 ± 1.7	7.1 ± 0.7	13.0 ± 2.4	8.2 ± 0.8
19.86	Geranial	1739	MS, KI, ST, PCI	144.0 ± 5.8a	96.9 ± 11.6b	82.7 ± 13.5	80.1 ± 11.8	142.1 ± 9.8a	102.7 ± 14.4b	132.7 ± 5.2	121.5 ± 3.8	131.5 ± 3.8a	78.2 ± 19.5b	159.2 ± 13.9	141.6 ± 11.3	129.9 ± 8.1	114.7 ± 6.2	175.6 ± 7.0	131.5 ± 16.6
20.92	(E,Z)-2,4-decadienal	1764	MS, KI, PCI	4.2 ± 0.4a	1.6 ± 0.2b	2.3 ± 0.4	1.8 ± 0.4	4.1 ± 0.7	2.4 ± 0.6	5.7 ± 0.5	3.5 ± 0.2	4.3 ± 0.4a	2.3 ± 0.4b	7.9 ± 1.0	5.4 ± 0.9	5.6 ± 0.7	4.9 ± 0.8	8.6 ± 0.7a	4.8 ± 0.5b
21.11	Methyl salicylate	1768	MS, KI, ST	55.9 ± 11.7	93.0 ± 23.4	73.9 ± 12.7b	154.5 ± 23.9a	195.3 ± 16.6a	114.1 ± 18.1b	6.0 ± 0.5	3.9 ± 0.1	117.6 ± 15.5	130.3 ± 38.6	102.2 ± 12.7b	214.1 ± 30.0a	109.2 ± 20.1	124.5 ± 18.5	8.2 ± 0.9	6.6 ± 0.9
22.34	(E,E)-2,4-decadienal	1795	MS, KI, PCI	3.5 ± 0.4	2.9 ± 0.3	4.6 ± 0.5a	2.7 ± 0.6b	5.5 ± 0.9	4.1 ± 1.0	5.7 ± 0.5	4.0 ± 0.2	5.2 ± 0.4a	3.8 ± 0.2b	13.8 ± 1.8	10.1 ± 1.9	6.6 ± 0.8	6.7 ± 0.8	8.9 ± 0.8a	5.5 ± 0.7b
22.7	*β*-damascenone	1874	MS, KI, ST, PCI	27.2 ± 0.	28.4 ± 4.0	24.4 ± 2.2	19.4 ± 2.1	37.0 ± 3.4	32.7 ± 2.4	16.5 ± 0.9	15.9 ± 0.8	15.1 ± 0.7	13.0 ± 2.7	44.4 ± 2.4	38.6 ± 3.6	16.1 ± 1.1	14.3 ± 0.9	30.1 ± 1.9	24.3 ± 2.2
23.72	Hexanoic acid	1897	MS, KI	6.8 ± 1.0a	1.9 ± 0.2b	2.8 ± 0.3	6.1 ± 2.1	11.3 ± 1.9	8.1 ± 1.3	4.3 ± 0.2	5.0 ± 0.4	5.7 ± 0.5	4.5 ± 1.2	7.2 ± 1.6	6.1 ± 0.7	4.8 ± 1.1	4.7 ± 0.4	7.7 ± 0.4	7.7 ± 1.1
23.89	Geranylacetone	1901	MS, KI, ST, PCI	256.2 ± 15.2a	199.2 ± 13.6b	272.9 ± 18.9a	123.9 ± 13.6b	392.3 ± 60.5a	191.2 ± 25.2b	225.3 ± 11.0	199.9 ± 4.1	196.6 ± 6.6a	98.3 ± 28.7b	421.9 ± 21.5	350.2 ± 41.8	136.7 ± 19.6	147.9 ± 9.5	348.7 ± 15.3a	182.5 ± 30.0b
25.76	2-phenylethanol	1940	MS, KI	4.5 ± 0.9	13.7 ± 4.9	2.9 ± 0.5	3.3 ± 0.5	3.4 ± 0.1	4.9 ± 1.2	1.9 ± 0.2b	2.4 ± 0.0a	2.9 ± 0.1	2.4 ± 0.5	25.0 ± 9.0	5.0 ± 1.1	4.1 ± 0.5	7.1 ± 0.0	4.6 ± 0.8	4.1 ± 1.0
26.45	*β*-ionone	1954	MS, KI, ST, PCI	23.4 ± 1.0	23.8 ± 3.4	31.7 ± 2.6a	21.5 ± 1.0b	31.1 ± 2.3	27.7 ± 1.3	13.2 ± 0.7b	17.6 ± 0.6a	24.0 ± 0.6	21.9 ± 4.4	23.3 ± 1.5	20.0 ± 1.7	20.2 ± 1.5b	26.3 ± 1.8a	18.8 ± 0.7	20.1 ± 2.8
26.78	Benzothiazole	1961	MS, KI, ST, PCI	5.2 ± 0.3	4.5 ± 0.2	5.0 ± 0.3	4.5 ± 0.2	5.9 ± 0.3	6.8 ± 0.6	7.1 ± 0.4	7.2 ± 0.4	7.6 ± 0.	7.4 ± 0.5	10.2 ± 0.9	11.0 ± 0.8	6.7 ± 0.7	8.56 ± 1.2	9.8 ± 0.5	11.6 ± 1.4
31.84	Pseudoionone	2149	MS, KT	24.8 ± 3.2	40.9 ± 9.6	24.4 ± 4.6	26.7 ± 8.0	24.0 ± 3.2	20.7 ± 3.0	14.7 ± 1.8	13.2 ± 2.1	27.9 ± 2.3a	17.3 ± 5.4b	82.5 ± 18.7	65.7 ± 19.5	24.0 ± 3.1	18.1 ± 2.0	23.9 ± 2.6a	15.4 ± 2.0b
33.58	Eugenol	2179	MS, KI, ST	0.6 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	2.0 ± 0.5	2.2 ± 0.2a	1.2 ± 0.3b	ns	ns	0.8 ± 0.1	1.8 ± 0.7	2.9 ± 0.2a	1.8 ± 0.1b	3.0 ± 0.7	2.5 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
36.55	Farnesyl acetone	2228	MS, KI, ST	16.8 ± 0.9	14.3 ± 1.0	25.5 ± 2.6a	10.4 ± 0.9b	30.0 ± 6.3	15.8 ± 1.7	17.3 ± 0.8	16.3 ± 0.3	15.2 ± 0.5a	7.7 ± 2.2b	33.2 ± 2.3	30.5 ± 4.2	8.5 ± 1.3	10.7 ± 0.7	24.7 ± 0.4a	13.1 ± 2.0b

[a]KI: Retention index, relative to n-alkanes (C8-C24) on the ZB-Wax Plus capillary column

[b]ID: Identification methods, MS: Mass spectra; KI values that agreed with the data reported in previous literature or the database on the web (http://www.nist.gov)

ST: Standard comparison, compounds identified using authentic standards; PCI indicated compounds identified using positive chemical ionization mode.

Table 4. Influence of genotype, production system, and genotype interaction on the contents of volatile compounds' classes with significant levels from ANOVA.

		Alcohol	Aldehydes	Esters	Fatty acids	Furans	Hydrocarbons	Ketones	Sulfur compounds
Production system (S)	Net-house	56.2b	666.9a	84.8	6.1	13.1	32.8b	1098.9a	29.2a
	Open-field	70.0a	572.7b	98.2	5.5	12.6	55.8a	904.1b	23.4b
	Significance	**	**	ns	ns	ns	***	**	***
Genotype (G)	TAM Hot-Ty	74.4	659.1abc	74.4bc	4.4bc	12.6	50.3ab	1057.7bc	23.4
	T3	56.0	576.5abc	114.2ab	3.8c	11.4	43.7ab	920.0bcd	23.0
	L501-55	68.2	726.8a	154.a	9.7a	13.3	32.7b	1087.5b	25.7
	SV8579TE	74.8	586.2abc	5.0d	4.6bc	13.0	42.9ab	1015.7bc	24.8
	Shourouq	51.3	535.5bc	124.0ab	5.1bc	13.7	48.2ab	812.6cd	29.8
	Seri	77.4	676.8ab	136.8ab	6.7abc	13.9	59.3a	1441.3a	24.8
	Mykonos	48.7	502.8c	116.9ab	4.8bc	10.5	37.1ab	689.0d	27.0
	DRP-8551	56.6	707.7a	7.3cd	7.7ab	14.9	43.7ab	1058.0bc	31.9
	Significance	ns	***	***	***	ns	*	***	ns
Interaction	S x G	ns	ns	*	**	***	Ns	*	ns

Unit = concentration (ng/g of tomato sample, equivalent of 2-octanone). *, **, and *** indicates significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ respectively. ns = not significant.

Genotype-based variation in the volatile profiles, and identification of biomarkers

In agriculture, quality predictors determine the economic value of crops. These quality predictors could be biomarkers for selection of desired trait genotypes for cultivation. Generally, in metabolomics studies, chemometric methods such as partial least squares discriminant analysis (PLS-DA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) are applied to discriminate and classify the measured variables by chromatographic techniques to understand the pattern and identify molecular markers.[187] Figas et al. [180] analyzed the distinct organoleptic and functional traits of 69 local tomatoes by multivariate analysis and confirmed that enhancement and selection of local tomato varieties showed improved quality. However, univariate analysis is often considered suboptimal since it fails to take into account any correlations among variables. In this study, we compared volatile profiles of eight tomato genotypes grown in two conditions and used PLS-DA analysis of GC-MS data to identify metabolites contributing to the observed differences (Fig. 4).

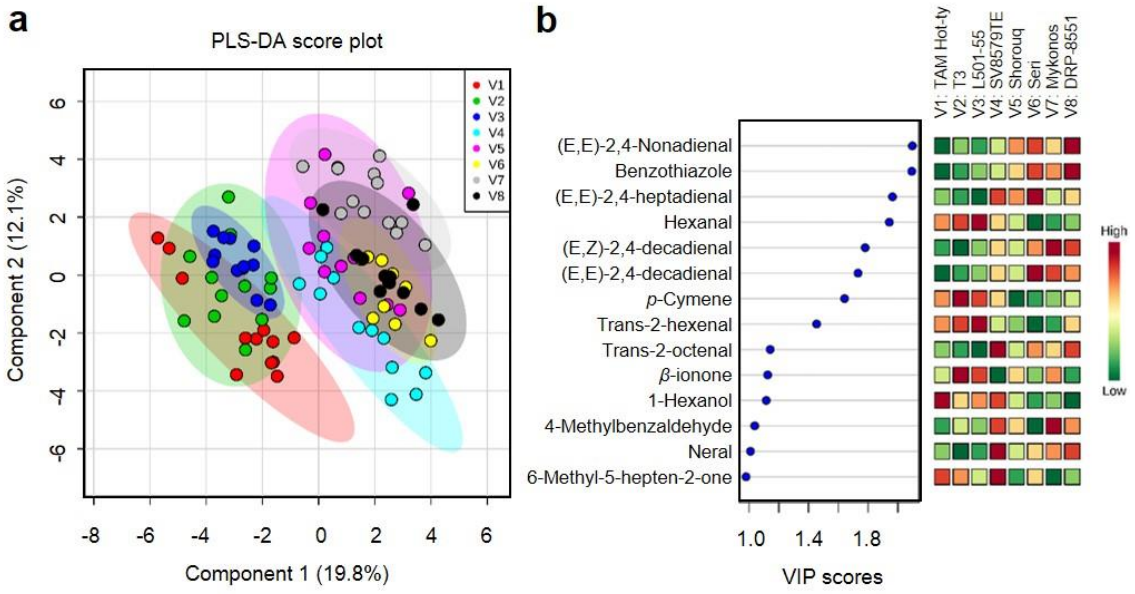

Figure 4. Multivariate analysis. (a) Partial least squares discriminant analysis (PLS-DA) scores plot corresponding to a model aimed at the discrimination between genotypes. The colored-ellipses denote 95% confidence intervals of each tomato variety. (b) Variable importance on projection (VIP) scores from the PLS-DA model indicating the most discriminating volatile metabolites between genotypes in descending order of importance with relative levels of the metabolite between genotypes.

PLS-DA was performed to develop a classification of tomato varieties based on the altered volatile metabolomic patterns aimed to discriminate between the net-house and open-field growing conditions (Fig. 4a). There were two main classes; three TAMU varieties (TAM Hot-Ty, T3, and L501-55) were more similar to each other, while the other five commercial varieties SV8579TE, Shourouq, Seri, Mykonos, and DRP-8551 from Seminis (St. Louis, MO, USA) were close to each other. To distinguish the volatile

profiles, we also produced a variable importance in projection (VIP score > 1.0) score plot from the PLS-DA model (Fig. 4b) to identify the top 15 metabolites contributing most significantly to the observed discrimination among the eight varieties. The levels of hexanal, *p*-cymene, and (E)-2-hexenal were higher in the first group of three TAMU varieties (Fig. 4b) whereas (E,E)-2,4-nonadienal, benzothiazole, (E,E)-2,4-heptadienal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal, (E)-2-octenal, and neral were higher in the commercial varieties. Furthermore, the significant levels of three potential volatiles from TAMU varieties were confirmed by the univariate analysis (Supplementary Fig. S4). This indicated that hexanal, *p*-cymene, and (E)-2-hexenal could be potential volatile markers to distinguish local Texas A&M University varieties from the other five commercial varieties.

Irrespective of the production system, levels of hexanal, *p*-cymene, and (E)-2-hexenal were considerably higher in TAMU varieties, compared to commercial tomato genotypes, and these could be considered as biomarkers to distinguish the TAMU varieties from the other commercial varieties. It was reported that six carbon (C_6) compounds such as hexanal and (E)-2-hexenal may mainly contribute to conferring tomato its fresh 'top-note'.[188] Moreover, several studies have indicated that these compounds function in combating biotic and abiotic stresses. Bate et al. [189] reported that (E)-2-hexenal generated in wounded plant tissues might induce defense-related genes. (E)-2-hexenal also exhibits a potent nematicidal activity against *Meloidogyne incognita* and can maintain the growth

of tomato plants.[190] In addition, hexanal found to extend tomato storage life.[191] Similarly, the monoterpene *p*-cymene was reported to have whitefly repellent potential.[192]

Influence of production system on tomato volatile profiles and identification of biomarkers

Genotype, growing conditions, maturity, growing season, harvest time, and post-harvest treatments affect the volatile profiles in tomatoes.[5, 28-29, 186, 193] However, no comprehensive reports have examined the individual and combined influences of production systems on fresh tomato volatiles.

Pattern recognition and multivariate statistical methods such as random forests (RF) and hierarchical clustering analysis (HCA) were applied to build a classification model for the two production systems (net-house and open-field) and the results are presented in Fig. 5a and 2b. RF is a multitude of tree predictors based on the combination of individual trees determined by the values of a random vector sampled independently and with the same distributions for all the trees in the forest.[194] The developed RF model showed the distinct effects of net-house and open-field conditions on tomato volatiles. In addition, a heat map was constructed to provide an intuitive overview of volatile profiles of eight tomato varieties grown in net-house and open-field conditions (Fig. 5b).

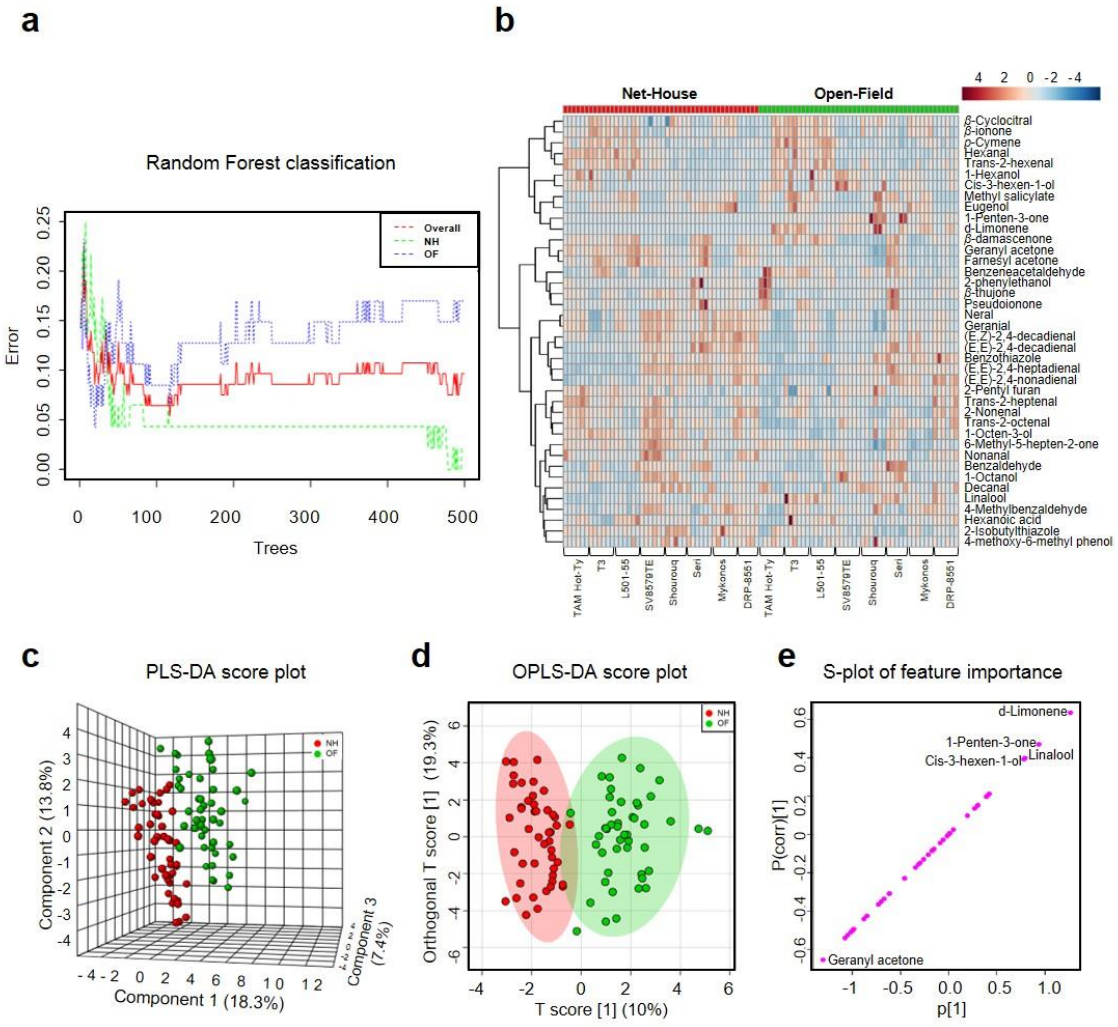

Figure 5. The metabolomic differences of tomato volatile compounds revealed the influence of the two production systems. (a) The Random Forests machine-learning algorithm classification was used for unsupervised clustering of volatiles from net-house (NH) and open-field (OF) tomatoes. (b) The heat map and hierarchical clustering analysis of metabolic profiles from tomatoes. Rows: sample; columns: metabolites. The degree of color saturation indicates the level of metabolites, with blue: lowest; red: highest (net-house and open-field). Correlation matrix of differential metabolites created using Pearson's correlation analysis based on the Random Forests classification model. (c) The figure represents the score plots of PLS-DA analysis of different production systems. (d) OPLS-DA score plot and (e) its corresponding S-plot based on GC-MS profiling data of tomato samples. All these models were analyzed by Metaboanalyst (http://www.metaboanalyst.ca/).

In the present study, box and whisker plots demonstrated that the relative levels of 16 tomato volatile metabolites were altered according to production systems with significant differences (Fig. 6). We identified 18 volatiles which were reported as important for the flavor of tomatoes in the previous study.[195] The levels of 10 out of these 18 volatile metabolites (1-penten-3-one, (E)-2-heptenal, 6-methyl-5-hepten-2-one, (Z)-3-hexen-1-ol, nonanal, 2-isobutylthiazole, linalool, neral, geranial, and geranylacetone) were significantly altered under net-house and open-field growing conditions (Fig. 6).

The earlier study reported that no significant influence of the environment on tomato volatiles was found (Cebolla-Cornejo et al. 2011). However, in this study, 16 out of 40 volatile metabolites were significantly influenced by growing systems and geranylacetone was identified as a potential metabolite marker based on the production system. The previous study demonstrated that different types of photo-selective netting affected the significant variation of geranylacetone levels from tomato fruits due to the light quality (Tinyane et al. 2013). It could be the reason that production systems as an environmental control might vary based on the covering material types and the feature of growing locations. Therefore, further investigation may need to be required for comprehensive understandings.

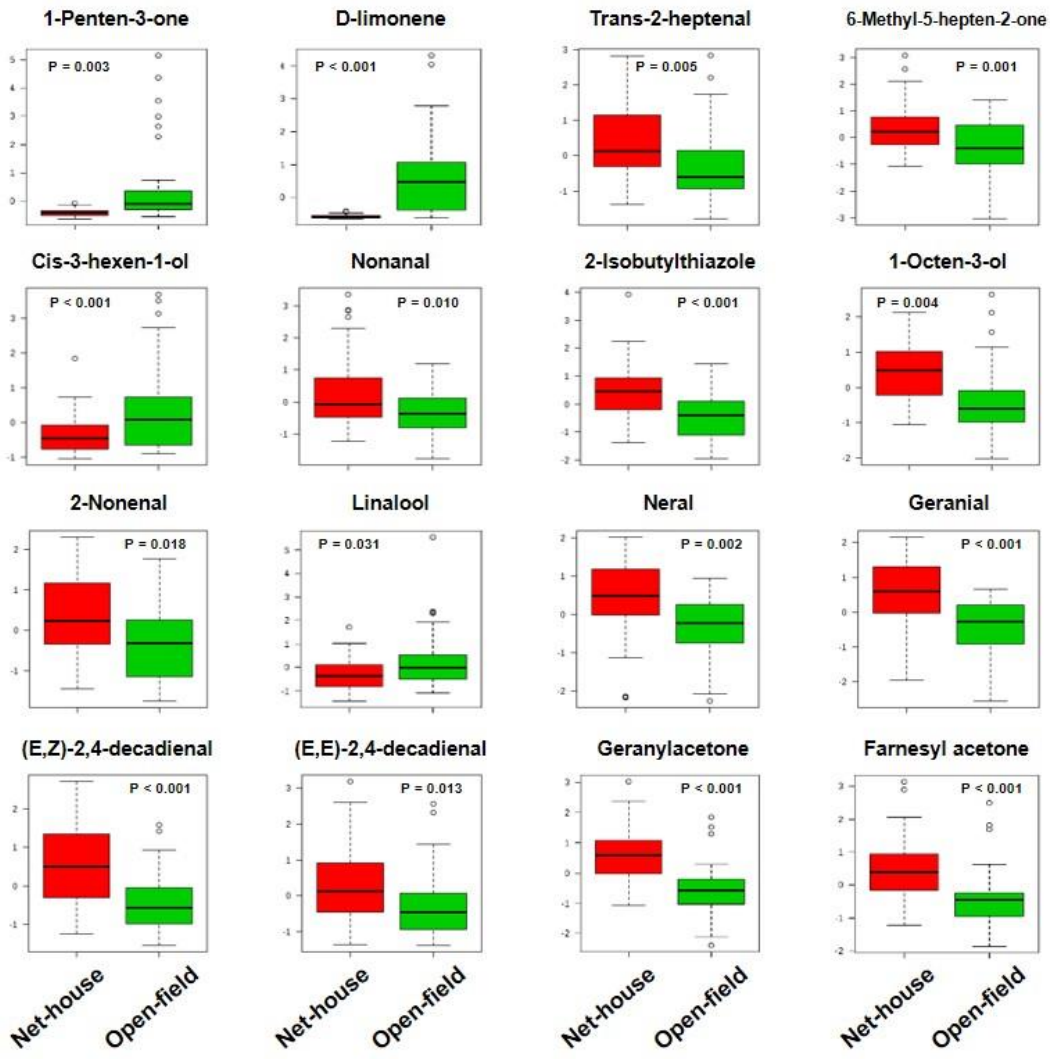

Figure 6. Relative abundance of significantly changed metabolites are described using box-and-whisker plot in tomato samples grown in the different production systems. Normalized concentrations of 16 volatile metabolites of tomato samples grown in the net-house (red) and open-field (green) with the *p*-value.

To discover potential biomarkers specific to the studied production systems, we conducted further multivariate analysis by partial least squares discriminant analysis (PLS-DA) and orthogonal projection to latent structures discriminant analysis (OPLS-DA) of the GC-MS dataset. The PLS-DA 3D plot showed two distinct clusters for two production systems (Fig. 5c and 5d). Significantly, characteristic differential metabolites or metabolic features between production systems were screened using the S-plot of the OPLS-DA model (Fig. 5e). The S-plot showed that compounds such as d-limonene and geranylacetone could be potential markers for tomatoes grown in different production systems. The observed level of d-limonene was higher in open-field-grown tomatoes, whereas, geranylacetone was higher in net-house-grown tomatoes. In addition, the univariate analysis showed that five varieties (TAM Hot-ty, T3, L501-55, Shourouq, and DRP-8551) had significantly higher amounts of geranylacetone from net-house tomatoes than open-field tomatoes. However, no significant changes were found between production systems for the remaining varieties (Supplementary Fig. S5).

Metabolic network of the key tomatoes volatiles that were significantly altered by the production system

Baldwin et al. [29] also reported the significant effects of tomato genotypes and growing conditions (cultivation year and growing season) on the levels of tomato volatiles and overall flavors. UV, sunlight, and temperature may be the key critical contributors that cause changes in the aroma volatiles.[196-197] Moreover, isoprenoid volatiles enhance

plant photoprotection and thermotolerance at raised temperatures, protecting plants from environmental damage and maintaining photosynthetic rates.[198-199]

Net-house cultivation provides a protected production system and season-extension tool, creating a hybrid of field and greenhouse growing conditions.[179] Indeed, most flavor-associated volatiles were significantly higher in all net-house grown tomatoes. The levels of 12 out of 16 volatile metabolites from the groups, including derived from carotenoids, phenolics, fatty acids, and isoprenoids, were significantly higher in the net-house-grown tomatoes (Fig. 7). This finding indicates that cultivating tomatoes in net-house conditions may be useful to improve tomato flavor. Metabolic pathways of the 16 key tomato volatiles that were significantly altered depending on the production systems ($P < 0.05$) were adapted from the previous study by Zhang et al. [175] (Fig. 7 and Table S6). The compounds were divided into four groups: carotenoids, isoprenoids, phenolics, and fatty acids, according to their biosynthetic pathways.

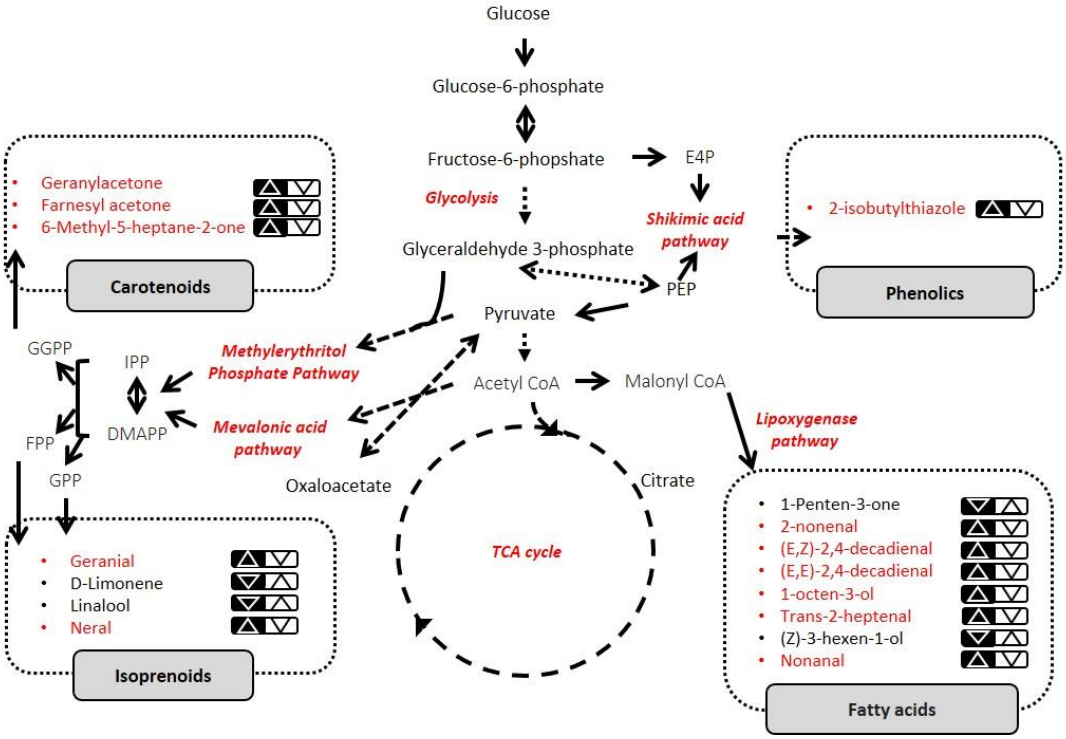

Figure 7. Metabolic network of the key tomatoes volatiles that were significantly ($p < 0.05$) altered in the net-house and open-field production systems. The red colored metabolites with the upward facing triangles in the black rectangle were present at higher levels in the net-house tomatoes and black-colored metabolites with downward facing triangles in the white rectangle were present at higher levels in the open-field tomatoes. (Abbreviations: PEP: phosphoenolpyruvate, E4P: erythrose 4-phosphate, IPP: isopentenyl pyrophosphate, DMAPP: dimethylallyl pyrophosphate, GPP: geranyl pyrophosphate, FPP-: farnesyl pyrophosphate, GGPP: geranylgeranyl pyrophosphate).

The net-house tomatoes showed higher levels of the phenolics-derived volatile 2-isobutylthiazole, compared with open-field tomatoes. Among phenolic volatiles, only the 2-isobutylthiazole level was significantly influenced depending on production systems, and a higher level was observed in net-house tomatoes. Consistent with our results, a

previous study demonstrated that this volatile was significantly affected by environmental factors such as temperature and radiation between two different cultivation seasons [28].

In addition, some fatty acid-derived volatiles, including 2-nonenal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal, 1-octen-3-ol, (E)-2-heptenal, and nonanal, had higher amounts in tomatoes grown in the net-house, whereas 1-penten-3-one and (Z)-3-hexen-1-ol were higher in the tomatoes grown in open fields. Among fatty acid-derived volatiles, the levels of eight compounds were significantly changed between the two production systems. In particular, 1-penten-3-one and (Z)-3-hexen-1-ol concentration levels were significantly higher in open-field tomatoes. Jiménez et al. [200] reported that 1-penten-3-one was initially generated by sunlight and hydroxyl radicals. In addition, (Z)-3-hexen-1-ol, a green leaf volatile, was higher in open-field fruit, as stress-triggered volatiles are produced by a range of wounded plants.[200-201] The concentrations of these two compounds were lower in the protected growing system than in open-field conditions.

For isoprenoid-derived volatiles, the concentrations of geranial and neral were higher from net-house tomatoes, whereas d-limonene and linalool were higher from the open-field tomatoes. Neral and geranial are geometrical isomers of citral and responsible for the aroma changes during sunlight exposure. These volatiles were reported to be susceptible to degradation under UV irradiation and UV-induced deterioration of citral could lead to the accumulation of d-limonene.[196] However, lower concentrations of both geranial and neral were observed from open-field-grown tomatoes in our study. Furthermore, Sasaki et al. [202] determined the essential effects of sunlight and UV exposure

on linalool biosynthesis in berries by comparing different covering materials. According to Sun et al.[196] linalool was confirmed as one of the products of d-limonene after UV irradiation. Our result of higher linalool levels in open-field tomatoes is consistent with these earlier studies.

Carotenoid-derived volatiles are important contributors to fruity and flowery tomato flavor-related odors.[28] We observed significantly enhanced levels of three carotenoid-derived volatiles (geranylacetone, 6-methyl-5-hepten-2-one (MHO), and farnesylaetone) from net-house tomatoes than the open-field tomatoes. Geranylacetone has a fruity/floral odor and is reported to be generated by oxidative cleavage of phytoene, phytofluene, ζ-carotene, and neurosporene, and the former two compounds are responsible for producing farnesylacetone, whereas lycopene was reported as a precursor of MHO.[4] The generation of carotenoid-derived compounds is influenced by various factors, including the type and levels of their precursors, enzymatic or non-enzymatic processes, and different growing conditions.[203] For instance, a relatively lower level of lycopene from off-season tomatoes might also cause higher MHO concentrations without its conversion into other compounds by endogenous enzymes.[28, 203] Therefore, extended tomato cultivation seasons due to the improvement of cultivation systems, and the effects of season on varieties need to be further studied.

Recently, it has been reported that 33 chemicals are correlated with consumers' liking and flavor intensity. Among them, five compounds, including 1-penten-3-one, (E)-2-heptenal, (E)-3-hexen-1-ol, 2-isobutylthiazole, and 6-methyl-5-penten-3-one from

tomato fruits were significantly influenced by production systems in this study (Tieman et al. 2017). The GC-MS data for the eight varieties were further collectively analyzed by three supervised methods (RF classification, PLS-DA, and OPLS-DA) to elucidate the underlying alterations of volatile profiles based on the production system and to identify metabolites (i.e. biomarkers) that contribute to these differences among the production systems. Results of multivariate analysis confirmed that the net-house and open-field tomatoes had distinct volatile profiles, and geranylacetone could be a biomarker for the production system. Geranylacetone is a linear apocarotenoid volatile compound and reported as mainly contributing to the fruity or floral flavor.[204]

According to the results, that the levels of some volatiles reported as influenced by environmental effects as stress-triggered were not significantly increased under the net-house growing condition. Moreover, majority compounds which altered by production system effects were increased in net-house grown tomato volatiles. Therefore, net-house can be considered as a good option as an environmentally controlled cultivation system.

CHAPTER V

EFFECTS OF VARIETY AND PRODUCTION SYSTEMS ON QUALITY OF TOMATO FRUITS AND IN VITRO BILE ACID BINDING CAPACITY

Introduction

Tomato fruit is routinely added to various cuisines to provide taste, flavor, and color.[205] Recent studies demonstrated that genetic and growing environmental factors greatly impact on the bioactive components in tomatoes.[62, 206-208] The fruit quality is usually evaluated based on its visual appearance, texture, organoleptic properties, and health-promoting compounds.[209] For instance, color, and taste are critical for fruit quality that determines consumers' buying preferences.[210] In addition, sugars and acid content contribute to the sweetness and overall aroma of fresh tomato.[211] Many studies reported that the health benefits of tomato fruits are found to be associated with its antioxidant components such as ascorbic acid and carotenoids.[212] Ascorbic acid has been reported to play a supportive role in the human immune system against oxidative stress, cardiovascular diseases, eye cataract, and certain types of cancer.[213] Carotenoids such as lycopene and ß-carotene have been extensively highlighted due to their interesting physiological capacities as provitamins, and antioxidant effects, particularly in powerful scavenging singlet oxygen produced from light initiated lipid oxidation or radiation.[214] In recent years, both consumers and growers are demanding tomato varieties with high yield but also fulfill requirements for good organoleptic traits and high nutritional values.[215] In

addition, there is a growing demand for locally grown food. In view of this, the protective cultivation practices such as greenhouse along with use of high yield cultivars are preferred practices.[92] In recent years, high-tunnel cultivation as a relatively low-cost system, the focus of season extension technology and enhancement of supplying regionally grown food to local markets.[216] However, these protective practices are also found to be considerable impact on yield and quality of tomato fruits.[100, 217] The color of tomatoes is a simple indicator to assess their ripeness and post-harvest life.[218] Cultivation environment has a direct impact on the synthesis of the primary and secondary metabolites of tomato fruit. Similarly, several research reports underline the fact that growing conditions such as greenhouse and high tunnel also have a remarkable impact on total phenolic and flavonoid composition of tomato fruits.[141, 215, 219] Cebolla-Cornejo and co-workers[159] showed that the screenhouse cultivation tends to alter the organoleptic quality and increases the variation of the content in taste-related variables. Tudor-Radu et al.[215] studied four tomato cultivars grown in the high-tunnel by comparing the contents of bioactive compounds and found the significant difference of lycopene levels. However, there is no reports on individual and combined effects of growing conditions and genotype on tomato fruit physicochemical characteristics, as well as on ascorbic acid and carotenoid contents.

Bile acids are steroid molecules synthesized in the liver from cholesterol and excreted into the bile.[220] The bile acids function as signaling molecules and regulate their synthesis and other metabolic processes such as glucose, lipids, and energy

homeostasis.[221] Furthermore, bile acids are final product of cholesterol and may cause cancer, hence maintenance of bile acid homeostasis is essential to achieve their physiologic functions and avoid their toxicity due to their detergent activity against biological membranes.[222-223] As signaling molecules, bile acids have been reported to regulate the inflammation and metabolism of lipids and carbohydrates by cascade controlling gene expression.[224] Tomato is a rich source of dietary fiber[225] and several studies have shown that the dietary fiber binds to bile acids[226] and increases their fecal excretion and consequently, cholesterol and the risk of colon cancer can be reduced.[227-228] In the present study, eight tomato varieties grown in the net-house and open-field were evaluated for tomato quality parameters such as peel color, total soluble solids (TSS), total acidity (TA), ascorbic acid, carotenoids and the bio-functional property in terms of in vitro bile acids binding capacity for the first time. This study will help to understand if specific cultivar and production systems are essential to produce tomatoes with desired quality traits and rich in health-promoting compounds.

Materials and methods

Chemicals

Metaphosphoric acid, phosphoric acid, ascorbic acid, lycopene, β-carotene, butylated hydroxytoluene (BHT), analytical grade solvents, cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), glycochenodeoxycholic acid (GCDCA), glycocholic acid (GCA), and glycodeoxycholic acid (GDCA), ammonium

nitrate, potassium dihydrogen phosphate, potassium chloride, potassium citrate, uric acid sodium salt, urea, lactic acid sodium salt, porcine gastric mucin, α-amylase, pepsin, and pancreatin were obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). All other chemicals were used in analytical grade.

Plant material and production systems

The study was conducted at the Texas A&M AgriLife Research and Extension Center, Weslaco, TX. The eight tomato varieties such as Texas A&M F1 hybrids (TAM Hot-Ty, T3, and L501-55) and commercial variety (SV8579TE, Shourouq, Seri, Mykonos, and DRP-8551) were grown in the net-house (NH) and open-field (OF) production systems and harvested in June 2016. The details of experimental design with production system conditions are described in our previous literature.[229] The fruits of TAM Hot-Ty, T3, and L501-55, Shourouq, Mykonos were round-shaped tomatoes and those of SV8579TE, Seri, and DRP-8551 had the oval types. For each analysis, three fruits with six replications per genotype were analyzed in the present study. To estimate tomato fruit quality, fresh tomatoes were sliced into six pieces, blended for 30 sec and stored at -20 °C until further analysis.

Peel color measurement

The peel color of tomato fruits was measured with a colorimeter (Minolta CR-400 Chroma Meter, Konica Minolta Sensing, INC., Osaka Japan). Before recording the sample

measurements, the instrument was calibrated using the white calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan). Tomato fruit peel color was measured on three equatorial regions of the tomato fruits. The CIE Lab values of L* (lightness/darkness), a* (redness/greenness), b* (yellowness/blueness), and C* (Chroma) were obtained.

Total soluble solids (TSS), total acidity (TA), and pH

The TSS content was determined using a hand refractometer (American Optical Corp., South Bridge, MA, USA) at 25 ºC, and the results were expressed as °Brix. To determine the TA contents, tomato samples (5 g) were mixed with 45 mL of nanopure water and titrated with 0.1N sodium hydroxide, and total acidity was expressed as percent citric acid through a DL 22 Food and beverage analyzer (Mettler Toledo, Columbus, OH, USA).

Estimation of ascorbic acid (AA) content

To measure AA content, 1 mL 3% meta-phosphoric acid was added to 1 g of tomato puree. The reaction mixture was vortexed and sonicated for 30 min. Then, it centrifuged (4500 rpm, 15 min), and finally, the supernatant was collected and filtered through a 0.45 µm PTFE filter before HPLC quantitative analysis. The HPLC analysis was performed on the HPLC system of Thermo Finnigan HPLC (San Jose, CA, USA) equipped with an autosampler, quaternary HPLC pump, photodiode array detector, and

ChromQuest for chromatography data analysis. The 10 µL sample was injected, and the chromatographic separation was conducted using C_{18} Gemini 5 µm column (250 mm x 4.6 mm i.d., Phenomenex, USA). As an isocratic mobile phase, 30 mM phosphoric acid in nanopure water was used with a 0.8 mL/min flow rate. The content of AA was calculated from the external calibration curve of their corresponding commercial standards at 254 nm. The results were expressed as µg/g FW.

Quantification of carotenoids

The extraction of carotenoids from tomatoes was carried out using acetone-hexane (4:3, v/v) with 0.1% BHT) to prevent oxidation. Tomato puree (10 g) was extracted with 10 mL of extraction solvent by homogenization for 2 min, organic layer was separated, the remaining residue was re-extracted twice using 10 mL and 5 mL, respectively. All three organic layers were pooled, passed through 0.45 µm PTFE filter and used for HPLC analysis. To identify and quantify carotenoids from tomato samples, the extract (30 µL) was injected into Waters HPLC (Milford, MA, USA) equipped with a PDA detector (2996), a binary HPLC pump 1525, and 171 plus autosampler. The chromatographic separations were conducted on a YMC C_{30} reversed-phase column (150 x 3.0 mm i.d., 3 µm, YMC Europe, Dinslaken, Germany). The gradient mobile phases of methanol (A) and hexane/isopropyl alcohol (1:1, v/v) with flow rate of 0.4 mL/min were used for the separation of carotenoids. The gradient program was set as follows: 0 min: 90% A; 4 min 60% A; 6 min: 45% A; 17 min 45% A; 18 min: 18% A; 37 min: 18% A; 40 min 90% A;

45 min: 90% A. The UV-Vis spectrum was collected in the range 210 to 700 nm with detection wavelength 450 nm.

Identification of carotenoids

Mass spectral analysis was conducted on the ultrahigh-performance liquid chromatography (Agilent 1290 system, Waldbronn, Germany) coupled to atmospheric pressure positive chemical ionization high-resolution quadrupole time-of-flight mass spectrometry (Maxis Impact, Bruker Daltonics, Billerica, MA). Carotenoids from tomato samples were separated on YMC C_{30} column (50 x 2.0 mm i.d., 3 µm, YMC Europe, Dinslaken, Germany). The mass spectra were acquired at positive mode using atmospheric pressure chemical ionization (APCI) on a maXis impact mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The mass spectrometer operating parameters were set as follows: nebulizer pressure 2.5 Bar; dry gas flow, 6.0 L/min; ion source temperature, 350 °C; corona current, 4000 nA; HV capillary, 3500 V. Data acquisition and processing were conducted using DataAnalysis Software version 4.3 (Bruker Daltonics, Billerica, MA, USA). The UV-Vis scanning spectra were obtained between 200 and 800 nm. The identification of compounds was achieved by matching UV absorptions at 450 nm, pseudomolecular ion mass values and MS/MS fragmentation patterns with authentic standards and data reported in the literature[230-231] The concentration of ß-carotene and lycopene were calculated from the external calibration curve of their corresponding commercial standards. All isomers of the carotenoids were quantified according to the

corresponding curves of their all-trans commercial standards.[232] Concentration values were expressed as micrograms of carotenoids per gram of fresh weight of tomato fruits (μg/g FW).

Determination of In vitro bile acid binding assay

For bile acid binding study, *in vitro* digestion of samples was performed as per established protocols with slight modifications.[233] For the oral digestion, freeze-dried tomato samples (0.1 g) were mixed with 1 mL simulated saliva fluid (Table S7) and vortexed for 1 min, followed by incubation in a shaking water bath at 37 °C for 5 min, at 180 rpm. For gastric digestion, the chyme pH was adjusted to 2.0 with 1 N HCl, then 60 μL pepsin buffer (200 μg pepsin in 1 mL 0.1 M HCl) was added to each sample, followed by vortexing for 30 s and incubated on shaking water bath at 37 °C for 1 h. Then, chyme pH was adjusted to 6.8 with 1 N NaOH for intestinal digestion followed by the addition of 0.5 mL pancreatin (6.25 mg/mL in 50 mM phosphate buffer), 0.2 mL bile acid mixture (2.5 mM sodium glycodeoxycholate, 12.1 mM sodium cholate, 7.4 mM sodium deoxycholate, 5.1 mM sodium glycochenodeoxycholate, 1.24 mM sodium glycocholate, 11.6 mM sodium chenodeoxycholate), then vortexed for 30 s and incubated in a shaking water bath at 37 °C for 3 h. Subsequently, digestion was terminated by inactivating enzymes at 78 °C in a water bath for 7 min. centrifuged at 800g for 30 min and the supernatant was carefully collected and filtered through Whatman No 1 filter paper. The remaining residue was rinsed with 5 mL nano-pure water in a water bath to remove

adhering bile acids. To evaluate the bile acids binding capacity, the residue was extracted twice two mL of 80% methanol by sonication, centrifugation, and filtration. Both the extracts were pooled, passed through a 0.45μm filter before HPLC analysis. Bound bile acids were quantified using an Agilent 1200 HPLC system (Foster City, CA, USA) and a Gemini C18 column (250mm× 4.6 mm 5 μm) with a guard column (Phenomenex, Torrance, CA, USA). The separation was performed using a binary mobile phase of (A) 30 mM phosphoric acid and (B) acetonitrile at a flow rate of 0.7 mL/min. For each sample, 20 μL was injected, and peaks were monitored at 210 nm.

Determination of total, insoluble, and soluble dietary fiber

Total, insoluble, and soluble dietary fiber was analyzed using AOAC Official Method 991.43.[234] In brief, a duplicate of frozen-dried tomato samples (one gram) was prepared and digested with three enzymes such as α-amylase, protease, and amyloglucosidase to remove starch and protein. After digestion steps, residue particles were considered insoluble dietary fiber, and ethanol was used to precipitate and obtain soluble dietary fiber. Total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber which were estimated by gravimetric analysis.

Data analysis

The univariate statistical analysis was performed using the SPSS software (v. 23, BM SPSS Statistics, IBM Corp., Chicago, IL, USA). Significant differences between

treatments were assessed with a student t-test (p-value < 0.05). One-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test was performed for the multiple mean comparisons (p-value < 0.05). Furthermore, multivariate statistical analysis, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed using MetaboAnalyst 4.0 (http://www.metaboanlayst.ca/).

Results and discussion

Production system and genotype effect on fruit quality traits of tomatoes

Table 5 shows the color indices of tomato cultivars grown in the NH and OF. Significant variations were observed in the values of L* (lightness), a* (redness), b* (yellowness), C* (chroma), and h (hue) among tomato fruits of studied varieties and production systems. The production system effect on these color index values was varying based on the varieties. Three tomato varieties such as T3, Shourouq, and DRP-8551 had considerably higher values of L* from OF-grown tomatoes. The significantly higher values of a* were found from NH-grown T3, Shourouq, and DRP-8551 varieties than these of field-grown ones, whereas TAM Hot-ty and SV8579TE showed substantially greater values from the OF than those of tomatoes from the NH. In the case of b* value, five tomato varieties, including T3, L501-55, SV8579TE, Shourouq, and DRP-8551 were significantly affected by the production systems and higher values were found from the OF grown tomatoes than the values from tomatoes grown in the NH system. Similarly,

considerably higher values of C* were found from the three varieties such as TAM Hot-ty, T3, and SV8579TE of fields-grown tomatoes. Finally, substantially higher h values were observed from four tomatoes such as T3, SV8579TE, Shourouq, and DRP-8551 of tomatoes grown in the OF than those from the NH grown ones. In addition, the significantly higher influence of genotype, production system and their interaction on the color index values ($p < 0.001$), except the interactive effect between variety and production system on the C* values (Table 5).

Table 5. The influence of variety and production systems (HT and OF) and their interaction on the tomato peel color characteristics from the eight varieties.

	L*		a*		b*		C*		h	
Cultivar	HT	OF	HT	OF	HT	OF	HT	OF	HT	OF
TAM Hot-Ty	50.19 ± 0.64a	51.25 ± 0.77a	91.48 ± 0.71 b	94.09 ± 0.74 a	75.73 ± 1.15a	77.28 ± 1.03a	119.01 ± 0.85 b	121.95 ± 0.85 a	39.52 ± 0.51a	39.34 ± 0.45a
T3	49.63 ± 0.78 b	55.83 ± 0.83 a	87.68 ± 0.62 a	83.17 ± 1.19 b	73.43 ± 1.20 b	84.78 ± 1.19 a	114.64 ± 0.80 b	119.24 ± 0.83 a	39.83 ± 0.54 b	45.53 ± 0.71 a
L501-55	53.21 ± 0.94 a	56.64 ± 0.87 a	89.41 ± 1.16 a	89.25 ± 1.07 a	79.21 ± 1.45 b	83.39 ± 1.20 a	119.86 ± 1.11 a	122.47 ± 0.93a	41.45 ± 0.72a	43.05 ± 0.61a
SV8579TE	52.59 ± 0.70a	52.14 ± 0.54a	91.95 ± 0.88 b	95.13 ± 0.46 a	75.13 ± 1.28 b	80.38 ± 0.80 a	118.86 ± 1.36 b	124.63 ± 0.68 a	39.13 ± 0.36 b	40.15 ± 0.29 a
Shourouq	49.51 ± 0.53 b	69.32 ± 2.54 a	90.84 ± 0.61 a	69.06 ± 3.47 b	74.84 ± 1.05 b	93.79 ± 2.37 a	117.44 ± 1.00 a	120.02 ± 1.43 a	39.21 ± 0.39 b	53.87 ± 1.93 a
Seri	50.44 ± 0.52 a	49.71 ± 0.61 a	98.20 ± 0.50 a	99.92 ± 0.44 a	74.07 ± 0.95 a	75.31 ± 0.99 a	123.08 ± 0.86 a	125.23 ± 0.81 a	36.94 ± 0.30 a	36.92 ± 0.33 a
Mykonos	56.94 ± 0.76 a	57.67 ± 0.64a	87.27 ± 0.89 a	88.02 ± 0.77 a	86.47 ± 1.06 a	87.91 ± 0.91 a	123.07 ± 0.97 a	124.36 ± 0.85 a	44.70 ± 0.47 a	44.94 ± 0.38 a
DRP-8551	49.12 ± 0.40 b	53.76 ± 0.64 a	96.47 ± 0.33 a	92.85 ± 0.64 b	75.10 ± 0.80 b	81.19 ± 1.02 a	122.38 ± 0.62 a	123.46 ± 0.97 a	37.84 ± 0.28 b	41.09 ± 0.33 a

Data present means ± S.E. and different letters in the column according to production systems and their interaction indicate significant differences at $p < 0.05$ (Tukey HSD test).

TSS and TA are the main components responsible for tomato flavor.[235] High TSS and TA contents are desirable traits in both processing and fresh-market tomato cultivars.[236] However, little is known about the influence of NH growing on physicochemical traits of tomato fruit, in relation to genotypic variation. The results of the influence of variety and production systems (NH and OF) and their interaction on TSS, TA, pH, and ascorbic acid (AA) content of tomato fruit are shown in Table 6. Almost all tomato varieties grown in NH had significantly high total TSS, except variety TAM Hot-Ty. In particular, NH grown L501-55 variety had the highest TSS value, and OF grown SV8579TE variety had the lowest value of TSS (Table 6). Similarly, results of TA levels of eight tomato varieties grown in NH and OF were statistically significant between the variety and production systems. All studied tomato varieties grown in NH had significantly higher levels of TA than grown in OF (Table 6). Among these, the L501-55 variety grown in NH showed the highest value of TA, and the lowest value was observed in Mykonos variety grown in OF (v). Remarkably, three local TAMU varieties (TAM Hot-Ty, T3, and L501-55) showed comparatively higher values of TA than those of other commercial varieties. Alike to TSS and TA, pH levels of eight tomato varieties grown in NH and OF were also measured and found that both variety and production system significantly influence pH levels of tomato fruits. Significantly higher pH values were observed for OF-grown TAM Hot-ty, SV8579TE, and Seri tomato varieties.

It has been reported that AA content in tomato fruits can be affected by various factors such as genotype, pre-harvest climatic conditions and cultural practices, and postharvest handling procedures and their interactions.[36] Moreover, the light intensity was

considered as one of the crucial factors for the formation and high yield of AA content of tomato fruits.[42] We found that the studied cultivar had a significant effect on AA content. Conversely, the observed influence of production systems on AA contents was non-significant. However, the influence of interaction between variety and production system on AA content was found to be significant (Table 6). The varieties, TAM-Hot-ty, T3, Mykonos, and DRP-8551 showed no significant change in the AA contents between production systems. The AA levels were higher in NH grown varieties, L501-55 and Seri (Table 6). Conversely, varieties, SV8579TE and Shourouq had significantly higher levels of AA in OF. Rosello et al.[237] also found similar findings and reported that the genotypic effects were observed as major contributors to tomato fruits along with interaction with the environment. Taken together, the selection of varieties and cultivation practices are crucial for improving tomato fruit physicochemical quality traits and ascorbic acid.

Table 6. Quality characteristics such as total soluble solids, total acidity, pH, and the contents of ascorbic acid of the eight varieties of tomato grown in the net-house (NH) and open-field (OF).

Variety	Total soluble solid (TSS) (°Brix)		Total acidity (TA) (%)		pH		Ascorbic acid (µg of FW)	
	NH	OF	NH	OF	NH	OF	NH	OF
TAM Hot-Ty	5.2 ± 0.1^{a}	5.0 ± 0.1^{a}	3.3 ± 0.1^{a}	2.8 ± 0.1^{b}	4.25 ± 0.02^{b}	4.37 ± 0.03^{a}	64.02 ± 5.50^{a}	56.15 ± 4.26^{a}
T3	4.8 ± 0.1^{a}	4.5 ± 0.1^{b}	3.1 ± 0.1^{a}	2.2 ± 0.2^{b}	4.27 ± 0.01^{a}	4.34 ± 0.04^{a}	65.76 ± 2.54^{a}	59.63 ± 2.58^{a}
L501-55	6.1 ± 0.2^{a}	5.0 ± 0.1^{b}	3.9 ± 0.2^{a}	3.1 ± 0.1^{b}	4.21 ± 0.02^{a}	4.24 ± 0.02^{a}	60.37 ± 3.92^{a}	46.12 ± 3.02^{b}
SV8579TE	4.6 ± 0.0^{a}	3.8 ± 0.1^{b}	2.1 ± 0.0^{a}	1.7 ± 0.1^{b}	4.46 ± 0.03^{b}	4.61 ± 0.02^{a}	52.58 ± 3.25^{b}	66.96 ± 2.03^{a}
Shourouq	4.7 ± 0.0^{a}	4.1 ± 0.1^{b}	2.3 ± 0.1^{a}	1.8 ± 0.1^{b}	4.62 ± 0.02^{a}	4.68 ± 0.03^{a}	85.89 ± 3.09^{b}	101.69 ± 4.99^{a}
Seri	4.8 ± 0.1^{a}	4.3 ± 0.0^{b}	2.1 ± 0.1^{a}	1.8 ± 0.1^{b}	4.49 ± 0.02^{b}	4.69 ± 0.02^{a}	136.28 ± 3.06^{a}	114.37 ± 2.53^{b}
Mykonos	5.2 ± 0.1^{a}	4.5 ± 0.1^{b}	2.0 ± 0.1^{a}	1.6 ± 0.1^{b}	4.59 ± 0.02^{a}	4.64 ± 0.03^{a}	103.85 ± 3.03^{a}	106.96 ± 3.12^{a}
DRP-8551	4.8 ± 0.1^{a}	3.9 ± 0.0^{b}	2.5 ± 0.1^{a}	1.8 ± 0.1^{b}	4.50 ± 0.02^{a}	4.51 ± 0.02^{a}	113.05 ± 4.49^{b}	125.93 ± 4.57^{a}

Data present means ± S.E. and means with different letters between production systems indicate significant differences between production systems at $p < 0.05$.

Identification and quantification of carotenoids

In the present study, UHPLC-APCI-HR-QTOFMS was used to identify carotenoids from tomato fruits. We have identified five carotenoids such as all-trans-ß-carotene, 13-cis-lycopene, 9-cis-lycopene, all-trans-lycopene, and 5-cis-lycopene based on elution order, UV-Visible and mass spectral data. The chromatographic elution characteristics and the UV−Vis spectrum provides crucial evidence for identifying these geometric forms of carotenoids and mass spectral data helped to confirm the molecular structure, based on fragmentation patterns. This information was compared with published data.[232] The results of this study were showing that all-trans-lycopene and its isomer compounds showed protonated molecular ion fragment at *m/z* 537.4454 and all-trans-ß-carotene (536.4382), corresponds to their molar mass (Table 6).

Table 7. Tentative identification of chromatographic, UV-Vis and mass spectrometry characteristics of carotenoids from tomato, obtained by HPLC-PDA and APCI-MS.

No.	Tentative identification	λ (nm)				Molecular formula	Exact mass	Mass adducts	Theoretical mass	Mass error (ppm)	MS/MS fragment ions (*m/z*)
		cis-peak	I	II	III						
1	all-trans-ß-carotene	nd	425	451	480	$C_{40}H_{56}$	536.4395	[M]+	536.4382	nd	439, 383, (327), 251
2	13-cis-lycopene	360	439	465	496	$C_{40}H_{56}$	537.4545	[M+H]+	537.4454	-16.9	495, 439, 383, (327), 251
3	9-cis-lycopene	367	439	467	497	$C_{40}H_{56}$	537.4500	[M+H]+	537.4454	-8.5	495, 439, 383, (327), 251
4	all-trans-lycopene	369	444	471	502	$C_{40}H_{56}$	537.4495	[M+H]+	537.4454	-7.6	495, 439, 383, (327), 251
5	5-cis-lycopene	351	445	472	503	$C_{40}H_{56}$	537.4471	[M+H]+	537.4454	-3.1	495, 439, 383, (327), 251

nd: not detected

Table 8. The content (µg/g Fresh weight) of carotenoids in eight tomato varieties grown in different production systems such as the net-house (NH) and open-field (OF).

Variety	all-trans-*ß*-carotene		13-cis-lycopene		9-cis-lycopene		all-trans-lycopene		5-cis-lycopene	
	NH	OF	NH	OF	NH	OF	NH	OF	NH	OF
TAM Hot-Ty	0.78 ± 0.04b	1.06 ± 0.02^{a}	1.88 ± 0.19^{a}	2.21 ± 0.13^{a}	0.29 ± 0.04^{a}	0.28 ± 0.02^{a}	93.86 ± 6.74^{a}	103.95 ± 4.80^{a}	6.19 ± 0.44^{b}	8.35 ± 0.32^{a}
T3	1.20 ± 0.05^{b}	1.45 ± 0.08^{a}	2.40 ± 0.16^{a}	2.49 ± 0.19^{a}	0.28 ± 0.04^{a}	0.26 ± 0.04^{a}	104.07 ± 8.39^{a}	82.58 ± 5.40^{b}	5.39 ± 0.44^{a}	4.33 ± 0.30^{a}
L501-55	1.42 ± 0.03^{b}	1.86 ± 0.10^{a}	3.93 ± 0.52^{a}	3.91 ± 0.41^{a}	0.58 ± 0.08^{a}	0.52 ± 0.06^{a}	139.84 ± 18.00^{a}	139.66 ± 14.86^{a}	7.81 ± 1.14^{a}	7.28 ± 0.79^{a}
SV8579TE	0.74 ± 0.07^{b}	1.10 ± 0.04^{a}	2.28 ± 0.25^{b}	4.77 ± 0.19^{a}	0.31 ± 0.05^{b}	0.64 ± 0.03^{a}	128.78 ± 6.36^{b}	190.90 ± 8.02^{a}	5.39 ± 0.56^{b}	9.69 ± 0.42^{a}
Shourouq	1.09 ± 0.07^{b}	1.53 ± 0.09^{a}	2.17 ± 0.25^{a}	1.84 ± 0.19^{a}	0.32 ± 0.04^{a}	0.22 ± 0.03^{b}	102.88 ± 11.14^{a}	66.77 ± 7.17^{b}	4.52 ± 0.50^{a}	2.87 ± 0.34^{b}
Seri	0.82 ± 0.05^{b}	1.01 ± 0.05^{a}	3.31 ± 0.20^{b}	4.89 ± 0.42^{a}	0.45 ± 0.03^{b}	0.62 ± 0.06^{a}	156.61 ± 9.26^{b}	195.35 ± 15.00^{a}	7.68 ± 0.50^{b}	9.93 ± 0.81^{a}
Mykonos	1.31 ± 0.06^{a}	1.41 ± 0.04^{a}	2.64 ± 0.48^{a}	3.82 ± 0.37^{a}	0.32 ± 0.06b	0.43 ± 0.04^{a}	101.22 ± 18.98^{a}	113.11 ± 10.30^{a}	5.25 ± 1.05^{a}	6.06 ± 0.56^{a}
DRP-8551	0.83 ± 0.06^{b}	1.05 ± 0.05^{a}	3.62 ± 0.23^{a}	3.31 ± 0.37^{a}	0.49 ± 0.05^{a}	0.46 ± 0.07^{a}	171.32 ± 10.09^{a}	133.75 ± 10.59^{b}	8.72 ± 0.60^{a}	8.27 ± 1.48^{a}

Data present means ± S.E. of two replications, each replication containing three subsamples (n = 6). Different letters indicate significant differences between production systems at $p < 0.05$.

The results of HPLC was used for the quantification of these compounds, and shown in Table 8. The observed level of all-trans-ß-carotene of tomatoes was ranged from 0.74 to 1.42 and from 1.01 to 1.86 µg/g of fresh weight (FW) grown in the NH and OF conditions. The ranges of 13-cis-lycopene contents were 1.88–3.93 and 1.84–4.89 µg/g FW. The levels of 9-cis-lycopene of tomatoes from NH and OF conditions were ranged from 0.29 to 0.58 and from 0.22 to 0.64 µg/g FW, respectively. Regard to all-trans-lycopene, the detected levels of NH-grown tomatoes were range from 93.86 to 171.32, whereas OF-grown tomatoes had values ranged from 66.77 to 195.35 µg/g FW. Lastly, the ranges of 5-cis-lycopene of tomatoes grown in the NH and OF conditions were 4.52–8.72 and 2.87–9.93 µg/g FW, respectively.

In addition, the significantly different effects of the production system on the content of studied compounds were found to be genotype-specific. Interestingly, most varieties had considerably higher levels of all-trans-β-carotene in the field conditions than the observed values of tomatoes grown in the NH condition but no significant difference was detected for Mykonos. The contents of 13-cis-lycopene of SV8579TE and Seri grown in the OF conditions were substantially higher than the levels of NH-grown tomatoes. The considerably different influence of cultivation system on the level of 9-cis-lycopene was observed, and three varieties such as SV8579TE, Seri, and Mykonos had substantially increased levels from OF conditions, whereas Shourouq showed the considerably higher level was found from the NH condition. The contents of all-trans-lycopene T3, Shourouq, and DRP-8551 were observed as significantly higher in NH conditions, and two varieties such as SV8579TE and Seri had substantially enhanced levels from OF conditions. The

considerably higher value of 5-cis-lycopene was found from NH-grown Shourouq, whereas TAM Hot-Ty, SV8579TE, and Seri showed significantly higher values from the OF than the observed levels from NH conditions (Table 8).

In addition to genetic factors, two main abiotic factors such as temperature and light can govern carotenoids levels.[238] The rates of biosynthesis of lycopene and *β*-carotene can be increased during the ripening of the fruit. Moreover, direct sunlight exposure during its development will influence higher carotenoid levels than those of shaded fruits.[239] In addition, It has been reported that temperature played a vital role in lycopene and *β*-carotene accumulation.[240] The favorable temperatures for lycopene and *β*-carotene synthesis in fresh tomatoes suggested to be 22 to 25°C, and the rate of lycopene synthesis can be entirely inhibited at 32°C. The content of lycopene drastically reduced at 30–35°C, but not that of *β*-carotene.[241-242] Therefore, higher levels of all-trans-*β*-carotene in the tomatoes were observed in OF compare to NH.

In vitro Bile acid binding capacities of tomatoes grown in the net-house and open-field

In the present study, six bile acids were used for estimating the binding capacity via in vitro digestion of tomatoes of eight varieties grown in NH and OF (Fig. 8). The bile acid binding capacities of various vegetables have been reported to be influenced by genotype, growing environment, harvest time, and cooking style.[226, 243-244] Here we found that the production system effect on the percentage of bound bile acid varied based on the tomato varieties. Among eight varieties from NH and OF samples, significantly higher

binding capacities were found in OF tomatoes of three varieties such as TAM Hot-ty, T3, and Seri. CDCA and DCA binding capacities were higher in OF-grown TAM Hot-ty, GCDCA, CDCA, and DCA binding capacities were found to be higher in T3 variety grown in the OF-grown tomato. The bile acid binding capacities of GDCA, CDCA, and DCA were observed as higher in the OF-grown Seri varieties. Conversely, two tomato varieties such as L501-55 and SV8579TE showed significantly greater capacity of binding bile acid by CA, GCDCA, and GDCA with NH-grown L501-11, whereas CDCA binding capacity was detected as higher from NH-grown SV8579TE.

Two tomato varieties had significantly different binding activities of GCDCA and GDCA according to production systems, NH-grown L501-55 had higher binding capacity than those of tomatoes grown in the OF, whereas Seri had greater capacities from field-grown tomatoes. Bound CDCA and DCA levels were considerably influenced according to production systems. The CDCA and DCA are reported to be toxic at higher concentrations.[245] Among the studied tomato varieties, the bile acid binding capacities of three varieties, including Shourouq, Mykonos, and DRP-88551 were not significantly affected by production systems. Similar to our findings, Gomez et al. also reported that growing conditions such as locations may not significantly affect *in vitro* bile acids binding capacities of garnet stem dandelion.[244] In addition, maintaining the rich dietary fiber in diet has been reported to have health benefits .[227] Tomato is one of the principle sources in fresh and processed forms containing high levels of dietary fiber for a daily meal. Dietary fibers consist of soluble and insoluble dietary fiber, and they may play a

role in health-promoting effects related to reducing serum cholesterol by binding to bile acid slats and eliminating them from the body.[246] In the present study, the ranges of estimated soluble, insoluble, and total dietary fibers were 6.6–10.1 %, 22.0–39.7 %, and 30.2–49.0 % from tomatoes grown in the NH systems, whereas 6.3–11.0 %, 40.1–51.9 %, and 46.4–58.5 % from OF-grown tomatoes on dry weight basis (Table S8).

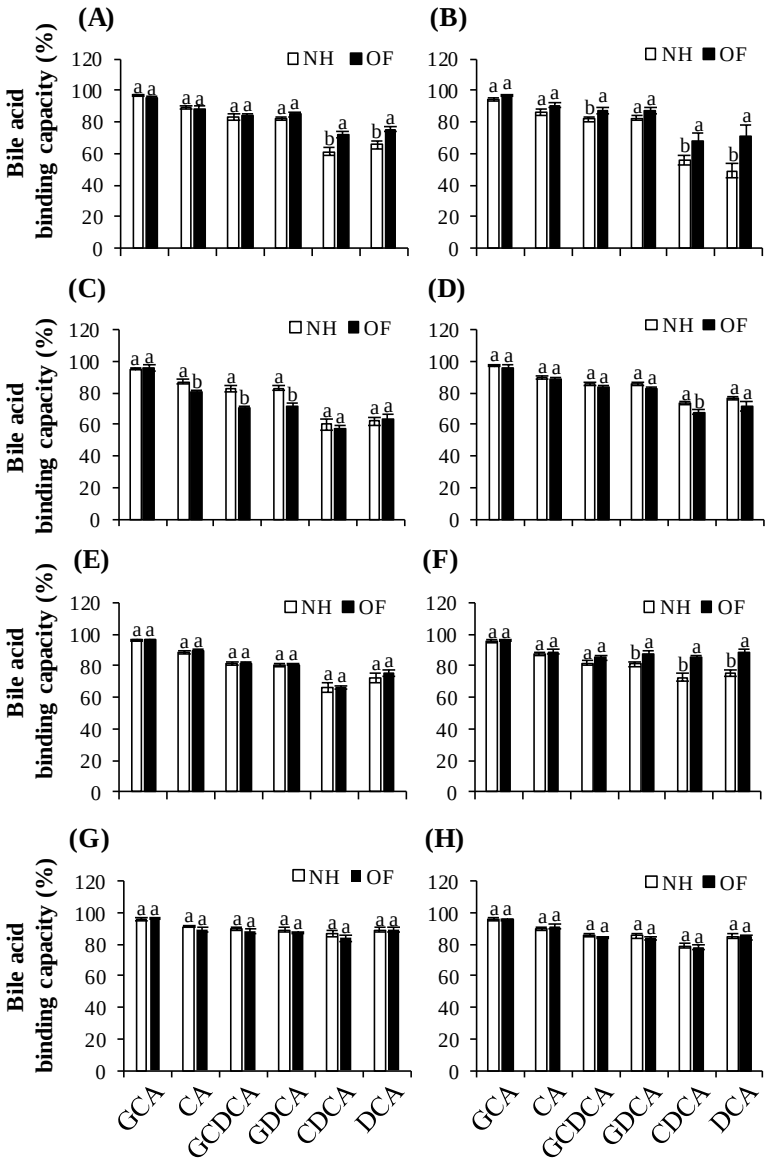

Figure 8. The influence of production systems such as net-house (NH) and open-field (OF) on the bile acid binding capacities of tomatoes. (A) TAM Hot-Ty (B) T3 (C) L501-55 (D) SV8579TE, (E) Shourouq (F) Seri (G) Mykonos, and (H) DRP-8551. Different letters in each bile acid indicate significant difference between production systems according to student t-test ($P < 0.05$). (GCA, glycocholic acid; CA, cholic acid; GCDCA, glycochenodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid).

CHAPTER VI

GENOTYPE AND PRODUCTION SYSTEM EFFECT ON MELATONIN, SEROTONIN, PHENOLICS, AND ANTIOXIDANT ACTIVITIES OF TOMATOES

Introduction

Nutrition security is an essential and integral element in food security. Consequently, maintaining the diversity of macro- and micronutrients in foods is necessary for enough energy, good health, and disease prevention.[247] In this way, the Food and Agriculture Organization (FAO) has set as one of the main goals in worldwide food security to prevent all types of malnutrition, enhance health, and terminate hunger by ensuring nutritious and ample food by 2030.[248] Considering these expectations, the choice of cultivars and agronomic conditions play a predominant role as a basic approach to achieve nutritious food production.[249] Tomato is one of the key ingredients of today's diet that is routinely used in cuisines to provide main color and taste worldwide. It is consumed in raw, cooked, and processed product forms such as juice, puree, paste, and ketchup.[250-251] Tomato is a rich source of several health-promoting compounds such as carotenoids, vitamins, phenolic acids, and flavonoids, and its consumption may be helpful for prevention of several diet-related chronica diseases.[252-253] Consequently, studies regarding factors that influence tomato health-promoting components have grown these days.[254]

Liquid chromatography-mass spectrometry (LC-MS) has been considered as an accurate and useful method for identification and quantification of several bioactive

components including phenolics, melatonin, and serotonin, even when low amounts are present in foods.[250, 255] Melatonin (N-acetyl-5-methoxy-tryptamine) and serotonin (5-hydroxytryptamine) are categorized as indoleamines, synthesized from tryptophan, and reported to possess neurotransmitter activities involved in the central nervous system.[256] So far, a wide range of phenolic compounds such as hydroxybenzoic acid (gallic acid, protocatechuic acid, and 4-hydroxybenzoic acid), hydroxycinnamic acid (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid), flavonols (rutin and quercetin), flavanone (naringin and naringenin), and flavone (apigenin) have been reported in tomatoes.[257-259] These phenolic constituents are accountable for tomato reputation as a health-beneficial source of dietary antioxidants.[260] Available literature demonstrated that the indoleamine and phenolics contents and antioxidant capacities of tomato are considerably influenced by its genotype and factors like growing system, weather condition, fertilizers, irrigation, and postharvest handlings.[261-264]

Recently, demand for higher health-promoting compounds containing horticultural crops has been increased. In that context, the selection of precise cultivar and cultivation practices are becoming critical to improving the phytochemical content of tomatoes (Slimestad et al., 2009). In the present study, quantitative profiles of indoleamines, phenolic compounds, and antioxidant activities in eight different tomato varieties grown in open-field and net-house were analyzed by UHPLC-HR-QTOF-MS for the first time.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 4-hydroxybenzoic acid, apigenin, ascorbic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, melatonin, naringenin, naringin, p-coumaric acid, protocatechuic acid, quercetin, rutin, serotonin, and sinapic acid were procured from Sigma-Aldrich, St. Louis, MO, USA. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was obtained from Chem-Impex Int'l. Inc. (Bensenville, IL, USA). All other chemicals used were of analytical grade.

Plant materials and sample preparation

Three Texas A&M University (TAMU) developed varieties- TAM Hot-Ty, T3, and L501-55 and five commercial varieties- as Shourouq, Mykonos, SV8579TE, Seri, and DRP-8551 were grown in the net-house and open-field conditions (Weslaco, Texas, USA). Experimental conditions were the same as described by Lee and co-authors (2019).[265] Briefly, a net-house type structure was completely covered with 50-mesh insect screen. The open-field plots were set next to the net-house to reduce soil variation. Three replicated plots per cultivar at either the net-house or open-field were tested in a complete randomized design with 8 plants/plot at 0.46 m spacing between plants and 1.5 m between rows. Irrigation, fertilization, and pesticide applications were consistent between production systems.

Five uniform vine-ripe tomatoes per plot were harvested, sliced into pieces and blended for 30 sec and stored at -20 °C until further use. To estimate the total phenolics, antioxidant activities, and contents of phenolics, melatonin, and serotonin, 100 mg of freeze-dried tomato sample (LabconcoFreeZone, Kansas City, MO, USA) was extracted with 1.5 mL of acidified methanol (methanol: water: acetic acid, 80:19:1, v/v/v). All tubes were vortexed for 30 s, sonicated (Cole-Parmer Ultrasonic 8893, Vernon Hills, IL) for one h at 47 kHz and cold conditions and centrifuged at 10,621 × g for 10 min (Eppendorf 5417C, Eppendorf, Hamburg, Germany). Finally, the filtered extracts were stored at -20 °C until further analysis.

UHPLC-HR/QTOF-MS based estimation of melatonin, serotonin and phenolics

The supernatant was injected into UHPLC-HR-QTOF-MS-equipped with Eclipse Plus C_{18} RRHD (1.8 μm, 50 × 2.1 mm) was obtained using our published method.[266] Binary mobile phase, 0.1% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B) was used with gradient program for pump B as follows: 0 min, 0%; 2 min, 0%; 15 min, 80%; 18 min, 0%; 20 min, 100% at the flow rate of 0.2 mL min^{-1}. The mass spectra were acquired in a positive mode using the ESI interface (Bruker Daltonics, Billerica, MA, USA). The mass spectrometer operating parameters were: nebulizer gas pressure, 2.8 bar; nebulizer gas flow, 8 L min^{-1}; sheath nebulizer gas temperature, 220 °C; sheath gas heater temperature, 220 °C. DataAnalysis Software (version 4.3) was used to control the

instruments and for data acquisition and processing. Authentic standards of melatonin, and phenolic acids were used for quantitative profiling.

Total phenolics and antioxidant capacity assays

Determination of total phenolics

The Folin–Ciocalteu reagent (FCR) method was used for the estimation of total phenolic contents of tomato samples.[267] An aliquot of 10 μL of the sample was added to a 96-well microplate, and the volume was adjusted to 200 μL with nanopure water. Then, 20 μL of FCR was added to each well, and the microplate was kept at room temperature for 10 min. After the incubation, 50 μL of sodium carbonate was added and incubated for 20 min. The absorbance was read at 760 nm, and the value of total phenolic contents was calculated and expressed as gallic acid equivalents (mg GAE/g DW) from the gallic acid (0, 10, 20, 30, 40, 50, 75 and 100μg) standard curve.

DPPH free radical-scavenging activity

The ability of the extracts to scavenge DPPH radicals was estimated using the method described by the previous report.[267] Initially, 20 μL of samples were added to a 96-well microplate, and the total volume in each well was adjusted to 100 μL with methanol. Afterward, 180 μL of DPPH was added to each well, and the absorbance was recorded at 515 nm using a microplate reader. Results were expressed as μg ascorbic acid equivalent in g DW of the sample.

ABTS assay

Free radical scavenging activity of tomato samples was measured by ABTS radical cation decolorization assay.[267] To prepare ABTS solution, 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) were prepared and stored in the dark at room temperature for 14 h before use. The addition of 10 μL of plant extract to each well of a microplate and the volume was adjusted to 100 μL with methanol. The absorbance at 734 nm was recorded by adding 180 μL of ABTS, and results were obtained by drawing an ascorbic acid standard curve (0.25, 0.5, 1, 1.5, 2, 2.5 μg).

Statistical analysis

The influence of production systems between net-house and open-field was carried out using a Student's *t*-test (*P*-value ≤ 0.05). For conducting a multiple mean comparison (*P*-value ≤ 0.05) analysis, one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test was performed. In addition, the correlation among all observed components according to the production system was conducted by Pearson correlation with the significant level at 0.05 and 0.01 using SPSS software (v. 23, BM SPSS Statistics, IBMCorp., Chicago, IL, USA). Further investigation on the genotype and cultivation system effects on tomato metabolites, the chemometric analysis was performed by exporting LC-MS data using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/).

Results and discussion

Untargeted metabolomic analysis.

The PLS-DA and PCA score plots of the eight tomato varieties grown in the net-house and open-field are presented in Figure 9, S6 and S7. An untargeted UPLC/ESI-HR-QTOFMS metabolomics approach was used to understand the effect of the production system and genotype on the global metabolic response of tomato fruits. To inspect the overall influential pattern, all datasets were initially classified according to production systems regardless of genotypes. The dataset was analyzed using an unsupervised multivariate method, principal component analysis (PCA) (Figure S6A and S6C). However, this model did not provide clear separation among production systems effect, whereas PLS-DA score plots showed a clear separation between production systems (Figure S6B and 6A). Evaluated cultivars were distinguished irrespective of the production system and resulted in both PCA and PLS-DA score plots that depict mainly three clusters. The first group consists of TAM Hot-Ty, T3, and L501-55, the second cluster includes SV8579TE, Shourouq, Seri, and Mykonos, and DRP-8551 variety shows distinctively separation from the other two groups (Figure S6C). In addition, PLS-DA scores plot was performed to confirm the distinctive influence of production system on each variety, and it was confirmed that metabolic profiles of all studied varieties were influenced by net-house and open-field conditions (Figure S7). Similarly, earlier studies reported that PCA and PLS-DA are useful to elucidate the relation between observed variables based on the treatment when comparing volatile profiles of net-house and open

field grown tomatoes.[265] Similarly, tomato polyphenols and antioxidant capacity have been studied for the identification of chemotaxonomic markers to distinguish between tomatoes according to genotype.[268]

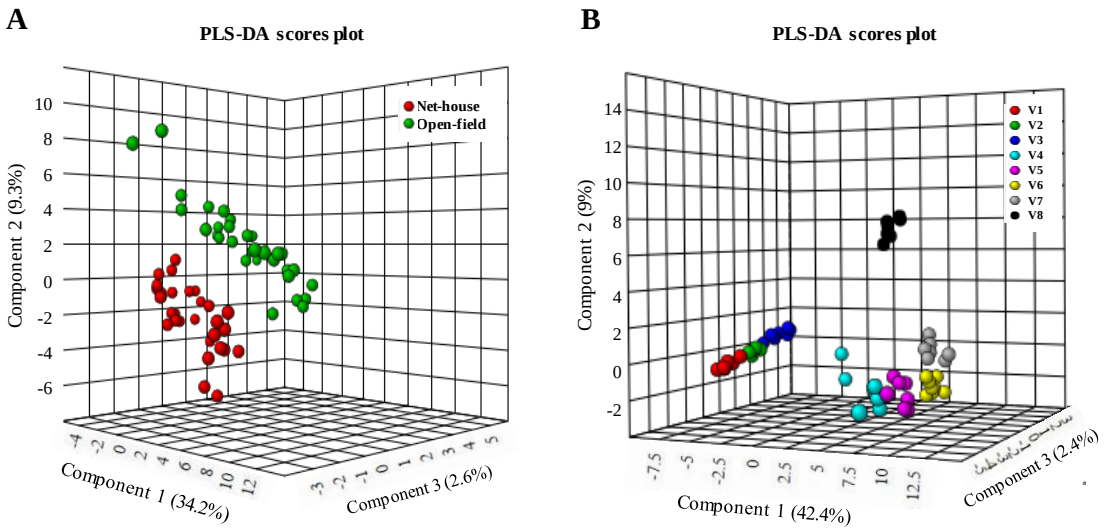

Figure 9. Multivariate statistical analysis based on metabolite profile dataset obtained from UPLC-HR-TOF-MS with positive ESI mode. PLS-DA 3D scores plot of the influence of production system (NH: net-house, OF: open-field) regardless of eight studied tomato varieties (A) and the genotype effect (B) (V1: TAM Hot-ty, V2: T3, V3: L501-55, V4: SV8579TE, V5: Shourouq, V6: Seri, V7: Mykonos, and V8: DRP-8551).

Identification of phenolics and indoleamine compounds

UPLC-QTOF-MS and LC/MS are considered as reliable and rapid methods for the identification of phenolic compounds in complex sample matrices. In this study, tomato samples were analyzed by UPLC-HR-QTOF-MS using positive ionization mode. Fig. 10

shows the UPLC separation of phenolic compounds by C_{18} column. Table 9 exhibit the 16 identified compounds, UV maxima, accurate mass, mass error, and MS/MS fragments. We have identified 9 phenolic acid compounds, including gallic acid (2.1 min, *m/z* 171.0292), protocatechuic acid (4.5 min, *m/z* 155.0339), 4-hydroxy-benzoic acid (6.4 min, *m/z* 139.0391), chlorogenic acid (8.2 min, *m/z* 355.1038), caffeic acid (8.3 min, *m/z* 181.0497), p-coumaric acid (10.1 min, *m/z* 165.0543), ferulic acid (11.3 min, *m/z* 195.0651), sinapic acid (11.7 min, *m/z* 225.0758), and t-cinnamic acid (16.3 min, *m/z* 149.0591). The result of identified flavonoids are rutin (12.5 min, *m/z* 611.1625), naringin (14.0 min, *m/z* 581.1902), quercetin (17.0 min, *m/z* 303.0509), naringenin (18.5 min, *m/z* 273.0760), and apigenin (18.8 min, *m/z* 271.0601). In addition, the peaks eluted at 3.3 and 13.3 min were identified as serotonin (*m/z* 177.1028) and melatonin (*m/z* 233.1293), respectively. The characteristics of these compounds were confirmed by comparison with retention time and MS^2 fragmentation of the standard and the results of the published literature.[269-273]

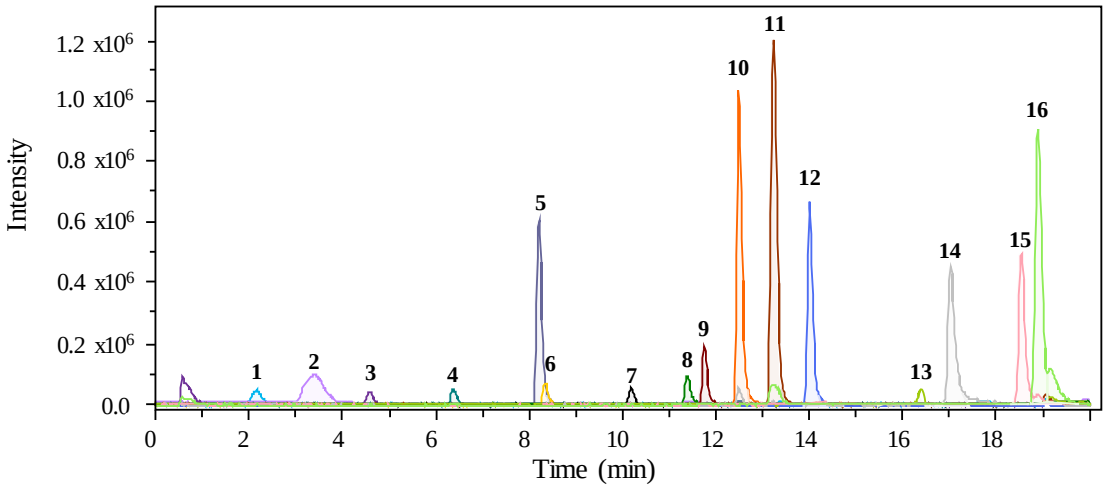

Figure 10. Chromatograph of standards solution obtained by UPLC-MS/MS analysis. (1) gallic acid (2) serotonin (3) protocatechuic acid (4) 4-hydroxybenzoic acid (5) chlorogenic acid (6) caffeic acid (7) p-coumaric acid (8) ferulic acid (9) sinapic acid (10) rutin (11) melatonin (12) naringin (13) t-cinnamic acid (14) quercetin (15) naringenin (16) apigenin.

Table 9. Identification of phenolic compounds in tomatoes by LC-DAD, UPLC-HR-ESI-TOF-MS, and MS/MS data.

Rt (min)	Compound	Experimental mass (*m/z*)	UV λ_{max} (nm)	MS/MS fragments at positive mode (*m/z*)	Molecular formula	Theoretical mass (m/z)	Mass error (ppm)
2.1	Gallic acid	171.0292	214, 270	153, 107, 81	$C_7H_6O_5$	171.0288	2.3
3.3	Serotonin	177.1028	276, 296	160, 115, 117	$C_{10}H_{12}N_2O$	177.1022	3.4
4.5	Protocatechuic acid	155.0339	259, 296	137, 84	$C_7H_6O_4$	155.0339	0.1
6.4	4-hydroxybenzoic acid	139.0391	255	121, 84	$C_7H_6O_3$	139.0390	0.7
8.2	Chlorogenic acid	355.1038	326	163, 135, 89, 117	$C_{16}H_{18}O_9$	355.1024	3.9
8.3	Caffeic acid	181.0497	322	163, 135, 107	$C_9H_8O_4$	181.0495	1.1
10.1	P-Coumaric acid	165.0543	310	91, 119	C9H8O3	165.0546	-1.8
11.3	Ferulic acid	195.0651	322	89, 177, 117	$C_{10}H_{10}O_4$	195.0652	-0.5
11.7	Sinapic acid	225.0758	324	207	$C_{11}H_{12}O_5$	225.0758	0.0
12.5	Rutin	611.1625	354	303	$C_{27}H_{30}O_{16}$	611.1607	2.9
13.3	Melatonin	233.1293	278	174, 159, 143	$C_{13}H_{16}N_2O_2$	233.1285	3.4
14.0	Naringin	581.1902	284, 334	153, 147, 85	$C_{27}H_{32}O_{14}$	581.1865	6.4
16.3	T-Cinnamic acid	149.0591	278	103, 131	$C_9H_8O_2$	149.0597	-4.0
17.0	Quercetin	303.0509	372	153., 137	$C_{15}H_{10}O_7$	303.0499	3.3
18.5	Naringenin	273.0760	284, 333	153, 91, 119	$C_{15}H_{12}O_5$	273.0758	0.7
18.8	Apigenin	271.0601	267, 338	153, 91, 119	$C_{15}H_{10}O_5$	271.0601	0.0

The genotype and production system effect on quantitative profiles of tomatoes metabolites

Levels of melatonin and serotonin

The identification and quantification of melatonin and serotonin content in eight tomato varieties grown in net-house and open-field are shown in Fig. 11. The melatonin levels ranged from 94.8 to 214.4 μg g^{-1} of dry weight (DW) and 104.3 to 281.8 μg g^{-1} DW in net-house and open-field systems, respectively. Our results indicate that the effect of the production system on melatonin content is genotype-specific among evaluated

varieties. The melatonin level of Mykonos and DRP-8551 were found to be significantly influenced by the production system, however melatonin content was higher in cultivar Mykonos in the open-field while DRP-8551 showed higher content under net-house conditions (Fig. 11). The serotonin levels ranged from 37.2–129.7 μg g^{-1} DW and 39.2–128.9 μg g^{-1} DW in net-house and open-field conditions, respectively (Fig. 11B). A previous study reported higher serotonin levels in round tomato (221.9 μg g^{-1} DW) and cherry tomato (156.1 μg g^{-1} DW).[274] Differences in serotonin content between studies can be attributed to cultivar and environmental conditions. Our findings showed that T3 and L501-55 cultivars showed significantly higher levels of serotonin from open-field conditions. Consequently, tomato is regarded for its therapeutic potential due to considerable amount of serotonin.[275] To take a further insight irrespective of genotype and production systems on studied compounds, the abundance is expressed as a heatmap in Fig. S8A and 8B, respectively. We could not observe a significantly different effect of production system on melatonin and serotonin contents (Fig. S8A). Regarding variety, Shourouq and Mykonos had considerably higher melatonin, whereas the highest level of melatonin was found in SV8579TE and significantly lower amounts were detected in TAM Hot-Ty and L501-55 among studied tomato varieties (Fig. S8B).

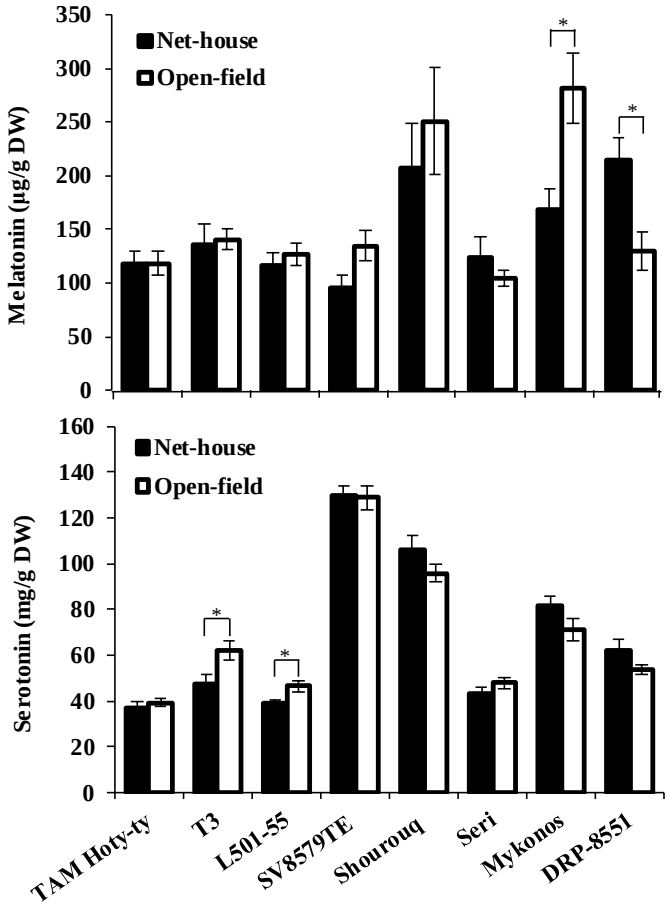

Figure 11. The influence of production system (net-house and open-field) on the contents of melatonin and serotonin of eight tomato varieties. Results are expressed mean ± standard error, and different letter indicates significant differences at the level of 0.05 (*) and 0.01 (**) between production systems.

It has been reported that melatonin and serotonin play significant roles in quality, yield, and protection in plant defensive response to biotic and abiotic stresses.[276-277] Moreover, melatonin has been reported to have health-associated advantages such as

ameliorating problems related to sleep by regulating the circadian clock.[278] Previous literature has examined that melatonin levels may vary according to development stages, genotype, and growing conditions.[261-262, 279] For instance, Riga et al., reported that the shade effect on melatonin levels are distinctively different based on the genotype, and these significantly influenced tomato genotypes are more likely to be sensitive to the shading condition.[279] It has been reported that fresh tomato contains more serotonin than processed tomato products, and accumulating evidence suggests that it may possess health beneficial effects such as anti-obesity capacity and neurotransmitter activity such as appetite, sleep, and anxiety related to the central nervous system.[275, 280]

The levels of phenolic acid compounds

Several reports have shown tomato's health beneficial properties due to the presence of phenolic compounds with antioxidant activities.[260, 281] Our present work identified and quantified nine phenolic compounds, including three hydroxybenzoic acid (gallic acid, protocatechuic acid, and 4-hydroxybenzoic acid) and six hydroxycinnamic acids (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, and t-cinnamic acid), to assess the influence of production system, genotype, and their interaction on the tomato fruits (Table 10). The observed gallic acid levels of eight net-house and open-field-grown tomato varieties ranged from 119.8–190.8 μg g^{-1} DW and 090.8–119.- μg g^{-1} DW, respectively. Only tomatoes from cultivar SV8579TE had a significantly higher level of gallic acid when grown in the net-house as compared to open-

field ones. In addition, the heatmaps depict the abundance of phenolic acids of eight tomato varieties, and the richest level of gallic acid was observed from Mykonos variety, while L501-55 had the lowest value ($P < 0.05$) (Fig. S8B). The range of protocatechuic acid levels from net-house grown tomatoes ranged 2.8–7.8 μg g^{-1} DW, whereas a range of 3.5–13.5 μg g^{-1} DW from tomatoes grown in the field condition. Considerably greater levels of protocatechuic acid were found in SV8579TE and Mykonos varieties from the open fields than those from the net-house. Besides variety, growing conditions and the interaction with a variety were found to have significant effects on the level of protocatechuic acid. (Table 10 and Fig. S8A). Moreover, irrespective of the production system, the highest and lowest levels of the compounds were found in SV8579TE and TAM Hot-ty varieties respectively (Fig. S8B). The contents of 4-hydroxy benzoic acid in tomatoes were ranged 3.8–6.5 μg g^{-1} DW and 3.7–5.1 μg g^{-1} DW of eight tomato varieties from net-house and open-field conditions, respectively. Particularly, T3, L501-55, and DRP-8551 had substantially higher amounts of 4-hydroxy benzoic acid from net-house-cultivated fruits compared to those of field-grown tomatoes. Furthermore, the considerable influence of the cultivation system, variety, and their interaction was observed in the 4-hydroxy benzoic acid levels. Notably, the net-house system may affect the considerable amount of 4-hydroxy benzoic acid than the value from the open-field condition. Regard to genotype, the greatest levels of 4-hydroxy benzoic acid were obtained from TAM Hot-ty and T3 varieties, while its level of Shourouq and Mykonos present the smallest value.

The range of chlorogenic acid content from net-house and open-field-grown tomatoes ranged from 10.7 to 36.2 µg g^{-1} DW and from 10.5 to 33.6 µg g^{-1} DW, respectively, and only Shourouq variety was significantly influenced by production systems with its higher level from the net-house condition (Table 10). Regardless of the cultivation system, T3 variety produced the richest abundance of chlorogenic acid (Fig. S8B). The caffeic acid level of tomatoes in the net-house and open-field system ranged from 11.0 to 28.5 µg g^{-1} DW and 11.5 to 33.5 µg g^{-1} DW, respectively. T3 variety was significantly affected based on production systems, and the considerably increased caffeic acid content was found from the open-field condition. Among different varieties, T3 variety had the largest amount of caffeic acid. The p-coumaric acid contents from net-house-grown tomatoes of eight varieties ranged from 9.2 to 17.5 µg g^{-1} DW, whereas the range of 9.6–19.3 µg g^{-1} DW was observed from tomatoes cultivated in the field. In addition, the significant difference of the p-coumaric acid amount was detected based on genotype, and maximum value was observed from the Seri variety. The ferulic acid levels produced from eight tomato varieties ranged from 12.4 to 30.4 µg g^{-1} DW in the net-house condition and from 9.6 to 27.4 µg g^{-1} DW in the open fields. There was no significance of growing conditions, but genotype and its interaction with the production system were observed to have a considerable impact on the level of ferulic acid. The ranges of assessed sinapic acid levels of tomatoes were 3.3–4.8 µg g^{-1} DW and 3.0–6.8 µg g^{-1} DW from net-house and open-field conditions, respectively. It is noteworthy that five tomato varieties, including TAM Hot-ty, T3, L501-55, Shourouq, and Mykonos had significantly higher

contents from tomatoes grown in open fields than those of net-house-grown ones. Moreover, considerable effects of cultivation system, variety, and their interaction were observed on the sinapic acid. Finally, the considerably increased t-cinnamic acid level was found in net-house-cultivated T3 variety. The abundance of t-cinnamic acid ranged from 3.7 to 9.8 μg g^{-1} DW and 3.4 to 7.0 μg g^{-1} DW from eight tomato varieties grown in the net-house and open-field conditions. Only T3 variety was influenced according to the cultivation system and a significantly higher level of t-cinnamic acid was detected from net-house-grown tomatoes than the content from the open-field. In regards to variety, the maximum amount was found in Mykonos variety.

Sinapic acid is commonly found in fruits and vegetables. Sinapic acid has been reported to possess health beneficial properties, including antioxidant, antimicrobial, anti-inflammatory, anticancer, neuroprotective, and anti-anxiety activity.[282-283] In consistent with our findings, the increased sinapic acid level was observed in tomatoes after UV-B irradiation.[264] The previous study reported that sinapic acid may play a protective role against ultraviolet irradiation.[284] It may explain our findings of the relatively enhanced amount of sinapic acid from the open-field-grown tomatoes than those of from the controlled environment such as net-house [264].

Table 10. The influence of production systems such as net-house (NH) and open-field (OF), varitey, and their interaction on the contents of phenolic acid compounds of eight tomato varieties (mean ± S.E., µg g-1 DW).

VARIETY	SYSTEM	Gallic Acid	Protocatechuic Acid	4-Hydroxy-Benzoic Acid	Chlorogenic Acid	Caffeic Acid	P-Coumaric Acid	Ferulic Acid	Sinapic Acid	T-Cinnamic Acid
TAM Hoty-ty	*NH*	139.0 ± 7.3a	2.8 ± 0.3a	5.9 ± 0.5a	11.8 ± 1.3a	21.8 ± 1.6a	9.2 ± 1.0a	12.4 ± 1.0a	3.9 ± 0.4b	4.0 ± 0.5a
	OF	133.9 ± 9.9a	3.5 ± 0.7a	5.1 ± 0.8a	13.4 ± 1.5a	24.2 ± 3.2a	10.3 ± 0.8a	9.6 ± 1.1a	6.8 ± 0.7a	3.9 ± 0.6a
T3	*NH*	145.6 ± 8.4a	5.3 ± 0.4a	6.5 ± 0.6a	36.2 ± 3.9a	28.5 ± 0.8b	11.4 ± 0.9a	24.3 ± 1.1a	4.8 ± 0.3b	5.1 ± 0.7a
	OF	183.5 ± 19.3a	7.1 ± 1.1a	4.5 ± 0.3b	33.6 ± 3.6a	33.5 ± 1.7a	11.6 ± 0.9a	27.4 ± 1.9a	6.6 ± 0.2a	3.4 ± 0.3b
L501-55	*NH*	119.8 ± 7.6a	6.3 ± 0.4a	5.8 ± 0.3a	14.6 ± 2.4a	28.0 ± 1.2a	10.1 ± 1.0a	25.2 ± 3.2a	4.4 ± 0.4b	4.6 ± 0.4a
	OF	100.4 ± 7.6a	5.2 ± 0.4a	4.5 ± 0.2b	18.1 ± 3.8a	28.1 ± 1.2a	9.6 ± 0.7a	13.7 ± 1.6b	6.0 ± 0.6a	4.1 ± 0.6a
SV8579TE	*NH*	142.0 ± 5.9a	7.0 ± 0.7b	4.2 ± 0.4a	14.2 ±1.6a	13.0 ± 1.2a	15.0 ± 1.1a	19.1 ± 1.5a	3.3 ± 0.3a	5.4 ± 1.4a
	OF	112.5 ± 11.2b	13.5 ± 2.0a	4.7 ± 0.6a	10.5 ± 1.2a	13.2 ± 1.8a	18.0 ± 1.2a	20.9 ± 2.0a	3.0 ± 0.4a	6.5 ± 1.4a
Shourouq	*NH*	158.6 ± 14.6a	6.8 ± 0.7a	3.8 ± 0.6a	17.8 ± 2.0a	14.0 ± 2.1a	15.4 ± 0.9a	22.2 ± 1.3a	4.0 ± 0.2b	3.7 ± 1.0a
	OF	158.2 ± 23.4a	7.8 ± 0.9a	3.7 ± 0.2a	11.8 ± 1.3b	11.5 ± 1.4a	15.6 ± 2.8a	25.6 ± 3.0a	5.6 ± 0.4a	4.1 ± 1.3a
Seri	*NH*	134.0 ± 22.2a	7.8 ± 1.2a	3.9 ± 0.3a	15.3 ± 1.3a	15.6 ± 1.4a	17.5 ± 1.4a	12.7 ± 1.8a	4.1 ± 0.3a	5.3 ± 0.6a
	OF	100.2 ± 9.0a	8.4 ± 0.8a	4.4 ± 0.6a	11.1 ± 2.0a	14.3 ± 1.2a	19.3 ± 2.4a	15.2 ± 2.0a	4.5 ± 0.3a	7.0 ± 1.2a
Mykonos	*NH*	190.8 ± 14.8a	6.8 ± 0.8b	3.9 ± 0.6a	10.7 ± 1.1a	11.0 ± 1.2a	12.9 ± 1.1a	30.4 ± 1.8a	4.5 ± 0.3b	9.8 ± 2.0a
	OF	175.7 ± 14.5a	10.3 ± 1.5a	3.8 ± 0.4a	12.2 ± 1.8a	14.3 ± 1.7a	12.8 ± 0.9a	25.2 ± 2.0a	6.1 ± 0.5a	6.2 ± 1.3a

Table 10 Continued

VARIETY	SYSTEM	Gallic Acid	Protocatechuic Acid	4-Hydroxy-Benzoic Acid	Chlorogenic Acid	Caffeic Acid	P-Coumaric Acid	Ferulic Acid	Sinapic Acid	T-Cinnamic Acid
DRP-8551	*NH*	151.5 ± 6.5a	7.8 ± 0.5a	6.0 ± 0.4a	14.7 ± 1.1a	23.5 ± 1.6a	14.6 ± 0.9a	21.2 ± 1.4a	3.7 ± 0.3a	4.5 ± 0.5a
	OF	156.8 ± 1.6a	8.0 ± 0.7a	3.9 ± 0.5b	14.0 ± 0.8a	20.5 ± 1.3a	13.6 ± 1.4a	17.9 ± 1.1a	4.4 ± 0.5a	4.1 ± 0.5a
ANOVA	Production system (P)	0.248	0.009	0.009	0.250	0.537	0.255	0.093	<0.001	0.377
	Variety (V)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	P * V	0.096	0.045	0.045	0.419	0.174	0.960	<0.001	0.014	0.140

The levels of flavonoids

The flavonoids have been considered as exerting health-promoting properties such as antioxidant activity.[260] In addition, various epidemiologic data have reported that the association between the consumption of polyphenol-rich food such as tomato and the reduced risk of cardiovascular diseases.[285] In the present study, the five identified flavonoids were categorized in flavonols (rutin and quercetin), flavanone (naringin and naringenin), and flavone (apigenin) and quantified (Table 11 and Fig. S8). The range of rutin content from eight tomato varieties was varied as 17.8–191.5 μg g^{-1} DW and from 5.4–255.7 μg g^{-1} DW in net-house and open-field conditions, respectively. The considerably elevated level of rutin was found in TAM Hot-ty from open-field-grown, whereas SV8579TE and Seri had significantly enhanced rutin content from the net-house (Table 11). The heatmap depicts the genotypic effect on rutin, and the richest level was detected from T3, whereas the lowest levels were observed from SV8579TE and Mykonos varieties (Fig. S8B). Naringin level ranged in eight tomato varieties from 0.9-3.2 μg g^{-1} DW in the net-house and from 0.6-2.8 μg g^{-1} DW in the open fields. The significant impact of the production system was found in four tomato varieties, for instance, TAM hot-ty and Mykonos had enhanced levels of naringin from open field-grown samples whereas, net-house-grown SV8579TE and Shourouq produced a higher level of naringin than those from tomatoes grown in the field (Table 11). Irrespective of the production system, a significantly higher level of naringin was detected from T3 variety among the studied varieties (Fig. S8B). The range of quercetin levels from net-house and open-field-grown tomatoes were 0.6825.4 μg g^{-1} DW and 0.5–14.8 μg g^{-1} DW, respectively. Only Seri

variety had a significant impact on the quercetin level, showing significantly greater levels from the open-field condition (Table 11). The substantial variety effect on quercetin levels was assessed, and the richest was found in the Seri variety (Fig. S8B). The abundance of naringenin of net-house-cultivated tomato ranged from 9.1 to 41.7 μg g^{-1} DW, whereas the range of field-grown tomatoes was 12.9–33.5 μg g^{-1} DW. The considerable impact of production system on naringenin levels was observed, and the net-house system produced higher levels of naringenin from the T3 variety, whereas L501-55, SV8579TE, and Mykonos had a considerably richer level of naringenin from the field-grown ones. Regardless of the cultivation system, Seri variety had the highest content of naringenin (Table 11 and Fig. S8B). Lastly, apigenin levels of eight tomato varieties grown in the net-house condition ranged from 0.1 to 0.4 μg g^{-1} DW and 0.2–0.4 μg g^{-1} DW of tomatoes grown in the field condition. There were no significant effects of production system and genotype, but their interaction seemed to be considerably influencing apigenin contents (Table 11).

Table 11. The influence of production systems such as net-house (NH) and open-field (OF), variety, and their interaction on the contents of flavonoid compounds of eight tomato varieties (mean ± S.E., µg g-1 DW).

Variety	Rutin	Naringin	Quercetin	Naringenin	Apigenin
TAM Hoty-ty					
NH	104.2 ± 11.0b	0.87 ± 0.17b	2.2 ± 0.4a	12.0 ± 1.6a	0.22 ± 0.03a
OF	160.8 ± 16.9a	1.92 ± 0.17a	1.8 ± 0.3a	12.9 ± 1.4a	0.24 ± 0.03a
T3					
NH	191.5 ± 9.7a	3.19 ± 0.52a	3.4 ± 0.6a	27.4 ± 2.2a	0.38 ± 0.05a
OF	255.7 ± 30.1a	2.79 ± 0.46a	5.9 ± 1.2a	18.8 ± 2.6b	0.16 ± 0.03b
L501-55					
NH	117.7 ± 13.9a	0.99 ± 0.23a	2.3 ± 0.5a	18.2 ± 2.3b	0.22 ± 0.03a
OF	125.5 ± 4.6a	0.87 ± 0.17a	3.0 ± 0.6a	23.2 ± 2.1a	0.19 ± 0.03a
SV8579TE					
NH	36.0 ± 3.5a	1.10 ± 0.23a	9.6 ± 0.5a	9.1 ± 1.0b	0.22 ± 0.03a
OF	5.4 ± 0.6b	0.58 ± 0.06b	8.5 ± 1.5a	15.1 ± 1.7a	0.22 ± 0.03a
Shourouq					
NH	76.1 ± 10.0a	3.08 ± 0.70a	12.4 ± 1.1a	24.8 ± 3.4a	0.24 ± 0.03a
OF	77.9 ± 12.2a	0.58 ± 0.06b	14.5 ± 2.7a	15.6 ± 3.4a	0.24 ± 0.05a
Seri					
NH	98.8 ± 12.3a	0.70 ± 0.06a	25.4 ± 1.1a	41.7 ± 3.0a	0.38 ± 0.08a
OF	55.7 ± 12.0b	0.58 ± 0.06a	14.8 ± 1.9b	33.5 ± 4.1a	0.22 ± 0.03a
Mykonos					
NH	17.8 ± 6.8a	0.64 ± 0.06b	0.6 ± 0.1a	18.2 ± 1.2b	0.11 ± 0.03a
OF	16.2 ± 5.3a	0.99 ± 0.12a	0.5 ± 0.1a	25.2 ± 2.3a	0.35 ± 0.16a
DRP-8551					
NH	53.3 ± 5.2a	0.70 ± 0.12a	9.5 ± 0.8a	22.1 ± 2.0a	0.27 ± 0.08a
OF	60.9 ± 14.3a	0.75 ± 0.06a	11.8 ± 0.9a	16.7 ± 2.2a	0.22 ± 0.03a
ANOVA					
Production system (P)	0.300	0.494	0.431	0.225	0.201
Variety (V)	<0.001	<0.001	<0.001	<0.001	0.515
P * V	0.001	0.054	<0.001	0.001	<0.001

The different letters indicate the significant effect of the production system on the variety at $p < 0.05$ (Student t-test). The multiple mean comparison analysis was carried out by using one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test.

The total phenolics and antioxidant activities

Total phenolics and antioxidant activity (in DPPH and ABTS assays) of eight tomato cultivars grown in net-house and open-field were measured (Figure 5). The results of total phenolics were expressed as gallic acid equivalents and ranged from 3.4-3.9 mg g^{-1} DW from tomatoes in the net-house system, whereas 3.6–4.5 mg g^{-1} DW was observed from open-field grown tomatoes. Similarly, it has been reported that total phenolics of tomatoes ranged from 1.3 to 2.5 mg g^{-1} DW from different cultivars.[286] Notably, individual cultivars were distinctively influenced by the production system. For example, when cultivars and field-grown tomatoes of four varieties, including T3, SV8579TE, Seri, and Mykonos were grown in open field conditions, had significantly higher levels of total phenolics than those of tomatoes cultivated in the net-house (Fig. S8A). Total phenolics were considerably influenced by the production system and genotype. Similarly, for the further insight of the production system and genotype effects were compared in Fig. S8. The levels of total phenolics were higher from tomatoes grown in the open-field than the level from the net-house condition ($P < 0.01$), independently of genotype (Fig. S8A). In variety-wise, Seri and L501-55 showed the maximum and minimum abundance of total phenolic contents according to the genotypic effect, respectively (Fig. S8B). It has been reported that different total phenolics are attributed to various influencing factors such as genotype, part of the fruit, and growing condition such as covering materials in protected culture.[27, 287] In DPPH assay, the antioxidant activity of tomatoes from net-house and open-field conditions ranged from 639.4 µg g^{-1} DW to 714.3 µg g^{-1} DW and 634.9 µg g^{-1}

DW to 759.5 µg g^{-1} DW of ascorbic acid equivalents, respectively. Considerably higher antioxidant activities were found in two tomato varieties such as TAM-Hot-ty and T3 from open-field-grown tomatoes than those of tomatoes grown in protected culture (Fig. S8B). The antioxidant activity of tomatoes using DPPH assay based on the production system was not significantly affected, whereas genotypic-specific effects were observed and T3 had the highest antioxidant activity among studied tomato varieties (Fig. S3B). Finally, ABTS assay ranged as 1.7–2.1 mg g^{-1} DW in the net-house-grown tomatoes, whereas 1.7 –2.2 mg g^{-1} DW in the field-cultivated. According to the variety, the distinctively different influence of production systems was observed. The antioxidant capacity of four tomato varieties, including T3, L501-55, SV8579TE, and DRP-8551, was substantially higher in open-field grown tomatoes than those cultivated in the net-house. However, only SV8579TE variety had significantly elevated antioxidant capacity was observed in open fields (Fig. 4C). Moreover, variety-specific different antioxidant activity was observed for T3, SV8579TE, and Shourouq than the remained tomato varieties (Fig. S8B). It has been reported that ABTS assay is generally used for measuring the antioxidant capacity of fruit and vegetable compared to the DPPH assay, since ABTS assay represented the antioxidant potential of more hydrophilic and lipophilic compounds, whereas DPPH assay represents more lipophilic compounds.[266] This may explain our findings that the higher values resulted in ABTS assay than those of DPPH assay.

It was noteworthy that considerably enhanced caffeic acid and sinapic acid levels of tomatoes from open fields may be accountable for the higher values total phenolic

contents and antioxidant activities of T3 variety from the open-field condition than from net-house system (Fig. 12 and Table 10). Similar with our findings, the previous study demonstrated that the variation of antioxidant activity and their potential components of tomatoes might vary according to genotypes.[27] Furthermore, the growing environment including photosynthetically active radiation as well as UV light transmission properties may affect phenolic compounds and antioxidant activity based on tomato cultivar.[288]

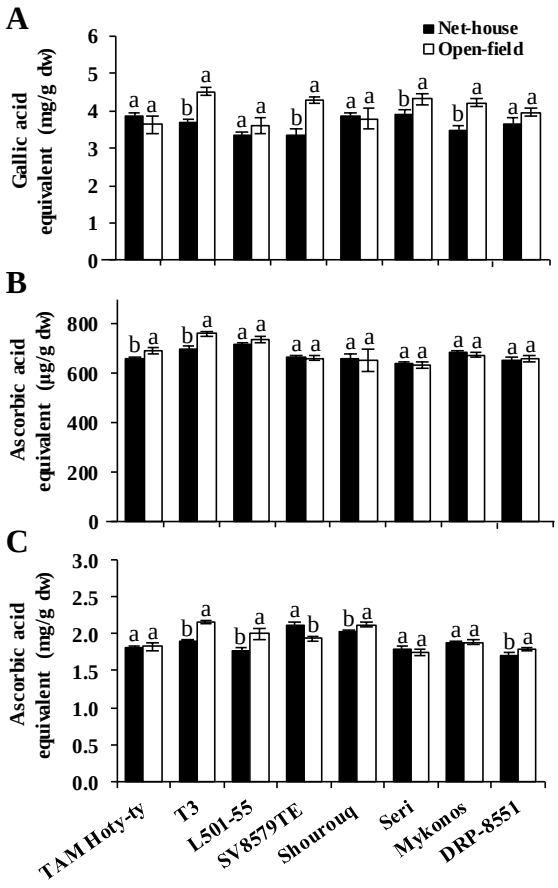

Figure 12. The contents of total phenolics (A) and DPPH (B) and ABTS (C), free radical-scavenging activities, from eight tomato varieties grown in the net-house and open-field. Results are expressed mean ± standard error, and different letter indicates significant differences ($P \leq 0.05$) between production systems.

CHAPTER VII

EFFECT OF PRODUCTION SYSTEM AND INHIBITORY POTENTIAL OF AROMA VOLATILES ON POLYPHENOL OXIDASE AND PEROXIDASE ACTIVITIES OF TOMATOES

Introduction

Tomato (*Solanum lycopersicum*) is one of the most consumed horticultural crops in fresh and processed forms worldwide.[23] Tomato is a rich source of biofunctional nutrients, including lycopene, *β*-carotene, ascorbic acid, and polyphenols, which have been reported to provide several health benefits.[289] It has been reported that tomato-based products accounted for 75% of total tomato consumption.[23] However, the nutrient contents and the quality of tomatoes are influenced by several parameters, including genotype, cultivation practices, and postharvest storage.[290] In addition, the most essential fruit quality attributes such as color, constituent, and viscosity are changed during tomato processing, mainly, due to the enzymes, polyphenol oxidase (PPO) and peroxidase (POD).[24] The PPO and POD activities of tomatoes are variable according to different developmental stages, cultivar, and postharvest conditions.[289, 291]

Both PPO and POD are considered as vital enzymes and play considerable roles in the plant defense and several metabolic processes.[292-293] The economic importance of these enzymes is noted due to their involvement in the quality deterioration during the processing and storage of foods.[25] The PPO enzyme catalyzes the oxidation of

polyphenols to *o*-quinones, which subsequently polymerize into undesirable brown, red, or black pigments in the presence of oxygen.[25] The POD enzyme, an antioxidant enzyme, catalyzes the conversion of hydrogen peroxide to water using polyphenols as a hydrogen donor.[294] Consequently, POD is considered as an indicator of food quality reduction by acting synergistically with PPO which generates H_2O_2 during the oxidation of polyphenols.[114, 294] Therefore, PPO and POD activities of plant-based foods have been assessed during postharvest and processing handling to maintain the quality, and to reduce deterioration reactions.[113-114] However, it has been reported that the difficulty of detecting enzyme reactions, since the outcomes are highly dependent on defined conditions.[295] Hence, optimizing the influencing factors such as enzyme extraction conditions, pH, temperature, or substrate concentrations are prioritized to maximize the efficiency of measuring enzyme activities.[295]

Several studies have been conducted to inhibit and inactivate the PPO and POD enzymes. For instance, various inhibitory chemicals, including ascorbic acid, cysteine, or volatile compounds, have been shown to reduce the enzymatic browning reaction of fruits and vegetables.[25, 114, 296-299] In addition, in tomato PPO and POD activities have been studied in the context of stress resistance against pathogens, and fruit browning.[24, 300-302] Spagna et al. reported that tomato color changes during the storage due to PPO activity associated with browning and lycopene degradation.[24] However, only a few studies examined the effects of tomato PPO and POD inhibition.

In recent years, the cultivation of tomato in the protected environment has increased to maintain the demand and supply throughout the year. The controlled growing conditions such as production under plastic tunnels and net houses also was found to have a potential impact on biotic and abiotic stresses, which may affect productivity and quality.[303] However, the influence of growing conditions on tomato PPO and POD in connection with genotypes has not been well studied. The present study reports the optimization of PPO and POD extraction and enzymatic activity using high-throughput 96 well plates to evaluate the inhibitory potential of tomato volatile compounds. Finally, we evaluated the effect of the production system on PPO and POD activities using eight tomato cultivars.

Materials and methods

Reagents and materials

Catechol, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H_2O_2), guaiacol, polyvinylpolypyrrolidone (PVPP), sodium monophosphate, sodium diphosphate, and authentic volatile standards (1-penten-3-one, d-limonene, (E)-2-heptenal, (Z)-3-hexen-1-ol, linalool, β-damascenone, geranylacetone, and farnesylacetone) were procured from Sigma-Aldrich (St. Louis, MO). Pierce® BCA protein assay kit was obtained from Thermo Scientific (Rockford, IL). All other reagents were analytical grade. The vine-ripe tomato fruits were obtained from a local supermarket (HEB, College Station, TX).

Extraction of PPO and POD enzymes from fruits

Initially, PPO and POD enzyme extraction and partial purification conditions were optimized using the following variables: extraction buffer pH (4.5–8.0) with or without the addition of supplement (5% PVPP containing 5 mM EDTA), and precipitation with acetone. Briefly, all fruits were washed with distilled water and dried with paper towels. Later on, samples were blended for one min using a commercial blender. An aliquot of 3 mL of sodium phosphate buffer (100 mM, pH 4.5–8.0) with or without supplement (5% PVPP containing 5 mM of EDTA) was added to the blended sample (3 g). The mixture was centrifuged at 8496 *g* (4 °C, 15 min). The resultant supernatant was used as the crude extract for optimization of tomato PPO and POD extraction conditions and further used for acetone precipitation. For protein precipitation, cold acetone (-20 °C) was added to crude enzyme extract in a 1:1 ratio (v/v).[304] The precipitate was separated after centrifugation at 8496 *g* for 15 min. Finally, the precipitate was dissolved in 2 mL of 0.1 M phosphate buffer, and used for PPO and POD activity assays. 2.3. Microplate-based PPO and POD assays

The activity of PPO and POD enzymes was measured using microplate reader. In this study, catechol and guaiacol were used as the substrates for tomato PPO and POD assays, respectively.[305-306] For the PPO assay, 100 µL of sodium phosphate buffer (100 mM, pH 7) and 50 µL 150 mM catechol solution were added to each microplate well. An enzyme extract (50 µL) was added to each well to initiate the reaction. The change in absorbance was monitored at the wavelength of 404 nm at 25 °C in the microplate reader

(Synergy-HT, BioTek, Winooski, VT). For the POD assay, 125 µL of sodium phosphate buffer (100 mM, pH 7), 25 µL of guaiacol (24 mM), and 25 µL of 12 mM hydrogen peroxide solution were added to a microplate well. Subsequently, 25 µL of enzyme extract was added to initiate the reaction, and the change in absorbance was recorded at 465 nm in the microplate reader. For both assays, one unit of enzymatic activity (U) was defined as the absorbance increase of 0.001 per minute under the assay conditions.[307] The specific activity was determined by expressing PPO or POD activity/mg protein.

Microplate-based PPO and POD assays

The activity of PPO and POD enzymes was measured using microplate reader. In this study, catechol and guaiacol were used as the substrates for tomato PPO and POD assays, respectively.[305-306] For the PPO assay, 100 µL of sodium phosphate buffer (100 mM, pH 7) and 50 µL 150 mM catechol solution were added to each microplate well. An enzyme extract (50 µL) was added to each well to initiate the reaction. The change in absorbance was monitored at a wavelength of 404 nm at 25 °C in the microplate reader (Synergy-HT, BioTek, Winooski, VT). For the POD assay, 125 µL of sodium phosphate buffer (100 mM, pH 7), 25 µL of guaiacol (24 mM), and 25 µL of 12 mM hydrogen peroxide solution were added to a microplate well. Subsequently, 25 µL of enzyme extract was added to initiate the reaction, and the change in absorbance was recorded at 465 nm in the microplate reader. For both assays, one unit of enzymatic activity (U) was defined

as the absorbance increase of 0.001 per minute under the assay conditions.[307] The specific activity was determined by expressing PPO or POD activity/mg protein.

Estimation of protein content

The protein content 5 to 250 µg/mL ($y = 0.0011x$, $R^2 = 0.9982$).

of crude enzyme, extract was measured using Pierce™ BCA Protein Assay Kit, according to the manufacturers' directions and measured at 562 nm after incubating at 37 °C for 30 min (Thermo Scientific, IL). The bovine serum albumin (BSA) was used as a standard protein, and standard calibration was generated with varying concentrations of BSA ranging from 5 to 250 µg/mL ($y = 0.0011x$, $R2 = 0.9982$).

Kinetic properties of tomato PPO and POD

To estimate the Michaelis–Menten constant (K_m) and maximum velocity (V_{max}), the PPO and POD activities were measured at different concentrations of catechol (25–200 mM) and guaiacol (4–32 mM), respectively. For the POD assay, hydrogen peroxide concentrations (4–60 mM) were tested for optimal activity. Lineweaver and Burk plot was used to calculate K_m and V_{max} values for catechol and guaiacol as substrates for PPO and POD activities, respectively.

Effect of pH and temperature on the enzyme activities

The acetone precipitate of crude enzymes was dissolved with 100 mM phosphate buffer with pH range 3.5–8.0 to determine the optimal pH. The effects of pH on enzyme activities were examined at 25 °C using 150 mM catechol and 24 mM guaiacol as substrates for PPO and POD, respectively and 12 mM hydrogen peroxide was also used for the POD reaction. Further, using the above optimum pH and substrate concentrations, the optimal temperatures (25–60 °C) for PPO and POD activities were determined. Briefly, the standard reaction mixtures without PPO and POD enzymes were heated to the appropriate temperature for 2 min in the water bath. After the attainment of the designated reaction temperature, enzymes were added, and the activity was measured. The results were expressed as percent relative activity in comparison to maximum enzyme activities (100%).

Thermal stability of PPO and POD

Tomato PPO and POD (0.3 mL) enzymes were heated in water baths maintained at 55, 65, 75, and 85 °C separately. After heating for different times (0, 5, 10, 20, 30, and 60 min), the vials were put in an ice bath until the PPO and POD activities were assayed. The results were expressed by residual enzyme activity (RA) in heat-treated samples as a fraction of initial activity using the following equation: Residual enzyme activity (RA) = $A_i/A_0 \times 100$, where A_i and A_0 are measured enzyme activity after heating for a different time and time zero, respectively.

The thermal denaturation kinetics of tomato PPO and POD were described by using a first-order inactivation constant (k_d) which was calculated from the slope of the natural logarithm (ln) of A_i/A_0 versus time graph. The half-life time of the enzyme ($t_{1/2}$) and decimal reduction time (D-value) to reduce the initial activity up to 90% were estimated based on k_d value by using the reported equations.[308] In addition, the energy of activation of denaturation (ΔE) was calculated based on the slope of the Arrhenius plot (natural logarithm of k_d values (ln(K)) vs. reciprocal of absolute temperatures (1/T)) by using the universal gas constant, R (8.314 J/mol). Other activation parameters such as ΔG (Gibbs free energy for enzyme inactivation), ΔH (enthalpy change, a measure of the number of non-covalent bonds broken), and ΔS (entropy change, a measure of net enzyme and solvent disorder) were determined based on reported equations.[24]

Inhibitory effect of aroma volatiles on PPO enzyme activities

The inhibitory effect of pure volatile compounds such as 1-penten-3-one, (Z)-3-hexen1-ol, ß-damascenone, geranylacetone, linalool, d-limonene, (E)-2-heptenal, and farnesylacetone on PPO and POD activities were studied at different concentrations (1–80 mM). Each volatile compound (800 mM) was prepared in ethanol and serially diluted with 100 mM phosphate buffer (pH 7). The inhibition percentage of tomato PPO activity was calculated using the following equation: $I\ (\%) = [(A_c – A_i)/A_c] \times 100$, where A_c is the absorbance in control and A_i is the absorbance in treatment.[110]

Effect of production system on enzyme activities

Eight tomato cultivars were grown in net-house and open-field production systems in Weslaco, Texas, USA and were harvested in June 2016. The Beefsteak round type of fruits was TAM Hot-Ty, T3, L501-55, Shourouq, and Mykonos. The oval-shaped fruits were SV8579TE, Seri, and DRP-8551. The net-house type hoop house structure consisted of a 2000-ft^2 and was covered with 50-mesh insect screen (20–29% shade) as per previously published.[265] The harvested fruits were sliced into pieces and blended for 30 s and stored at -20 °C until further analysis for PPO and POD enzyme activities. The results were expressed as U/g of fresh weight.

Statistical analysis

All of the data were reported as the mean ± standard error. Significant differences between production systems were assessed with a Student's t-test (P-value ≤ 0.001). The multiple mean comparisons (P-value ≤ 0.05) analysis was carried out by using one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test using SPSS software (v. 23, BM SPSS Statistics, IBM Corp., Chicago, IL).

Results and discussion

PPO and POD enzymes play an essential role in fruits and vegetables by improving and maintaining their qualities. We have previously shown that the levels of flavor-associated volatile compounds can be influenced by the production system.[265, 309]

However, the influence of production systems and volatiles on tomato PPO and POD activities is not known yet. Our present work indicates the influence of OF and NH growing on tomatoes PPO and POD activities of different genotypes, and also uniquely demonstrated PPO inhibitory potential tomato certain volatiles

Optimization of PPO and POD extraction conditions

Initially, the efficiency of extraction of PPO and POD enzymes from tomato fruits was investigated by considering pH and the role of the supplement (5% PVPP containing 5 mM EDTA), using sodium phosphate buffer. Results show that pH and addition of the supplement in the extraction buffer had significant impacts on enzyme activity (Fig. 13). The activity of PPO crude extract was highest at pH 8.0 with supplement (Fig. 13a). Conversely, POD showed maximum activity at pH 7.0 without supplement (Fig. 13b). The addition of PVPP and EDTA in the enzyme extraction buffer is routinely advised to promote the separation of phenolic compounds during the enzyme extraction.[310] Similarly, we also observed that adding supplements (PVPP and EDTA) in the extraction buffer increased the activity of tomato PPO, which underscores the inhibitory role of certain tomato phenols during PPO extraction. By contrast, the addition of 5% PVPP containing 5 mM EDTA substitute in the extraction buffer had a negative impact on the POD activity (Fig. 13b). These findings indicate that pH and supplement contents of the extraction buffer are critical parameters, which need to be considered to achieve the optimal yield of PPO and POD enzymes.

Casado et al. [311] reported the challenge in measuring PPO activity from tomato due to its undetectable levels of enzymes. In view of this, the protein precipitation method could be helpful to enrich the enzyme concentration by removing impurities.[312] The effect of acetone precipitation on the final yield of PPO and POD was also assessed as a partial enzyme purification method in comparison with those of crude extraction, and the results of these studies are shown in Table 12. After acetone precipitation, 95.9% and 98.0% of total proteins were removed, and specific activities PPO and POD were increased roughly 8.2 and 21.3 folds, respectively. This result indicates that the partial purification step with acetone precipitation is critical for optimal PPO and POD activities. Few studies have been conducted in the context of optimization PPO and POD extraction conditions in tomato fruits.[24] The observed purification factor for tomato PPO with acetone precipitation was 8.2, which was relatively higher in comparison with literature reported 5 and 4.6 folds purification factors by using ammonium sulfate and Triton X-114, respectively.[311]

Table 12. Partial purification of polyphenol oxidase (PPO) and peroxidase (POD) from tomatoes.

Purification steps	Total volume (mL)	Activity (U/mL)	Total activity (U)	Protein (mg/mL)	Total protein (mg)	Specific activity (U^{-1} mg $protein^{-1}$)	Yield (%)	Purification (fold)
PPO								
Crude extract	5.5	231.1	1271.1	7.1	39.3	32.4	100.0	1.0
Acetone precipitate	2.0	211.9	423.7	0.8	1.6	265.1	33.3	8.2
POD								
Crude extract	5.5	13226.7	72746.7	6.3	34.6	2100.0	100.0	1.0
Acetone precipitate	2.0	15764.4	31528.9	0.4	0.7	44733.2	43.3	21.3

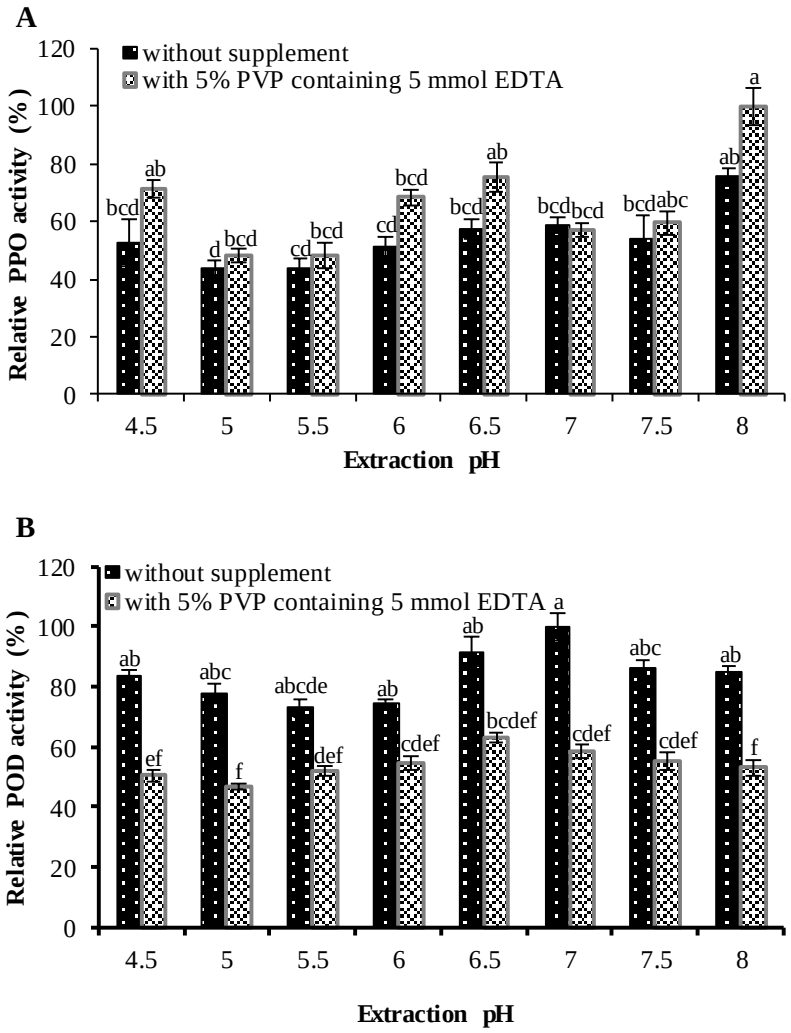

Figure 13. Effects extraction conditions with or without 5% PVPP containing 5 mmol EDTA at different pH (4.5–8) on PPO (A) and POD (B) activities. Bars ± SD having different letter are statistically different at $\alpha = 0.05$.

Characterization of partially purified tomato PPO and POD

In this study, we found that the oxidized products of catechol and guaiacol substrate-based PPO and POD enzymatic reactions had maximum absorptions at 404 and 465 nm, respectively (Fig. S9). In addition, optimal substrate concentrations for the maximum activity were determined for both partially purified enzymes. Finally, kinetic properties were also estimated using the K_m as an indicator of the enzyme affinity for the substrate and the V_{max}, which signifies the maximum rate where the substrate will be converted to a product.[313]

The observed PPO activity increased gradually from 25 to 150 mM of catechol concentrations and decreased after 150 mM (Fig. 14A). The Lineweaver–Burk plot for tomato PPO is depicted in Fig. 14B. The K_m and V_{max} values for tomato PPO were 62.47 mM and 263.16 U/mL/min, respectively. Similarly, the optimal activity of tomato POD was obtained by using 24 mM of guaiacol among studied concentrations ranged from 4 to 32 mM (Fig. 14C). The resultant K_m and V_{max} values for tomato POD were determined as 37 mM and 10,000 U/mL/min, respectively (Fig. 14D). In addition, the optimal concentration of H_2O_2 was selected as 12 mM among different studied concentrations (4 to 60 mM). Based on results, 150 mM of catechol was optimized for PPO assay, and 24 mM guaiacol with 12 mM H_2O_2 was finalized for POD assay.

The pH and temperature have been reported to have considerable roles in catalytic activities of enzymes, mainly in the context of substrate affinity and enzyme denaturation.[107] .[110] Therefore, the effect of different pH on PPO and POD activities were

investigated at defined substrate concentrations. The maximum activity of tomato PPO and POD was observed at pH 7.0 and 5.5, respectively (Fig. 15A). In plants, the pH value for maximum enzyme activity was found to vary according to the plant source, its varieties, and substrates used.[295] These results were different from previous reports, in which the optimal pH of tomato PPO and POD were pH 7.0 and 6.2 with catechol and o-dianisidine as substrates, respectively. pH 4.8 was determined for tomato PPO by using 3,4-dihydroxyphenylacetic acid as a substrate.[295, 302]

The effect of temperature on tomato PPO and POD activities were further investigated within the range of 25–60 °C (Fig. 15B). The maximum activity for PPO and POD enzymes was observed at 50 and 45 °C, respectively. Similarly, we also found that the activity tomato PPO enzyme declined as the temperature increased above 55 °C.[24]

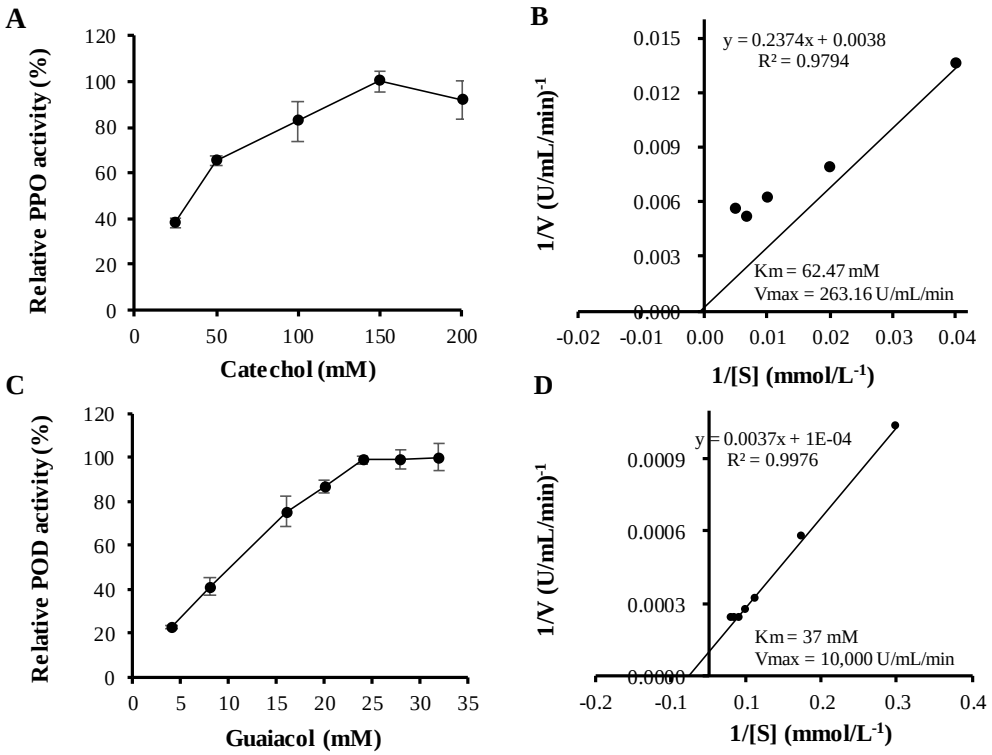

Figure 14. Effects of parameters on tomato PPO and POD activities. (A) Effect of substrate concentration (catechol) on PPO activity. (B) Lineweaver-Burk plots for catechol. (C) Effect of substrate concentration (guaiacol) on POD activity. (D) Lineweaver-Burk plots for guaiacol. (E) Effect of hydrogen peroxide on POD activity.

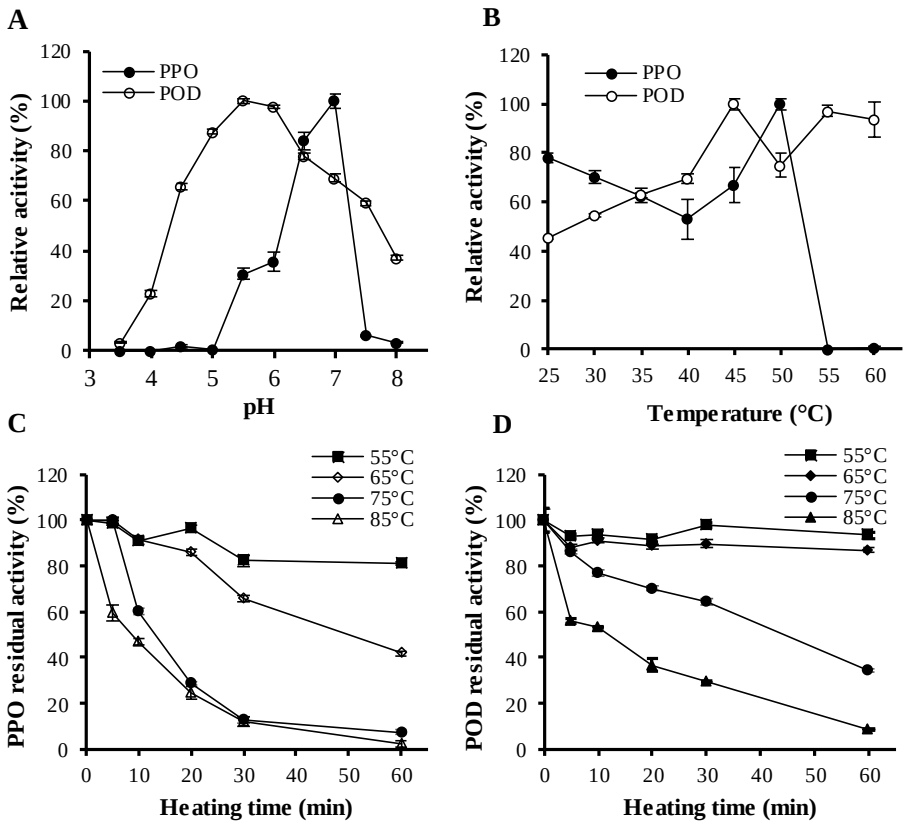

Figure 15. Effects of pH on PPO and POD activity (A) and the influence of different temperatures on PPO and POD activity (B). Thermal inactivation profiles of PPO (C) and POD activities (D) at different heating temperatures (55, 65, 75, and 85 °C) and times (0, 5, 10, 20, 30, and 60 min) (Activity evaluated three replications, vertical bars represent standard error).

Thermal inactivation PPO and POD enzymes

The thermal stability profiles of partially purified tomato PPO and POD enzymes were performed by heating at various temperatures (55, 65, 75, and 85 °C) for 0, 5, 10, 20, 30, and 60 min (Fig.15C and D). The inactivation of endogenous enzymes of fruits

and vegetables is considered as one of the most important steps in food preservation operations to prevent the loss of quality attributes such as color, flavor, texture, and nutritional characteristics.[117] Markedly, the one-fourth of total tomato consumption has been reported as processed products such as puree.[23] During the processing of tomato products such as puree, thermal treatment is the most favored method to inactivate oxidative enzymes.[302] However, little is known about the difference in thermal inactivation between tomato PPO and POD using thermodynamic and kinetic parameters. The results of residual enzyme activity showed that the PPO and POD activities were continuously reduced over time. Around 90% of PPO and POD activities were lost at 75°C for 30 min and 85°C for 60 min treatment, respectively (Fig. 15C and 15D).

To study detailed kinetic parameters for thermal inactivation of tomato PPO and POD, the denaturation constants (k_d), half-life ($t_{1/2}$), and the decimal reduction time (D-value) were estimated for characterization of enzyme stability, and results were shown in Table S9. The denaturation constants (k_d) increased with increasing temperature. The higher k_d specifies that the enzyme is less thermostable at a higher temperature.[308] In general, D-value indicates stability and sensitivity of enzymes to heat.[308] Our results showed that both D and $t_{1/2}$ values of tomato PPO and POD decreased with increase in the temperature of heat treatment (Table S9). Altogether, results of this study showed that PPO and POD activities decreased faster at higher temperatures, and the tomato POD was more thermostable than PPO. The activation energy (ΔE) for the heat inactivation of PPO and POD was calculated by Arrhenius equation. The observed values of ΔE were 95.1 (r^2

= 0.94) and 157.6 kJ mol^{-1} (r^2 = 0.97) for tomato PPO and POD, respectively (Table 13, Fig. S10).

The observed Δ*G* of tomato PPO and POD were 394.37 ± 7.47 and 395.8 ± 7.5 KJ mol^{-1} was for POD heat inactivation. The values of Δ*H* were 92.28 ± 0.05 and 154.7 ± 0.05 KJ mol^{-1} for PPO and POD heat inactivation, respectively. Δ*S* values of PPO and POD heat inactivation were -880.04 ± 5.39 and -702.1 ± 8.82 J mol^{-1}, respectively (Table 13). The higher Δ*E* and Δ*H* values of POD indicated that the tomato POD is more resistant to heat than PPO. Similar to our findings, strawberry POD was found to be more thermostable than PPO.[25] However, existing literature also suggests that the thermostability PPO and POD enzymes of plant and plant products considerably vary due to the presence of their different molecular forms, as well.[24, 297, 314]

Table 13. Transition state parameters for the heat inactivation of tomato PPO and POD (means ± standard error for the triplicate experiment).

Enzyme extract	ΔE (KJ mol^{-1})	ΔG (KJ mol^{-1})	ΔH (KJ mol^{-1})	ΔS (J mol^{-1})
PPO	95.1	394.37 ± 7.47	92.28 ± 0.05	-880.04 ± 5.39
POD	157.6	395.8 ± 7.50	154.7 ± 0.05	-702.1 ± 8.82

*ΔE, activation energy for PPO and POD heat inactivation; ΔG, Gibbs free energy for enzyme inactivation; ΔH, enthalpy change; ΔS, entropy change. Results were calculated based on the previous report.[24]

Inhibitory effects of aroma-volatile on PPO activity

So far more than 400 volatile compounds were reported in tomato fruits, and 16 compounds were considered as flavor-associated compounds.[143, 265] In the present study, PPO of tomato aroma-associated volatiles such as 1-penten-3-one, (Z)-3-hexen-1-ol, linalool, *β*-damascenone, geranylacetone, d-limonene, (E)-2-heptenal, and farnesylacetone were investigated (Table 14). Moreover, amongst these, six volatiles such as 1-penten-3-one, (Z)-3-hexen-1-ol, linalool, *β*-damascenone, geranylacetone, and d-limonene had inhibitory effects on tomato PPO. Remarkably, *β*-damascenone and d-limonene showed over 50% inhibition of PPO at the concentration of 40 and 80 mM, respectively (Table 14). However, 1-penten-3-one, (Z)-3-hexen-1-ol, linalool, and geranylacetone exhibited PPO inhibition in the range 3.4–28 % at their highest concentrations (80 mM) (Table 14). Notably, in our previous studies, the aroma volatile compounds of tomato fruit were found to be significantly influenced by genotype and cultivating practices such as open-field and net-house, and these factors distinctly modulate *β*-damascenone and d-limonene.[265, 309] Previous reports also demonstrated that volatile compounds inhibited the activities of PPO and POD enzymes.[24, 296, 298-299] However, to the best of our knowledge, herein, we have for the first time demonstrated the PPO inhibitory potentials of aroma volatiles, *β*-damascenone and d-limonene.

Table 14. The inhibitory effect of volatile compounds at different concentrations on tomato PPO activity (% catechol inhibition).

Compounds	1 mM	5 mM	10 mM	20 mM	40 mM	80 mM
1-Penten-3-one	0.0	0.8 ± 0.0^{bc}	1.3 ± 0.7^{bc}	1.2 ± 0.3^{bc}	2.3 ± 0.5^{ab}	3.4 ± 0.8^{a}
(Z)-3-Hexenol	0.0	3.2 ± 1.1^{cd}	6.2 ± 1.4^{cd}	9.0 ± 1.5^{bc}	13.8 ± 1.8^{b}	27.7 ± 1.6^{s}
β-damascenone	2.9 ± 0.6^{c}	12.4 ± 0.6^{c}	26.9 ± 5.7^{b}	34.3 ± 1.7^{b}	52.2 ± 1.3^{a}	58.0 ± 3.4^{a}
Geranylacetone	0.0	0.1 ± 0.1^{c}	2.5 ± 0.3^{bc}	5.1 ± 0.7^{ab}	5.4 ± 0.8^{a}	5.7 ± 1.3^{a}
Linalool	0.0	0.0	3.9 ± 0.6^{c}	6.6 ± 0.5^{c}	15.7 ± 0.9^{b}	28.0 ± 1.4^{a}
D-Limonene	0.3 ± 0.3^{d}	2.9 ± 0.6^{d}	5.3 ± 0.3^{d}	13.1 ± 0.7^{c}	25.6 ± 1.0^{b}	50.3 ± 3.4^{a}
(E)-2-Heptenal	0.0	0.0	0.0	0.0	0.0	0.0
Farnesylacetone	0.0	0.0	0.0	0.0	0.0	0.0

Means in the same row followed by the same letter are not significantly different at the $P \leq 0.05$ level according to Tukey's Honestly Significant Difference (HSD) Test (means ± standard error, n=6).
0.0: No inhibition.

Effect of production system on PPO and POD activities

PPO and POD are defense-related enzymes and found to improve the resistance of tomato plants against pathogens. Moreover, these enzymes play a key role in the storability of fruits and vegetables.[296, 315] Considering this, monitoring the activities of PPO and POD activities during agronomical practices is becoming vital. The results of PPO and POD activities of tomato fruits of eight varieties grown in the net-house and open-field are shown in Fig. 16. The open-field grown tomatoes from three tomato varieties such as TAM Hot-ty, L501-55, and SV8579TE showed significantly higher PPO activities than fruit grown in the net-house ($P \leq 0.05$). By contrast, net-house tomatoes of T3, Seri, and DRP-8551 had higher PPO activity than open-field fruits. No significant difference in tomato PPO activity was found in Shourouq and Mykonos varieties grown in both studied production systems. Conversely, net-house grown tomato fruits of TAM

Hot-ty and SV8579TE varieties had significantly higher POD activity levels compared to their open-field grown tomatoes. However, net-house grown tomato fruits of varieties, L501-55, Shourouq, Seri, and DRP-8551 showed significantly lower activities than their open-field grown tomatoes. The non-significant changes in POD activity levels of T3 and Mykonos were observed between production systems. The PPO and POD activities of tomato varieties Mykonos were not affected by production systems. In addition, four studied varieties, TAM Hot-ty, SV8579TE, Seri, and DRP-8551 exhibited comparatively similar patterns of PPO and POD activities. These findings collectively indicated that production systems have a considerable impact on tomato fruit PPO and POD activities. However, this feature is strictly associated with tomato genotype. Similar to our findings, tomato PPO and POD activities were also found to vary with genotype and different growing conditions such as UV radiation and irrigation.[316-317]

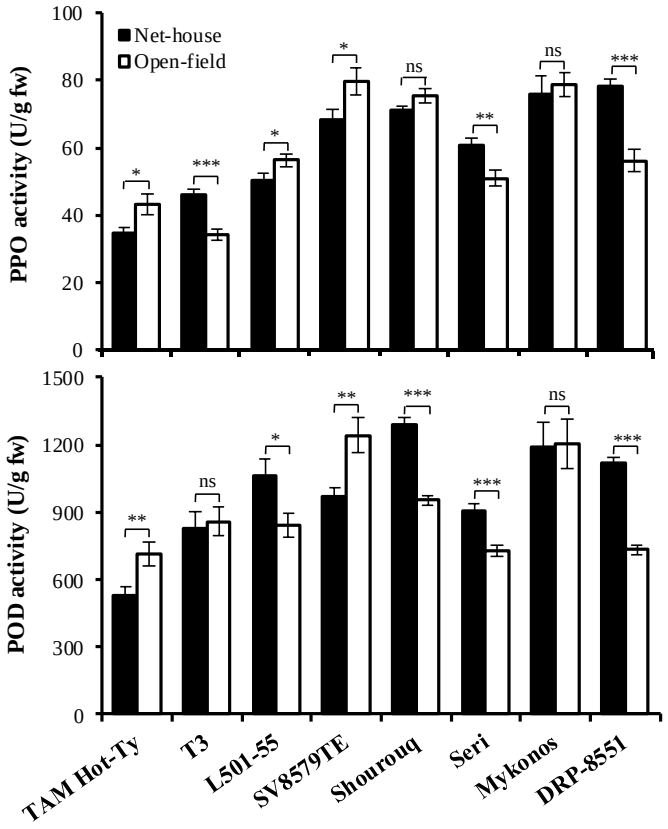

Figure 16. The influence of production systems (net-house and open-field) on PPO and POD activities of different tomato varieties (Asterisks indicate statistical difference of the values, *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$, ns: no significance).

CHAPTER VIII

METABOLIC CHANGES OF TOMATO IN RESPONSE TO INFESTATION WITH TOMATO POTATO PSYLLID VECTORED BUT NOT CANDIDATUS LIBERIBACTER SOLANACEARUM

Introduction

The phloem-limited bacterium *'Candidatus* Liberibacter solanacearum' (Lso) is transmitted by the tomato-potato psyllid (TPP, *Bactericera cockerelli*), and both the pathogen and the vector cause severely destructive symptoms on *Solanaceae* crops such as zebra chip disease in potato (*Solanum tuberosum*) and vein greening in tomato (*Solanum lycopersicum*).[126, 318-319] In tomato, resistance to the insect vector TPP has been found in wild-relative species *Solanum habrochaites*.[320] Furthermore, a recent study on recombinant inbred lines (RILs) developed from the cross of resistant *S. habrochaites* with cultivated tomato identified several major quantitative trait loci (QTLs) responsible for adult TPP mortality and fecundity. Analysis of the major resistance QTL found in RIL LA3952 carrying *S. habrochaites* insertion on chromosome 8 revealed that the presence of Lso is required to increase adult TPP mortality. By contrast, the reduced TPP oviposition trait in LA3952 is independent of Lso indicating that the presence of the pathogen influence plant-insect interactions.

Consequently, understanding the effect of TPP carries or free of Lso on crops may be prerequisite to advance on the development of resistant cultivars to reduce economical

loss.[136] So far, substantial genomic and metabolic work has been focused to elucidate the influence of the TPP-Lso complex in susceptible potato genotypes.[321-323] However, little information is known about the metabolic changes response to TPP, vectoring or not the Lso in resistant genotypes.[130, 324] Several reports indicate that changes in metabolic profiles including phenolics, hormone, and volatile compounds in response to plants interaction with herbivores and/or pathogens, are involved in the plant defensive mechanisms.[130, 134-136] Moreover, constitutive and elicited phytohormones have been reported to play crucial roles in the hosting plant deploying defensive signaling against herbivore and pathogen. Mainly, defense-signaling pathways involve plant phytohormones jasmonic acid and salicylic acid which regulate resistance in the host.[130, 324] Previous reports revealed that the regulation of phytohormones in response to stress is specific, varying accordingly to the host plant and pathogenic strain.[325] Furthermore, differently regulated gene expressions involved in hormonal pathways were observed in Lso-infected potatoes based on the varieties, and the distinction may have association with their susceptibility and resistance.[322] In addition to phytohormones, volatile metabolites have also been studied for their role in defensive responses to herbivore or pathogen infection since the can act as indirect plant defensive mechanisms attracting carnivorous predator against herbivory attacks.[134, 326-327] However, herbivore or pathogen-induced metabolic changes can be influenced by several factors, including the interacting species, developmental stages of plants, insect densities, and the different period after inoculation may be accountable for the different pattern of metabolic profile.[135, 328-330] Furthermore,

the additional interaction of the pathogen in interaction with its vector has been suggested to influence host-insect vector interaction.[331]

Non-targeted or targeted metabolomics approaches using LC/MS and GC/MS datasets are considered as a useful tool for distinguishing the influence of herbivore/pathogen-infected plants on metabolic changes and filtering the biomarkers.[332] In particular, understanding plant innate immune responses to vector colonization and pathogen infection could lead to novel strategies for the management of plant diseases.[130] Therefore, in the present study, we report a comparative tomato metabolic profile in response to TPP carrying or not the Lso pathogen in resistant and susceptible genotypes to identify putative metabolites involved in defensive signaling in response to TPP attack.

Materials and methods

Chemicals

Standards of plant hormones (abscisic acid (ABA), zeatin (ZA), gibberellic acid (GA), jasmonic acid (JA), and salicylic acid (SA) and phenolic acids (4-hydroxy-benzoic acid, benzoic acid, caffeic acid, gallic acid, protocatechuic and phthalic acid) were procured from Sigma (St. Louis, MO). The plant hormone 12-oxo phytodienoic acid (OPDA) was obtained Cayman Chemical (Ann Arbor, MI). All other chemicals, solvents used were of analytical and mass spec grade were obtained from Sigma-Aldrich (St. Louis, MO).

Plant materials and experimental design

The tomato TPP-susceptible cultivar CastleMart (CM) and the TPP-resistant recombinant inbred line LA3952, were grown at the Texas A&M AgriLife Research and Extension Center at Weslaco, TX. Tomato plants were grown in the controlled conditions with 16-hrs light at 23°C in 500-cc pots. Full factorial experimental design consisted on two genotypes (CM vs LA 3952) and three insect treatments [TPP-Lso(-), TPP-Lso(+), and mock control]. The six treatment combinations were imposed at the 5th week after transplanting. Ten adult psyllids were infested on the second fully opened leaf from the top using organza bag cages to prevent them to escape, while mock-inoculated consisted on empty cages. Five replications per treatment combination were performed. Tissue was collected 48 h after infestation and stored at -80 °C until further analysis of volatile and non-volatile components. The typical workflow is shown in Fig. 17.

Insect colonies

Lso-free TPP colonies and TPP colonies carrying Lso haplotype B of the Western biotype were reared in confining cages containing tomato and pepper plants. The colonies were tested for Lso before infestation by PCR using primer set OA2 forward 5′-GCGCTTATTTTTAATAGGAGCGGC-3′ [333] and OI2c reverse 5′-GCCTCGCGACTTCGCAACCCAT-3′ [334] targeting the 16S rRNA gene of Lso to detect its presence. The Lso haplotype was also tested by PCR using SSR primer pairs Lso-SSR-1F forward 5′-TTATTTTGAGATGGTTTGTTAAATG-3′ and Lso-SSR-1R reverse 5′-

TATTATCATTCTATTGCCTATTTCG-3′.[335] Amplification were performed as described by Avila et al 2019 (data not shown).

Analysis of plant phenolics by UPLC-QTOF-MS

Frozen leaf material was ground in liquid nitrogen, and 1 mL methanol was added to 50 mg of leaf sample. Each sample tube was vortexed (30 s), sonicated (1 h at 4 °C), and centrifuged (10,621 x g) for 10 min. The supernatant was passed through 0.45 micro filters and injected to UPLC-ESI-HR-QTOFMS, and the separation of phenolic acids was achieved using our published method.[266] The separated supernatant was injected into a UPLC-ESI-HR-QTOFMS equipped with Eclipse Plus C_{18} Rapid Resolution High Definition (1.8 μm, 50 × 2.1 mm) column. The gradient mobile phase, 0.1% aqueous formic acid (A) and 0.1% formic acid in acetonitrile (B) was used with gradient program for pump B as follows: 0-2 min, 0%; 2-15 min, 0-80%; 18-20 min, 80-100%. The separation was achieved at the flow rate of 0.2 mL min^{-1}. Mass spectral analysis was performed using high-resolution mass spectrometer (maXis impact, Bruker Daltonics, Bellerica, MA) using electrospray positive ionization mode. The operating parameters of the mass spectrometer were nebulizer gas pressure, 2.8 bar; nebulizer gas flow, 8 L min^{-1}; sheath nebulizer gas temperature, 220 °C; sheath gas heater temperature, 220 °C. DataAnalysis Software (version 4.3) was used to processes the data. Authenticate standards of phenolic acids were used for quantitative profiling.

Estimation of plant phytohormones by UPLC-QTOF-MS

Frozen crushed materials (50 mg) in fresh weight (FW) were weighed and transferred into 1.5 mL microfuge tubes. Then, 1 mL extraction solvent, 2-propanol: water: acetic acid (80:19:1, v/v), was added to each tube. The samples were vortexed, sonicated (1 h) and centrifuged (10,621 x g) for 10 min. The supernatant was separated and filtered samples were used for UHPLC-HR-QTOF-MS analysis of phytohormones using authentic standards. The separation of plant hormones was performed on Eclipse Plus C_{18} RRHD column (1.8 μm, 50 × 2.1 mm) with a flow rate of 0.15 mL min^{-1}. The mass spectral conditions and HPLC gradient separation was acquired according to our recent publication.[336]

Analysis of volatile metabolic profiles by HS-SPME/GC-MS

Sample preparation

Plant samples were ground with liquid nitrogen and 100 mg were placed in 20 mL SPME screw top amber vials with 1 mL of saturated calcium chloride and 200 ng of camphor dissolved in ethanol as an internal standard. The sample was vortexed for one min and sonicated for 30 min, before GC-MS analysis (Austin, TX).

HS-SPME/GC–MS analysis conditions

Tomato volatile compounds were extracted by headspace-solid phase microextraction (HS-SPME) equipped with a 50/30μm

Carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber (Sigma-Aldrich, St.Louis, MO). The samples were incubated and extracted for 2 and 30 min at 60 °C, respectively. The SPME fiber was desorbed at 225 °C for 2min, fiber conditioning was followed for 7 min, by placing into the injector of gas chromatography equipped with an electron ionization source with a Dual-Stage Quadrupole (DSQ II) mass spectrometer (Thermo Scientific, Austin, TX). Chromatographic separation was achieved with a Zebron ZB-5MS plus capillary column coated with 5% diphenyl-95% dimethylpolysiloxane (30m×0.25 mm) (Phenomenex, Inc. Torrance, CA). The conditions applied for the GC–MS were an initial oven temperature of 40 °C, held for 1 min, then increased to 90 °C at a rate of 10 °C/min, and increased to 175 °C at a rate of 3 °C/min. Finally, it was increased to 230 °C at a rate of 35 °C/min and held for 2 min at the final temperature, with a total run time of 38 min. Electron impact (EI) data from *m/z* 40 to 450 were acquired at a scanning speed of 11.5 scans per sec and with an ionization voltage of 70 eV. The ion source temperature and mass transfer line temperature were maintained at 280 °C. The data were recorded and processed using Xcalibur software (v. 2.0.7., Thermo-Fisher Scientific, San Jose, CA, USA).

Identification and quantification volatile metabolites

Volatile compounds of tomato leaf were identified by comparing their mass spectra, Kovats indices (KI), and retention times of authentic standards. The KI values were determined using the number of carbons and their retention times of *n*-alkane

standards (C_{10}–C_{24}) achieved from the same analysis condition as of samples. Each mass spectrum was also compared in Wiley 8 and NIST05 mass spectral library. Quantifying the relative changes in tomato volatile metabolites was conducted using internal standard, camphor, based on the previous literature.[309, 337]

Statistical analysis

The univariate statistical analysis was performed to assess the significant difference between treatments with a student t-test (p-value < 0.05), and one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test was carried out for multiple mean comparison analysis (v. 23, BM SPSS Statistics, IBMCorp., Chicago, IL). Further investigation on the influence of genotype and cultivation system on tomato metabolites, the chemometric analysis was performed by MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) using LC-MS and GC-MS data sets.

Results

Untargeted metabolomic analysis.

Untargeted metabolomic analysis by partial least squares-discriminant analysis (PLS-DA) score plots using a dataset derived from UPLC-ESI-HR-QTOFMS was used to understand metabolic regulation of resistant (LA3952) and susceptible (CM) tomato genotypes in the response to Lso-free and Lso-positive TTP. PLS-DA score plots show that tomato genotypes were had a differential metabolite profile in response to TPP

treatments independently of susceptibility or resistance 48 hrs after insect infestation (Fig. 18A and 18B). However, both herbivore and pathogen-infected susceptible tomato plants caused a slightly different metabolic profile than those from other test groups, whereas comparatively far distance between control and TPP-Lso (+) test groups by having TPP-Lso (-) infected plant between them in insect-resistant variety. Further metabolomic insights were observed based on plant resistance to the insect, PLS-DA scores plot using combined all test groups and three clusters were presented based on studied groups (Figure 18C). The main separation was due to the effect of the variety, revealing exceptional metabolic changes induced by TPP-Lso (+)-infested Castlemart plants among test groups. Together, the findings indicate that non-volatile metabolic changes in tomato plants can be attributed to the response to both, the insect and its vectored pathogen. Moreover, the significance of metabolic influence can vary accordingly to plant resistance against the insect vector.

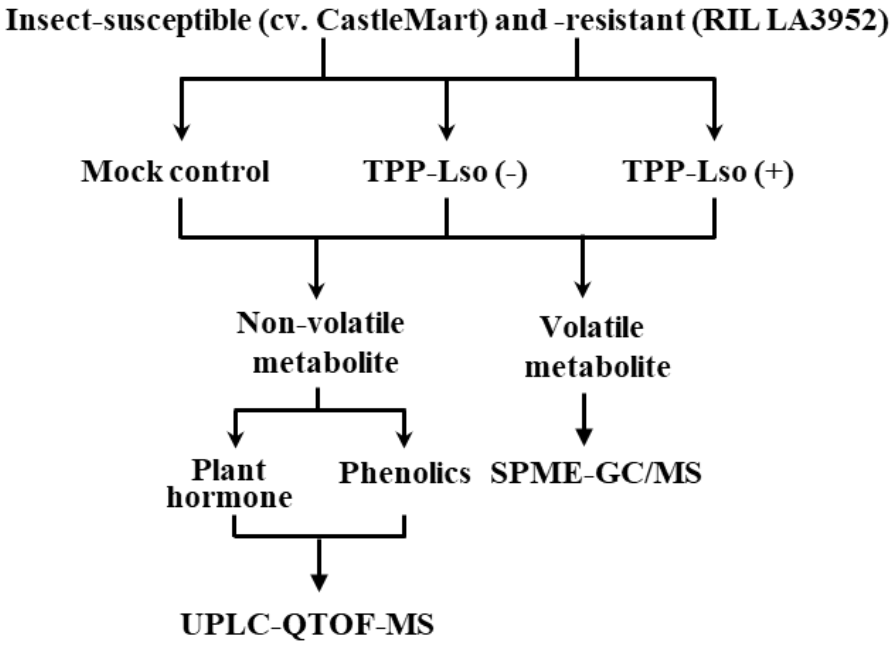

Figure 17. Experimental design for investigating the response of insect-susceptible (CM) and resistant (LA3952) tomato varieties inoculated with tomato potato psyllid (TPP) carrying or not *Candidatus* Liberibacter Solanacearum (Lso). The five-week-old tomato seedlings were infested and harvested after two days. Five replications were used for each treatment.

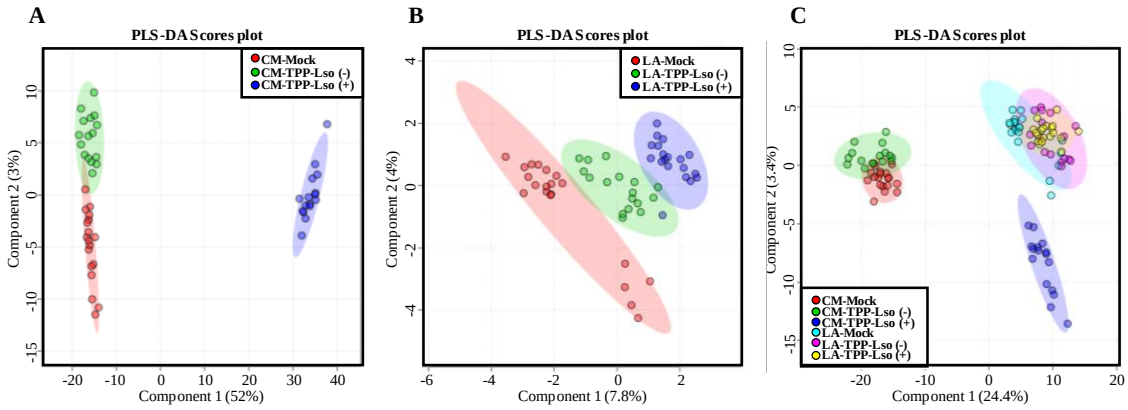

Figure 18. Partial least squares discriminate analysis (PLS-DA) of untargeted metabolomics using dataset of methanolic extract obtained by UPLC-HR-TOF-MS with positive ESI mode from tomato plants inoculated with mock control, TPP-Lso (-), and TPP-Lso (+). The two-dimensional PLS-DA score plots depict the claustration according to the metabolic response to inoculations of insect-susceptible CM tomato variety (A), insect-resistant LA3952 variety (B), and combined all test groups (C).

Phenolics response based on treatments

Nine phenolic compounds including gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, phthalic acid, chlorogenic acid, p-coumaric acid, ferulic acid, rutin, and naringenin were identified and quantified using UPLC-QTOF-MS (Table S10). The phenolic levels per treatment are presented in Fig. 19. There were significant genotypic effects on seven phenolic compounds except for protocatechuic acid and ferulic acid. The higher levels of gallic acid ($P < 0.01$), p-coumaric acid ($P < 0.001$), and naringenin ($P < 0.05$) were observed from resistant LA3952 as compared to the values observed in susceptible tomato plants (CM). Conversely, the susceptible plants had significantly

higher contents of four phenolic compounds such as 4-hydroxybenzoic acid ($P < 0.001$), phthalic acid ($P < 0.05$), chlorogenic acid ($P < 0.001$), and rutin ($P < 0.001$) than those level of resistant plants. Moreover, differences between treatments were observed. For example, the substantially increased contents of gallic acid were observed regardless of genotypes infested with TPP-Lso (-) than control plants. Notably, this elevated level of LA3952 was even substantially higher than the gallic acid level of TPP-Lso (+) treated one. The level of p-coumaric was not detectable in CM plants inoculated with TPP-Lso (+), and no significant difference was found between control and TPP-Lso (-)-inoculated CM plants. Meanwhile, p-coumaric acid levels of LA3952 plants were not influenced based on treatments. The naringenin levels were considerably enhanced in response to TPP-Lso (+) than the levels of control and TPP-Lso (-)-treated ones in both susceptible and resistant plants. Substantially enhanced phthalic acid levels were found in TPP-Lso (-) and TPP-Lso (+) inoculated LA3951 in comparison to the control plant, but not between two treatments, whereas no significant effect of pathogenic treatment on CM plants. Similarly, resistant LA3952 plants had increased amounts of chlorogenic acid in TPP-Lso (-) and TPP-Lso (+) inoculated plants without the observed difference between two treatments. In CM plants, TPP-Lso (+) treated plants showed a significantly higher level of chlorogenic acid than those of control and TPP-Lso (-) inoculated plants. However, no considerable changes were detected on 4-hydroxybenzoic acid and rutin between the treatments.

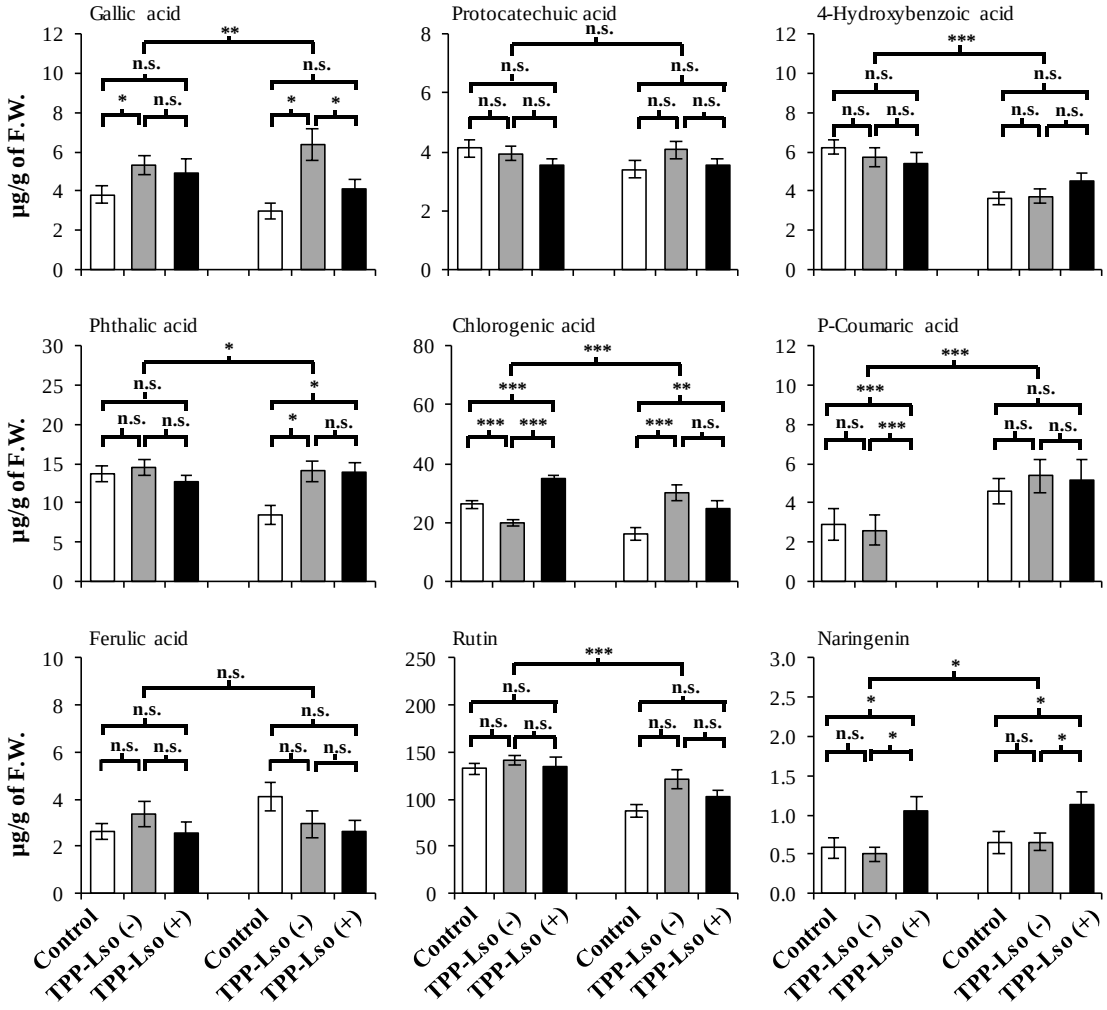

Figure 19. The levels of phenolics of CM and LA3952 tomato plant, susceptible and resistant varieties, against with or without *Candidatus* Liberibacter Solanacearum (Lso) transmitted by tomato-potato psyllid (TPP). Results are presented as mean ± S.E., and letters mean the significant difference ($P \leq 0.05$) among tested inoculations in tomato plants based on a post hoc Tukey test.

Comparative analysis of plant hormone between susceptible and resistant tomato plants against TPP-Lso

In the present study, identification and quantification of nine plant hormones such as zeatin, gibberellic acid, indole acetic acid, abscisic acid, salicylic acid, jasmonic acid, and 12-oxo phytodienoic acid (OPDA), melatonin, and serotonin from tomato plants were analyzed using UPLC-QTOF-MS (Fig. 20 and Table S11). Amongst them, genotypical effects were found in five hormonal compounds. For example, zeatin ($P < 0.001$), indole-3-acetic acid ($P < 0.001$), and salicylic acid ($P < 0.01$) contents were higher in the susceptible CM plants, however, no significant difference was not found according to treatments. notably, indole-3-acetic acid levels were not detectable in resistant plants. Conversely, the resistant LA3952 showed considerably higher levels of gibberellic acid ($P < 0.001$) and jasmonic acid ($P < 0.001$) than the observed values in the susceptible plants, and differences of these levels were observed between treatments. For example, the significantly and/or constitutively increased gibberellic acid contents of TPP-Lso (+) inoculated susceptible than the observed value of control and TPP-Lso (-) inoculated plants, whereas the considerably reduced level was observed in resistant plants infested with TPP-Lso (+) plants than other two treatments. Regard to jasmonic acid contents, the LA 3952 plants showed a substantially reduced level in response to the TPP-Lso (-) tested plants. However, no significant changes were detected in the CM plats among the tests. However, no significant genotypic effects were found in the levels of melatonin, abscisic acid, serotonin, and 12-Oxi-phytodienoic acid. Taken together, five compounds, including

zeatin, gibberellic acid, indole acetic acid, salicylic acid, and jasmonic acid can be considered as their involvement related to susceptibility and resistance of tomato plants against either TPP or both pathogen and its vector psyllid.

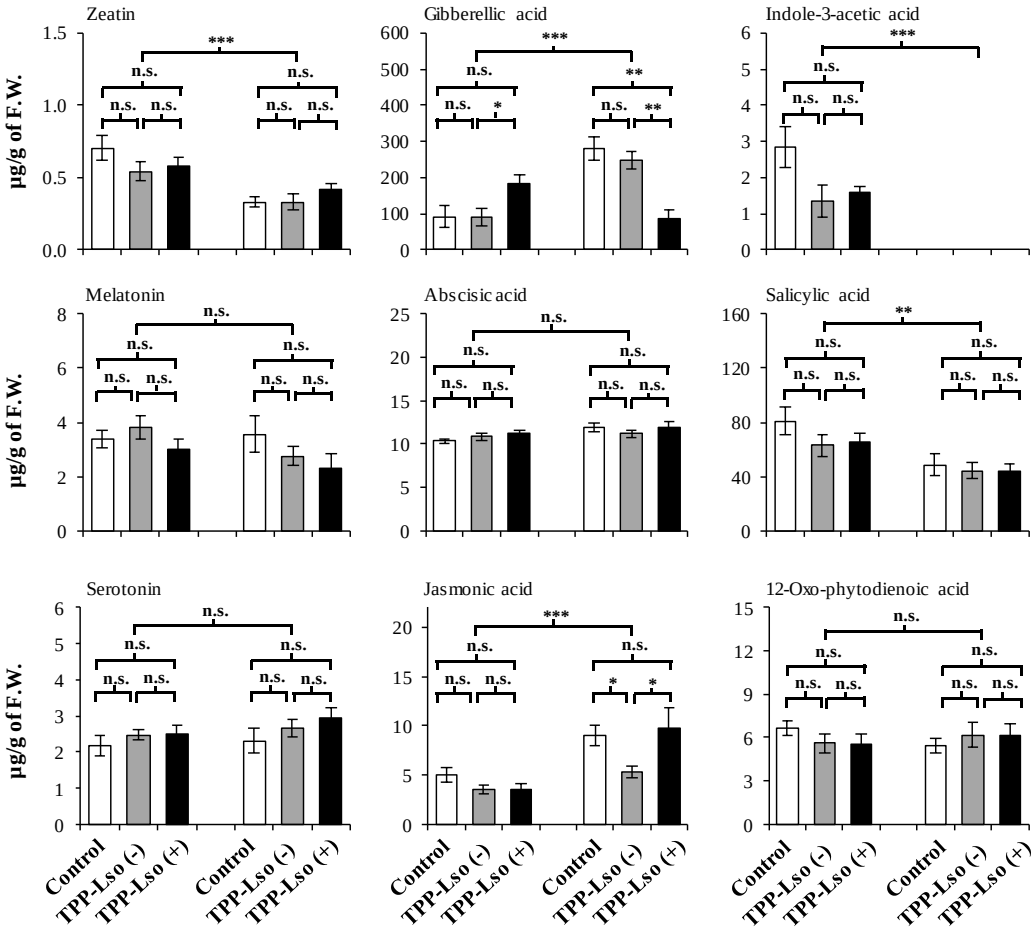

Figure 20. The levels of hormones and melatonin of susceptible (CM) and resistant tomato varieties (LA3952) against tomato-potato psyllid (TPP) carrying or free of bacteria, *Candidatus* Liberibacter Solanacearum (Lso). Results are expressed as mean ± S.E., and letters indicate the statistical significance ($P \leq 0.05$) among treatment in two tomato varieties according to a post hoc Tukey's test.

Analysis of volatile metabolites of tomato plant using HS-SPME/GC-MS

Identification and quantification of volatile metabolites

The profiles of volatile metabolites of insect-susceptible and -resistant tomato plants measured at 48 h after infestation are shown in Table 16. In total, 43 volatile metabolites were identified, quantified, and classified into five chemical classes such as monoterpene, sesquiterpene, fatty acids-derived, norisoprenoids, and phenylpropanoids. Notably, significantly different metabolite profile was detected for measured volatiles between treatments except for twelve compounds including as 2,4-hexadienal, 2-carene, 3-carene, α-terpinene, β-phellandrene, β-ocimene, terpinolen, α-campholenal, ethyl salicylate, valencene, dihydroactinidiolide, and farnesylacetone (Table 16). When comparing tomato genotypes, the abundance of five volatile compounds, including the monoterpene β-pinene and four sesquiterpenes β-elemene, β-caryophyllene, α-humulene, and caryophyllene oxide were considerably higher in the resistant LA3952 variety than those in susceptible CM variety. Conversely, only hexanal level was higher in CM as compared to LA3952.

Table 15. Identification and qunatification of volatile metabolites of mock, TPP-Lso (-), and TPP-Lso (+) inoculted insect-susceptible and -resistant tomato plants, CM (CM) and LA3952 (LA), respectively (ng/100 mg of fresh weight, mean ± S.E.).

No.	RT	Compounds	KI	ID	CM			LA		
					Mock control	TPP-Lso (-)	TPP-Lsp (+)	Mock control	TPP-Lso (-)	TPP-Lsp (+)
1	4.63	Hexanal	830	MS, KI, ST	10.57 ± 0.77a	3.96 ± 1.02b	1.48 ± 0.27b	2.57 ± 0.34b	2.72 ± 0.86b	14.92 ± 2.65a
2	5.71	(E)-2-Hexenal	872	MS, KI, ST	230.63 ± 22.94bc	223.99 ± 42.87bc	141.97 ± 17.01c	309.03 ± 43.91b	257.62 ± 31.03bc	492.11 ± 51.39a
3	7.50	2,4-Hexadienal	928	MS, KI	0.99 ± 0.19a	0.85 ± 0.17a	0.74 ± 0.16a	1.32 ± 0.38a	0.91 ± 0.17a	1.72 ± 0.57a
4	8.13	α-Pinene	944	MS, KI, ST	4.74 ± 0.44b	7.98 ± 1.12a	5.76 ± 0.92ab	4.42 ± 0.50b	6.40 ± 0.47ab	5.48 ± 0.62ab
5	10.19	β-Pinene	986	MS, KI	6.45 ± 1.29c	9.96 ± 1.10bc	5.37 ± 1.06c	18.8 ± 1.31a	13.53 ± 2.01ab	14.60 ± 2.38ab
6	10.56	2-Carene	996	MS, KI	93.43 ± 8.60a	141.57 ± 15.49a	103.56 ± 14.01a	103.45 ± 10.52a	103.99 ± 9.37a	114.66 ± 12.03a
7	10.86	3-Carene	1004	MS, KI	40.80 ± 4.27a	62.07 ± 7.64a	47.89 ± 7.00a	47.42 ± 4.92a	44.15 ± 4.39a	45.18 ± 5.43a
8	11.41	α-Terpinene	1019	MS, KI, ST	9.01 ± 1.30a	13.27 ± 1.69a	11.72 ± 1.75a	12.79 ± 0.39a	14.87 ± 1.58a	14.37 ± 1.55a
9	11.79	P-Cymene	1029	MS, KI, ST	3.62 ± 0.58c	5.11 ± 0.68bc	3.50 ± 0.61c	4.25 ± 0.46c	10.58 ± 1.55a	8.55 ± 0.96ab
10	12.07	β-Phellandrene	1036	MS, KI, ST	476.35 ± 40.23a	701.74 ± 75.28a	519.46 ± 74.65a	538.26 ± 51.54a	503.77 ± 39.98a	493.63 ± 47.56a
11	12.92	β-Ocimene	1056	MS, KI	3.52 ± 0.33a	5.05 ± 0.53a	4.63 ± 0.99a	5.59 ± 0.83a	3.15 ± 0.41a	3.62 ± 0.47a
12	13.43	γ-Terpinen	1067	MS, KI, ST	0.64 ± 0.08b	1.08 ± 0.11a	0.69 ± 0.10b	0.69 ± 0.09b	0.80 ± 0.05ab	0.74 ± 0.09ab
13	14.02	3,5-Octadien-2-one	1080	MS, KI	1.17 ± 0.24a	1.35 ± 0.25a	0.47 ± 0.10ab	1.23 ± 0.42a	0.21 ± 0.04b	0.73 ± 0.09ab
14	14.15	2-Octanol	1083	MS, KI	1.97 ± 0.25ab	2.61 ± 0.12a	1.27 ± 0.17bc	1.58 ± 0.41bc	0.75 ± 0.08c	1.00 ± 0.14c
15	14.75	Terpinolen	1095	MS, KI, ST	1.23 ± 0.15a	2.15 ± 0.30a	1.62 ± 0.33a	1.66 ± 0.20a	1.24 ± 0.14a	1.27 ± 0.15a
16	15.70	α-Campholenal	1114	MS, KI	2.75 ± 0.09a	3.01 ± 0.14a	3.50 ± 0.62a	3.46 ± 0.15a	3.29 ± 0.11a	3.53 ± 0.05a
17	16.27	Alloocimene	1124	MS, KI	0.43 ± 0.10abc	0.78 ± 0.15a	0.72 ± 0.12ab	0.39 ± 0.05abc	0.30 ± 0.04c	0.31 ± 0.05bc
18	19.90	Methyl salicylate	1184	MS, KI, ST	10.17 ± 1.52a	9.38 ± 1.08ab	7.22 ± 1.14ab	5.99 ± 0.63ab	2.78 ± 0.47b	2.76 ± 0.22b
19	20.81	Decanal	1197	MS, KI, ST	2.63 ± 0.14a	2.53 ± 0.10bc	2.41 ± 0.16bc	3.09 ± 0.27a	1.85 ± 0.11b	2.93 ± 0.22a
20	21.22	β-Cyclocitral	1206	MS, KI, ST	4.53 ± 0.48bc	5.18 ± 0.74bc	3.17 ± 0.35c	6.33 ± 0.89b	4.29 ± 0.42bc	9.23 ± 0.94a
21	21.34	Benzothiazole	1209	MS, KI, ST	2.97 ± 0.19a	2.90 ± 0.13a	2.66 ± 0.15a	2.91 ± 0.27a	1.36 ± 0.08b	1.35 ± 0.09b
22	22.32	Cuminal	1234	MS, KI	0.67 ± 0.05b	1.01 ± 0.08a	1.14 ± 0.15a	0.66 ± 0.06b	0.17 ± 0.01c	0.31 ± 0.02c
23	22.85	Piperitone	1247	MS, KI	0.43 ± 0.03a	0.42 ± 0.03ab	0.45 ± 0.06a	0.31 ± 0.03abc	0.28 ± 0.03bc	0.25 ± 0.03c
24	22.93	β-Cyclohomocitral	1249	MS, KI	0.54 ± 0.07ab	0.60 ± 0.12ab	0.42 ± 0.02b	0.71 ± 0.10ab	0.42 ± 0.03b	0.79 ± 0.06a
25	23.52	Ethyl salicylate	1263	MS, KI	0.34 ± 0.08a	0.86 ± 0.40a	0.28 ± 0.03a	0.35 ± 0.07a	0.09 ± 0.03a	0.21 ± 0.05a
26	26.63	δ-Elemene	1331	MS, KI	2.91 ± 0.37cd	5.90 ± 0.81a	5.51 ± 0.79ab	3.30 ± 0.50bc	0.92 ± 0.42d	2.56 ± 0.26cd
27	27.31	Eugenol	1345	MS, KI	2.09 ± 0.24a	1.59 ± 0.12ab	1.17 ± 0.18bcd	1.48 ± 0.23abc	0.64 ± 0.05d	0.85 ± 0.14cd
28	29.00	β-Elemene	1379	MS, KI	3.07 ± 0.23bc	3.97 ± 0.37abc	2.83 ± 0.38c	4.49 ± 0.50a	3.02 ± 0.23bc	4.27 ± 0.25ab
29	30.02	Dodecanal	1398	MS, KI	11.69 ± 0.96a	9.51 ± 0.66ab	9.30 ± 0.91ab	12.28 ± 1.06a	6.32 ± 0.44b	9.31 ± 0.55ab
30	30.17	β-Caryophyllene	1401	MS, KI, ST	29.97 ± 4.09b	48.28 ± 5.07ab	42.93 ± 7.83ab	56.07 ± 7.73a	36.42 ± 4.14ab	37.16 ± 2.95ab
31	30.39	α-Ionone	1407	MS, KI	1.53 ± 0.22ab	1.75 ± 0.35ab	0.98 ± 0.08b	2.54 ± 0.34a	1.70 ± 0.23ab	2.56 ± 0.38a
32	30.71	γ-Elemene	1416	MS, KI	1.10 ± 0.16ab	1.37 ± 0.24ab	0.75 ± 0.10b	1.71 ± 0.34b	1.22 ± 0.19ab	1.19 ± 0.25ab
33	31.15	Aristolene	1428	MS, KI	1.10 ± 0.15a	1.10 ± 0.14a	0.97 ± 0.17ab	0.98 ± 0.12ab	0.55 ± 0.06b	0.57 ± 0.05b
34	31.67	α-Humulene	1442	MS, KI	8.54 ± 1.24b	11.76 ± 1.24ab	11.99 ± 2.11ab	14.59 ± 1.61a	9.49 ± 1.04ab	9.44 ± 0.77ab
35	32.71	β-Ionone	1469	MS, KI, ST	18.77 ± 2.28abc	19.48 ± 2.70abc	11.33 ± 1.34c	22.01 ± 3.41ab	14.81 ± 1.42bc	26.93 ± 2.36a
36	32.83	β-Ionone-5,6-epoxide	1472	MS, KI	2.37 ± 0.33bc	2.37 ± 0.43bc	1.53 ± 0.17c	3.26 ± 0.53ab	2.27 ± 0.23bc	4.11 ± 0.38a
37	33.04	Valencene	1478	MS, KI, ST	0.35 ± 0.038a	0.50 ± 0.07a	0.51 ± 0.11a	0.35 ± 0.05a	0.26 ± 0.03a	0.28 ± 0.03a
38	33.74	β-Guaiene	1495	MS, KI	0.70 ± 0.06ab	0.93 ± 0.09a	0.91 ± 0.18a	0.55 ± 0.10ab	0.33 ± 0.08b	0.45 ± 0.07b
39	34.41	Dihydroactinidiolide	1512	MS, KI	2.46 ± 0.37a	2.27 ± 0.37a	1.80 ± 0.22a	2.50 ± 0.42a	2.08 ± 0.22a	3.17 ± 0.43a
40	36.68	Caryophyllene Oxide	1566	MS, KI	0.72 ± 0.12c	0.99 ± 0.13c	0.70 ± 0.12c	1.83 ± 0.25a	1.18 ± 0.12bc	1.63 ± 0.17ab
41	38.21	Tetradecanal	1601	MS, KI	1.62 ± 0.12a	1.32 ± 0.12abc	1.18 ± 0.13bcd	1.60 ± 0.10ab	0.80 ± 0.05d	1.10 ± 0.07cd
42	38.40	Benzophenone	1609	MS, KI	1.33 ± 0.07a	1.36 ± 0.03a	1.18 ± 0.07a	1.24 ± 0.07a	0.64 ± 0.04b	0.81 ± 0.04b
43	44.68	Farnesylacetone	1906	MS, KI, ST	0.25 ± 0.04a	0.16 ± 0.03a	0.37 ± 0.16a	0.20 ± 0.04a	0.17 ± 0.02a	0.19 ± 0.02a

KI: Retention index, relative to n-alkanes (C8-C24) on the ZB-5 capillary column. ID: Identification methods, MS: Mass spectra; KI values that agreed with the data reported in previous literature or the database on the web (http://www.nist.gov). ST: Standard comparison, compounds identified using authentic standards. Different letters in the same row indicated significant differences between production systems at 95%.

The influence of TPP vectored or not Lso on distinctive volatile distribution based on the susceptibility and resistance of tomato plants

The distinctive changes of volatile metabolites according to in response to infestations were analyzed in tomato TPP-resistant vs -susceptible plants (Fig. 21). The cumulative contents of volatile compounds in susceptible and resistant plants after inoculations present in Fig. 21A, it was observed that resistant LA3952 produced the highest levels of volatile compounds against psyllids carrying the Lso pathogen followed by susceptible CM group in response to inoculation with TPP-Lso (-). Conversely, CM variety inoculated with TPP-Lso (+) showed the lowest total value (Fig. 21A). Volatile compounds were classified into five different chemical classes, including monoterpene, sesquiterpene, fatty acid-derived, norisoprenoids, and phenylpropanoids (PA)-derived compounds. The constitutive and/or significant differences between treatments were observed based on chemical groups (Fig.21B–F). There were no substantially genotypic differences were observed in monoterpenes, sesquiterpenes, and norisoprenoids between susceptible and resistant plants. There were significantly genotypic differences in fatty acid-derived and PA-derived compounds. For example, higher levels of fatty acid-derived compounds in the resistant compound than the observed values of susceptible plants, whereas the higher level of PA-derived compounds were found in the susceptible plants than resistant LA3952 plants.

The total contents of volatile compounds based on chemical groups were present as relative levels in comparison to the highest levels. Notably, susceptible CM plant inoculated with TPP-Lso (-) had the constitutively higher level of total monoterpene than

other treatments in monoterpene group (Fig. 21B). The control group of LA3952 plants had constitutively higher total sesquiterpenes than the observed value of susceptible tomato plants. Moreover, the total level was relatively higher in susceptible CM plants in response to the TPP-Lso (-) inoculation (Fig. 21C). The considerably higher total fatty acid-derived compounds were observed in resistant plant than the value of susceptible plants. Interestingly, the significantly different response of genotypes against the inoculation with TPP-Lso (-) and TPP-Lso (+). The susceptible plants infected with TPP-Lso (+) had significantly reduced level of total fatty acid-derived compounds, whereas substantially higher level of this group of metabolites was detected from LA3952 plants than the level of TPP-Lso (-) treated plants (Fig. 21D). Similar to the fatty acid-derived group, the response of total norisoprenoids of susceptible and resistant genotypes against TPP-Lso (+) and TPP-Lso (-) inoculations were observed as constitutive reductions, respectively (Fig. 21E). Lastly, the substantial difference of total PA-derived compounds was detected based on genotypes, and both TPP-Lso (-) and TPP-Lso (+) treatments occurred significantly reduced levels in LA3952 resistant plants (Fig. 21F).

Furthermore, multivariate analysis using an sPLS-DA method shows that there are three clusters in both TPP resistant and susceptible plants attributed to the effect of mock, TPP-Lso (-), and TPP-Lso (+) inoculations (Fig. 21G and 21H). In addition, to obtain further insight into volatile metabolic distributions based on the difference of herbivore-defensive plant response to tested inoculations, all investigated 43 metabolites were depicted in heat maps and bar graphs (Fig. 21I, 21J, and Fig. S11). There were

genotypically different contents of individual volatile compounds, and several volatile metabolites such as two fatty acid-derived 3,5-octadien-2-one and dodecanal, 1-octanol, and tetradecanal, four PA-derived metabolites (eugenol, benzophenone, methyl salicylate, and benzothiazole), six monoterpenes (α-pinene, γ-terpinen, piperitone, alloocimene, cuminal, and terpinolene, and five sesquiterpenes (valencene, β-caryophyllene, aristolene, δ-elemene, and β-guaiene) were significantly higher in susceptible CM plants than these contents in resistant plants. Conversely, the abundances of three fatty acid-derived derivatives (hexanal, (E)-2-hexenal, decanal), four norisoprenoids (β-ionone, β-ionone-5,6-epoxide, β-cyclocitral, α-ionone), two monoterpenes (p-cymene, β-pinene), and two sesquiterpenes (caryophyllene oxide, and β-elemene) were richer in resistant LA3952 plants than the observed levels in CM plants (Fig. S11. In genotype-wise, the distinctive alteration between the treatment was also observed. In the susceptible cultivar CastleMart, most of the metabolites were upregulated when infested with Lso-free psyllids, while in TPP resistant LA3959 plants where downregulated as compared to their respective mock controls (Fig. 21I). Particularly, significantly elevated levels of 1-octanol, α-pine, and γ-terpinene in susceptible CM plants against TPP without carrying Lso than observed levels of mock and TPP-Lso (+) treated plants. Moreover, nine volatile metabolites, including β-phellandrene, 3-carene, cuminal, β-ocimene, terpinolene, 2-carene, β-caryophyllene, δ-elemene, and β -guaiene levels were detected as substantially higher in CM plants than the observed contents of its control plant (Fig. S11). On the other hand, a higher proportion of metabolites were upregulated in the LA3952 when plants where infested with Lso-

positive psyllids as compared to susceptible plants (Fig. 21J). It is noteworthy that the content of hexanal and (E)-2-hexenal was significantly enhanced in LA3952 plants in response to TPP-Lso (+) than control and TPP without carrying pathogen (Fig. S11).

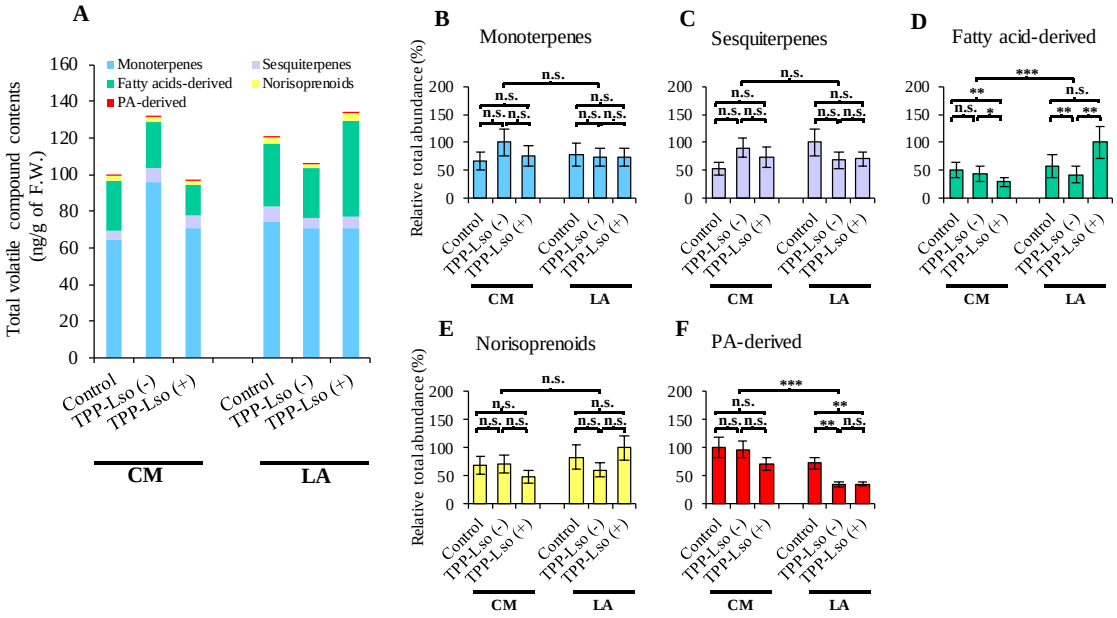

Figure 21. The influence of mock, TPP-Lso (-), and TPP-Lso (+) inoculation on insect–susceptible and –resistant tomato plants, CM (CM) and LA3952 (LA), respectively, in their voaltile metabolites. (A) Stack plots describing the total and relative abundance of observed classes of volatile metabolites according to the treatment. Bar graphs display the relative abundance of total volatiles in comparison to the maximum value based on chemical classes (B) monoterpenes (C) sesquiterpenes (D) fatty acid-derived (E) norisoterpenoid (F) phenylpropanoids phenylpropanoids (PA)-derived based on the volatile metabolic response of tomato plants based on test inoculation. (G) sPLS-DA scores plats of insect-susceptible and (H) insect–resistant tomato plants show the distinctively discriminated three clusters among studied groups. Bar graphs indicating the sum contents of detected compounds based on the chemical classes, including monoterpene (D), sesquiterpene (E), fatty acids-derived (F), norisoprenoids (G), and phenolics-derived (H). The heatmaps show the effect of treatment on the mean abundance of studied metabolites within groups based on the tomato varieties such as CM (I) and LA (J).

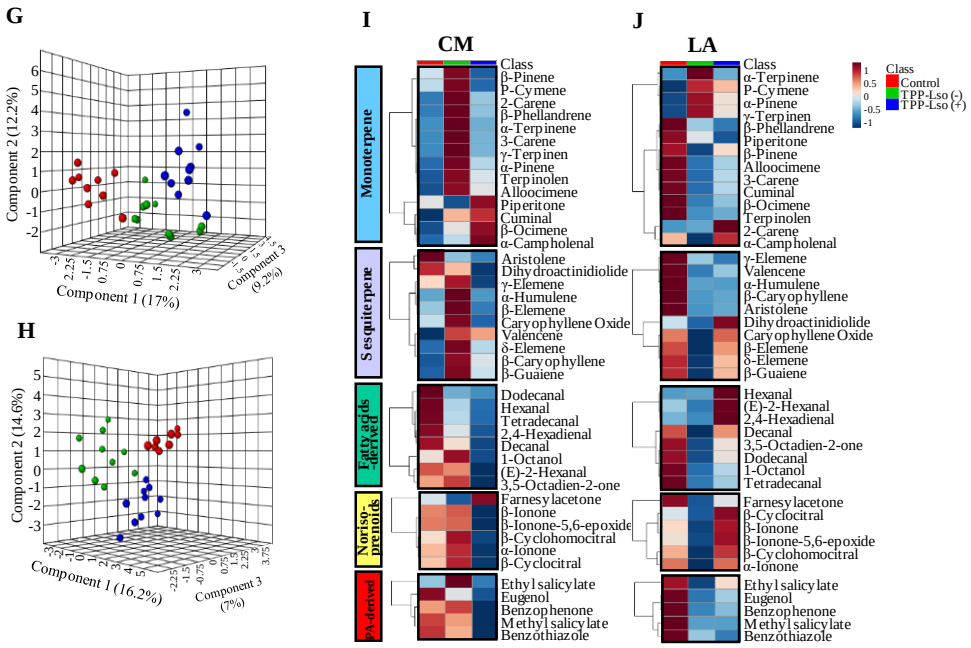

Figure 21 Continued.

Distinguished volatile metabolites after TPP harboring or not the pathogens using metabolomics approach

The identified and quantified tomato volatile compounds were subjected to multivariate analysis using unsupervised principal component analysis (PCA) and supervised methods such as partial least squares discriminant analysis (PLS-DA) and sparse PLS-DA (sPLS-DA) to understand the volatile metabolic profiles of tomato plants in response to mock, TPP-Lso (-), and TPP-Lso (+) inoculation in resistant and susceptible

plants (Fig. 22). The results show a distinct partition among test groups and mainly, two separated groups were more clearly perceived in sPLS-DA and PLS-DA plots than those of unsupervised PCA plot (Fig. 22A–22C). The first distinguished cluster consists of four test groups including CM variety inoculated with Mock, TPP-Lso (-), TPP-Lso (+), and mock-inoculated LA3952 control group, whereas the second cluster contains two LA3952 groups infected with TPP-Lso (-) and TPP-Lso (+). Furthermore, the variable influence on projection (VIP) scores plot (1.0 <) derived from an PSL-DA model presents that 17 volatile compounds are accountable for the separation pattern (Fig. 22D). Among these, 13 volatile compounds may contribute to classifying tested groups into two groups. In particular, six compound levels such as (E)-2-hexenal, β-cyclocitral, β-ionone-5,6-epoxide, p-cymene, β-elemene, and β-ionone were observed with relatively higher abundance, while seven volatile compounds, including methyl salicylate, eugenol, benzophenone, 1-octanol, benzothiazole, aristlene, and cuminal were present as lower abundance in the second cluster than those in the first group. In addition, the loading plot corresponding to the PLS-DA model illustrates that relative correlation among placed variables with marked with different color based on chemical classes. It is also confirmed that 13 compounds above mentioned are negatively correlated by placed in the distance (Fig. 22E).

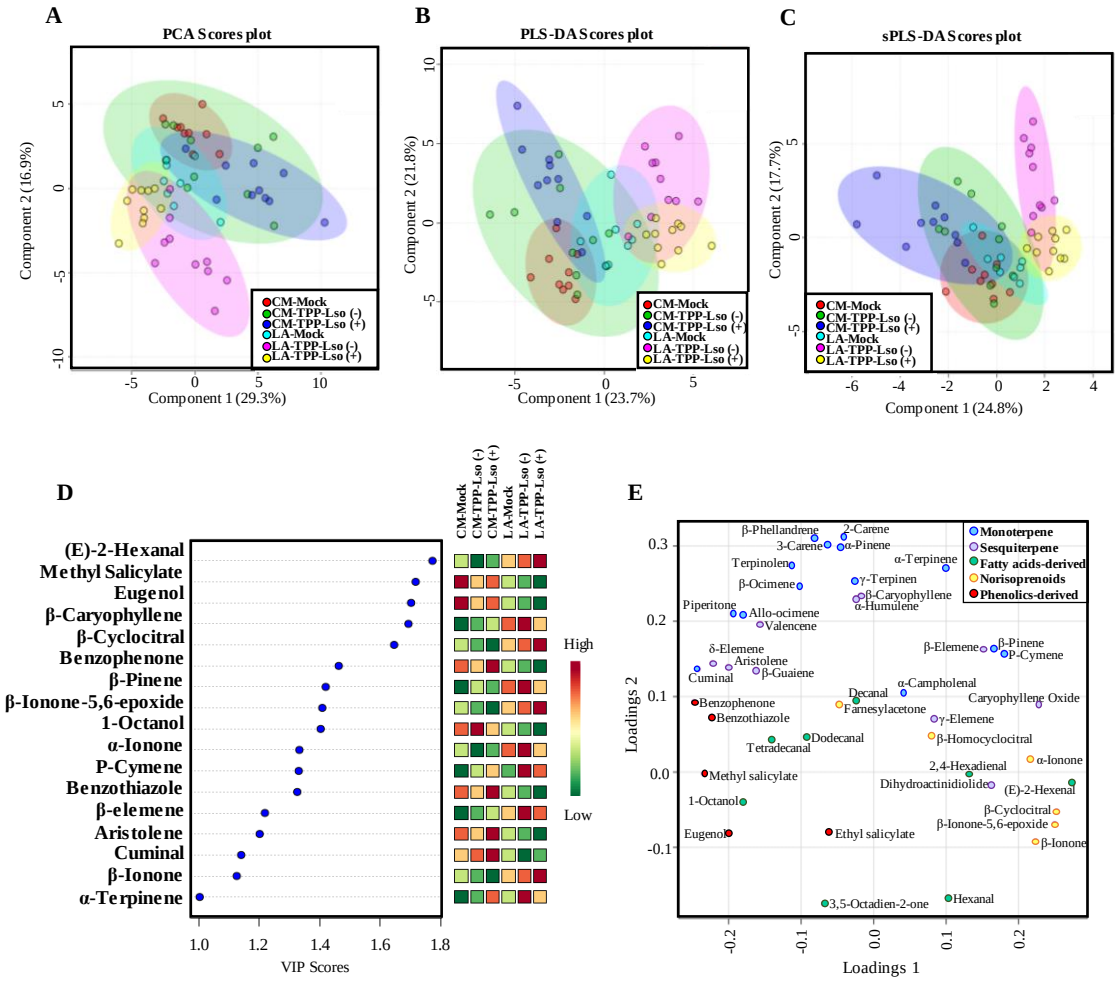

Figure 22. Multivariate analysis using GC-MS dataset to explore the different effect of mock control, TPP-Lso (-), and TPP-Lso (+) inoculations on the insect-susceptible and -resistant tomato varieties, CM (CM) and LA3952 (LA), respectively. Scores plots of (A) PCA, (B) PLS-DA, and (C) sPLS-DA indicate the discrimination between studied test groups. (D) Variable Importance for Projection (VIP) scores derived from a PLS-DA model to examine and filter the variables (VIP > 1.0) having influence on the PLS-DA scores plot. (E) The loading plot illustrates the variables responsible for the separating pattern in the PLS-DA model.

Discussion

The main objective of the present study was aimed to achieve an insight into the metabolic profiles of insect-susceptible and -resistant tomato plants in response to the infestation with tomato-potato psyllids carrying or not its vectored bacteria *Candidatus* Liberibacter solanacearum (Lso). Non-volatile and volatile compounds were identified and quantified using UPLC-QTOF-MS and HS-SPME-GC-MS, respectively.

Tomato phenolic composition is differentially regulated in susceptible and resistant plants in response to TPP and Lso infestation

The phenolic pathway has been previously implicated in the plant defensive mechanism against TPP carrying the Lso in *Solanaceae* crops.[338-339] However, the effect of Lso on TPP regulation of plant phenolic composition in tomato plants has not yet elucidated. Previous studies demonstrated that the difference of phenolic compositions may be genotype-specific [321], and we also found the significant difference of 4-hydroxybenzoic acid and rutin levels based on the control group of varieties (Fig. 17). Similar to our findings in LA3952 variety, significantly herbivore-induced levels of gallic acid and chlorogenic acid were observed in the previous reports.[340-341] Furthermore, the function of chlorogenic acid and rutin related to plant defense against herbivore by deterring insect growth has been reported.[341-342] In addition, regardless of insect resistance or susceptibility, considerably increased amounts of chlorogenic acid on infested plants with TPP-Lso (+) were observed (Fig. 17). Similarly, the level of chlorogenic acid of

Hypericum spp. (*H. perforatum and H. triquetrifolium*) was found to be considerably enhanced after fungal pathogens inoculation, as well as it has been proposed to enhance the tolerance of fungal infection in peach fruits.[329-330]. It has been reported that p-coumaric acid may contribute to plant resistance in response to the pathogen.[343]. In that context, we could not detect it in susceptible plants infested with pathogen-infected TPP, and the suppressed p-coumaric acid level may be involved in the susceptibility (Fig. 17). Meanwhile, in the present study, other compounds such as protocatechuic acid, phthalic acid, and ferulic acid were not substantially different between genotype nor TPP-Lso treatments although they have been implicated in defensive signaling in other plant-insect interaction systems. For example, Nissinen et al. reported that the level change of ferulic acid can be led based on experimental conditions such as insect density and plant growth stage.[328]

Plant defensive hormones play a differential role in resistant and susceptible plants

Phytohormone has been reported to play crucial roles involved in plant development and defense by mediating the interaction between herbivores and plant pathogens.[344] Several studies on the regulatory response of phytohormones-associated metabolic pathway to potato zebra chip disease have been conducted.[322, 345] However, little information is known about the exact role and alteration of these plant hormonal metabolites, and their related pathway in tomato plant in response to TPP carrying or not the Lso are not fully understood.[130] Salicylic acid and jasmonic acid have been considered

as their important functions related to the plant's defense response to the pathogenic attack and disease outbreak.[346] Similar to our findings as significant higher jasmonic acid level in mock and TPP-Lso (+)-inoculated resistant tomato plant than that of susceptible plant, and the previous study observed that highly induced jasmonic acid and its level was retained longer in resistant watermelon than that in the susceptible line.[347] However, exceptions to general rule of salicylic acid and jasmonic acid-mediated signaling pathways in plant defense have been illustrated.[344] On the other hand, certain hormones such as cytokinin, gibberellic acid, auxin, and abscisic acid have been defined with the regulatory role in plant development and growth.[348] In addition to this function, it has been shown to be used in relation to plant immune responses.[344] Yang et. al., reported that gibberellic acid may have a negative role in rice basal disease resistance to bacterial blight disease.[349] Similarly, we found the considerably down-regulated content of gibberellic acid in TPP-Lso (+)-infected LA3952 variety than observed values of its control and TPP-Lso (-) tested plants ($P < 0.01$), whereas the constitutively and significantly up-regulated level was observed in the susceptible plants infested with TPP carrying Lso than control and TPP-Lso (-) infested plant, respectively. In addition, gibberellic acid has been reported as produced by bacterial and fungal pathogens and this metabolite may play a virulent role.[349] Remarkably, indole-3-acetic acid was only detectable in susceptible CM variety, and was constitutively downregulated by TPP infestation independently of Lso. The involvement of indole-3-acetic acid in plant-pathogen interactions has been reported to be linked to plant defense or disease development, and its production can be regulated by plant and

microbes.[350] Similar to our findings, existing literature implicated that indole-3-acetic acid overproducing plants may increase the susceptibility and promote the pathogen growth.[351] However, the variation on defense-associated responses of host plants to psyllid carrying or not the pathogen may be attributed to several factors such as different stages of insect, plant variety, days after infestation, or types of herbivore and/or its harboring pathogens.[130, 324, 352-353] For example, a previous study showed that salicylic acid-mediated defense signaling by upregulating pathogenesis-related 4 (P4) may be elicited in susceptible *S. lycopersicum* C. Money-Maker in response to the pathogen Lso. However, resistant LA3952 plants may regulate P4 expression in response to TPP without vectoring Lso but not to the Lso. Notwithstanding these findings, we observed only genotypic difference of salicylic acid contests but not treated in the present study (Fig. 19).

The different response on the profile of volatile metabolites is elicited by susceptible and resistant plants in response to TPP vectoring or not the Lso.

Role of herbivore-induced plant volatiles involved in indirect defenses

Herbivore-induced plant volatiles have been reported to be involved in indirect defenses in responses to insect herbivory by acting as biochemical cues that attract natural predators [134]. Mainly, these volatiles are derived from terpenoids, fatty acid, and phenylalanine derivatives.[327] In the terpenoids pathway, the initial precursor is C_5 isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) derived from mevalonic acid (MVA) or 2C-methyl-D-erythrito 4-phosphate (MEP) pathways, respectively. Then,

monoterpene (C_{10}) and sesquiterpene (C_{15}) are catalyzed by terpene synthases via geranyl diphosphate (GPP and farnesyl diphosphate (FPP).[354] The different metabolic profiles between susceptible and resistant plants and the defensive function may be attributed to the composition of terpenoids against phloem-feeding insects.[331, 355-357] Similarly, significantly higher contents of five volatile compounds, categorized into monoterpene and sesquiterpene class, were observed in the insect-resistant LA3952 variety than those in the susceptible CM variety (Table 16). Therefore, volatile compounds may be involved in TPP LA3952 based resistance. Moreover, we observed that the blend of volatile metabolites of tomato plants is differently regulated in response to the infestation with TPP carrying or not the Lso (Fig. 20). Earlier literature also demonstrated that genetic and metabolic changes of host plants can be different according to co-infested or not. For example, tomato plants infected with TPP-Lso (-) upregulated genes involved plant defenses regardless of the time-point, whereas TPP-Lso (-)-infected plants showed the initial down-regulation and the delayed the up-regulation of defense-related genes.[130] In addition, terpenoids have been reported as one of the major groups as HIPVs [327], and we also observed that total monoterpene and sesquiterpene abundances were comparatively higher in Lso negative TPP-infested CM variety, and γ-terpinene and δ-elemene levels were significantly increased (Fig. 20B, C, and I). Moreover, γ-terpinene has been reported to possess insecticidal and larvicidal activity.[358-359] However, HIPVs were not emitted from TPP infestation in LA3952 variety, and it may imply that the herbivore attack does not significantly influence insect-resistant plants to produce HIPVs (Fig. 20J).

The response of volatile metabolites to plant pathogen

Vector-borne bacterial pathogens transmitted to host plants by insects as vectors, and earlier studies highlighted that pathogens transferred by insects may modulate both insect fitness and plant host defensive signaling. For instance, Lso may modulate gene expression in tomato plants, alter the blend of volatile compounds, and finally influence insect behavior as its vector for increasing inoculation and acquisition rates.[324] Moreover, plant pathogens interact with their insect vectors directly and indirectly via host plants and the interaction may be involved in their fitness benefits.[360] It has been reported that pathogens have been found to modulate volatile blends and promote the mutualism with their vectors by suppressing terpenoid synthesis, producing toxic or deterrent compounds to various types of organisms, in host plant, and, consequently, vectors can perform better and pathogen spread and transmit themselves onto plants.[327, 331] Conversely, β-ionone, β-cyclohomociral, hexanal, and (E)-2-hexenal levels were significantly elevated in the TPP-Lso (+)-infected LA3952 group (Fig. 20J). Amongst 20them, β-ionone and β-cyclohomociral are classified in norisoprenoids, which are mainly derived by cleaving carotenoid compounds by sharing IPP and DMAPP as primary precursors and their biosynthesis involved in methylerythritol phosphate (MEP) pathway.[361] Similarly, β-ionone promotes resistance of tobacco plant against pathogen by functioning either as a signal or as the inducer of signal release in treated plants.[362-363] Fatty acids-derived volatiles are produced from C_{18} unsaturated linoleic acid or linolenic acids as substrates, and green leaf volatiles (GLVs) such as hexanal and (E)-2-hexenal are reported to play

roles involved in plant fitness against herbivore or its interaction with pathogen.[364] For instance, earlier literature underlined that these C_6-aldehydes may inhibit the plant pathogen germination and protect plants from pathogenic infections.[365-366] Furthermore, pathogen-induced (E)-2-hexenal has been reported to possess antimycobacterial activity.[367] Finally, phenylpropanoids or benzenoids volatile compounds are mainly derived from phenylalanine as a substrate in the shikimic acid pathway and these volatiles and are also considered as involved in resistance to biotic and abiotic stresses.[327] In the present study, phenylpropanoids/benzenoids volatile metabolites were significantly decreased after infested with both TPP carrying or not the pathogen (Fig. 20J). In the same context, the previous study elucidated that the suppression of plant defense reactions was observed in pathogen-infected sugar beet (*Beta vulgaris* L.) leaves during the early disease development by the reduced gene expressions related in phenylpropanoids/benzenoids synthetic pathway.[368] Taken together, findings imply that insect-susceptible and resistant tomato plants in response of Lso negative or positive TPP may result in different defensive reaction of HIVPs by showing higher terpenoids (C_{10} and C_{15}) in TPP-Lso (-)-infected CM tomato plant, whereas the considerable reduction of volatile compounds categorized in fatty acid-derived GLVs, norisoprenoids, and phenylpropanoids/benzenoids in LA3952 variety with infected with TPP carrying or not the pathogen (Fig. 20I and 20J).

Multivariate analysis of TPP-Lso treatment on susceptible and resistant tomato plants

Metabolomic approaches have been considered as efficient techniques to examine the profiles of metabolic changes in response to herbivore or pathogen infection and identify responsible metabolite associated with resistance and susceptibility.[332, 369-371] Mainly, PCA is using as an unsupervised pattern recognition tool is used for a potential discrimination pattern, and supervised PLS-DA and sPLS-DA are applied to classify the assessed variables and identify metabolite markers.[332] In the present study, these chemometric methods were performed to investigate the influence of test inoculations in volatile metabolic changes of resistant and susceptible tomato plants, and major two clusters were distinguished between assigned groups of observations (Fig. 21A-21C). VIP scores plots filtered observed variables with higher relative abundances of six compound levels such as (E)-2-hexenal, β-cyclocitral, β-ionone-5,6-epoxide, p-cymene, β-elemene, and β-ionone in the second cluster (Fig. 21D). Amongst them, (E)-2-hexenal and β-cyclohomocitral, and β-ionone were confirmed that they were significantly induced after infection with TPP-Lso (+) in resistant plants (Fig. 20J). Therefore, these metabolites can be possible pathogen-responsive biomarkers to distinguish susceptible and resistant tomato in response to TPP carrying Lso or not. However, these potential biomarkers need to be further investigated on more tolerance variety and different stage of infection.

CHAPTER IX

SUMMARY AND CONCLUSIONS

In the present study, the effect genotypes and growing (OF and NH) conditions on phytochemical and biofunctional qualities of tomato fruits were investigated. Initially, HS-SPME/GC-MS method was optimized to prepare the volatile metabolite profiles of fresh-blended tomato samples. The optimal SPME parameters were found to be 2 g of tomato sample, 50/30 µm DVB/CAR/PDMS-coated fiber, and extraction at 60°C for 45 min. This optimized method in tandem with chemometrics was used to characterize the volatile compounds from four varieties grown under the OF and NH production systems to identify the production system-specific volatile markers. In this study, β-damascenone and geranylacetone were identified as potential volatile markers to distinguish high-tunnel and OF grown tomatoes. However, the production system specific markers may also be influenced by genotype, growing conditions, and harvest periods. Therefore, further studies focusing on more varieties grown in different locations are warranted for the discovery of other possible biomarkers.

In another study, the volatile profiles of three local Texas A&M University (TAMU) and five commercial tomato varieties grown in NH and OF conditions were analyzed by HS-SPME/GC-MS methods, and the individual and collective influences of genotype and the two production systems were studied using univariate and multivariate chemometric approaches. The results of this study indicated that local TAMU varieties

were distinguished from commercial varieties tested in terms of volatiles, and contents of consumers liking and flavor intensity related volatiles, (1-penten-3-one, (E)-2-heptenal, (E)-3-hexen-1-ol, 2-isobutylthiazole, and 6-methyl-5-penten-3-one) were significantly affected by production systems. In addition, this information may be valuable to recommend tomato varieties and cultivation practices to improve or preserve desirable tomato flavor components. Furthermore, season also may be influential factor and should be confirmed to evaluate volatile markers based on the harvest seasons.

In addition to tomato volatiles, we have evaluated changes in the physicochemical characteristics and health-promoting compounds of tomato fruits according to different varieties and production systems. The results of this study clearly showed tomato genotype governs the fruit quality traits individually as well as with cultivating practices. Moreover, production systems affected the tomato peel color, fruit qualities, and the levels of all-trans-ß-carotene and 13-cis-lycopene. However, no significant differences between the contents of ascorbic acid, 9-cis-lycopene, all-trans-lycopene, and 5-cis-lycopene were observed between the OF and NH production systems. Altogether, findings of this study underline the importance of the rational choice of variety and environmental conditions together to get desired quality traits in tomato fruits.

Similarly, we also have demonstrated that the OF and NH growing distinctively affected indoleamines, phenolic acids, and flavonoids in eight tomato varieties, including total phenolics and antioxidant activities. The range of melatonin and serotonin contents from all studied tomatoes was from 0.09 to 0.28 μg g^{-1} DW and 37.2–129.7 μg g^{-1} DW,

respectively. This finding indicates that the impact of OF and NH growing on tomato melatonin and serotonin levels may be distinct based on genotype. Further considerable increase in melatonin was detected from OF grown Mykonos, while DRP-8551 had significantly higher melatonin levels from the NH production system. In regard to serotonin, two tomato varieties, T3 and L501-55, were significantly enhanced in the OF condition than those of the NH system. The quantitative distinctions in phenolic composition among tomato genotypes underscore the significance of choosing variety and production system for cultivating tomatoes based on the desired constituent. Further research may be needed to provide more comprehensive information about tomato phenolic composition and their response to production systems.

Alike to phytochemicals, the impact of OF and NH on tomato PPO and POD activities including the inhibitory potential of certain volatile compounds were examined. Herein, we have described the extraction procedure for partially purified PPO and POD enzymes from tomato fruits. For the PPO assay, 150 mM of catechol and pH 7.0 were the optimal condition for maximum activity. Conversely, we found 24 mM guaiacol with 12 mM H_2O_2 and pH 6.0 was the best condition for the POD assay. Thermal inactivation studies confirmed that tomato POD is more resistant to heat than PPO, and both enzymes were found to be inactivated about 90% at 85 °C and 60 min and 75 °C and 30 min, respectively. Moreover, obtained kinetic parameters based on thermal inactivation from this study can be helpful and useful in developing processing tomato products in the industry. Uniquely, this study shows, tomato aroma volatiles such as β-damascenone and

d-limonene have considerable PPO inhibitory potential. The overall findings of this study highlighted the significant impacts of growing conditions on defense-related, and shelf-life linked enzymes, PPO and POD.

In another distinct study, the metabolic changes (volatiles, hormone, and phenolics) were evaluated in TPP carrying or not Lso infection of susceptible and resistant tomato plants. We found that different volatile profiles for susceptible and resistant varieties based on test inoculations. Regard to HIPVs such as monoterpene and sesquiterpene abundance had considerably enhanced in susceptible variety infected with TPP without pathogen infection. However, this phenomenon was not found in the resistant variety, instead that, GLVs, including hexenal and (E)-2-hexenal levels were substantially elevated in the TPP-Lso (+) inoculated resistant groups. The jasmonic acid level was found to be significantly increased in TPP-Lso (+)-inoculated LA3952 group than the level of only TPP-infested group. However, no significant changes of salicylic acid according to treatment in the present study. Multivariate analysis further confirmed that the influence of these compounds on differentiating the observed group separations, which may be helpful for screening and selecting TPP carrying or not the pathogen varieties.

REFERENCES

1. Peralta, I. E.; Spooner, D. M., History, origin and early cultivation of tomato (Solanaceae). *Genetic improvement of solanaceous crops* **2007,** *2*, 1-27.

2. Davies, J. N.; Hobson, G. E.; McGlasson, W. B., The constituents of tomato fruit — the influence of environment, nutrition, and genotype. *C R C Critical Reviews in Food Science and Nutrition* **1981,** *15* (3), 205-280.

3. FAOSTAT, Food and Agriculture Organization Corporate Statistical Database URL: http://www.fao.org/home. **2017**.

4. Tieman, D.; Zhu, G.; Resende, M. F., Jr.; Lin, T.; Nguyen, C.; Bies, D.; Rambla, J. L.; Beltran, K. S.; Taylor, M.; Zhang, B.; Ikeda, H.; Liu, Z.; Fisher, J.; Zemach, I.; Monforte, A.; Zamir, D.; Granell, A.; Kirst, M.; Huang, S.; Klee, H., A chemical genetic roadmap to improved tomato flavor. *Science* **2017,** *355* (6323), 391-394.

5. Wang, D.; Seymour, G. B., Tomato Flavor: Lost and Found? *Molecular Plant* **2017,** *10* (6), 782-784.

6. Slimestad, R.; Verheul, M. J., Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes. *Journal of Agricultural and Food Chemistry* **2005,** *53* (8), 3114-9.

7. Erland, L. A.; Murch, S. J.; Reiter, R. J.; Saxena, P. K., A new balancing act: the many roles of melatonin and serotonin in plant growth and development. *Plant signaling & behavior* **2015,** *10* (11), e1096469.

8. Khachik, F.; Carvalho, L.; Bernstein, P. S.; Muir, G. J.; Zhao, D.-Y.; Katz, N. B., Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Experimental Biology and Medicine* **2002,** *227* (10), 845-851.

9. Giovannucci, E., Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of the national cancer institute* **1999,** *91* (4), 317-331.

10. Marston, A.; Hostettmann, K.; Andersen, Ø.; Markham, K., Separation and quantification of flavonoids. *Flavonoids: Chemistry, biochemistry and applications* **2006**, 1-36.

11. McGuire, S., US Department of Agriculture and US Department of Health and Human Services, Dietary Guidelines for Americans, 2010. Washington, DC: US Government Printing Office, January 2011. *Advances in Nutrition: An International Review Journal* **2011,** *2* (3), 293-294.

12. Xiao, Z.; Wu, Q.; Niu, Y.; Liu, Q.; Chen, F.; Ma, N.; Zhou, X.; Zhu, J., Optimization of Headspace Solid-Phase Micro-Extraction and Its Application in Analysis of Volatile Compounds in Cherry Tomato by Gas Chromatography. *Food Analytical Methods* **2016**, 1-14.

13. Maul, F.; Sargent, S. A.; Sims, C. A.; Baldwin, E. A.; Balaban, M. O.; Huber, D. J., Tomato flavor and aroma quality as affected by storage temperature. *Journal of Food Science* **2000,** *65* (7), 1228-1237.

14. Klee, H. J., Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology. *New Phytologist* **2010,** *187* (1), 44-56.

15. Hayase, F.; Chung, T.-Y.; Kato, H., Changes of volatile components of tomato fruits during ripening. *Food Chemistry* **1984,** *14* (2), 113-124.

16. Mayer, F.; Takeoka, G.; Buttery, R.; Whitehand, L.; Bezman, Y.; Naim, M.; Rabinowitch, H.; Deibler, K.; Delwiche, J., Differences in the aroma of selected fresh tomato cultivars. *Handbook of flavor characterization. Sensory analysis, chemistry, and physiology* **2004**, 189-205.

17. Wright, D. H.; Harris, N. D., Effect of nitrogen and potassium fertilization on tomato flavor. *Journal of Agricultural and Food Chemistry* **1985,** *33* (3), 355-358.

18. Stern, D. J.; Buttery, R. G.; Teranishi, R.; Ling, L.; Scott, K.; Cantwell, M., Effect of storage and ripening on fresh tomato quality, Part I. *Food Chemistry* **1994,** *49* (3), 225-231.

19. Kandel, D. R.; Bedre, R. H.; Mandadi, K. K.; Crosby, K.; Avila, C. A., Genetic Diversity and Population Structure of Tomato (Solanum lycopersicum) Germplasm Developed by Texas A&M Breeding Programs. *American Journal of Plant Sciences* **2019,** *10* (7), 1154-1180.

20. Arin, L.; Ankara, S., Effect of low-tunnel, mulch and pruning on the yield and earliness of tomato in unheated glasshouse. *J. Appl. Hort* **2001,** *3* (1), 23-27.

21. Carey, E. E.; Jett, L.; Lamont, W. J.; Nennich, T. T.; Orzolek, M. D.; Williams, K. A., Horticultural crop production in high tunnels in the United States: A snapshot. *HortTechnology* **2009,** *19* (1), 37-43.

22. Wittwer, S. H.; Castilla, N., Protected cultivation of horticultural crops worldwide. *HortTechnology* **1995,** *5* (1), 6-23.

23. Del Giudice, R.; Petruk, G.; Raiola, A.; Barone, A.; Monti, D. M.; Rigano, M. M., Carotenoids in fresh and processed tomato (Solanum lycopersicum) fruits protect cells from oxidative stress injury. *J. Sci. Food Agric.* **2017,** *97* (5), 1616-1623.

24. Spagna, G.; Barbagallo, R. N.; Chisari, M.; Branca, F., Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *J. Agric. Food Chem.* **2005,** *53* (6), 2032-8.

25. Zhang, X. L.; Shao, X. F., Characterisation of Polyphenol Oxidase and Peroxidase and the Role in Browning of Loquat Fruit. *Czech J. Food Sci.* **2015,** *33* (2), 109-117.

26. Strange, R. N.; Scott, P. R., Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.* **2005,** *43*, 83-116.

27. Bhandari, S. R.; Cho, M. C.; Lee, J. G., Genotypic variation in carotenoid, ascorbic acid, total phenolic, and flavonoid contents, and antioxidant activity in selected tomato breeding lines. *Hortic Environ Biote* **2016,** *57* (5), 440-452.

28. Liu, T.; Zhu, W.; Huang, J.; Chen, H.; Nie, R.; Li, C.-m., Comparison of the nutritional as well as the volatile composition of in-season and off-season Hezuo 903 tomato at red stage. *Eur Food Res Technol* **2016,** *243* (2), 203-214.

29. Baldwin, E. A.; Scott, J. W.; Bai, J. H., Sensory and Chemical Flavor Analyses of Tomato Genotypes Grown in Florida during Three Different Growing Seasons in Multiple Years. *Journal of the American Society for Horticultural Science* **2015,** *140* (5), 490-503.

30. Torres, P.; Galleguillos, P.; Lissi, E.; López-Alarcón, C., Antioxidant capacity of human blood plasma and human urine: Simultaneous evaluation of the ORAC index and ascorbic acid concentration employing pyrogallol red as probe. *Bioorganic & medicinal chemistry* **2008,** *16* (20), 9171-9175.

31. Borguini, R. G.; Ferraz da Silva Torres, E. A., Tomatoes and tomato products as dietary sources of antioxidants. *Food Reviews International* **2009,** *25* (4), 313-325.

32. Vissers, M. C.; Bozonet, S. M.; Pearson, J. F.; Braithwaite, L. J., Dietary ascorbate intake affects steady state tissue concentrations in vitamin C–deficient mice: tissue deficiency after suboptimal intake and superior bioavailability from a food source (kiwifruit). *The American journal of clinical nutrition* **2010,** *93* (2), 292-301.

33. Gallie, D. R., The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. *Journal of experimental botany* **2013,** *64* (2), 433-443.

34. Singh, S.; Majumdar, D. K., Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil). *Journal of Ethnopharmacology* **1999,** *65* (1), 13-19.

35. Davey, M. W.; Montagu, M. v.; Inzé, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I. J. J.; Strain, J. J.; Favell, D.; Fletcher, J., Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture* **2000,** *80* (7), 825-860.

36. Lee, S. K.; Kader, A. A., Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest biology and technology* **2000,** *20* (3), 207-220.

37. Bhandari, S. R.; Lee, J. G., Ripening-Dependent Changes in Antioxidants, Color Attributes, and Antioxidant Activity of Seven Tomato (Solanum lycopersicum L.) Cultivars. *J Anal Methods Chem* **2016,** *2016*, 5498618.

38. Sass-Kiss, A.; Kiss, J.; Milotay, P.; Kerek, M.; Toth-Markus, M., Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International* **2005,** *38* (8-9), 1023-1029.

39. Perveen, R.; Suleria, H. A. R.; Anjum, F. M.; Butt, M. S.; Pasha, I.; Ahmad, S., Tomato (Solanum lycopersicum) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims—a comprehensive review. *Critical reviews in food science and nutrition* **2015,** *55* (7), 919-929.

40. Schieber, A.; Carle, R., Occurrence of carotenoid cis-isomers in food: technological, analytical, and nutritional implications. *Trends in Food Science & Technology* **2005,** *16* (9), 416-422.

41. Amiri-Rigi, A.; Abbasi, S., Microemulsion-based lycopene extraction: effect of surfactants, co-surfactants and pretreatments. *Food chemistry* **2016,** *197*, 1002-1007.

42. Dumas, Y.; Dadomo, M.; Di Lucca, G.; Grolier, P., Effects of environmental factors and agricultural techniques on antioxidantcontent of tomatoes. *Journal of the Science of Food and Agriculture* **2003,** *83* (5), 369-382.

43. Khachik, F.; Beecher, G. R.; Smith, J. C., Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *Journal of Cellular Biochemistry* **1995,** *59* (S22), 236-246.

44. Clinton, S. K.; Emenhiser, C.; Schwartz, S. J.; Bostwick, D. G.; Williams, A. W.; Moore, B. J.; Erdman, J. W., cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiology and Prevention Biomarkers* **1996,** *5* (10), 823-833.

45. Schmitz, H. H.; Poor, C. L.; Wellman, R.; Erdman Jr, J. W., Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. *J Nutr* **1991,** *121* (10), 1613-1621.

46. Gärtner, C.; Stahl, W.; Sies, H., Preferential increase in chylomicron levels of the xanthophylls lutein and zeaxanthin compared to beta-carotene in the human. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung. Journal international de vitaminologie et de nutrition* **1995,** *66* (2), 119-125.

47. Boileau, A. C.; Merchen, N. R.; Wasson, K.; Atkinson, C. A.; Erdman, J. W., Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. *The Journal of nutrition* **1999,** *129* (6), 1176-1181.

48. Martínez-Valverde, I.; Periago, M. J.; Provan, G.; Chesson, A., Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture* **2002,** *82* (3), 323-330.

49. Robak, J.; Gryglewski, R. J., Flavonoids are scavengers of superoxide anions. *Biochemical pharmacology* **1988,** *37* (5), 837-841.

50. So, F. V.; Guthrie, N.; Chambers, A. F.; Moussa, M.; Carroll, K. K., Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. **1996**.

51. Crozier, A.; Jaganath, I. B.; Clifford, M. N., Dietary phenolics: chemistry, bioavailability and effects on health. *Natural product reports* **2009,** *26* (8), 1001-1043.

52. Slimestad, R.; Verheul, M., Review of flavonoids and other phenolics from fruits of different tomato (Lycopersicon esculentum Mill.) cultivars. *Journal of the Science of Food and Agriculture* **2009,** *89* (8), 1255-1270.

53. Slimestad, R.; Verheul, M. J., Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes. *Journal of agricultural and food chemistry* **2005,** *53* (8), 3114-3119.

54. Slimestad, R.; Verheul, M. J., Content of chalconaringenin and chlorogenic acid in cherry tomatoes is strongly reduced during postharvest ripening. *Journal of agricultural and food chemistry* **2005,** *53* (18), 7251-7256.

55. Muir, S. R.; Collins, G. J.; Robinson, S.; Hughes, S.; Bovy, A.; De Vos, C. R.; van Tunen, A. J.; Verhoeyen, M. E., Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nature biotechnology* **2001,** *19* (5), 470-474.

56. Krause, M.; Galensa, R., Determination of naringenin and naringenin-chalcone in tomato skins by reversed phase HPLC after solid-phase extraction. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **1992,** *194* (1), 29-32.

57. Farah, A.; Donangelo, C. M., Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology* **2006,** *18* (1), 23-36.

58. De Bruyn, J.; Garretsen, F.; Kooistra, E., Variation in taste and chemical composition of the tomato (Lycopersicon esculentum Mill.). *Euphytica* **1971,** *20* (2), 214-227.

59. Walker, J., Phenolic acids in ‚cloud' and normal tomato fruit wall tissue. *Journal of the Science of Food and Agriculture* **1962,** *13* (7), 363-367.

60. Wardale, D. A., Effect of phenolic compounds in Lycopersicon esculeutum on the synthesis of ethylene. *Phytochemistry* **1973,** *12* (7), 1523-1530.

61. Vallverdu-Queralt, A.; Medina-Remon, A.; Martínez-Huélamo, M.; Jáuregui, O.; Andres-Lacueva, C.; Lamuela-Raventos, R. M., Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *Journal of agricultural and food chemistry* **2011,** *59* (8), 3994-4001.

62. Luthria, D. L.; Mukhopadhyay, S.; Krizek, D. T., Content of total phenolics and phenolic acids in tomato (Lycopersicon esculentum Mill.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis* **2006,** *19* (8), 771-777.

63. Erland, L. A.; Turi, C. E.; Saxena, P. K., Serotonin: An ancient molecule and an important regulator of plant processes. *Biotechnology advances* **2016,** *34* (8), 1347-1361.

64. Erland, L. A. E.; Turi, C. E.; Saxena, P. K., Serotonin: An ancient molecule and an important regulator of plant processes. *Biotechnology Advances* **2016,** *34* (8), 1347-1361.

65. Arnao, M. B.; Hernández-Ruiz, J., Growth conditions influence the melatonin content of tomato plants. *Food chemistry* **2013,** *138* (2), 1212-1214.

66. Stürtz, M.; Cerezo, A. B.; Cantos-Villar, E.; Garcia-Parrilla, M. C., Determination of the melatonin content of different varieties of tomatoes (Lycopersicon esculentum) and strawberries (Fragaria ananassa). *Food Chemistry* **2011,** *127* (3), 1329-1334.

67. Riga, P.; Medina, S.; García-Flores, L. A.; Gil-Izquierdo, Á., Melatonin content of pepper and tomato fruits: Effects of cultivar and solar radiation. *Food Chemistry* **2014,** *156*, 347-352.

68. Hano, S.; Shibuya, T.; Imoto, N.; Ito, A.; Imanishi, S.; Aso, H.; Kanayama, Y., Serotonin content in fresh and processed tomatoes and its accumulation during fruit development. *Scientia Horticulturae* **2017,** *214*, 107-113.

69. Murch, S. J.; Saxena, P. K., Melatonin: a potential regulator of plant growth and development? *In Vitro Cellular & Developmental Biology-Plant* **2002,** *38* (6), 531-536.

70. Murch, S. J.; Campbell, S. S.; Saxena, P. K., The role of serotonin and melatonin in plant morphogenesis: regulation of auxin-induced root organogenesis in in vitro-cultured explants of St. John's wort (Hypericum perforatum L.). *In Vitro Cellular & Developmental Biology-Plant* **2001,** *37* (6), 786-793.

71. Baldwin, E. A.; Goodner, K.; Plotto, A., Interaction of Volatiles, Sugars, and Acids on Perception of Tomato Aroma and Flavor Descriptors. *Journal of Food Science* **2008,** *73* (6), S294-S307.

72. Petro-Turza, M., Flavor of tomato and tomato products. *Food Reviews International* **1986,** *2* (3), 309-351.

73. Buttery, R., Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. *Flavor science: Sensible principles and techniques* **1993**, 259-286.

74. Abegaz, E. G.; Tandon, K. S.; Scott, J. W.; Baldwin, E. A.; Shewfelt, R. L., Partitioning taste from aromatic flavor notes of fresh tomato (Lycopersicon esculentum, Mill) to develop predictive models as a function of volatile and nonvolatile components. *Postharvest Biology and Technology* **2004,** *34* (3), 227-235.

75. Tieman, D.; Bliss, P.; McIntyre, Lauren M.; Blandon-Ubeda, A.; Bies, D.; Odabasi, Asli Z.; Rodríguez, Gustavo R.; van der Knaap, E.; Taylor, Mark G.; Goulet, C.; Mageroy, Melissa H.; Snyder, Derek J.; Colquhoun, T.; Moskowitz, H.; Clark, David G.; Sims, C.; Bartoshuk, L.; Klee, Harry J., The Chemical Interactions Underlying Tomato Flavor Preferences. *Current Biology* **2012,** *22* (11), 1035-1039.

76. Klee, H. J.; Tieman, D. M., Genetic challenges of flavor improvement in tomato. *Trends in Genetics* **2013,** *29* (4), 257-262.

77. Alexander, L.; Grierson, D., Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany* **2002,** *53* (377), 2039-2055.

78. Buttery, R. G.; Ling, L. C. In *Volatile compounds of tomato fruit and plant parts: relationship and biogenesis*, ACS symposium series (USA), 1993.

79. Goff, S. A.; Klee, H. J., Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value? *Science* **2006,** *311* (5762), 815-819.

80. Brauss, M. S.; Linforth, R. S. T.; Taylor, A. J., Effect of Variety, Time of Eating, and Fruit-to-Fruit Variation on Volatile Release during Eating of Tomato Fruits (Lycopersicon esculentum). *Journal of Agricultural and Food Chemistry* **1998,** *46* (6), 2287-2292.

81. Galliard, T.; Matthew, J. A.; Wright, A. J.; Fishwick, M. J., The enzymic breakdown of lipids to volatile and non-volatile carbonyl fragments in disrupted tomato fruits. *Journal of the Science of Food and Agriculture* **1977,** *28* (9), 863-868.

82. Ayseli, M. T.; İpek Ayseli, Y., Flavors of the future: Health benefits of flavor precursors and volatile compounds in plant foods. *Trends in Food Science & Technology* **2016,** *48*, 69-77.

83. Rekha, K. R.; Selvakumar, G. P.; Sethupathy, S.; Santha, K.; Sivakamasundari, R. I., Geraniol Ameliorates the Motor Behavior and Neurotrophic Factors Inadequacy in MPTP-Induced Mice Model of Parkinson's Disease. *Journal of Molecular Neuroscience* **2013,** *51* (3), 851-862.

84. Boulogne, I.; Petit, P.; Ozier-Lafontaine, H.; Desfontaines, L.; Loranger-Merciris, G., Insecticidal and antifungal chemicals produced by plants: a review. *Environmental Chemistry Letters* **2012,** *10* (4), 325-347.

85. Hubert, J.; Münzbergová, Z.; Santino, A., Plant volatile aldehydes as natural insecticides against stored-product beetles. *Pest Management Science* **2008,** *64* (1), 57-64.

86. Fadida, T.; Selilat-Weiss, A.; Poverenov, E., N-hexylimine-chitosan, a biodegradable and covalently stabilized source of volatile, antimicrobial hexanal. Next generation controlled-release system. *Food Hydrocolloids* **2015,** *48*, 213-219.

87. Lanciotti, R.; Gianotti, A.; Patrignani, F.; Belletti, N.; Guerzoni, M. E.; Gardini, F., Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in Food Science & Technology* **2004,** *15* (3), 201-208.

88. Davidovich-Rikanati, R.; Sitrit, Y.; Tadmor, Y.; Iijima, Y.; Bilenko, N.; Bar, E.; Carmona, B.; Fallik, E.; Dudai, N.; Simon, J. E.; Pichersky, E.; Lewinsohn, E., Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. *Nature Biotechnology* **2007,** *25* (8), 899-901.

89. Keiler, A. M.; Zierau, O.; Kretzschmar, G., Hop extracts and hop substances in treatment of menopausal complaints. *Planta medica* **2013,** *79* (07), 576-579.

90. Li, Y.; Lv, O.; Zhou, F.; Li, Q.; Wu, Z.; Zheng, Y., Linalool Inhibits LPS-Induced Inflammation in BV2 Microglia Cells by Activating Nrf2. *Neurochemical Research* **2015,** *40* (7), 1520-1525.

91. Hunter, B.; Drost, D.; Black, B.; Ward, R., Improving growth and productivity of early-season high-tunnel tomatoes with targeted temperature additions. *HortScience* **2012,** *47* (6), 733-740.

92. Gosselin, A.; Trudel, M.-J., Influence of root-zone temperature on growth, development and yield of cucumber plants cv. Toska. *Plant and soil* **1985,** *85* (3), 327-336.

93. Hurewitz, J.; Maletta, M.; Janes, H. In *The effects of root-zone heating at normal and cool air temperatures on growth and photosynthetic rates of tomatoes*, III International Symposium on Energy in Protected Cultivation 148, 1983; pp 871-876.

94. Shedlosky, M.; White, J., Growth of bedding plants in response to root-zone heating anf night temperature regimes. *Journal of the American Society for Horticultural Science* **1987,** *112* (2), 290-295.

95. Healy, G.; Emerson, B.; Dawson, J., Tomato variety trials for productivity and quality in organic hoop house versus open field management. *Renewable Agriculture and Food Systems* **2017**, 1-11.

96. Wells, O., Guidelines for using high tunnels for tomato production. *Univ. of New Hampshire Coop. Ext. Publ* **1991,** *3*, 92-95.

97. Kadir, S.; Carey, E.; Ennahli, S., Influence of high tunnel and field conditions on strawberry growth and development. *HortScience* **2006,** *41* (2), 329-335.

98. Rowley, D.; Black, B. L.; Drost, D.; Feuz, D., Early-season extension using June-bearing 'Chandler'strawberry in high-elevation high tunnels. *HortScience* **2010,** *45* (10), 1464-1469.

99. Salamé-Donoso, T. P.; Santos, B. M.; Chandler, C. K.; Sargent, S. A., Effect of high tunnels on the growth, yields, and soluble solids of strawberry cultivars in Florida. *International Journal of Fruit Science* **2010,** *10* (3), 249-263.

100. O'Connell, S.; Rivard, C.; Peet, M. M.; Harlow, C.; Louws, F., High tunnel and field production of organic heirloom tomatoes: Yield, fruit quality, disease, and microclimate. *HortScience* **2012,** *47* (9), 1283-1290.

101. Lopez-Andreu, F.; Lamela, A.; Esteban, R.; Collado, J. In *Evolution of quality parameters in the maturation stage of tomato fruit*, Symposium on Protected Cultivation of Solanacea in Mild Winter Climates 191, 1985; pp 387-394.

102. Wilkens, R. T.; Spoerke, J. M.; Stamp, N. E., Differential responses of growth and two soluble phenolics of tomato to resource availability. *Ecology* **1996,** *77* (1), 247-258.

103. Abushita, A. A.; Daood, H. G.; Biacs, P. A., Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry* **2000,** *48* (6), 2075-2081.

104. Raffo, A.; Leonardi, C.; Fogliano, V.; Ambrosino, P.; Salucci, M.; Gennaro, L.; Bugianesi, R.; Giuffrida, F.; Quaglia, G., Nutritional value of cherry tomatoes (Lycopersicon esculentum cv. Naomi F1) harvested at different ripening stages. *Journal of Agricultural and Food Chemistry* **2002,** *50* (22), 6550-6556.

105. Sadler, G.; Davis, J.; Dezman, D., Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *Journal of food science* **1990,** *55* (5), 1460-1461.

106. Landl, A.; Abadias, M.; Sárraga, C.; Viñas, I.; Picouet, P., Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. *Innovative Food Science & Emerging Technologies* **2010,** *11* (4), 557-564.

107. Taranto, F.; Pasqualone, A.; Mangini, G.; Tripodi, P.; Miazzi, M. M.; Pavan, S.; Montemurro, C., Polyphenol Oxidases in Crops: Biochemical, Physiological and Genetic Aspects. *Int. J. Mol. Sci.* **2017,** *18* (2), 377.

108. Terefe, N. S.; Buckow, R.; Versteeg, C., Quality-related enzymes in fruit and vegetable products: effects of novel food processing technologies, part 1: high-pressure processing. *Critical Reviews in Food Science and Nutrition* **2014,** *54* (1), 24-63.

109. Oliveira, C. M.; Ferreira, A. C. S.; De Freitas, V.; Silva, A. M., Oxidation mechanisms occurring in wines. *Food Research International* **2011,** *44* (5), 1115-1126.

110. Batista, K. A.; Batista, G. L. A.; Alves, G. L.; Fernandes, K. F., Extraction, partial purification and characterization of polyphenol oxidase from Solanum lycocarpum fruits. *J. Mol. Catal. B Enzym.* **2014,** *102*, 211-217.

111. Taranto, F.; Pasqualone, A.; Mangini, G.; Tripodi, P.; Miazzi, M.; Pavan, S.; Montemurro, C., Polyphenol oxidases in crops: biochemical, physiological and genetic aspects. *International journal of molecular sciences* **2017,** *18* (2), 377.

112. Onsa, G. H.; bin Saari, N.; Selamat, J.; Bakar, J., Purification and characterization of membrane-bound peroxidases from Metroxylon sagu. *Food Chemistry* **2004,** *85* (3), 365-376.

113. Badawy, M. E.; Rabea, E. I., Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. *Postharvest Biology and Technology* **2009,** *51* (1), 110-117.

114. Jang, J. H.; Moon, K. D., Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. *Food Chem* **2011,** *124* (2), 444-449.

115. Friedman, M., Food browning and its prevention: an overview. *Journal of Agricultural and Food chemistry* **1996,** *44* (3), 631-653.

116. De Corato, U.; Cancellara, F. A., Measures, technologies, and incentives for cleaning the minimally processed fruits and vegetables supply chain in the Italian food industry. *Journal of Cleaner Production* **2019,** *237*, 117735.

117. Pankaj, S. K.; Misra, N. N.; Cullen, P. J., Kinetics of tomato peroxidase inactivation by atmospheric pressure cold plasma based on dielectric barrier discharge. *Innov. Food Sci. Emerg. Technol.* **2013,** *19*, 153-157.

118. Tinello, F.; Lante, A., Recent advances in controlling polyphenol oxidase activity of fruit and vegetable products. *Innovative food science & emerging technologies* **2018**.

119. Benaceur, F.; Gouzi, H.; Meddah, B.; Neifar, A.; Guergouri, A., Purification and characterization of catechol oxidase from Tadela (Phoenix dactylifera L.) date fruit. *International journal of biological macromolecules* **2019,** *125*, 1248-1256.

120. Sommano, S. In *Extraction of active polyphenol oxidase and peroxidase from a plant rich in phenolics: Backhousia myrtifolia*, II Southeast Asia Symposium on Quality Management in Postharvest Systems 1088, 2013; pp 547-551.

121. Mantovani, C.; Clemente, E., Peroxidase and polyphenoloxidase activity in tomato in natura and tomato purée. *Acta Scientiarum. Technology* **2010,** *32* (1), 91-97.

122. Hernández, A.; Cano, M. P., High-pressure and temperature effects on enzyme inactivation in tomato puree. *Journal of Agricultural and Food Chemistry* **1998,** *46* (1), 266-270.

123. Page-Weir, N.; Jamieson, L.; Chhagan, A.; Connolly, P.; Curtis, C., Efficacy of insecticides against the tomato/potato psyllid (Bactericera cockerelli). *New Zealand Plant Protection* **2011,** *64*, 276-281.

124. Brown, J. K.; Rehman, M.; Rogan, D.; Martin, R.; Idris, A., First report of "Candidatus Liberibacter psyllaurous"(synonym "Ca. L. solanacearum") associated with 'tomato vein-greening'and 'tomato psyllid yellows' diseases in commercial greenhouses in Arizona. *Plant Disease* **2010,** *94* (3), 376-376.

125. Sengoda, V. G.; Munyaneza, J. E.; Crosslin, J. M.; Buchman, J. L.; Pappu, H. R., Phenotypic and etiological differences between psyllid yellows and zebra chip diseases of potato. *American Journal of Potato Research* **2010,** *87* (1), 41-49.

126. Liu, D.; Trumble, J. T.; Stouthamer, R., Genetic differentiation between eastern populations and recent introductions of potato psyllid (Bactericera cockerelli) into western North America. *Entomologia experimentalis et applicata* **2006,** *118* (3), 177-183.

127. Liefting, L.; Perez-Egusquiza, Z.; Clover, G.; Anderson, J., A new 'Candidatus Liberibacter'species in Solanum tuberosum in New Zealand. *Plant Disease* **2008,** *92* (10), 1474-1474.

128. Haapalainen, M.; Wang, J.; Latvala, S.; Lehtonen, M. T.; Pirhonen, M.; Nissinen, A., Genetic variation of 'Candidatus Liberibacter solanacearum'haplotype C and identification of a novel haplotype from Trioza urticae and stinging nettle. *Phytopathology* **2018,** *108* (8), 925-934.

129. Munyaneza, J. E., Zebra chip disease of potato: biology, epidemiology, and management. *American Journal of Potato Research* **2012,** *89* (5), 329-350.

130. Huot, O. B.; Levy, J. G.; Tamborindeguy, C., Global gene regulation in tomato plant (Solanum lycopersicum) responding to vector (Bactericera cockerelli) feeding and

pathogen ('Candidatus Liberibacter solanacearum') infection. *Plant Mol Biol* **2018,** *97* (1-2), 57-72.

131. Casteel, C. L.; Hansen, A. K.; Walling, L. L.; Paine, T. D. J. P. o., Manipulation of plant defense responses by the tomato psyllid (Bactericerca cockerelli) and its associated endosymbiont Candidatus Liberibacter psyllaurous. **2012,** *7* (4), e35191.

132. Levy, J.; Ravindran, A.; Gross, D.; Tamborindeguy, C.; Pierson, E., Translocation of 'Candidatus Liberibacter solanacearum', the zebra chip pathogen, in potato and tomato. *Phytopathology* **2011,** *101* (11), 1285-1291.

133. Nachappa, P.; Shapiro, A. A.; Tamborindeguy, C., Effect of 'Candidatus Liberibacter solanacearum'on fitness of its insect vector, Bactericera cockerelli (Hemiptera: Triozidae), on tomato. *Phytopathology* **2012,** *102* (1), 41-46.

134. Gasmi, L.; Martínez-Solís, M.; Frattini, A.; Ye, M.; Collado, M. C.; Turlings, T. C.; Erb, M.; Herrero, S., Can herbivore-induced volatiles protect plants by increasing the herbivores' susceptibility to natural pathogens? *Appl. Environ. Microbiol.* **2019,** *85* (1), e01468-18.

135. Rashed, A.; Wallis, C. M.; Paetzold, L.; Workneh, F.; Rush, C. M., Zebra Chip Disease and Potato Biochemistry: Tuber Physiological Changes in Response to 'Candidatus Liberibacter solanacearum' Infection Over Time. *Phytopathology* **2013,** *103* (5), 419-426.

136. Mas, F.; Vereijssen, J.; Suckling, D. M., Influence of the pathogen Candidatus Liberibacter solanacearum on tomato host plant volatiles and psyllid vector settlement. *J. Chem. Ecol.* **2014,** *40* (11-12), 1197-202.

137. Munyaneza, J. E., Zebra chip disease, Candidatus Liberibacter, and potato psyllid: A global threat to the potato industry. *American Journal of Potato Research* **2015,** *92* (2), 230-235.

138. Casteel, C. L.; Walling, L. L.; Paine, T. D., Behavior and biology of the tomato psyllid, Bactericerca cockerelli, in response to the Mi-1.2 gene. *Entomologia experimentalis et applicata* **2006,** *121* (1), 67-72.

139. Liu, D.; Trumble, J., Ovipositional preferences, damage thresholds, and detection of the tomato–potato psyllid Bactericera cockerelli (Homoptera: Psyllidae) on selected tomato accessions. *Bulletin of Entomological Research* **2006,** *96* (2), 197-204.

140. Szczepaniec, A.; Varela, K. A.; Kiani, M.; Paetzold, L.; Rush, C. M., Incidence of resistance to neonicotinoid insecticides in Bactericera cockerelli across Southwest US. *Crop protection* **2019,** *116*, 188-195.

141. Davies, J. N.; Hobson, G. E.; McGlasson, W., The constituents of tomato fruit—the influence of environment, nutrition, and genotype. *Critical Reviews in Food Science & Nutrition* **1981,** *15* (3), 205-280.

142. USDA-NASS, Vegetables 2015 Summary (February 2016). **2016**.

143. Tieman, D.; Zhu, G.; Resende, M. F. R.; Lin, T.; Nguyen, C.; Bies, D.; Rambla, J. L.; Beltran, K. S. O.; Taylor, M.; Zhang, B.; Ikeda, H.; Liu, Z.; Fisher, J.; Zemach, I.; Monforte, A.; Zamir, D.; Granell, A.; Kirst, M.; Huang, S.; Klee, H., A chemical genetic roadmap to improved tomato flavor. *Science* **2017,** *355* (6323), 391-394.

144. Klee, H. J.; Tieman, D. M., The genetics of fruit flavour preferences. *Nature Reviews Genetics* **2018**, 1.

145. Cortina, P. R.; Santiago, A. N.; Sance, M. M.; Peralta, I. E.; Carrari, F.; Asis, R., Neuronal network analyses reveal novel associations between volatile organic compounds and sensory properties of tomato fruits. *Metabolomics* **2018,** *14* (5), 57.

146. Bauchet, G.; Grenier, S.; Samson, N.; Segura, V.; Kende, A.; Beekwilder, J.; Cankar, K.; Gallois, J. L.; Gricourt, J.; Bonnet, J., Identification of major loci and genomic regions controlling acid and volatile content in tomato fruit: implications for flavor improvement. *New Phytologist* **2017,** *215* (2), 624-641.

147. Tikunov, Y.; Lommen, A.; De Vos, C. R.; Verhoeven, H. A.; Bino, R. J.; Hall, R. D.; Bovy, A. G., A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant physiology* **2005,** *139* (3), 1125-1137.

148. Tikunov, Y. M.; de Vos, R. C.; Paramás, A. M. G.; Hall, R. D.; Bovy, A. G., A role for differential glycoconjugation in the emission of phenylpropanoid volatiles from tomato fruit discovered using a metabolic data fusion approach. *Plant physiology* **2010,** *152* (1), 55-70.

149. Rambla, J. L.; Alfaro, C.; Medina, A.; Zarzo, M.; Primo, J.; Granell, A., Tomato fruit volatile profiles are highly dependent on sample processing and capturing methods. *Metabolomics* **2015,** *11* (6), 1708-1720.

150. Figueira, J.; Câmara, H.; Pereira, J.; Câmara, J. S., Evaluation of volatile metabolites as markers in Lycopersicon esculentum L. cultivars discrimination by

multivariate analysis of headspace solid phase microextraction and mass spectrometry data. *Food chemistry* **2014,** *145*, 653-663.

151. Cortina, P. R.; Asis, R.; Peralta, I. E.; Asprelli, P. D.; Santiago, A. N., Determination of volatile organic compounds in Andean tomato landraces by headspace solid phase microextraction-gas chromatography-mass spectrometry. *Journal of the Brazilian Chemical Society* **2017,** *28* (1), 30-41.

152. Jayaprakasha, G.; Murthy, K.; Patil, B., Inhibition of pancreatic cancer cells by furocoumarins from *Poncirus trifoliata*. *Planta Medica* **2012,** *78* (11), PI175.

153. Adams, R., *Identification of essential oils by ion trap mass spectroscopy*. Academic Press: 1989.

154. Zhang, J.; Zhao, J.; Xu, Y.; Liang, J.; Chang, P.; Yan, F.; Li, M.; Liang, Y.; Zou, Z., Genome-wide association mapping for tomato volatiles positively contributing to tomato flavor. *Front. Plant Sci.* **2015,** *6*.

155. Sumner, L. W.; Amberg, A.; Barrett, D.; Beale, M. H.; Beger, R.; Daykin, C. A.; Fan, T. W.-M.; Fiehn, O.; Goodacre, R.; Griffin, J. L., Proposed minimum reporting standards for chemical analysis. *Metabolomics* **2007,** *3* (3), 211-221.

156. Wang, L. B.; Baldwin, E. A.; Bai, J. H., Recent Advance in Aromatic Volatile Research in Tomato Fruit: The Metabolisms and Regulations. *Food Bioprocess Tech* **2016,** *9* (2), 203-216.

157. Eriksson, L.; Johansson, E.; Antti, H.; Holmes, E., Multi-and megavariate data analysis. In *Metabonomics in toxicity assessment*, CRC Press: 2005; pp 263-336.

158. Farneti, B.; Alarcón, A. A.; Papasotiriou, F. G.; Samudrala, D.; Cristescu, S. M.; Costa, G.; Harren, F. J. M.; Woltering, E. J., Chilling-Induced Changes in Aroma Volatile Profiles in Tomato. *Food Bioprocess Tech* **2015,** *8* (7), 1442-1454.

159. Cebolla-Cornejo, J.; Roselló, S.; Valcárcel, M.; Serrano, E.; Beltrán, J.; Nuez, F., Evaluation of Genotype and Environment Effects on Taste and Aroma Flavor Components of Spanish Fresh Tomato Varieties. *Journal of Agricultural and Food Chemistry* **2011,** *59* (6), 2440-2450.

160. Fiehn, O.; Kopka, J.; Trethewey, R. N.; Willmitzer, L., Identification of Uncommon Plant Metabolites Based on Calculation of Elemental Compositions Using Gas Chromatography and Quadrupole Mass Spectrometry. *Analytical Chemistry* **2000,** *72* (15), 3573-3580.

161. Peterson, A. C.; Hauschild, J.-P.; Quarmby, S. T.; Krumwiede, D.; Lange, O.; Lemke, R. A. S.; Grosse-Coosmann, F.; Horning, S.; Donohue, T. J.; Westphall, M. S.; Coon, J. J.; Griep-Raming, J., Development of a GC/Quadrupole-Orbitrap Mass Spectrometer, Part I: Design and Characterization. *Analytical Chemistry* **2014,** *86* (20), 10036-10043.

162. Farneti, B.; Cristescu, S. M.; Costa, G.; Harren, F. J.; Woltering, E. J., Rapid Tomato Volatile Profiling by Using Proton-Transfer Reaction Mass Spectrometry (PTR-MS). *Journal of Food Science* **2012,** *77* (5).

163. Vandendriessche, T.; Nicolai, B. M.; Hertog, M. L. A. T. M., Optimization of HS SPME Fast GC-MS for High-Throughput Analysis of Strawberry Aroma. *Food Analytical Methods* **2013,** *6* (2), 512-520.

164. Spínola, V.; Perestrelo, R.; Câmara, J. S.; Castilho, P. C., Establishment of Monstera deliciosa fruit volatile metabolomic profile at different ripening stages using solid-phase microextraction combined with gas chromatography–mass spectrometry. *Food Research International* **2015,** *67*, 409-417.

165. Câmara, J. S.; Alves, M. A.; Marques, J. C., Classification of Boal, Malvazia, Sercial and Verdelho wines based on terpenoid patterns. *Food Chemistry* **2007,** *101* (2), 475-484.

166. Mendes, B.; Gonçalves, J.; Câmara, J. S., Effectiveness of high-throughput miniaturized sorbent- and solid phase microextraction techniques combined with gas chromatography–mass spectrometry analysis for a rapid screening of volatile and semi-volatile composition of wines—A comparative study. *Talanta* **2012,** *88*, 79-94.

167. Ma, Q. L.; Hamid, N.; Bekhit, A. E. D.; Robertson, J.; Law, T. F., Optimization of headspace solid phase microextraction (HS-SPME) for gas chromatography mass spectrometry (GC–MS) analysis of aroma compounds in cooked beef using response surface methodology. *Microchemical Journal* **2013,** *111*, 16-24.

168. Fernandez, O.; Urrutia, M.; Bernillon, S.; Giauffret, C.; Tardieu, F.; Le Gouis, J.; Langlade, N.; Charcosset, A.; Moing, A.; Gibon, Y., Fortune telling: metabolic markers of plant performance. *Metabolomics* **2016,** *12* (10), 158.

169. Kasote, D.; Suleman, T.; Chen, W.; Sandasi, M.; Viljoen, A.; van Vuuren, S., Chemical profiling and chemometric analysis of South African propolis. *Biochem. Syst. Ecol.* **2014,** *55*, 156-163.

170. Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D., Which impact for β-damascenone on red wines aroma? *Journal of agricultural and food chemistry* **2007,** *55* (10), 4103-4108.

171. Uddin, A. N.; Labuda, I.; Burns, F. J., A novel mechanism of filaggrin induction and sunburn prevention by β-damascenone in Skh-1 mice. *Toxicology and Applied Pharmacology* **2012,** *265* (3), 335-341.

172. Jirovetz, L.; Buchbauer, G.; Schmidt, E.; Stoyanova, A. S.; Denkova, Z.; Nikolova, R.; Geissler, M., Purity, Antimicrobial Activities and Olfactoric Evaluations of Geraniol/Nerol and Various of Their Derivatives. *Journal of Essential Oil Research* **2007,** *19* (3), 288-291.

173. Marumoto, S.; Okuno, Y.; Miyazawa, M., Inhibition of β-Secretase Activity by Monoterpenes, Sesquiterpenes, and C13 Norisoprenoids. *Journal of Oleo Science* **2017**, ess16188.

174. NASS, U., National Agricultural Statistics Service (NASS). *Washington, DC: US Department of Agriculture* **2016**.

175. Zhang, J.; Zhao, J.; Xu, Y.; Liang, J.; Chang, P.; Yan, F.; Li, M.; Liang, Y.; Zou, Z., Genome-Wide Association Mapping for Tomato Volatiles Positively Contributing to Tomato Flavor. *Frontiers in Plant Science* **2015,** *6*, 1042.

176. Tieman, D.; Bliss, P.; McIntyre, L. M.; Blandon-Ubeda, A.; Bies, D.; Odabasi, A. Z.; Rodriguez, G. R.; van der Knaap, E.; Taylor, M. G.; Goulet, C.; Mageroy, M. H.; Snyder, D. J.; Colquhoun, T.; Moskowitz, H.; Clark, D. G.; Sims, C.; Bartoshuk, L.; Klee, H. J., The chemical interactions underlying tomato flavor preferences. *Current Biology* **2012,** *22* (11), 1035-9.

177. Ruiz, J. J.; Alonso, A.; García-Martínez, S.; Valero, M.; Blasco, P.; Ruiz-Bevia, F., Quantitative analysis of flavour volatiles detects differences among closely related traditional cultivars of tomato. *Journal of the Science of Food and Agriculture* **2005,** *85* (1), 54-60.

178. Piombino, P.; Sinesio, F.; Moneta, E.; Cammareri, M.; Genovese, A.; Lisanti, M. T.; Mogno, M. R.; Peparaio, M.; Termolino, P.; Moio, L.; Grandillo, S., Investigating physicochemical, volatile and sensory parameters playing a positive or a negative role on tomato liking. *Food Research International* **2013,** *50* (1), 409-419.

179. Ingwell, L. L.; Thompson, S. L.; Kaplan, I.; Foster, R. E., High tunnels: protection for rather than from insect pests? *Pest Management Science* **2017,** *73* (12), 2439-2446.

180. Figas, M. R.; Prohens, J.; Raigon, M. D.; Fita, A.; Garcia-Martinez, M. D.; Casanova, C.; Borras, D.; Plazas, M.; Andujar, I.; Soler, S., Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups. *Food Chemistry* **2015,** *187* (Supplement C), 517-24.

181. Jayaprakasha, G. K.; Murthy, K. N. C.; Uckoo, R. M.; Patil, B. S., Chemical composition of volatile oil from Citrus limettioides and their inhibition of colon cancer cell proliferation. *Ind Crop Prod* **2013,** *45*, 200-207.

182. Van Lancker, F.; Adams, A.; Delmulle, B.; De Saeger, S.; Moretti, A.; Van Peteghem, C.; De Kimpe, N., Use of headspace SPME-GC-MS for the analysis of the volatiles produced by indoor molds grown on different substrates. *Journal of Environmental Monitoring* **2008,** *10* (10), 1127-1133.

183. Socaci, S. A.; Socaciu, C.; Muresan, C.; Farcas, A.; Tofana, M.; Vicas, S.; Pintea, A., Chemometric discrimination of different tomato cultivars based on their volatile fingerprint in relation to lycopene and total phenolics content. *Phytochem Analysis* **2014,** *25* (2), 161-9.

184. Tikunov, Y.; Lommen, A.; de Vos, C. H.; Verhoeven, H. A.; Bino, R. J.; Hall, R. D.; Bovy, A. G., A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiology* **2005,** *139* (3), 1125-37.

185. Fernie, A. R.; Schauer, N., Metabolomics-assisted breeding: a viable option for crop improvement? *Trends Genetics* **2009,** *25* (1), 39-48.

186. Cebolla-Cornejo, J.; Rosello, S.; Valcarcel, M.; Serrano, E.; Beltran, J.; Nuez, F., Evaluation of genotype and environment effects on taste and aroma flavor components of Spanish fresh tomato varieties. *Journal of Agricultural and Food Chemistry* **2011,** *59* (6), 2440-50.

187. Kasote, D.; Suleman, T.; Chen, W.; Sandasi, M.; Viljoen, A.; van Vuuren, S., Chemical profiling and chemometric analysis of South African propolis. *Biochemical Systematics and Ecology* **2014,** *55*, 156-163.

188. Boukobza, F.; Dunphy, P. J.; Taylor, A. J., Measurement of lipid oxidation-derived volatiles in fresh tomatoes. *Postharvest Biology and Technology* **2001,** *23* (2), 117-131.

189. Bate, N. J.; Rothstein, S. J., C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. *The Plant Journal* **1998,** *16* (5), 561-9.

190. Lu, H.; Xu, S.; Zhang, W.; Xu, C.; Li, B.; Zhang, D.; Mu, W.; Liu, F., Nematicidal Activity of trans-2-Hexenal against Southern Root-Knot Nematode (Meloidogyne incognita) on Tomato Plants. *Journal of Agricultural and Food Chemistry* **2017,** *65* (3), 544-550.

191. Utto, W.; Mawson, A. J.; Bronlund, J. E., Hexanal reduces infection of tomatoes by Botrytis cinerea whilst maintaining quality. *Postharvest Biology and Technology* **2008,** *47* (3), 434-437.

192. Bleeker, P. M.; Diergaarde, P. J.; Ament, K.; Guerra, J.; Weidner, M.; Schutz, S.; de Both, M. T.; Haring, M. A.; Schuurink, R. C., The role of specific tomato volatiles in tomato-whitefly interaction. *Plant Physiology* **2009,** *151* (2), 925-35.

193. Thybo, A. K.; Edelenbos, M.; Christensen, L. P.; Sørensen, J. N.; Thorup-Kristensen, K., Effect of organic growing systems on sensory quality and chemical composition of tomatoes. *Lwt-Food Sci Technol* **2006,** *39* (8), 835-843.

194. Breiman, L., Random forests. *Mach Learn* **2001,** *45* (1), 5-32.

195. Baldwin, E. A.; Scott, J. W.; Shewmaker, C. K.; Schuch, W., Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *Hortscience* **2000,** *35* (6), 1013-1022.

196. Sun, H.; Ni, H.; Yang, Y.; Wu, L.; Cai, H. N.; Xiao, A. F.; Chen, F., Investigation of sunlight-induced deterioration of aroma of pummelo (Citrus maxima) essential oil. *Journal of Agricultural and Food Chemistry* **2014,** *62* (49), 11818-30.

197. Holopainen, J. K.; Gershenzon, J., Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science* **2010,** *15* (3), 176-84.

198. Penuelas, J.; Llusia, J.; Asensio, D.; Munne-Bosch, S., Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. *Plant Cell Environ* **2005,** *28* (3), 278-286.

199. Copolovici, L. O.; Filella, I.; Llusia, J.; Niinemets, U.; Penuelas, J., The capacity for thermal protection of photosynthetic electron transport varies for different monoterpenes in Quercus ilex. *Plant Physiology* **2005,** *139* (1), 485-96.

200. Jiménez, E.; Lanza, B.; Antiñolo, M. a.; Albaladejo, J., Photooxidation of Leaf-Wound Oxygenated Compounds, 1-Penten-3-ol, (Z)-3-Hexen-1-ol, and 1-Penten-3-one, Initiated by OH Radicals and Sunlight. *Environmental Science & Technology* **2009,** *43* (6), 1831-1837.

201. Yi, S. Y.; Ku, S. S.; Sim, H. J.; Kim, S. K.; Park, J. H.; Lyu, J. I.; So, E. J.; Choi, S. Y.; Kim, J.; Ahn, M. S.; Kim, S. W.; Park, H.; Jeong, W. J.; Lim, Y. P.; Min, S. R.; Liu, J. R., An Alcohol Dehydrogenase Gene from Synechocystis sp. Confers Salt Tolerance in Transgenic Tobacco. *Frontiers in Plant Science* **2017,** *8* (1965), 1965.

202. Sasaki, K.; Takase, H.; Matsuyama, S.; Kobayashi, H.; Matsuo, H.; Ikoma, G.; Takata, R., Effect of light exposure on linalool biosynthesis and accumulation in grape berries. *Bioscience Biotechnology Biochemistry* **2016,** *80* (12), 2376-2382.

203. Lewinsohn, E.; Sitrit, Y.; Bar, E.; Azulay, Y.; Ibdah, M.; Meir, A.; Yosef, E.; Zamir, D.; Tadmor, Y., Not just colors—carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. *Trends in Food Science and Technology* **2005,** *16* (9), 407-415.

204. Wang, L.; Baldwin, E. A.; Bai, J., Recent Advance in Aromatic Volatile Research in Tomato Fruit: The Metabolisms and Regulations. *Food Bioprocess Tech* **2015,** *9* (2), 203-216.

205. Dan, Y.; Yousef, G.; Campbell, F. N.; Phelps, D. W.; Burnett, C.; Kekkonen, A.; Shockley, A.; Lila, M. A., Development, and genetic and metabolic characterization of new tomato mutants with enhanced and deficient carotenoid content. *The Journal of Horticultural Science and Biotechnology* **2017**, 1-9.

206. Coyago-Cruz, E.; Corell, M.; Stinco, C. M.; Hernanz, D.; Moriana, A.; Meléndez-Martínez, A. J., Effect of regulated deficit irrigation on quality parameters, carotenoids and phenolics of diverse tomato varieties (Solanum lycopersicum L.). *Food Research International* **2017,** *96*, 72-83.

207. Caris-Veyrat, C.; Amiot, M.-J.; Tyssandier, V.; Grasselly, D.; Buret, M.; Mikolajczak, M.; Guilland, J.-C.; Bouteloup-Demange, C.; Borel, P., Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *Journal of agricultural and food chemistry* **2004,** *52* (21), 6503-6509.

208. Jagadeesh, S.; Charles, M.; Gariepy, Y.; Goyette, B.; Raghavan, G.; Vigneault, C., Influence of postharvest UV-C hormesis on the bioactive components of tomato during post-treatment handling. *Food Bioprocess Tech* **2011,** *4* (8), 1463-1472.

209. Pinheiro, J.; Alegria, C.; Abreu, M.; Gonçalves, E. M.; Silva, C. L. M., Kinetics of changes in the physical quality parameters of fresh tomato fruits (Solanum lycopersicum, cv. 'Zinac') during storage. *Journal of Food Engineering* **2013,** *114* (3), 338-345.

210. Verheul, M. l. J.; Slimestad, R.; Tjøstheim, I. H., From producer to consumer: greenhouse tomato quality as affected by variety, maturity stage at harvest, transport conditions, and supermarket storage. *Journal of agricultural and food chemistry* **2015,** *63* (20), 5026-5034.

211. Baldwin, E.; Scott, J.; Einstein, M.; Malundo, T.; Carr, B.; Shewfelt, R.; Tandon, K., Relationship between sensory and instrumental analysis for tomato flavor. *Journal of the American Society for Horticultural Science* **1998,** *123* (5), 906-915.

212. Raiola, A.; Rigano, M. M.; Calafiore, R.; Frusciante, L.; Barone, A., Enhancing the health-promoting effects of tomato fruit for biofortified food. *Mediators of inflammation* **2014,** *2014*.

213. Adalid, A. M.; Roselló, S.; Nuez, F., Evaluation and selection of tomato accessions (Solanum section Lycopersicon) for content of lycopene, β-carotene and ascorbic acid. *Journal of food composition and analysis* **2010,** *23* (6), 613-618.

214. Liu, R. H., Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition* **2013,** *4* (3), 384S-392S.

215. Tudor-Radu, M.; Vijan, L. E.; Tudor-Radu, C. M.; Ion, T.; Rodica, S.; Mitrea, R., Assessment of Ascorbic Acid, Polyphenols, Flavonoids, Anthocyanins and Carotenoids Content in Tomato Fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **2016,** *44* (2).

216. Conner, D. S.; Montri, A. D.; Montri, D. N.; Hamm, M. W., Consumer demand for local produce at extended season farmers' markets: guiding farmer marketing strategies. *Renewable Agriculture and Food Systems* **2009,** *24* (4), 251-259.

217. Reeve, J.; Drost, D., Yields and soil quality under transitional organic high tunnel tomatoes. *HortScience* **2012,** *47* (1), 38-44.

218. Radzevičius, A.; Viškelis, P.; Viškelis, J.; Karklelienė, R.; Juškevičienė, D., Tomato fruit color changes during ripening on vine. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* **2014,** *8* (2), 112-114.

219. Marsic, N. K.; Gasperlin, L.; Abram, V.; Budic, M.; Vidrih, R., Quality parameters and total phenolic content in tomato fruits regarding cultivar and microclimatic conditions. *Turkish Journal of Agriculture and Forestry* **2011,** *35* (2), 185-194.

220. Lin, C.-H.; Kohli, R., Bile acid metabolism and signaling: potential therapeutic target for nonalcoholic fatty liver disease. *Clinical and translational gastroenterology* **2018,** *9* (6), 164.

221. Copple, B. L.; Li, T., Pharmacology of bile acid receptors: evolution of bile acids from simple detergents to complex signaling molecules. *Pharmacological research* **2016,** *104*, 9-21.

222. Thakare, R.; Alamoudi, J. A.; Gautam, N.; Rodrigues, A. D.; Alnouti, Y., Species differences in bile acids I. Plasma and urine bile acid composition. *Journal of Applied Toxicology* **2018**.

223. Perez, M. J.; Briz, O., Bile-acid-induced cell injury and protection. *World journal of gastroenterology: WJG* **2009,** *15* (14), 1677.

224. Chávez-Talavera, O.; Tailleux, A.; Lefebvre, P.; Staels, B., Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology* **2017,** *152* (7), 1679-1694. e3.

225. Hervert-Hernández, D.; García, O. P.; Rosado, J. L.; Goñi, I., The contribution of fruits and vegetables to dietary intake of polyphenols and antioxidant capacity in a Mexican rural diet: Importance of fruit and vegetable variety. *Food Research International* **2011,** *44* (5), 1182-1189.

226. Yang, I. F.; Jayaprakasha, G. K.; Patil, B. S., In vitro bile acid binding capacities of red leaf lettuce and cruciferous vegetables. *Journal of agricultural and food chemistry* **2017,** *65* (36), 8054-8062.

227. Zhang, N.; Huang, C.; Ou, S., In vitro binding capacities of three dietary fibers and their mixture for four toxic elements, cholesterol, and bile acid. *Journal of Hazardous Materials* **2011,** *186* (1), 236-239.

228. FRIEDMAN, M.; Fitch, T.; Levin, C.; Yokoyama, W., Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *Journal of food science* **2000,** *65* (5), 897-900.

229. Lee, J. H.; Jayaprakasha, G.; Avila, C. A.; Crosby, K. M.; Patil, B. S. J. F. c., Metabolomic studies of volatiles from tomatoes grown in net-house and open-field conditions. **2019,** *275*, 282-291.

230. De Faria, A.; De Rosso, V.; Mercadante, A., Carotenoid composition of jackfruit (Artocarpus heterophyllus), determined by HPLC-PDA-MS/MS. *Plant foods for human nutrition* **2009,** *64* (2), 108-115.

231. Rojas-Garbanzo, C.; Pérez, A. M.; Bustos-Carmona, J.; Vaillant, F., Identification and quantification of carotenoids by HPLC-DAD during the process of peach palm (Bactris gasipaes HBK) flour. *Food Research International* **2011,** *44* (7), 2377-2384.

232. Gentili, A.; Caretti, F.; Ventura, S.; Pérez-Fernández, V.; Venditti, A.; Curini, R., Screening of Carotenoids in Tomato Fruits by Using Liquid Chromatography with Diode Array–Linear Ion Trap Mass Spectrometry Detection. *Journal of Agricultural and Food Chemistry* **2015,** *63* (33), 7428-7439.

233. Yang, I.; Jayaprakasha, G. K.; Patil, B., In vitro digestion with bile acids enhances the bioaccessibility of kale polyphenols. *Food & function* **2018,** *9* (2), 1235-1244.

234. Helrich, K., Official Methods of analysis of the Association of Official Analytical Chemisty. **1990**.

235. Turhan, A.; Şeniz, V., Estimation of certain chemical constituents of fruits of selected tomato genotypes grown in Turkey. *African Journal of Agricultural Research* **2009,** *4* (10), 1086-1092.

236. Turhan, A.; Ozmen, N.; Serbeci, M.; Seniz, V., Effects of grafting on different rootstocks on tomato fruit yield and quality. *Hortic. Sci* **2011,** *38*, 142-149.

237. Roselló, S.; Adalid, A. M.; Cebolla-Cornejo, J.; Nuez, F., Evaluation of the genotype, environment and their interaction on carotenoid and ascorbic acid accumulation in tomato germplasm. *Journal of the Science of Food and Agriculture* **2011,** *91* (6), 1014-1021.

238. ILIĆ, Z. S.; MILENKOVIĆ, L.; ŠUNIĆ, L.; STANOJEVIĆ, L.; BODROŽA-SOLAROV, M.; MARINKOVIĆ, D. In *Tomato fruits quality as affected by light intensity using color shade nets*, Proceedings. 47th Croatian and 7th International Symposium on Agriculture. Opatija. Croatia, 2012; p 418.

239. McCollum, J., Effects of light on the formation of carotenoids in tomato fruits. *Journal of Food Science* **1954,** *19* (1-6), 182-189.

240. Liu, T.; Zhu, W.; Huang, J.; Chen, H.; Nie, R.; Li, C.-m., Comparison of the nutritional as well as the volatile composition of in-season and off-season Hezuo 903 tomato at red stage. *European Food Research and Technology* **2016**, 1-12.

241. Atherton, J.; Rudich, J., *The tomato crop: a scientific basis for improvement*. Springer Science & Business Media: 2012.

242. Baqar, M.; Lee, T., Interaction of CPTA and high temperature on carotenoid synthesis in tomato fruit. *Zeitschrift für Pflanzenphysiologie* **1978,** *88* (5), 431-435.

243. Barkat, N.; Singh, J.; Jayaprakasha, G. K.; Patil, B. S., Effect of harvest time on the levels of phytochemicals, free radical-scavenging activity, α-amylase inhibition and bile acid-binding capacity of spinach (Spinacia oleracea). *Journal of the Science of Food and Agriculture* **2018**.

244. Gomez, M. K.; Singh, J.; Acharya, P.; Jayaprakasha, G.; Patil, B. S., Identification and Quantification of Phytochemicals, Antioxidant Activity, and Bile Acid-Binding Capacity of Garnet Stem Dandelion (Taraxacum officinale). *Journal of food science* **2018**.

245. Hofmann, A. F. J. A. o. i. m., The continuing importance of bile acids in liver and intestinal disease. **1999,** *159* (22), 2647-2658.

246. Chandalia, M.; Garg, A.; Lutjohann, D.; von Bergmann, K.; Grundy, S. M.; Brinkley, L. J. J. N. E. J. o. M., Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. **2000,** *342* (19), 1392-1398.

247. Hwalla, N.; El Labban, S.; Bahn, R. A., Nutrition security is an integral component of food security. *Front Life Sci* **2016,** *9* (3), 167-172.

248. FAO, *The state of food security and nutrition in the world 2018: Building Climate Resilience for Food Security and Nutrition. Rome: Food and Agriculture Organization of the United Nations.* Food & Agriculture Org.: 2018.

249. Khoshgoftarmanesh, A. H.; Schulin, R.; Chaney, R. L.; Daneshbakhsh, B.; Afyuni, M., Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. *Agron Sustain Dev* **2010,** *30* (1), 83-107.

250. Di Lecce, G.; Martinez-Huelamo, M.; Tulipani, S.; Vallverdu-Queralt, A.; Lamuela-Raventos, R. M., Setup of a UHPLC-QqQ-MS method for the analysis of phenolic compounds in cherry tomatoes, tomato sauce, and tomato juice. *J. Agric. Food Chem.* **2013,** *61* (35), 8373-80.

251. Kwon, H.; Kim, T. K.; Hong, S. M.; Se, E. K.; Cho, N. J.; Kyung, K. S., Effect of household processing on pesticide residues in field-sprayed tomatoes. *Food Sci Biotechnol* **2015,** *24* (1), 1-6.

252. Ribas-Agustí, A.; Cáceres, R.; Gratacós-Cubarsí, M.; Sárraga, C.; Castellari, M. J. F. a. m., A validated HPLC-DAD method for routine determination of ten phenolic compounds in tomato fruits. **2012,** *5* (5), 1137-1144.

253. Strati, I. F.; Gogou, E.; Oreopoulou, V., Enzyme and high pressure assisted extraction of carotenoids from tomato waste. *Food Bioprod Process* **2015,** *94*, 668-674.

254. Martí, R.; Roselló, S.; Cebolla-Cornejo, J., Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *J. Cancers* **2016,** *8* (6), 58.

255. Huang, X.; Mazza, G., Application of LC and LC-MS to the analysis of melatonin and serotonin in edible plants. *Crit. Rev. Food Sci. Nutr.* **2011,** *51* (4), 269-84.

256. Rehmani, N.; Farhan, M.; Hadi, S., DNA binding and its degradation by the neurotransmitter serotonin and its structural analogues melatonin and tryptophan: Putative neurotoxic mechanism. *J. Mol. Genet. Med.* **2016**, 1747-0862.

257. Gomez-Romero, M.; Segura-Carretero, A.; Fernandez-Gutierrez, A., Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry* **2010,** *71* (16), 1848-64.

258. Moco, S.; Bino, R. J.; Vorst, O.; Verhoeven, H. A.; de Groot, J.; van Beek, T. A.; Vervoort, J.; de Vos, C. H., A liquid chromatography-mass spectrometry-based metabolome database for tomato. *Plant Physiol.* **2006,** *141* (4), 1205-18.

259. Liu, C. H.; Cai, L. Y.; Lu, X. Y.; Han, X. X.; Ying, T. J., Effect of Postharvest UV-C Irradiation on Phenolic Compound Content and Antioxidant Activity of Tomato Fruit During Storage. *J Integr Agr* **2012,** *11* (1), 159-165.

260. Bakir, S.; Kamiloglu, S.; Tomas, M.; Capanoglu, E., Tomato polyphenolics: putative applications to health and disease. In *Polyphenols: Mechanisms of Action in Human Health and Disease*, Elsevier: 2018; pp 93-102.

261. Okazaki, M.; Ezura, H., Profiling of melatonin in the model tomato (Solanum lycopersicum L.) cultivar Micro-Tom. *J. Pineal. Res.* **2009,** *46* (3), 338-43.

262. Sturtz, M.; Cerezo, A. B.; Cantos-Villar, E.; Garcia-Parrilla, M. C., Determination of the melatonin content of different varieties of tomatoes (Lycopersicon esculentum) and strawberries (Fragariaananassa). *Food Chem* **2011,** *127* (3), 1329-34.

263. Slimestad, R.; Verheul, M., Review of flavonoids and other phenolics from fruits of different tomato (Lycopersicon esculentum Mill.) cultivars. *J Sci Food Agr* **2009,** *89* (8), 1255-1270.

264. Castagna, A.; Dall'Asta, C.; Chiavaro, E.; Galaverna, G.; Ranieri, A., Effect of post-harvest UV-B irradiation on polyphenol profile and antioxidant activity in flesh and peel of tomato fruits. *Food Bioproc. Tech.* **2014,** *7* (8), 2241-2250.

265. Lee, J. H. J.; Jayaprakasha, G. K.; Avila, C. A.; Crosby, K. M.; Patil, B. S., Metabolomic studies of volatiles from tomatoes grown in net-house and open-field conditions. *Food Chem* **2019,** *275*, 282-291.

266. Kasote, D. M.; Jayaprakasha, G. K.; Patil, B. S., Leaf disc assays for rapid measurement of antioxidant activity. *Sci. Rep.* **2019,** *9* (1), 1884.

267. Barkat, N.; Singh, J.; Jayaprakasha, G. K.; Patil, B. S., Effect of harvest time on the levels of phytochemicals, free radical-scavenging activity, alpha-amylase inhibition and bile acid-binding capacity of spinach (Spinacia oleracea). *J. Sci. Food Agric.* **2018,** *98* (9), 3468-3477.

268. Vallverdu-Queralt, A.; Medina-Remon, A.; Martinez-Huelamo, M.; Jauregui, O.; Andres-Lacueva, C.; Lamuela-Raventos, R. M., Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *J. Agric. Food Chem.* **2011,** *59* (8), 3994-4001.

269. Biesaga, M.; Pyrzynska, K., Liquid chromatography/tandem mass spectrometry studies of the phenolic compounds in honey. *J. Chromatogr. A* **2009,** *1216* (38), 6620-6.

270. Ferreira, M. S.; de Oliveira, D. N.; Mesquita, C. C.; de Lima Barbosa, A. P.; Anhê, G. F.; Catharino, R. R., MALDI-MSI: a fast and reliable method for direct melatonin quantification in biological fluids. *Journal of Analytical Science and Technology* **2016,** *7* (1), 28.

271. Kasote, D. M.; Lee, J. H.; Jayaprakasha, G.; Patil, B. S., Seed priming with iron oxide nanoparticles modulate antioxidant potential and defense-linked hormones in watermelon seedlings. *ACS Sustainable Chemistry & Engineering* **2019,** *7* (5), 5142-5151.

272. Tivendale, N. D.; Davies, N. W.; Molesworth, P. P.; Davidson, S. E.; Smith, J. A.; Lowe, E. K.; Reid, J. B.; Ross, J. J., Reassessing the role of N-hydroxytryptamine in auxin biosynthesis. *Plant physiology* **2010,** *154* (4), 1957-1965.

273. Zhao, L. J.; Ni, Y.; Su, M. M.; Li, H. S.; Dong, F. C.; Chen, W. L.; Wei, R. M.; Zhang, L. L.; Guiraud, S. P.; Martin, F. P.; Rajani, C.; Xie, G. X.; Jia, W., High throughput and quantitative measurement of microbial metabolome by gas chromatography/mass spectrometry using automated alkyl chloroformate derivatization. *Anal Chem* **2017,** *89* (10), 5565-5577.

274. Ly, D.; Kang, K.; Choi, J. Y.; Ishihara, A.; Back, K.; Lee, S. G., HPLC analysis of serotonin, tryptamine, tyramine, and the hydroxycinnamic acid amides of serotonin and tyramine in food vegetables. *J. Med. Food* **2008,** *11* (2), 385-9.

275. Islam, J.; Shirakawa, H.; Nguyen, T. K.; Aso, H.; Komai, M., Simultaneous analysis of serotonin, tryptophan and tryptamine levels in common fresh fruits and vegetables in Japan using fluorescence HPLC. *Food Biosci* **2016,** *13*, 56-59.

276. Liu, J. L.; Zhang, R. M.; Sun, Y. K.; Liu, Z. Y.; Jin, W.; Sun, Y., The beneficial effects of exogenous melatonin on tomato fruit properties. *Sci Hortic-Amsterdam* **2016,** *207*, 14-20.

277. Arnao, M. B.; Hernandez-Ruiz, J., Functions of melatonin in plants: a review. *J. Pineal. Res.* **2015,** *59* (2), 133-50.

278. Shukla, M.; Chinchalongporn, V.; Govitrapong, P.; Reiter, R. J., The role of melatonin in targeting cell signaling pathways in neurodegeneration. *Annals of the New York Academy of Sciences* **2019,** *1443* (1), 75-96.

279. Riga, P.; Medina, S.; Garcia-Flores, L. A.; Gil-Izquierdo, A., Melatonin content of pepper and tomato fruits: effects of cultivar and solar radiation. *Food Chem* **2014,** *156*, 347-52.

280. Hano, S.; Shibuya, T.; Imoto, N.; Ito, A.; Imanishi, S.; Aso, H.; Kanayama, Y., Serotonin content in fresh and processed tomatoes and its accumulation during fruit development. *Sci Hortic-Amsterdam* **2017,** *214*, 107-113.

281. de Alvarenga, J. F. R.; Lozano-Castellón, J.; Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Lamuela-Raventós, R. M., Cooking practice and the matrix effect on the health properties of mediterranean diet: A study in tomato sauce. In *Advances in Plant Phenolics: From Chemistry to Human Health*, ACS Publications: 2018; pp 305-314.

282. Nićiforović, N.; Abramovič, H., Sinapic acid and its derivatives: natural sources and bioactivity. *Compr. Rev. Food Sci. Food Saf.* **2014,** *13* (1), 34-51.

283. Lee, H. E.; Kim, D. H.; Park, S. J.; Kim, J. M.; Lee, Y. W.; Jung, J. M.; Lee, C. H.; Hong, J. G.; Liu, X.; Cai, M., Neuroprotective effect of sinapic acid in a mouse model of amyloid β1–42 protein-induced Alzheimer's disease. *Pharmacol. Biochem. Behav.* **2012,** *103* (2), 260-266.

284. Bi, B.; Tang, J.; Han, S.; Guo, J.; Miao, Y., Sinapic acid or its derivatives interfere with abscisic acid homeostasis during Arabidopsis thaliana seed germination. *BMC Plant Biol.* **2017,** *17* (1), 99.

285. Martínez-Huélamo, M.; Tulipani, S.; Estruch, R.; Escribano, E.; Illán, M.; Corella, D.; Lamuela-Raventós, R. M., The tomato sauce making process affects the

bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food chemistry* **2015,** *173*, 864-872.

286. Erdinc, C.; Ekincialp, A.; Gundogdu, M.; Eser, F.; Sensoy, S., Bioactive components and antioxidant capacities of different miniature tomato cultivars grown by altered fertilizer applications. *J Food Meas Charact* **2018,** *12* (3), 1519-1529.

287. Luthria, D. L.; Mukhopadhyay, S.; Krizek, D. T., Content of total phenolics and phenolic acids in tomato (Lycopersicon esculentum Mill.) fruits as influenced by cultivar and solar UV radiation. *J Food Compos Anal* **2006,** *19* (8), 771-777.

288. Ahmadi, L.; Hao, X.; Tsao, R., The effect of greenhouse covering materials on phytochemical composition and antioxidant capacity of tomato cultivars. *J. Sci. Food Agric.* **2018,** *98* (12), 4427-4435.

289. Castagna, A.; Chiavaro, E.; Dall'asta, C.; Rinaldi, M.; Galaverna, G.; Ranieri, A., Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. *Food Chem* **2013,** *137* (1-4), 151-8.

290. Ayala-Zavala, J. F.; Wang, S. Y.; Wang, C. Y.; González-Aguilar, G. A., Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *Lebensm-Wiss Technol* **2004,** *37* (7), 687-695.

291. Casado-Vela, J.; Selles, S.; Bru, R., Influence of developmental stage, cultivar, and hexapeptide and cyclodextrin inhibitors on polyphenol oxidase activity from tomato fruits. *Journal of food biochemistry* **2006,** *30* (6), 623-640.

292. Aquino-Bolaños, E. N.; Mercado-Silva, E., Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. *Postharvest Biol. Technol.* **2004,** *33* (3), 275-283.

293. Liang, X.; Chen, Q.; Lu, H.; Wu, C.; Lu, F.; Tang, J., Increased activities of peroxidase and polyphenol oxidase enhance cassava resistance to Tetranychus urticae. *Exp. Appl. Acarol.* **2017,** *71* (3), 195-209.

294. Dicko, M. H.; Hilhorst, R.; Gruppen, H.; Traore, A. S.; Laane, C.; van Berkel, W. J.; Voragen, A. G., Comparison of content in phenolic compounds, polyphenol oxidase, and peroxidase in grains of fifty sorghum varieties from burkina faso. *J. Agric. Food Chem.* **2002,** *50* (13), 3780-8.

295. Arnnok, P.; Ruangviriyachai, C.; Mahachai, R.; Techawongstien, S.; Chanthai, S., Optimization and determination of polyphenol oxidase and peroxidase activities in hot pepper (Capsicum annuum L.) pericarb. *Int. Food Res. J.* **2010,** *17*, 385-392.

296. Gandia-Herrero, F.; Jimenez, M.; Cabanes, J.; Garcia-Carmona, F.; Escribano, J., Tyrosinase inhibitory activity of cucumber compounds: enzymes responsible for browning in cucumber. *J. Agric. Food Chem.* **2003,** *51* (26), 7764-9.

297. Zhou, X.; Xiao, Y.; Meng, X.; Liu, B., Full inhibition of Whangkeumbae pear polyphenol oxidase enzymatic browning reaction by l-cysteine. *Food Chem* **2018,** *266*, 1-8.

298. Kajiwara, T.; Matsui, K.; Akakabe, Y.; Murakawa, T.; Arai, C., Antimicrobial browning-inhibitory effect of flavor compounds in seaweeds. *J. Appl. Phycol.* **2006,** *18* (3-5), 413-422.

299. Marei, G. I. K.; Rasoul, M. A. A.; Abdelgaleil, S. A. M., Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. *Pestic. Biochem. Physiol.* **2012,** *103* (1), 56-61.

300. Bashan, Y.; Okon, Y.; Henis, Y., Peroxidase, polyphenoloxidase, and phenols in relation to resistance against Pseudomonas syringae pv. tomato in tomato plants. *Can. J. Bot.* **1987,** *65* (2), 366-372.

301. Li, L.; Steffens, J. C., Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. *Planta* **2002,** *215* (2), 239-47.

302. Clemente, E.; Mantovani, C., Peroxidase and polyphenoloxidase activity in tomato in natura and tomato purée. *Acta Sci. Technol.* **2010,** *32* (1), 91-97.

303. Ilic, Z. S.; Milenkovic, L.; Sunic, L.; Fallik, E., Effect of coloured shade-nets on plant leaf parameters and tomato fruit quality. *J. Sci. Food Agric.* **2015,** *95* (13), 2660-7.

304. Bravo, K.; Osorio, E., Characterization of polyphenol oxidase from Cape gooseberry (Physalis peruviana L.) fruit. *Food Chem* **2016,** *197* (Pt A), 185-90.

305. Liu, J.; Tian, S.; Meng, X.; Xu, Y., Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biol. Technol.* **2007,** *44* (3), 300-306.

306. Chen, J. P.; Zou, X.; Liu, Q.; Wang, F.; Feng, W.; Wan, N., Combination effect of chitosan and methyl jasmonate on controlling Alternaria alternata and enhancing activity of cherry tomato fruit defense mechanisms. *Crop Prot.* **2014,** *56*, 31-36.

307. Siguemoto, É. S.; Gut, J. A. W., Validation of spectrophotometric microplate methods for polyphenol oxidase and peroxidase activities analysis in fruits and vegetables. *Food Sci. Technol.* **2017,** *37* (suppl 1), 148-153.

308. Deylami, M. Z.; Rahman, R. A.; Tan, C. P.; Bakar, J.; Olusegun, I., Thermodynamics and kinetics of thermal inactivation of peroxidase from mangosteen (garcinia mangostana l.) Pericarp. *J. Eng. Sci. Technol.* **2014,** *9* (3), 374-383.

309. Lee, J. H. J.; Jayaprakasha, G. K.; Rush, C. M.; Crosby, K. M.; Patil, B. S., Production system influences volatile biomarkers in tomato. *Metabolomics* **2018,** *14* (7), 99.

310. Sommano, S. R., Determination of polyphenol oxidase and peroxidase activity in plants rich in phenolics and chlorophylls. *J. Postharvest Technol.* **2018,** *6* (4), 44-54.

311. Casado-Vela, J.; Sellés, S.; Bru, R., Purification and kinetic characterization of polyphenol oxidase from tomato fruits (Lycopersicon esculentum cv. Muchamiel). *Journal of Food Biochemistry* **2005,** *29* (4), 381-401.

312. Marcellin, E.; Gruber, C. W.; Archer, C.; Craik, D. J.; Nielsen, L. K., Proteome analysis of the hyaluronic acid-producing bacterium, Streptococcus zooepidemicus. *Proteome Sci.* **2009,** *7* (1), 13.

313. Niphadkar, S. S.; Vetal, M. D.; Rathod, V. K., Purification and characterization of polyphenol oxidase from waste potato peel by aqueous two-phase extraction. *Prep. Biochem. Biotechnol.* **2015,** *45* (7), 632-49.

314. Chisari, M.; Barbagallo, R. N.; Spagna, G., Characterization and role of polyphenol oxidase and peroxidase in browning of fresh-cut melon. *J. Agric. Food Chem.* **2008,** *56* (1), 132-8.

315. Zheng, Y.; Sheng, J.; Zhao, R.; Zhang, J.; Lv, S.; Liu, L.; Shen, L., Preharvest L-arginine treatment induced postharvest disease resistance to Botrysis cinerea in tomato fruits. *J. Agric. Food Chem.* **2011,** *59* (12), 6543-9.

316. Agarwal, S., Increased antioxidant activity in Cassia seedlings under UV-B radiation. *Biol. Plant.* **2007,** *51* (1), 157-160.

317. Tegelberg, R.; Julkunen-Tiitto, R.; Vartiainen, M.; Paunonen, R.; Rousi, M.; Kellomäki, S., Exposures to elevated CO2, elevated temperature and enhanced UV-B radiation modify activities of polyphenol oxidase and guaiacol peroxidase and concentrations of chlorophylls, polyamines and soluble proteins in the leaves of Betula pendula seedlings. *Environ. Exp. Bot.* **2008,** *62* (3), 308-315.

318. Butler, C. D.; Trumble, J. T., The potato psyllid, Bactericera cockerelli (Sulc)(Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. *Terrestrial Arthropod Reviews* **2012,** *5* (2), 87-111.

319. Munyaneza, J. E.; Mustafa, T.; Fisher, T. W.; Sengoda, V. G.; Horton, D. R., Assessing the Likelihood of Transmission of Candidatus Liberibacter solanacearum to Carrot by Potato Psyllid, Bactericera cockerelli (Hemiptera: Triozidae). *PLoS One* **2016,** *11* (8), e0161016.

320. Levy, J.; Tamborindeguy, C., Solanum habrochaites, a potential source of resistance against Bactericera cockerelli (Hemiptera: Triozidae) and "Candidatus Liberibacter solanacearum". *Journal of economic entomology* **2014,** *107* (3), 1187-1193.

321. Wallis, C. M.; Munyaneza, J. E.; Chen, J.; Novy, R.; Bester, G.; Buchman, J. L.; Nordgaard, J.; van Hest, P., 'Candidatus Liberibacter solanacearum' Titers in and Infection Effects on Potato Tuber Chemistry of Promising Germplasm Exhibiting Tolerance to Zebra Chip Disease. *Phytopathology* **2015,** *105* (12), 1573-84.

322. Levy, J. G.; Mendoza, A.; Miller, J. C.; Tamborindeguy, C.; Pierson, E. A., Global gene expression in two potato cultivars in response to 'Candidatus Liberibacter solanacearum' infection. *Bmc Genomics* **2017,** *18* (1), 960.

323. Harrison, K.; Tamborindeguy, C.; Scheuring, D. C.; Herrera, A. M.; Silva, A.; Badillo-Vargas, I. E.; Miller, J. C.; Levy, J. G., Differences in Zebra Chip Severity between "Candidatus Liberibacter Solanacearum' Haplotypes in Texas. *Am J Potato Res* **2019,** *96* (1), 86-93.

324. Casteel, C. L.; Hansen, A. K.; Walling, L. L.; Paine, T. D., Manipulation of plant defense responses by the tomato psyllid (Bactericerca cockerelli) and its associated endosymbiont Candidatus Liberibacter psyllaurous. *PLoS One* **2012,** *7* (4), e35191.

325. Di, X.; Takken, F. L.; Tintor, N., How phytohormones shape interactions between plants and the soil-borne fungus Fusarium oxysporum. *Frontiers in plant science* **2016,** *7*, 170.

326. Bautista-Lozada, A.; Espinosa-Garcia, F. J., Odor Uniformity among Tomato Individuals in Response to Herbivore Depends on Insect Species. *Plos One* **2013,** *8* (10), e77199.

327. Mumm, R.; Dicke, M., Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Can J Zool* **2010,** *88* (7), 628-667.

328. Nissinen, A. I.; Lemmetty, A.; Pihlava, J. M.; Jauhiainen, L.; Munyaneza, J. E.; Vanhala, P., Effects of carrot psyllid (Trioza apicalis) feeding on carrot yield and content of sugars and phenolic compounds. *Ann Appl Biol* **2012,** *161* (1), 68-80.

329. Cirak, C.; Radusiene, J.; Aksoy, H. M.; Mackinaite, R.; Stanius, Z.; Camas, N.; Odabas, M. S., Differential phenolic accumulation in two Hypericum species in response to inoculation with Diploceras hypericinum and Pseudomonasputida. *Plant Protect. Sci.* **2014,** *50* (3), 119-128.

330. Villarino, M.; Sandin-Espana, P.; Melgarejo, P.; De Cal, A., High chlorogenic and neochlorogenic acid levels in immature peaches reduce Monilinia laxa infection by interfering with fungal melanin biosynthesis. *J. Agric. Food Chem.* **2011,** *59* (7), 3205-13.

331. Luan, J. B.; Yao, D. M.; Zhang, T.; Walling, L. L.; Yang, M.; Wang, Y. J.; Liu, S. S., Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecol. Lett.* **2013,** *16* (3), 390-8.

332. Galeano Garcia, P.; Neves Dos Santos, F.; Zanotta, S.; Eberlin, M. N.; Carazzone, C., Metabolomics of Solanum lycopersicum Infected with Phytophthora infestans Leads to Early Detection of Late Blight in Asymptomatic Plants. *Molecules* **2018,** *23* (12), 3330.

333. Liefting, L.; Sutherland, P.; Ward, L.; Paice, K.; Weir, B.; Clover, G., A new "Candidatus Liberibacter" species associated with diseases of solanaceous crops. *Plant Disease* **2009,** *93*, 208-214.

334. Jagoueix, S.; Bové, J. M.; Garnier, M., PCR detection of the two «Candidatus» liberobacter species associated with greening disease of citrus. *Molecular and cellular probes* **1996,** *10* (1), 43-50.

335. Lin, H.; Islam, M. S.; Bai, Y.; Wen, A.; Lan, S.; Gudmestad, N. C.; Civerolo, E. L., Genetic diversity of 'Cadidatus Liberibacter solanacearum'strains in the United States and Mexico revealed by simple sequence repeat markers. *European Journal of Plant Pathology* **2012,** *132* (2), 297-308.

336. Kasote, D. M.; Lee, J. H. J.; Jayaprakasha, G. K.; Patil, B. S., Seed Priming with Iron Oxide Nanoparticles Modulate Antioxidant Potential and Defense-Linked Hormones in Watermelon Seedlings. *ACS Sustainable Chemistry & Engineering* **2019,** *7* (5), 5142-5151.

337. Zhang, J.; Zhao, J.; Xu, Y.; Liang, J.; Chang, P.; Yan, F.; Li, M.; Liang, Y.; Zou, Z., Genome-Wide Association Mapping for Tomato Volatiles Positively Contributing to Tomato Flavor. *Front. Plant Sci.* **2015,** *6*, 1042.

338. Rashed, A.; Wallis, C.; Paetzold, L.; Workneh, F.; Rush, C. J. P., Zebra chip disease and potato biochemistry: tuber physiological changes in response to 'Candidatus Liberibacter solanacearum'infection over time. **2013,** *103* (5), 419-426.

339. Mas, F.; Vereijssen, J.; Suckling, D. M. J. J. o. c. e., Influence of the pathogen Candidatus Liberibacter solanacearum on tomato host plant volatiles and psyllid vector settlement. **2014,** *40* (11-12), 1197-1202.

340. Dixit, G.; Praveen, A.; Tripathi, T.; Yadav, V. K.; Verma, P. C., Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. *J Asia-Pac Entomol* **2017,** *20* (2), 341-351.

341. Kundu, A.; Vadassery, J., Chlorogenic acid-mediated chemical defence of plants against insect herbivores. *Plant Biol.* **2019,** *21* (2), 185-189.

342. Bi, J.; Felton, G.; Murphy, J.; Howles, P.; Dixon, R.; Lamb, C., Do plant phenolics confer resistance to specialist and generalist insect herbivores? *J. Agric. Food Chem.* **1997,** *45* (11), 4500-4504.

343. McKeehen, J. D.; Busch, R. H.; Fulcher, R. G., Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and their contribution to Fusarium resistance. *J. Agric. Food Chem.* **1999,** *47* (4), 1476-1482.

344. Robert-Seilaniantz, A.; Grant, M.; Jones, J. D. G., Hormone Crosstalk in Plant Disease and Defense: More Than Just JASMONATE-SALICYLATE Antagonism. *Annu. Rev. Phytopathol.* **2011,** *49*, 317-343.

345. Navarre, D. A.; Shakya, R.; Holden, J.; Crosslin, J. M., LC-MS Analysis of Phenolic Compounds in Tubers Showing Zebra Chip Symptoms. *Am J Potato Res* **2009,** *86* (2), 88-95.

346. Pandey, S. P.; Somssich, I. E., The role of WRKY transcription factors in plant immunity. *Plant Physiol.* **2009,** *150* (4), 1648-55.

347. Lü, G.; Guo, S.; Zhang, H.; Geng, L.; Song, F.; Fei, Z.; Xu, Y., Transcriptional profiling of watermelon during its incompatible interaction with Fusarium oxysporum f. sp. niveum. *Eur. J. Plant Pathol.* **2011,** *131* (4), 585.

348. Vanstraelen, M.; Benkova, E., Hormonal interactions in the regulation of plant development. *Annu. Rev. Cell Dev. Biol.* **2012,** *28*, 463-87.

349. Yang, D. L.; Li, Q.; Deng, Y. W.; Lou, Y. G.; Wang, M. Y.; Zhou, G. X.; Zhang, Y. Y.; He, Z. H., Altered disease development in the eui mutants and Eui overexpressors indicates that gibberellins negatively regulate rice basal disease resistance. *Mol Plant* **2008,** *1* (3), 528-537.

350. Wang, S.; Fu, J., Insights into auxin signaling in plant–pathogen interactions. *Frontiers in Plant Science* **2011,** *2*, 74.

351. Mutka, A. M.; Fawley, S.; Tsao, T.; Kunkel, B. N., Auxin promotes susceptibility to P seudomonas syringae via a mechanism independent of suppression of salicylic acid-mediated defenses. *The Plant Journal* **2013,** *74* (5), 746-754.

352. Hao, G. X.; Pitino, M.; Ding, F.; Lin, H.; Stover, E.; Duan, Y. P., Induction of innate immune responses by flagellin from the intracellular bacterium, 'Candidatus Liberibacter solanacearum'. *Bmc Plant Biol.* **2014,** *14* (1), 211.

353. Cooper, W. R.; Bamberg, J. B., Variation in Susceptibility to Potato Psyllid, Bactericera cockerelli (Hemiptera: Triozidae), among Solanum verrucosum Germplasm Accessions. *Am J Potato Res* **2016,** *93* (4), 386-391.

354. Lange, B. M.; Ahkami, A., Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes-current status and future opportunities. *Plant Biotechnol J* **2013,** *11* (2), 169-196.

355. Sade, D.; Shriki, O.; Cuadros-Inostroza, A.; Tohge, T.; Semel, Y.; Haviv, Y.; Willmitzer, L.; Fernie, A. R.; Czosnek, H.; Brotman, Y., Comparative metabolomics and transcriptomics of plant response to Tomato yellow leaf curl virus infection in resistant and susceptible tomato cultivars. *Metabolomics* **2015,** *11* (1), 81-97.

356. Schmidt, D. D.; Voelckel, C.; Hartl, M.; Schmidt, S.; Baldwin, I. T., Specificity in ecological interactions: attack from the same lepidopteran herbivore results in species-specific transcriptional responses in two solanaceous host plants. *Plant Physiol.* **2005,** *138* (3), 1763-73.

357. Bleeker, P. M.; Mirabella, R.; Diergaarde, P. J.; VanDoorn, A.; Tissier, A.; Kant, M. R.; Prins, M.; de Vos, M.; Haring, M. A.; Schuurink, R. C., Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *PNAS* **2012,** *109* (49), 20124-9.

358. Kordali, S.; Kesdek, M.; Cakir, A., Toxicity of monoterpenes against larvae and adults of Colorado potato beetle, Leptinotarsa decemlineata Say (Coleoptera : Chrysomelidae). *Ind. Crops Prod.* **2007,** *26* (3), 278-297.

359. Lucia, A.; Zerba, E.; Masuh, H., Knockdown and larvicidal activity of six monoterpenes against Aedes aegypti (Diptera: Culicidae) and their structure-activity relationships. *Parasitol Res* **2013,** *112* (12), 4267-4272.

360. He, W. B.; Li, J.; Liu, S. S., Differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus. *Sci. Rep.* **2015,** *5*, 7682.

361. Rodriguez-Bustamante, E.; Sanchez, S., Microbial production of C13-norisoprenoids and other aroma compounds via carotenoid cleavage. *Crit. Rev. Microbiol.* **2007,** *33* (3), 211-30.

362. Dennis, J.; Guest, D., Acetylsalicylic acid and β-ionone decrease the susceptibility of tobacco to tobacco necrosis virus and Phytophthora parasitica var. nicotianae. *Australas. Plant Pathol.* **1995,** *24* (1), 57-64.

363. Salt, S.; Tuzun, S.; Kuć, J., Effects of β-ionone and abscisic acid on the growth of tobacco and resistance to blue mold. Mimicry of effects of stem infection by Peronospora tabacina Adam. *Physiol. Mol. Plant Pathol.* **1986,** *28* (2), 287-297.

364. Scala, A.; Allmann, S.; Mirabella, R.; Haring, M. A.; Schuurink, R. C., Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. *Int. J. Mol. Sci.* **2013,** *14* (9), 17781-811.

365. Shiojiri, K.; Kishimoto, K.; Ozawa, R.; Kugimiya, S.; Urashimo, S.; Arimura, G.; Horiuchi, J.; Nishioka, T.; Matsui, K.; Takabayashi, J., Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *PNAS* **2006,** *103* (45), 16672-16676.

366. Brown, G. C.; Prochaska, G. L.; Hildebrand, D. F.; Nordin, G. L.; Jackson, D. M., Green leaf volatiles inhibit conidial germination of the entomopathogen Pandora neoaphidis (Entomopthorales: Entomophthoraceae). *Environ Entomol* **1995,** *24* (6), 1637-1643.

367. Croft, K.; Juttner, F.; Slusarenko, A. J., Volatile Products of the Lipoxygenase Pathway Evolved from Phaseolus vulgaris (L.) Leaves Inoculated with Pseudomonas syringae pv phaseolicola. *Plant Physiol.* **1993,** *101* (1), 13-24.

368. Schmidt, K.; Heberle, B.; Kurrasch, J.; Nehls, R.; Stahl, D. J., Suppression of phenylalanine ammonia lyase expression in sugar beet by the fungal pathogen Cercospora beticola is mediated at the core promoter of the gene. *Plant Mol Biol* **2004,** *55* (6), 835-852.

369. Valenzuela-Soto, J. H.; Estrada-Hernandez, M. G.; Ibarra-Laclette, E.; Delano-Frier, J. P., Inoculation of tomato plants (Solanum lycopersicum) with growth-promoting Bacillus subtilis retards whitefly Bemisia tabaci development. *Planta* **2010,** *231* (2), 397-410.

370. Cajka, T.; Vaclavikova, M.; Dzuman, Z.; Vaclavik, L.; Ovesna, J.; Hajslova, J., Rapid LC-MS-based metabolomics method to study the Fusarium infection of barley. *J. Sep. Sci.* **2014,** *37* (8), 912-9.

371. Augustyn, W. A.; Regnier, T.; Combrinck, S.; Botha, B. M., Metabolic profiling of mango cultivars to identify biomarkers for resistance against Fusarium infection. *Phytochem Lett* **2014,** *10*, CIV-cx.

372. Espinal-Ruiz, M.; Parada-Alfonso, F.; Restrepo-Sánchez, L.-P.; Narváez-Cuenca, C.-E.; McClements, D. J. J. F.; function, Impact of dietary fibers [methyl cellulose, chitosan, and pectin] on digestion of lipids under simulated gastrointestinal conditions. **2014,** *5* (12), 3083-3095.

APPENDIX A

Figure S1. Fruits from the four studied cultivars.

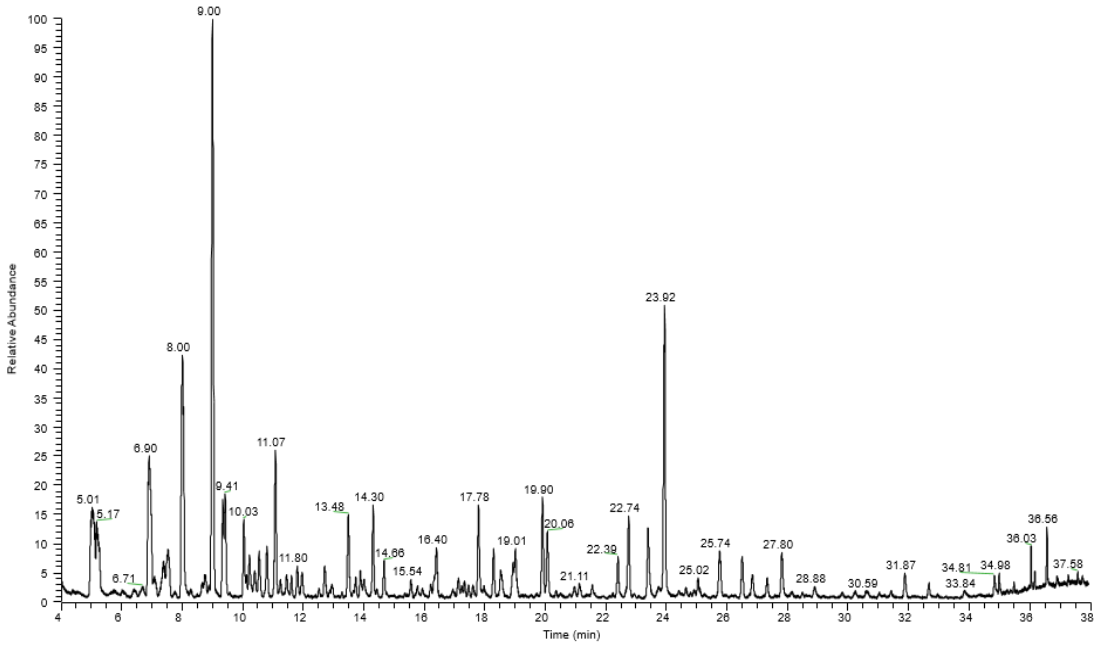

Figure S2. GC-MS profile of the volatile fraction from tomato fruits by HS-SPME/GC-MS, using a DVB/CAR/PDMS fiber at 60 C for 45 min (for peak identification, please see Table 1).

Table S1. List of identified and metabolites common to all tomato varieties with Metabolomics Standards Initiative (MSI) level.[155]

No.	RT	Metabolites	Library Match	Formula	Similarity	MSI level
1	4.38	1-Penten-3-one	wileyregistry8e	C_5H_8O	850	1
2	5.01	Hexanal	wileyregistry8e	$C_6H_{12}O$	930	1
3	6.9	Trans-2-hexenal	replib	$C_6H_{10}O$	900	2
4	7.07	2-Pentyl furan	wileyregistry8e	$C_9H_{14}O$	924	1
5	7.78	p-Cymene	wileyregistry8e	$C_{10}H_{16}$	893	2
6	8.75	Trans-2-heptenal	mainlib	$C_7H_{12}O$	844	1
7	8.87	1-Nitro-3-methylbutane	wileyregistry8e	$C_5H_{11}NO_2$	837	2
8	8.99	6-Methyl-5-hepten-2-one	wileyregistry8e	$C_8H_{14}O$	957	1
9	9.33	1-Hexanol	wileyregistry8e	$C_6H_{14}O$	930	1
10	10.04	Cis-3-hexen-1-ol	mainlib	$C_6H_{12}O$	924	1
11	10.22	Nonanal	wileyregistry8e	$C_9H_{18}O$	950	2
12	10.57	2-Isobutylthiazole	mainlib	$C_7H_{11}NS$	913	1
13	11.07	Trans-2-octenal	wileyregistry8e	$C_8H_{14}O$	873	2
14	11.61	1-Octen-3-ol	wileyregistry8e	$C_8H_{16}O$	972	2
15	11.96	β-thujone	wileyregistry8e	$C_{10}H_{16}O$	813	2
16	12.71	(E,E)-2,4-heptadienal	wileyregistry8e	$C_7H_{10}O$	869	2
17	12.86	Decanal	wileyregistry8e	$C_{10}H_{20}O$	952	2
18	13.48	Benzaldehyde	wileyregistry8e	C_7H_6O	904	1
19	13.89	2-Nonenal	wileyregistry8e	$C_9H_{16}O$	958	2
20	14.01	Cis-4-decenal	wileyregistry8e	$C_{10}H_{18}O$	845	2
21	14.31	Linalool	wileyregistry8e	$C_{10}H_{18}O$	910	1
22	14.66	1-Octanol	wileyregistry8e	$C_8H_{18}O$	942	2
23	15.35	(E,Z)-2,6-nonadienal	wileyregistry8e	$C_9H_{14}O$	900	2
24	16.39	β-Cyclocitral	replib	$C_{10}H_{16}O$	885	2
25	16.95	Benzeneacetaldehyde	wileyregistry8e	C_8H_8O	900	2
26	17.12	4-Methylbenzaldehyde	wileyregistry8e	C_8H_8O	922	1
27	17.23	1-phenylethanone	wileyregistry8e	C_8H_8O	889	1
28	18.29	Neral	wileyregistry8e	$C_{10}H_{16}O$	969	1
29	18.91	(E,E)-2,4-Nonadienal	replib	$C_9H_{14}O$	864	2
30	19.01	4-methoxy-6-methyl phenol	mainlib	$C_9H_{10}O$	842	2
31	19.9	Geranial	wileyregistry8e	$C_{10}H_{16}O$	940	1

Table S1 Continued

No.	RT	Metabolites	Library Match	Formula	Similarity	MSI level
32	20.95	(E,Z)-2,4-decadienal	wileyregistry8e	$C_{10}H_{16}O$	894	2
34	22.74	β-damascenone	wileyregistry8e	$C_{13}H_{18}O$	896	1
35	23.72	Hexanoic acid	wileyregistry8e	$C_6H_{12}O_2$	982	2
36	23.92	Geranyl acetone	wileyregistry8e	$C_{13}H_{22}O$	983	1
37	26.49	β-ionone	wileyregistry8e	$C_{13}H_{20}O$	911	1
38	26.82	Benzothiazole	wileyregistry8e	C_7H_5NS	904	1
39	30.58	Octanoic acid	wileyregistry8e	$C_8H_{16}O_2$	967	2
40	33.58	Eugenol	replib	$C_{10}H_{12}O_2$	927	1
41	36.56	Farnesyl acetone	wileyregistry8e	$C_{18}H_{30}O$	951	1

Table S2. Identifiers of the commonly shared metabolites, which includes CAS, PubChem CID, ChEBI, KEGG, and METLIN IDs. NA indicates metabolites without assigned DB identifiers.

No.	RT	Metabolites	Match	CAS	PubChem CID	ChEBI	KEGG	METLIN
1	4.38	1-Penten-3-one	Ethyl vinyl ketone	1629-58-9	15394	89945	NA	87803
2	5.01	Hexanal	Hexanal	66-25-1	6184	88528	NA	269119
3	6.9	Trans-2-hexenal	(E)-2-hexenal	6728-26-3	10690	28913	C08497	303260
4	7.07	2-Pentyl furan	2-Pentylfuran	3777-69-3	19602	89197	NA	85277
5	7.78	p-Cymene	Para-cymene	99-87-6	7463	28768	C06575	41091
6	8.75	Trans-2-heptenal	(E)-2-heptenal	18829-55-5	5283316	61724	NA	75301
7	8.87	1-Nitro-3-methylbutane	3-Methyl-1-nitrobutane	627-67-8	69396	NA	NA	335293
8	8.99	6-Methyl-5-hepten-2-one	6-Methyl-5-hepten-2-one	110-93-0	9496	16310	C07287	265042
9	9.33	1-Hexanol	Hexan-1-ol	111-27-3	8103	87393	NA	46070
10	10.04	Cis-3-hexen-1-ol	(Z)-3-hexen-1-ol	544-12-7	5281167	28857	C08492	271729
11	10.22	Nonanal	Nonanal	124-19-6	31289	84268	NA	269312
12	10.57	2-Isobutylthiazole	2-Isobutylthiazole	18640-74-9	62725	133683	NA	87981
13	11.07	Trans-2-octenal	(E)-2-octenal	2548-87-0	5283324	61748	C21138	75309
14	11.61	1-Octen-3-ol	1-Octen-3-ol	3391-86-4	18827	34118	C14272	46045
15	11.96	β-thujone	Beta-thujone	471-15-8	91456	50044	C20260	53340
16	12.71	(E,E)-2,4-heptadienal	(E,E)-2,4-heptadienal	4313-03-5	5283321	132837	NA	75306
17	12.86	Decanal	Decanal	112-31-2	8175	31457	C12307	36572
18	13.48	Benzaldehyde	Benzaldehyde	100-52-7	240	17169	C00261	58358
19	13.89	2-Nonenal	2-Nonenal	18829-56-6	5283335	61726	NA	316992
20	14.01	Cis-4-decenal	(Z)-4-decenal	21662-09-9	5362620	90056	NA	46438
21	14.31	Linalool	Linalool	78-70-6	6549	17580	C03985	41084
22	14.66	1-Octanol	1-Octanol	111-87-5	957	16188	C00756	6063
23	15.35	(E,Z)-2,6-nonadienal	(E,Z)-2,6-nonadienal	557-48-2	643731	7610	C08499	46414
24	16.39	β-Cyclocitral	Beta-cyclocitral	432-25-7	9895	53177	C20425	95447
25	16.95	Benzeneacetaldehyde	Phenylacetaldehyde	122-78-1	998	16424	C00601	58372
26	17.12	4-Methylbenzaldehyde	p-Tolualdehyde	104-87-0	7725	28617	C06758	66526
27	17.23	1-phenylethanone	Acetophenone	98-86-2	7410	27632	C07113	44734
28	18.29	Neral	(Z)-Citral	106-26-3	643779	29020	C09847	41099
29	18.91	(E,E)-2,4-Nonadienal	(E,E)-2,4-Nonadienal	5910-87-2	5283339	NA	NA	NA
30	19.01	4-methoxy-6-methyl phenol	2,6-Dimethylbenzaldehyde	1123-56-4	583841	NA	NA	371480
31	19.9	Geranial	(E)-Citral	141-27-5	638011	16980	C01499	41069

Table S2 Continued

No.	RT	Metabolites	Match	CAS	PubChem CID	ChEBI	KEGG	METLIN
32	20.95	(E,Z)-2,4-decadienal	(E,Z)-2,4-decadienal	25152-83-4	6427087	NA	NA	NA
33	22.39	(E,E)-2,4-decadienal	(E,E)-2,4-decadienal	25152-84-5	5283349	NA	NA	283433
34	22.74	β-damascenone	Beta-damascenone	23726-93-4	5366074	67251	NA	91646
35	23.72	Hexanoic acid	Hexanoic acid	142-62-1	8892	30776	C01585	111
36	23.92	Geranyl acetone	Geranyl acetone	3796-70-1	1549778	67206	NA	NA
37	26.49	β-ionone	Beta-ionone	79-77-6	638014	32325	C12287	69413
38	26.82	Benzothiazole	Benzothiazole	95-16-9	7222	45993	NA	88833
39	30.58	Octanoic acid	Octanoic acid	124-07-2	379	28837	C06423	112
40	33.58	Eugenol	Eugenol	97-53-0	3314	4917	C10453	4022
41	36.56	Farnesyl acetone	Farnesyl acetone	1117-52-8	1711945	67252	NA	265036

Table S3 Statistical values associated with partial least squares-discriminant analysis by using 'Leave one out' as the cross-validation method.

Variety	Components	Model statistics		
		Accuracy	R^2	Q^2
TAM Hot-Ty	4	0.9	0.996	0.629
AM Exp 1	2	1.0	0.991	0.772
TAM Exp 2	3	1.0	0.996	0.830
USAT 0121	3	1.0	0.993	0.821

Table S4. The metabolite features based on variable importance on projection (VIP) scores from partial least squares discriminant analysis (PLS-DA) of each tomato variety.

	TAM Hot-Ty	TAM Exp1	TAM Exp 2	USAT 0121
1-Penten-3-one	0.7	1.0	0.2	0.5
Hexanal	1.1	0.5	1.2	1.1
Trans-2-hexenal	1.1	1.3	0.6	0.9
2-Pentylfuran	0.7	1.2	0.6	0.4
p-Cymene	0.8	1.2	1.0	0.1
Trans-2-heptenal	1.3	0.0	1.2	1.1
1-Nitro-3-methylbutane	1.4	1.2	0.4	0.3
6-Methyl-5-hepten-2-one	1.4	0.5	1.1	0.6
1-Hexanol	0.9	1.1	0.1	0.8
Cis-3-hexen-1-ol	1.4	0.9	1.2	0.0
Nonanal	1.4	0.4	0.1	0.9
2-Isobutylthiazole	0.6	0.6	1.2	0.1
Trans-2-octenal	1.1	0.0	1.3	1.1
1-Octen-3-ol	0.9	0.1	1.0	0.8
Beta-thujone	0.7	1.1	1.7	1.1
E,E-2,4-heptadienal	0.6	0.8	1.2	1.5
Decanal	0.9	1.1	1.4	1.1
Benzaldehyde	0.4	0.1	0.1	1.2
2-Nonenal	0.5	1.1	1.0	1.2
Cis-4-decenal	0.7	0.3	0.7	1.4
Linalool	0.6	1.1	0.9	1.5
1-Octanol	1.4	1.0	1.6	0.3
E,Z-2,6-nonadienal	0.4	0.3	0.8	0.2
Beta-Cyclocitral	0.4	0.1	0.2	0.3
Benzeneacetaldehyde	0.2	0.6	0.6	1.0
4-Methylbenzaldehyde	1.1	0.8	0.5	0.1
Alpha-Acetophenone	1.4	1.6	1.7	1.6
Cis-citral	1.0	1.5	0.9	1.5
E,E-2,4-Nonadienal	0.1	1.1	1.1	1.6
2,4-Dimethylbenzaldehyde	1.7	1.3	1.4	1.1
Citral	0.3	1.2	0.1	1.2
E,Z-2,4-decadienal	1.1	1.1	0.6	0.1
E,E-2,4-decadienal	0.2	0.9	0.9	0.7
Beta-damascenone	1.2	1.6	1.5	1.6
Hexanoic acid	0.7	1.6	0.8	0.4
Geranyl acetone	1.7	1.3	1.2	1.5
Beta-ionone	1.1	1.0	0.3	0.2
Benzothiazole	0.9	1.2	0.8	1.2
Octanoic acid	0.4	0.8	1.2	1.4
Eugenol	0.1	1.5	0.8	0.6
Farnesyl acetone	1.7	0.4	1.5	1.0

Table S5. Total soluble solids, total acidity, and ripening ratio values of four tomato varieties cultivated in the high-tunnel and open-field (harvested in October of 2016).

Variety	TSS (Brix°)		TA (%)		Ripening ratio (%)	
	High-tunnel	Open-field	High-tunnel	Open-field	High-tunnel	Open-field
TAM Hot-Ty	5.5 ± 0.23a	5.5 ± 0.18a	0.41 ± 0.02a	0.45 ± 0.00a	13.42 ± 0.23a	12.43 ± 0.43a
TAM Exp 1	5.4 ± 0.20a	5.6 ± 0.23a	0.37 ± 0.01a	0.44 ± 0.05a	14.69 ± 0.80a	12.97 ± 1.26a
TAM Exp 2	5.4 ± 0.15a	5.6 ± 0.18a	0.37 ± 0.03a	0.45 ± 0.03a	14.78 ± 0.77a	12.38 ± 0.71a
USAT 0121	5.4 ± 0.11a	5.7 ± 0.21a	0.28 ± 0.02a	0.32 ± 0.03a	19.61 ± 1.14a	18.24 ± 1.34a

Total soluble solids (TSS), total acidity (TA), Ripening ratio= TSS/TA. [a] Each value is average of four replications ± standard error values. Mean with different letters between production system (high-tunnel and open-field) are significantly different by using a Student's *t*-test ($P < 0.05$).

Cultivar	Type	Source	Picture
TAM HOT-Ty	Round	Texas A&M University	
T3	Round	Texas A&M University	
L501-55	Round	Texas A&M University	
SV8579TE	Roma	Seminis	
Shorouq	Round	Seminis	
Seri	Roma	Seminis	
Mykonos	Round	Seminis	
DRP-8551	Roma	Seminis	

Figure S3. The eight tomato varieties examined in this study

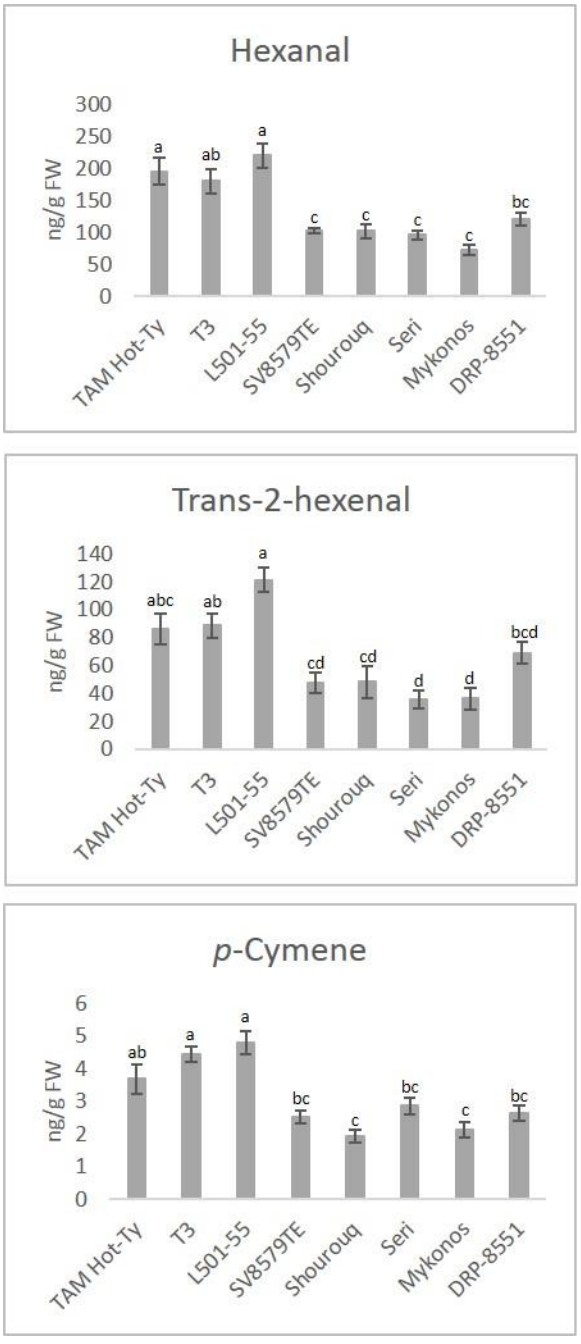

Figure S4. Univariate analysis of three potential genotype biomarkers of local TAMU varieties.

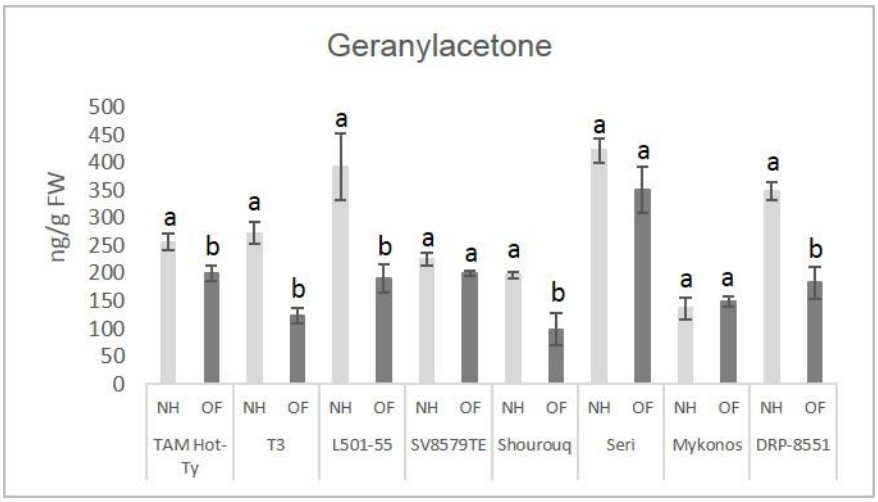

Figure S5. Univariate analysis of production systems effect on geranylacetone as a as a biomarker of production system from all eight studied varieties

Table S6. Identified volatile compounds and their concentrations (ng/g) in four tomato varieties grown in the net-house and open-field conditions.

No.	Identified metabolites	Net-house	Open-field	*t* test (*p*-value)	VIP[a]
C1	1-Penten-3-one	8.0 ± 1.1	21.3 ± 4.0	.003	1.4
C2	Hexanal	148.6 ± 11.6	123.4 ± 8.1	.079	0.5
C3	d-Limonene	1.8 ± 0.3	25.2 ± 3.0	.000	2.1
C4	Trans-2-hexenal	69.7 ± 6.0	63.8 ± 6.0	.486	0.2
C5	2-Pentyl furan	12.9 ± 0.6	13.5 ± 0.6	.520	0.1
C6	*p*-Cymene	3.1 ± 0.2	3.1 ± 0.2	.972	0.2
C7	Trans-2-heptenal	22.3 ± 0.9	18.7 ± 0.9	.005	1.2
C8	6-Methyl-5-hepten-2-one	717.6 ± 16.5	613.3 ± 26.5	.001	1.4
C9	1-Hexanol	10.0 ± 1.6	13.4 ± 1.6	.131	0.6
C10	Cis-3-hexen-1-ol	9.1 ± 1.1	19.0 ± 2.5	.000	1.2
C11	Nonanal	16.8 ± 0.8	14.1 ± 0.7	.010	1.1
C12	2-Isobutylthiazole	21.9 ± 1.0	15.8 ± 0.9	.000	1.7
C13	Trans-2-octenal	50.5 ± 1.7	44.9 ± 2.4	.062	1.1
C14	1-Octen-3-ol	7.1 ± 0.2	6.1 ± 0.3	.004	1.6
C15	*β*-thujone	28.4 ± 1.3	27.6 ± 2.1	.731	0.1
C16	(E,E)-2,4-heptadienal	26.3 ± 1.5	23.5 ± 1.7	.223	0.6
C17	Decanal	6.0 ± 0.3	6.7 ± 0.5	.264	0.4
C18	Benzaldehyde	21.9 ± 1.3	21.1 ± 1.6	.721	0.1
C19	2-Nonenal	10.9 ± 0.5	9.2 ± 0.5	.018	1.1
C20	Linalool	17.5 ± 0.8	20.1 ± 0.9	.031	1.1
C21	1-Octanol	6.1 ± 0.2	5.9 ± 0.3	.503	0.3
C22	*β*-Cyclocitral	19.9 ± 0.8	21.1 ± 0.7	.262	0.6
C23	Benzeneacetaldehyde	1.6 ± 0.2	2.0 ± 0.2	.094	0.9
C24	4-Methylbenzaldehyde	33.8 ± 0.8	33.0 ± 1.1	.542	0.2
C25	Neral	57.4 ± 2.3	46.4 ± 2.5	.002	1.4
C26	(E,E)-2,4-Nonadienal	20.8 ± 1.0	20.9 ± 1.2	.935	0.1
C27	4-methoxy-6-methyl phenol	9.2 ± 0.7	8.0 ± 0.5	.162	0.3
C28	Geranial	135.9 ± 4.6	107.7 ± 5.2	.000	1.6
C29	(E,Z)-2,4-decadienal	5.2 ± 0.3	3.3 ± 0.3	.000	1.7
C30	Methyl salicylate	87.2 ± 9.8	100.4 ± 12.0	.396	0.5
C31	(E,E)-2,4-decadienal	6.5 ± 0.5	4.8 ± 0.4	.013	1.1
C32	*β*-damascenone	26.1 ± 1.6	23.0 ± 1.5	.150	0.4
C33	Hexanoic acid	6.4 ± 0.5	5.5 ± 0.5	.185	0.2
C34	Geranyl acetone	277.6 ± 15.9	183.2 ± 12.4	.000	2.0
C35	2-phenylethanol	12.3 ± 7.1	9.9 ± 4.8	.779	0.0
C36	*β*-ionone	23.1 ± 1.0	22.4 ± 0.9	.585	0.1
C37	Benzothiazole	7.2 ± 0.3	7.6 ± 0.4	.412	0.5
C38	Pseudoionone	30.9 ± 3.4	26.4 ± 3.4	.374	0.3
C39	Eugenol	2.0 ± 0.2	1.8 ± 0.2	.655	0.2
C40	Farnesyl acetone	21.1 ± 1.4	14.5 ± 1.1	.000	1.5

Table S7. Chemical composition of simulated saliva fluid prepared for the bile acid binding.

Compound	Concentration (g/L)
Sodium chloride	1.594
Ammonium nitrate	0.328
Potassium dihydrogen phosphate	0.636
Potassium chloride	0.202
Potassium citrate	0.308
Uric acid sodium salt	0.021
Urea	0.198
Lactic acid sodium salt	0.146
Porcine gastric mucin	1.000

Impact of dietary fibers [methyl cellulose, chitosan, and pectin] on digestion of lipids under simulated gastrointestinal conditions.[372]

Table S8. Levels of dietary fiber in tomatoes grown in the net-house and open-field.

Variety	Sample weight (DW)	Soluble fiber (%)		Insoluble fiber (%)		Total fiber (%)	
		Net-house	Open-field	Net-house	Open-field	Net-house	Open-field
TAM Hot-ty	1.0	9.6	6.6	31.7	51.9	41.3	58.5
T3	1.0	10.0	6.7	25.9	50.6	35.9	57.3
L501-55	1.0	8.2	6.8	22.0	30.8	30.2	37.6
SV8579TE	1.0	6.6	6.3	34.8	40.1	41.4	46.4
Shourouq	1.0	8.7	9.5	35.0	43.0	43.7	52.5
Seri	1.0	10.1	11.0	38.9	45.0	49.0	56.0
Mykonos	1.0	7.8	7.4	39.7	40.9	47.5	48.4
DRP-8551	1.0	6.6	8.8	34.2	49.1	40.8	57.8

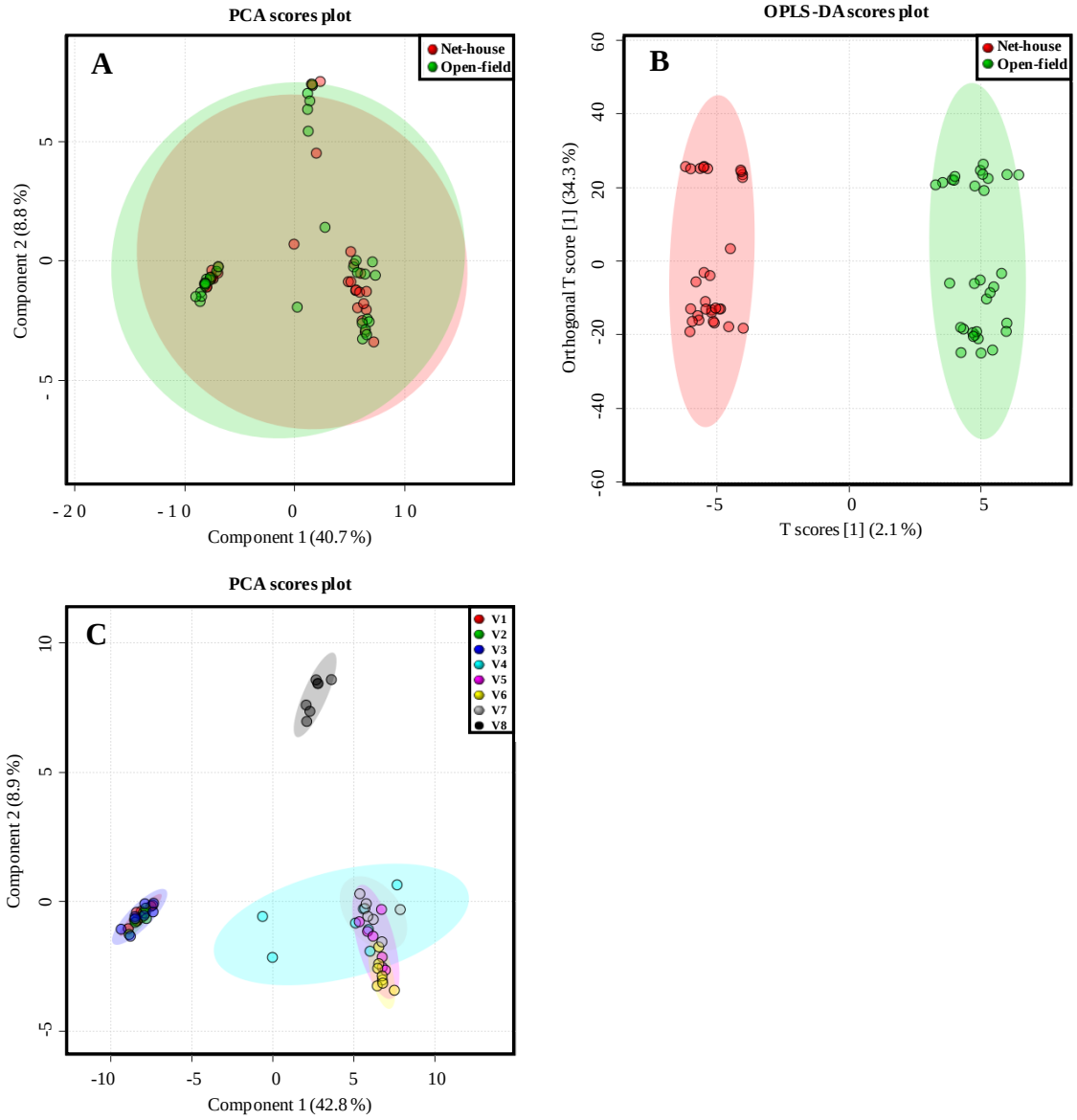

Figure S6. Multivariate statistical analysis on the dataset obtained by UPLC-HR-TOF-MS with positive ESI mode. The production system effect between net-house and open-field on tomatoes resulted in PCA (A) and OPLS-DA (B) scores plots. PCA analysis was perfumed on genotype effect (C) (V1: TAM Hot-ty, V2: T3, V3: L501-55, V4: SV8579TE, V5: Shourouq, V6: Seri, V7: Mykonos, and V8: DRP-8551).

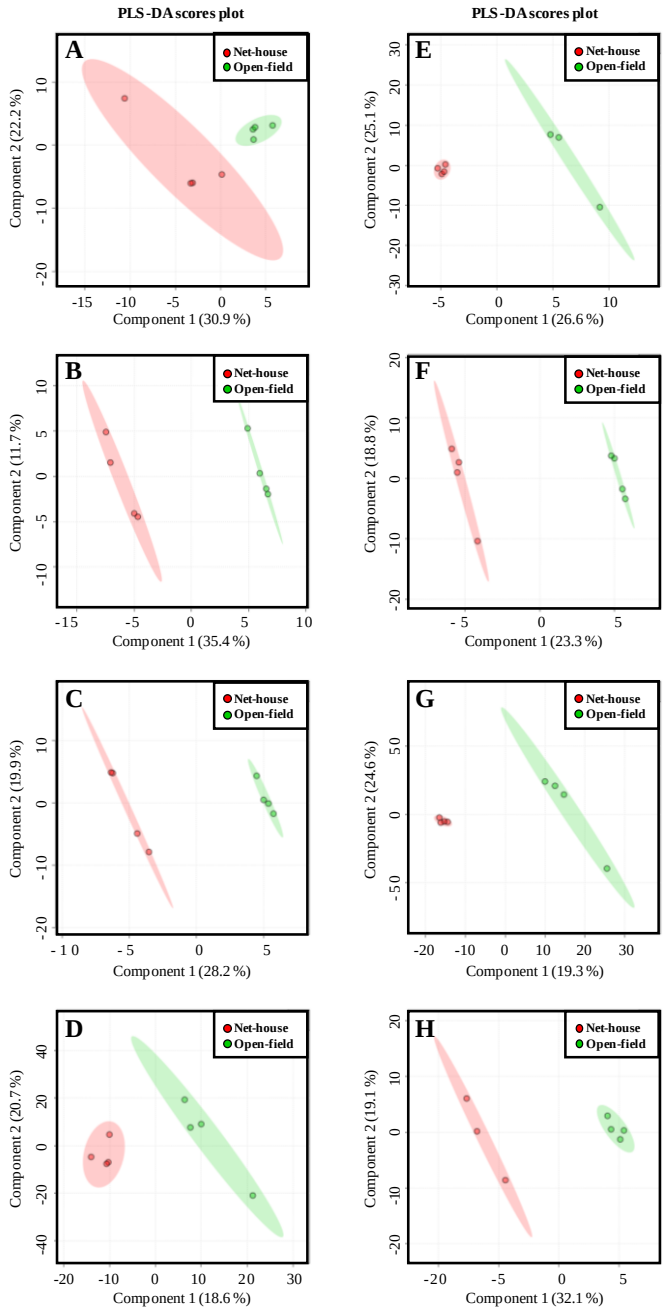

Figure S7. Partial least-squares discriminant analysis (PLS-DA) scores plots using datasets derived by UPLC-HR-TOF-MS with positive ESI mode. (A) TAM Hot-Ty (B) T3 (C) L501-55 (D) SV8579TE (E) Shourouq (F) Seri (G) Mykonos (H) DRP-8551 tomato varieties to different production systems between net-house and open-field. Ellipses represent 95% confidence intervals.

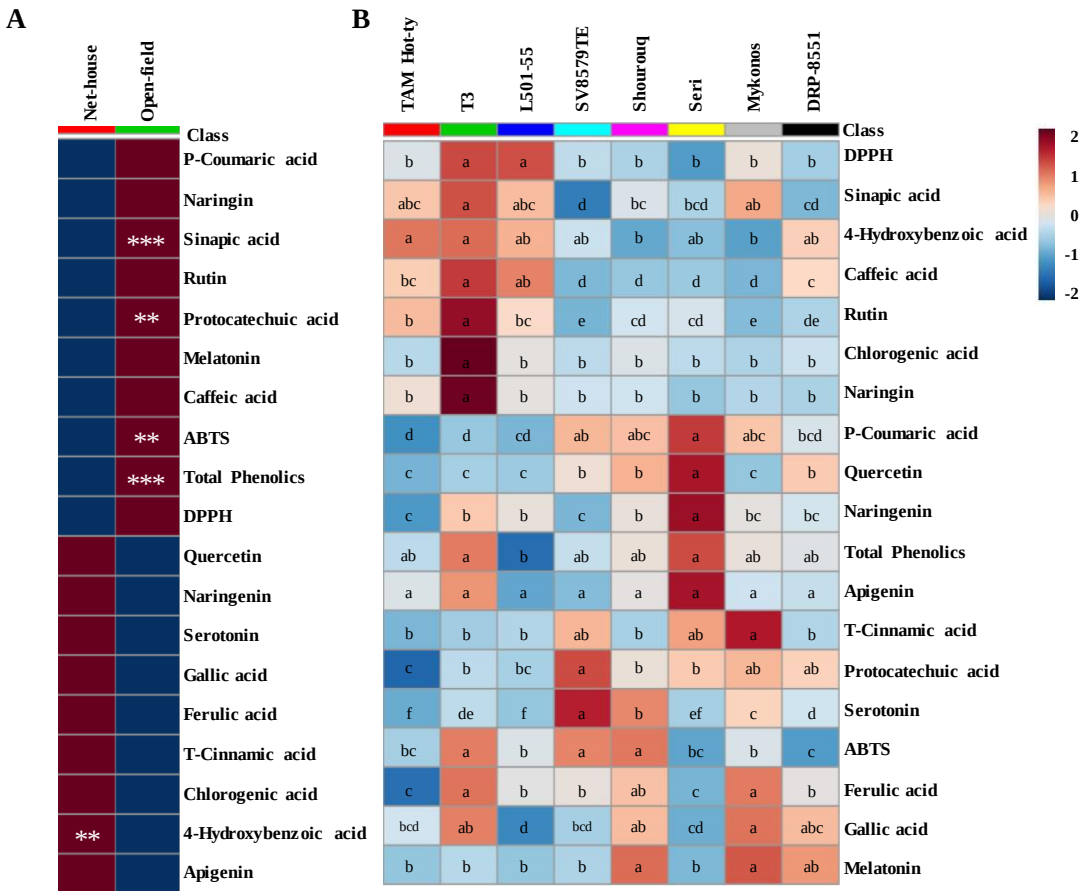

Figure S8. Heatmaps present the overall effects of the production system (A) and varieties (B) on the studied constituents. (Asterisks indicate a statistically significant difference between production systems at the level of $p < 0.01$ (**) and $p < 0.001$ (***), and the significant differences between the genotypes with different letters at $p < 0.05$).

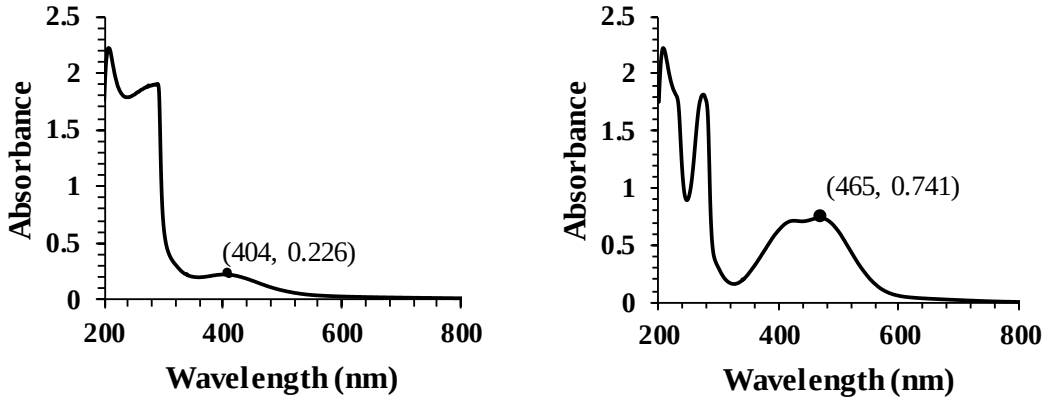

Figure S9. Scanning PPO and POD assays at wavelengths of 200-800 nm using catechol (a) and guaiacol (b) as substrates, respectively.

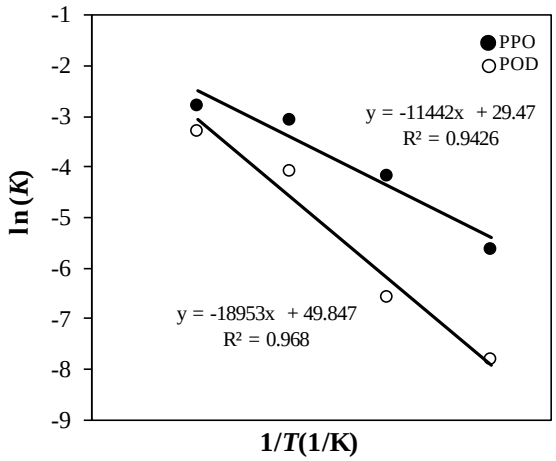

Figure S10. Arrhenius plot for heat inactivation of tomato PPO and POD enzymes.

Table S9. Kinetic parameters for the thermal inactivation of tomato PPO and POD at different temperature.

Temperature	PPO			POD		
(°C)	k_d (min^{-1})	D-value (min)	$t_{1/2\ (min)}$ (min)	k_d (min^{-1})	D-value (min)	$t_{1/2\ (min)}$ (min)
55	0.0036	639.6	192.5	0.0004	5756.5	1732.9
65	0.0153	150.5	45.3	0.0014	1644.7	495.1
75	0.0469	49.1	14.8	0.0166	138.7	41.8
85	0.0623	37.0	11.1	0.0377	61.1	18.4

Values were determined based on the reported equations.[308]

Table S10. Identification of phenolics using UPLC-QTOF-MS.

Rt (min)	Compound	Experimental mass (*m/z*)	UV λmax (nm)	MS/MS fragments at positive mode (*m/z*)	Molecular formula	Experimental mass (*m/z*)	Theoretical mass (m/z)	Mass error (ppm)
1.1	Gallic acid	171.0299	270	153, 107	$C_7H_6O_5$	171.0299	171.0288	6.4
1.4	Serotonin	177.1033	274	160, 117, 115	$C_{10}H_{12}N_2O$	177.1033	177.1022	6.0
2.1	Protocatechuic acid	155.0345	258	137, 84	$C_7H_6O_4$	155.0345	155.0339	4.0
3.5	4-hydroxybenzoic acid	139.0397	254	121, 84	$C_7H_6O_3$	139.0397	139.0390	5.0
5.3	Phtalic acid	167.0329	279	149	$C_8H_6O_4$	167.0329	167.0339	-6.0
6.4	Chlorogenic acid	355.1047	326	163, 135, 117, 89	$C_{16}H_{18}O_9$	355.1047	355.1024	6.5
7.4	p-Coumaric acid	165.0557	308	119, 91	$C_9H_8O_3$	165.0557	165.0546	6.7
9.2	Ferulic acid	195.0659	322	177, 117, 89	$C_{10}H_{10}O_4$	195.0659	195.0652	3.6
10.9	Rutin	611.1649	354	303	$C_{27}H_{30}O_{16}$	611.1649	611.1607	6.9
15.9	naringenin	273.0782	289	153	$C_{15}H_{12}O_5$	273.0782	273.0758	8.8

Table S11. Identification of plant hormones and melatonin in tomatoes by LC-DAD, LC-HR-TOF-MS with ESI positive ionization mode, and MS/MS data.

Rt (min)	Compound	Molecular formula	Theoretical mass (m/z)	Experimental mass (m/z)	Mass error (ppm)
4.3	Zeatin	$C_{10}H_{13}N_5O$	220.1193	220.1193	0.0
6.4	Gibberellin	$C_{19}H_{22}O_6$	347.1489	347.1479	-2.9
7.2	Melatonin	$C_{13}H_{16}N_2O_2$	233.1285	233.1283	-0.9
7.6	Indole-3-acetic acid	$C_{10}H_9NO_2$	176.0706	176.0698	-4.5
7.8	Salicylic acid	$C_7H_6O_3$	139.0390	139.0382	-5.8
7.8	Abscisic acid	$C_{15}H_{20}O_4$	265.1434	265.1430	-1.5
8.8	Jasmonic Acid	$C_{12}H_{18}O_3$	211.1329	211.1326	-1.4
11.9	12-Oxo-phytodienoic acid	$C_{18}H_{28}O_3$	293.2111	293.2110	-0.3

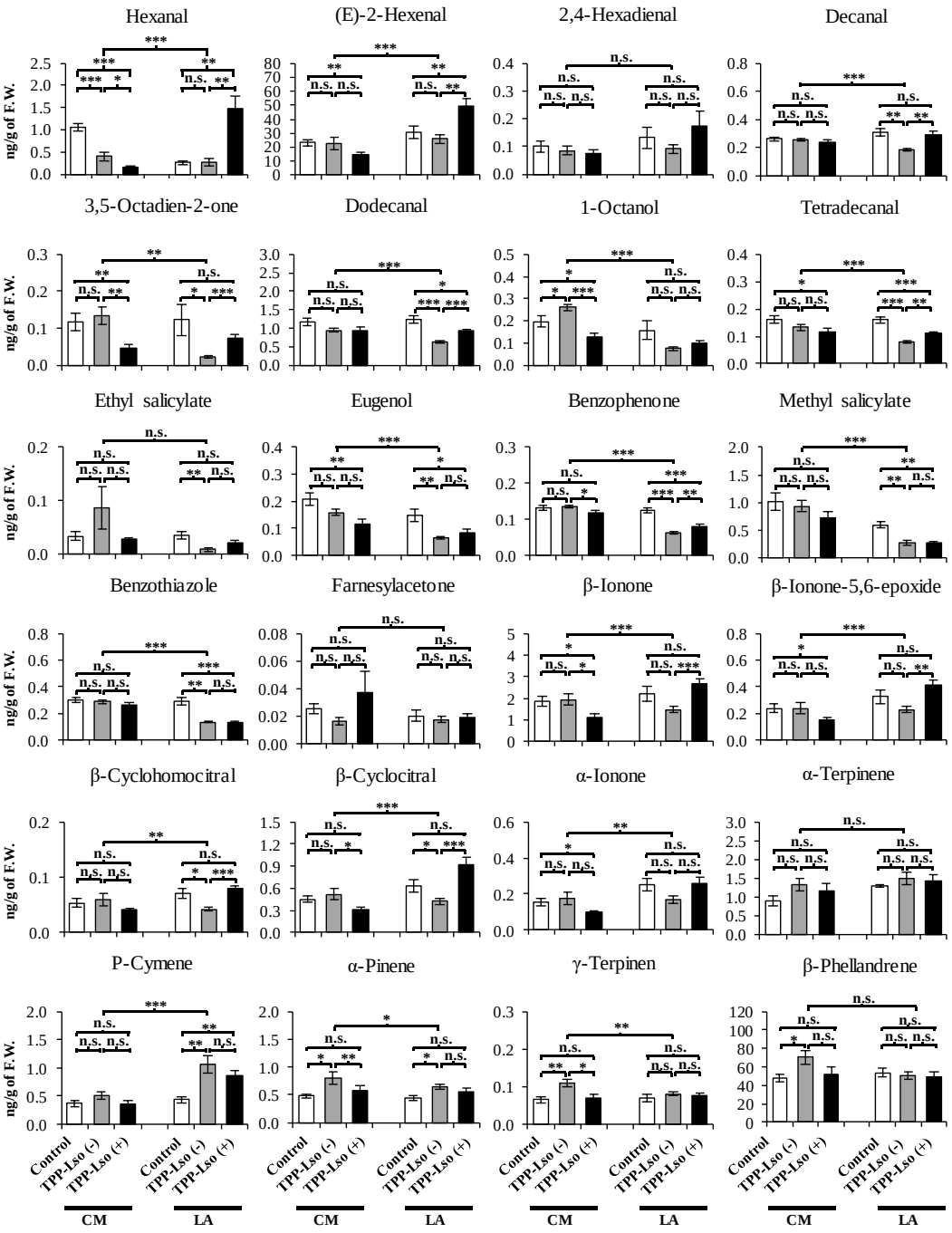

Fig. S11. The concentration of volatile metabolites according to the impact of genotype and treatment and asterisk indicates the significant difference based on student t-test and post hoc Tukey test (*, P < 0.05; **, P < 0.01; and ***, P < 0.001). n.s. indicates no significance.

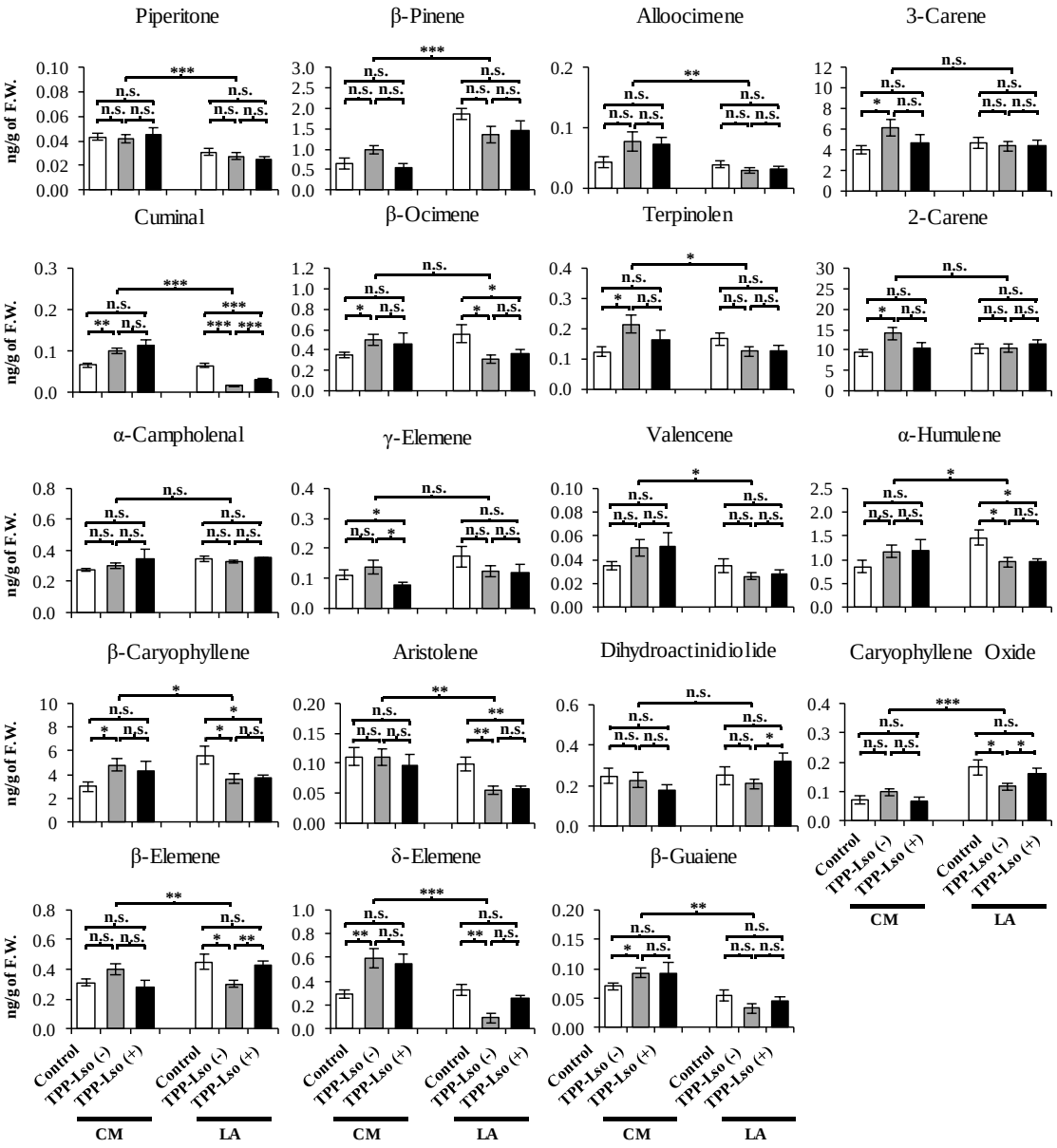

Fig. S11 Continued