Environmental Effects on Tea Quality

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Abstract

Plant-based products are highly complex samples that contain hundreds of volatile secondary metabolites. These natural products are often used as flavoring agents in foods, beverages, and pharmaceuticals and as odorants in cleaning supply, personal care and other consumer products. Plant secondary metabolites play a vial and important role in plant defense and are thought to be response for sensory and nutritional quality of the plant. The composition of plant volatile extracts is affected by plant species, geographical origin, cultivar, plant organ, maturity, and environmental factors, making identification of individual constituents challenging.

The complex nature of plant-based products makes it difficult to identify all of the constituents by gas chromatography/mass spectrometry (GC/MS) alone. The work described herein employs automated sequential, multidimensional gas chromatography/mass spectrometry (GC–GC/MS) to obtain a matrix-specific retention time/index and mass spectrometry database. Once the targeted metabolite database is produced, it can be used with spectral deconvolution and mass spectral subtraction of routine GC/MS data to reveal untargeted metabolites, providing an efficient, reliable, and unambiguous means to identify all constituents. Specifically, metabolites were used to track how climate variations affected teas harvested in Yunnan and Fujian Province in China. Striking differences in concentration were observed in response to elevational, seasonal,

and yearly differences. In addition, a field-practical volatile sample collection method was developed to measure plant response to environmental stress *in situ*.

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List of Acronyms

ANOVA – analysis of variance

C – (+)-catechin

C1 – column 1

C2 – column 2

CG – (-)-catechin gallate

CIS – cooled injection system

CTS – cryogenic trapping system

DAD – diode array detector

DCSE – direct contact sorptive extraction

DHS – dynamic headspace

EC – (-)-epicatechin

ECG – (-)-epicatechin gallate

EGC – (-)-epigallocatechin

EGCG – (-)-epigallocatechin gallate

GC – (-)-gallocatechin

GCG – (-)-gallocatechin gallate

GC/MS – gas chromatography/mass spectrometry

GC-GC – automated sequential, multidimensional gas chromatography

(heartcutting GC)

GC×GC – comprehensive gas chromatography

GC-O – gas chromatography-olfactometry

LC/MS – liquid chromatography/mass spectrometry

MANOVA – multivariate analysis of variance

MCS – multi column switching device

MeJA – methyl jasmonate

NIST – National Institute of Standards and Technology

ODP – olfactometry detection port

OPLS-DA – orthogonal projection to latent structures-discriminant analysis

PCA – principal components analysis

PDMS - polydimethylsiloxane

PERMANOVA – permutational multivariate analysis of variance

PET – polyethylene terephthalate

PLS-DA – partial least squares-discriminant analysis

RI – retention index

RIC – reconstructed ion current

RPA – relative peak area

RPD – relative percent difference

RSD – relative standard deviation

SHS – static headspace

SSV – scan to scan variance

TB – theobromine

TDU – thermal desorption unit

TIC – total ion current

VIP – variable importance projection

VOCs – volatile organic compound

Chapter 1. Introduction and Background

1.1 Climate Change and Crop Quality

Increased variation in climate patterns has significantly impacted the agriculture sector, challenging farmers to adapt farming practices and technology. Previous studies on the effects of climate change on agricultural systems have primarily focused on crop yields.[1-4] However, the effect on crop quality is poorly understood.[5-7]

Secondary metabolites are produced by plants primarily as defense compounds; however they are also thought to be responsible for plant quality, i.e. flavor, aroma, and nutrition. The production and concentration of plant metabolites are affected by both abiotic (temperature, rainfall, UV radiation) and biotic (insects, microbes) stresses, resulting in changes in sensory and nutritional quality of crops.[8-9] For example, tea (*Camellia sinensis* (L.) O. Kuntze) is highly dependent on cultivating conditions for optimal quality and growth. Ideally, tea is grown under temperate conditions; however, rising temperatures, changing precipitation patterns, and increased herbivory stress are a huge concern for future sustainability.[10-11]

Consumed primarily for its sensory properties, cultural significance and claimed health benefits, tea is the second most consumed beverage in the world, after water, with an estimated 3 billion cups consumed daily.[12] Growing knowledge of the health benefits has led to increased consumption worldwide. In addition to the

stimulant and relaxing properties provided by the caffeine and amino acid content, various studies have suggested that tea has antibacterial, anticancer, anti-inflammatory, antioxidant, antiviral, cardioprotective, and neuroprotective properties among others.[13-16] Health benefits aside, tea supports millions of farmers worldwide with a growing market of over $20 billion USD, with China responsible for over 38% of production.[17]

In China, the tea harvest is divided into four main seasons, based on the East Asian Monsoon cycle. The highest quality tea is obtained during the spring harvest, with a dramatic decrease in quality observed at the monsoon onset. Historical trends show the East Asian Monsoon is starting earlier and lasting longer, resulting in a narrower harvest window to obtain high quality tea.[18-19] In addition, the mean global temperature is projected to increase over the next few decades and is expected to negatively impact tea quality.[3, 20] Since tea is harvested seasonally, it is an ideal agricultural system to study crop quality as a function of climate variability.

Previously, we showed that under extreme rainfall conditions such as the East Asian Monsoon, the quality of tea harvested from Bulang Mountain in Yunnan Province, China changes significantly.[21-22] Concentration differences were observed for hundreds of volatile and non-volatile compounds only five days after the onset of the monsoon rains. For example, concentrations of catechins (catechin, catechin gallate, epicatechin 3-gallate, epigallocatechin,

epigallocatechin 3-galalte, gallic acid, gallocatechin, and gallocatechin 3-gallate) and methylxanthines (caffeine, theobromine, and theophylline) decreased by more than 50% during the transition from spring to summer (monsoon) rainfalls.[22] Both chemical families contain astringent, bitter compounds associated with many of the health-beneficial effects of tea.[23-25] In contrast, total polyphenol content and antioxidant potential were significantly higher, meaning other phenolic compounds such as flavones, flavonols, phenolic acids, and their derivatives [26] increased in concentration as the catechins decreased. In addition, to variations in non-volatile metabolites, differences were observed in the composition of the volatile fraction containing over 200 metabolites. Many increased, others decreased by hundreds of percent, while some exhibited no change in concentration.[21] Metabolites such as (*Z*)-jasmone (48%), (*Z*)-methyl jasmonate (84%), and phenylethyl alcohol (74%) were significantly higher in the spring harvest. All three are described as floral while the second is also sweet and the third honey-like.[27] On the other hand, 5,6-*epoxy*-β-ionone (90%), β-bourbonene (51%), and (2*E*)-hexenal (172%) increased from spring to monsoon. The first is characterized as fruity and woody, the second as woody and herbal, and the last as herbal and green. Collectively, these findings are consistent with farmer perceptions in the region, namely, that spring tea is of higher quality exhibiting sweet, floral aroma compared to green, earthy characteristics monsoon teas.[22] Consequently, farmers typically receive 50% less income for teas harvested during the monsoon season.[22]

Since tea is a quality-based resource, whose quality varies with changing environmental conditions and management practices, consumer purchasing decisions and market value for the farmer are ultimately affected. As a result, research is necessary to study the impact of climate change on tea quality over time to help farmers adapt to changing weather patterns and create a more sustainable agricultural system. However, the complexity of tea makes identification of chemical and functional constituents difficult.

1.2 Multidimensional Gas Chromatography

One-dimensional gas chromatography/mass spectrometry (GC/MS) is the most frequently used technique to analyze the volatile fraction of tea. However, a single dimension does not provide the resolving power required to fully separate all of the components of tea. Researchers have looked towards improving separation through advances in GC technology such computerized temperature control, pneumatically controlled flow, programmed temperature vaporizing inlets, and novel stationary phases. Despite technological advances, limitations still exist with respect to the number of compounds that can be separated during a single-column analysis. According to Davis and Giddings' statistical model of overlap,[28] in order to resolve 95% of components in a complex sample matrix, a peak capacity 39-times greater than the number of components present in the sample is required – which is practically impossible by GC/MS.

Chemical similarities between sample components lead to crowding in certain parts of the chromatogram while leaving empty space in others. This ordered/disordered distribution of peaks after separation of a complex sample can be described by Giddings' concept of sample dimensionality vs. system dimensionality.[29] Sample dimensionality is a measure of the sample complexity, whereas system dimensionality is the number stages in the separation. Figure 1-1 demonstrates this concept with a sample consisting of compounds that differ by size, shape, and color – a total of 3 sample dimensions.[30] Separation of these compounds based on a single system dimension leads to interferences due to other sample characteristics resulting in an incomplete separation. Increasing the system dimensionality to separate first by size then by color or shape improves separation resulting in an ordered chromatogram.

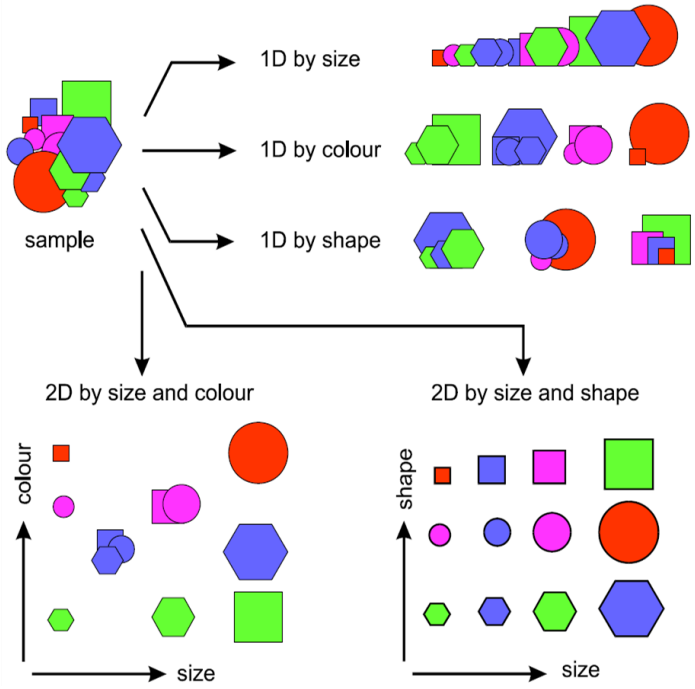

Figure 1-1. Visualization of sample dimensionality vs. system dimensionality. If a mixture containing components with different sizes, shapes, and colors is separated based on a single dimension, interferences will occur. Separation based on two dimensions, first size then shape or color improves separation and results in an ordered chromatogram.[30]

Multidimensional GC separations are far more efficient than a single column, where two orthogonal (dissimilar) separation mechanisms are employed. A multidimensional separation requires that the separation on the first dimension must not be lost on the second dimension.[31-32] There are two types of multidimensional GC techniques, heartcutting (GC-GC) and comprehensive (GCxGC). In GC-GC, selected sample portions (heartcuts) are transferred from the first dimension column onto the second dimension column for further separation.[33] The columns have different phases (e.g. polar vs. non-polar) and are connected by a flow-controlled switching device. On the other hand, GC×GC is seen as the limiting case of GC-GC when the width of the heart-cut approaches zero.[34] Small sample portions are continuously transferred from the first to the second column resulting in an increased separation space on a shorter time-scale. It is not the purpose of this work to compare multidimensional techniques, for that see current reviews.[35-38] For the work described herein, GC×GC will not be discussed further.

1.3 Gas Chromatography/Mass Spectrometry

GC/MS is unmatched it its ability to identify and quantify low molecular weight, low boiling point and thermally stable plant metabolites, but only if the mass spectra and are known and matrix components do not interfere with compound identification. Samples are identified by comparing spectra to those of authentic reference standard or mass spectral libraries such as the National Institute of Standards and Technology (NIST), Adams and Wiley. However, identification

fails when multiple compounds exit the GC at the same time. In this case, each compound's ion signal is recorded and, if more than one compound produces the same *m/z* ion, signals become additive. This confounds data interpretation since ion signals are no longer indicative of a single compound. To obtain mass spectral information from coeluting compounds mathematical algorithms are required to separate, or deconvolve, each compound's fragmentation pattern from all others.

In the following work, automated sequential, multidimensional gas chromatography/mass spectrometry (GC-GC/MS) and spectral deconvolution was used to increase resolution to analyze complex tea samples. In contrast to studies that transfer select sample portions onto the second column, we transferred the entire first dimension in 1-minute heartcuts, each as a separate injection. Subsequent injections are made only after the preceding heartcut has completely eluted off both columns. Utilizing the technique in this manner enables the construction of comprehensive metabolite databases containing retention time and mass spectral information. However, the gain in resolution over other separation choices, makes the technique extremely time consuming and not practical for routine analyses. Therefore, once the database is constructed, it can be used with GC/MS and spectral deconvolution for routine analyses.

Chapter 2. Optimizing Target/Nontarget GC/MS Workflows to Differentiate Tea Quality

2.1 Introduction

While GC-GC/MS is excellent at producing retention time and mass spectral data, it is extremely time-consuming. For example, if the 1st column separation employs a 40-min temperature program and 1-min sample portions are transferred from the 1st to the 2nd column, a total of 40, 2nd dimension data files are produced. If the 2nd column is a 50-min separation, the analysis of a single sample takes days. In addition, despite the increase in separation space GC-GC/MS offers, coelution still occurs due to the complexity of natural products. Also, high concentration analytes such as limonene in citrus oils will appear in multiple data files due to flow switch imprecision, which means the same compound must be reconciled to eliminate redundancies in the database. The total time we spend creating one library takes months to accomplish. To overcome these deficiencies, we developed new data analysis software that automatically inspects each peak in the data file, subtracts the mass spectrum of a compound from the total ion current (TIC) chromatogram, and evaluates whether the residual signal approximates background noise. When this occurs, compound identity, retention time, mass spectrum, and deconvolution ions are uploaded to the software. The same software can then be used to track these compounds from sample-to-sample by GC/MS.[39-41]

Rasmussen and Isenhour[42] first assessed the efficiency of library search algorithms to identify unknowns, followed by Stein and Scott[43] and McLafferty et

al.[44] Recently, Stein[45] reviewed the basic principles and factors that affect compound identification using mass spectral reference libraries while Sparkman[46], Koo[47] and Samokhin[48] compared the performance of newer library-matching algorithms[49-52] to those of Rasmussen, Stein, and McLafferty. The development of early mass spectral deconvolution software aimed at untangling spectra of coeluting compounds was investigated by Champan[53] and Likic.[54] More recent deconvolution software was reviewed by Putri[55], Du[56] and Yi[57], including vendor-specific software such as ChromaTOF (LECO), MassHunter Profinder (Agilent), and MassLynx (Waters) as well as ADAP-GC 2.0[58], AutoDecon[59], AMDIS[60], MetaboliteDetector[61], MetaboAnalyst[62], MetabolomeExpress Project[63], MetAlign[64], mMass[65], MZmine[66], OpenChrom[67], PyMS[68], PYQAN[69], SpectConnect[70], and TagFinder.[71] The latter group operates on a wide range of data files. All of these solutions provide spectral matching between library and sample data. Until BinBase, none of the aforementioned software included database functions that allowed analysts to add new information, compare sample outputs, or track compounds across multiple samples.[72] Although BinBase and Mass Profiler (Agilent) can compare data sets, they rely on high resolution MS data to differentiate samples. In addition, BinBase is reliant on LECO's ChromaTOF software to deconvolve spectra, limiting its application to LECO instruments. To our knowledge no software program exists that can differentiate MS fragmentation patterns to automatically subtract a full MS spectrum from the TIC signal to reveal and identify coeluting compounds.

We present new data analysis software, Ion Analytics, which works on all instrument data files that produce an industry standard .cdf extension.[73-75] The software automatically investigates each peak, determines mass spectral constancy at each scan across the peak. If invariant, uploads the retention time, mass spectrum, and relative abundance of three to six fragmentation ions used for deconvolution as well as the identity of the compound after searching the analyst's library, NIST, Wiley, Adams or any other spectral library that can be saved in NIST format. If peak scans are not constant, the software automatically differentiates fragmentation patterns, subtracts the "clean" mass spectrum from the TIC signal at each scan. After spectral subtraction the residual signal is compared to background noise. If the two signals approximate one another, the above mentioned information is uploaded into the database. Once the database is constructed, it can be used with spectral deconvolution and MS subtraction to track the compounds from one sample to the next by GC/MS. The work herein will be presented in two parts: 1. Automated method (database) construction by GC-GC/MS and 2. Chemical profiling of target and nontarget compounds by GC/MS.

Part 1: Automated Method Construction by GC-GC/MS

2.2 Experimental

2.2.1 Sample Collection and Extraction

Tea samples were collected in 2013 from Yunnan Province, China at high (1400 m) and low (650 m) elevation from the same mountain. Tea extracts were prepared by simultaneous distillation-extraction.[21] 10 g of tea was brewed in 100 mL of deionized water at 90 °C, and then cooled in a sealed container for 30 min. Both the infusion and 12 mL of methylene chloride were distilled at 100 °C and 60 °C, respectively, for 2 hr with volatiles collected in the organic phase. Anhydrous sodium sulfate was added to the distillate and concentrated to 500 μL under a stream of purified nitrogen.

2.2.2 Automated, Sequential 2D GC-GC/MS

The instrument configuration and heartcutting process have been described in detail elsewhere.[21] Briefly, the first GC (Agilent model 6890, Santa Clara, CA) housed the 1st column (C1, 30 m × 250 μm × 0.25 μm RTX-Wax, Restek, Bellefonte, PA) and was equipped with a flame ionization detector. Operating conditions were: 40 °C for 1 min, then ramped to 240 °C at 5 °C/min. C1 was connected to a programmable temperature, vaporization inlet (CIS, Gerstel, Mülheim an der Ruhr, Germany), operating in splitless mode, on one end and to a 5-port crosspiece (Gerstel) on the other. The 2nd column (C2, 30 m x 250 μm x 0.25 μm RXI-5MS, Restek) was housed in GC 2 (Agilent model 6890), which was connected to the crosspiece through a cryogenic freeze trap (CTS1, Gerstel) on one end and to an Agilent mass spectrometer (model 5975) on the other. C2 operating conditions were: 40 °C for 1 min, and ramped to 280 °C at 5 °C/min. Both columns operated at 1.2 mL/min of constant helium flow. A multi-column

switching device (MCS, Gerstel) supplied countercurrent flow to the crosspiece. Based on 1 min sample portions, a total of 40 heartcut data files were obtained. Because each heartcut was an independent analysis, subsequent injections were made after each preceding sample portion eluted from both columns. As a result, total analysis time was 3.5 days for each sample. MS operating conditions were: 230 °C and 150 °C for the ion source and quadrupole, respectively, 70 eV electron impact voltage, and 50 to 350 mass range, 12 scans/sec. A standard mixture of C_7-C_{30} *n*-alkanes (Sigma-Aldrich, St. Louis, MO) was used to calculate the retention index (RI) for each compound.

2.2.3 Tea Analysis

GC/MS operating conditions were as described in system 2. Concentrations were calculated as relative peak areas (RPA) using naphthalene-d_8 as the internal standard, except for four compounds. Calibration curves were produced for pentanol, terpinolene (TCI, Nihonbashi-honco, Japan), trans-linalool oxide (Sigma-Aldrich), and toluene (Supelco, Bellefonte, PA) from 0.5ug/ml to 50ug/ml. Response factors were calculated for each compound as follows:

$$RF = \frac{A_i C_{IS}}{A_{IS} C_i}$$

where subscripts *i* and *IS* refer to calibration compounds and internal standard, and C and A refer to their corresponding concentration and peak area. Calibration curves were acceptable when the average response factor, relative standard deviation (RSD), over the concentration range was ≤ 15 %, with $r^2 \geq 0.99$.

2.2.4 Data Analysis

New data analysis software (Ion Analytics, Andover, MA) was used to automatically inspect and record compound identities, peak retention times, and mass spectra of GC-GC/MS data for untargeted compounds. For MS subtraction, each software parameter defined below is set by the user. First, each peak was screened to determine if the spectrum at each scan was constant (± 20%). If so, the software computed the match between sample and library spectra (e.g., NIST, Wiley, Adams). If the fit was acceptable, compound name, CAS #, retention time, reference spectrum, 3-6 target ions and relative abundances were recorded in the database. In contrast, comparison of the sample and library or literature [76-79] RI was manual. Approximately 250 reference compounds were used to confirm compound identity by comparing sample and reference compound spectra and RI. These standards were purchased from Sigma-Aldrich, TCI, Acros Organics (Pittsburgh, PA), Alfa Aesar (Ward Hill, MA), MP Biomedicals (Santa Ana, CA), SPEX CertiPrep (Metuchen, NJ) and AccuStandard (New Haven, CT). If sample spectra and reference or library spectra did not match, the above information was uploaded into the database with a numeric identifier. Second, if the spectra across the peak varied, the software employed MS subtraction algorithms to search for constant scans, where the number of contiguous scans that must be constant is no fewer than three, average the mass spectrum from these scans, and then subtract that spectrum from the TIC signal. Once subtracted, the software automatically inspected residual ion signals to determine if the resulting peak scans were constant or approximated background noise, which was determined by inspecting

the highest baseline m/z signal. If constant, the mass spectra of the second compound was subjected to the treatment described above, with identities, retention time, mass spectra, and deconvolution ions uploaded into the database. If not (unresolved peak), the software repeated the subtraction process until the residual signal approximated background signal. If the resulting signal does not meet the user-defined criteria, no additional information is obtained.

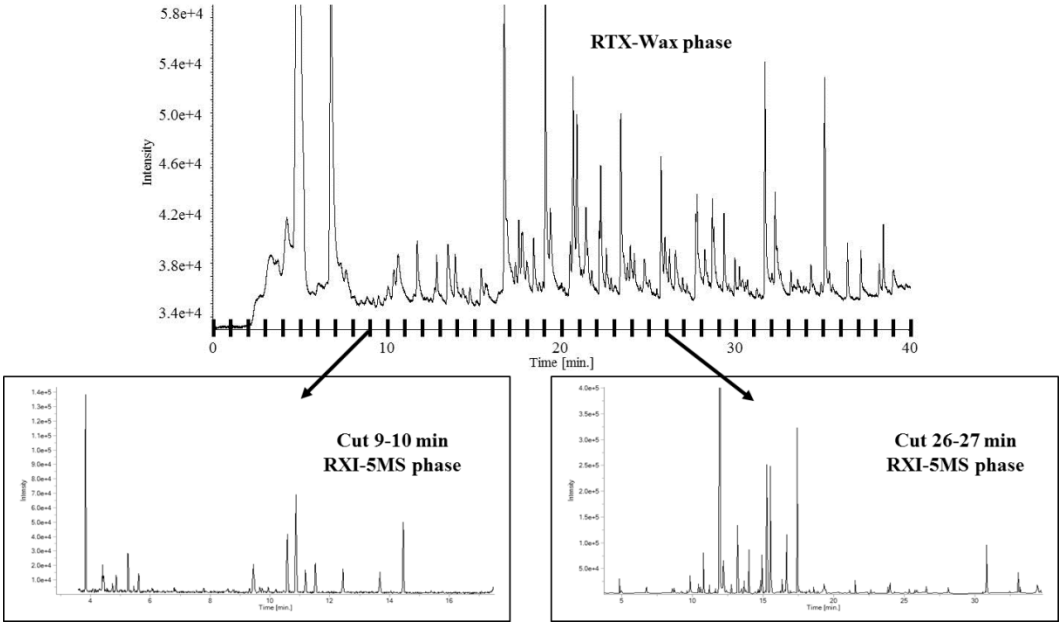

Figure 2-1. GC-GC/MS analysis of high elevation tea from Yunnan, China. The top chromatogram is the separation on Rtx-Wax (C1). The bottom chromatograms are 1 min heartcuts at 9 and 26 min on Rxi-5MS (C2).[80]

Once the database is constructed, it is used with spectral deconvolution to identify target compounds. The analyst can also set each spectral deconvolution parameter. First, the deviation in mass spectra must be ≤20% for five or more consecutive scans. Second, the scan-to-scan variance (SSV) must be <5. The SSV algorithm calculates the relative error by comparing the mass spectrum at each peak scan against one another. The smaller the difference, the closer SSV is to zero, the better the spectral agreement. Third, the Q-value must be ≥93. The Q-

value is an integer between 1 and 100 that measures the total ion ratio deviation of the absolute value of the expected minus observed ion ratio divided by the expected ion ration times 100 for each ion across the peak. The closer the value is to 100, the higher the certainty between database and sample spectra. Fourth, the Q-ratio compares the ratio of main ion intensity to confirming ion intensity across the peak. The acceptability limit for this criterion is ±20%. The software assigns a compound name or numerical identifier when the four compound acceptance criteria are met, establishing a single acceptance criterion. [21, 41, 81-82]

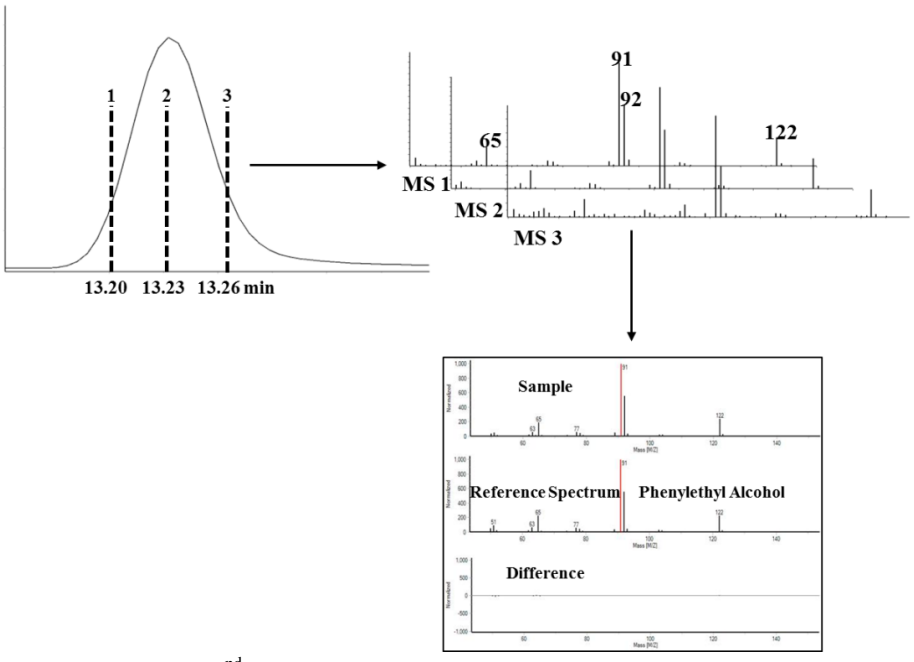

Figure 2-2. Inspection of 2nd dimension peak at 13.23 min. When the mass spectrum is constant across the peak, the software compares the sample spectrum to reference compound and commercial library spectra to assign identity, in this case, phenylethyl alcohol.[80]

2.3 Results and Discussion

Although GC-GC/MS is time-consuming, it is the best technique for producing comprehensive libraries of chemical constituents in complex samples. An illustrative example is shown in Figure 2-1. The top chromatogram is the

separation of high elevation green tea on the 1st column, while the bottom two chromatograms depict 1 min separations at 9 and 26 min. Evident is the increase in separation space, since the first sample portion corresponds to an unresolved region of the chromatogram while the second reveals a few compounds on the wax column. More than 50 compounds have been identified from these two heartcuts.

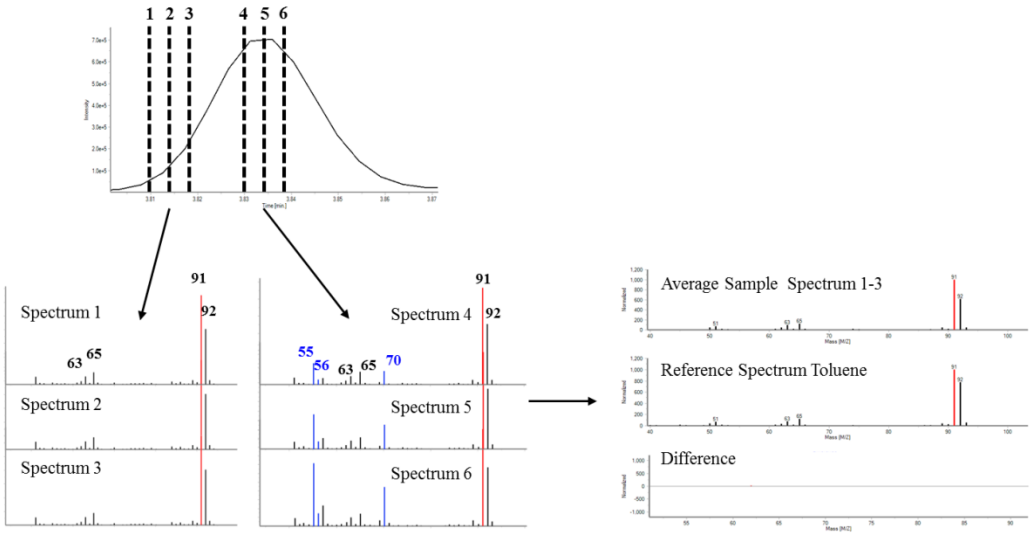

Figure 2-3. Inspection of 2nd dimension peak at 3.83 min. If the mass spectra vary across the peak, due to coeluting compounds, the software searches 3-5 invariant scans and averages them to compare reference and/or library spectra. Spectra 1-3 correspond to toluene and spectra 4-6 correspond to toluene with a coeluting compound (blue ions).[80]

2.3.1 Library Creation

First, the Automated Method Construction command is used to inspect all 40 data files. If the mass spectrum is constant at each peak scan, see Figure 2-2, the software compares the sample mass spectrum and retention time against the user and commercial libraries. When the compound acceptance criterion is met, compound name, CAS #, RT, mass spectrum, and 3-6 target ions and their abundances are uploaded to the database. For example, phenylethyl alcohol elutes

at 13.23 min on the RXI-5MS phase in sample portion 26, with reference compound data confirming compound identity. In all other cases, e.g., where NIST/Wiley/Adams spectra meet the similarity factor match criterion, compounds are considered tentatively identified. If the mass spectrum cannot be matched to a library spectrum but all other peak confirmation criteria are met, the compound is assigned a unique number that can be updated when reference compounds become available.

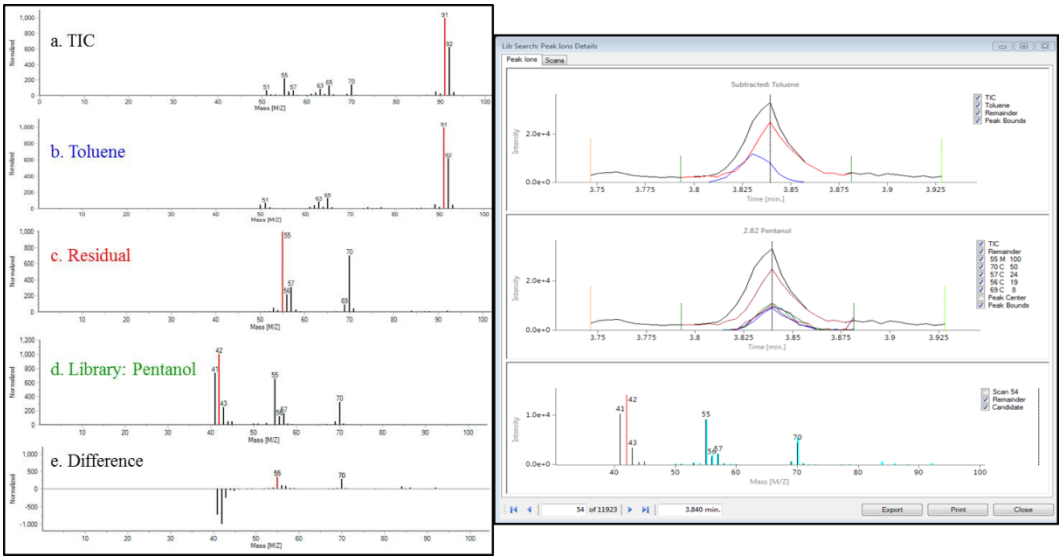

Figure 2-4. MS subtraction of toluene spectrum (b) from the total ion current (TIC) chromatogram (a) yields residual spectrum (c). If the residual spectrum (c) is constant it is compared to reference and/or library spectra (d) to assign identity. Since library spectra include <50 mass unites, see experimental section, the residual spectra (e) is based on the base ion at *m/z* 42, hence the resulting signal. The peak ion detail view shown in the top right depicts the TIC (black), toluene (blue) and residual (red) peaks after toluene subtraction whereas the middle box illustrates co-maximization of each residual ion trace. The bottom box shows the spectrum match of the residual (blue) and library (black) for pentanol.[80]

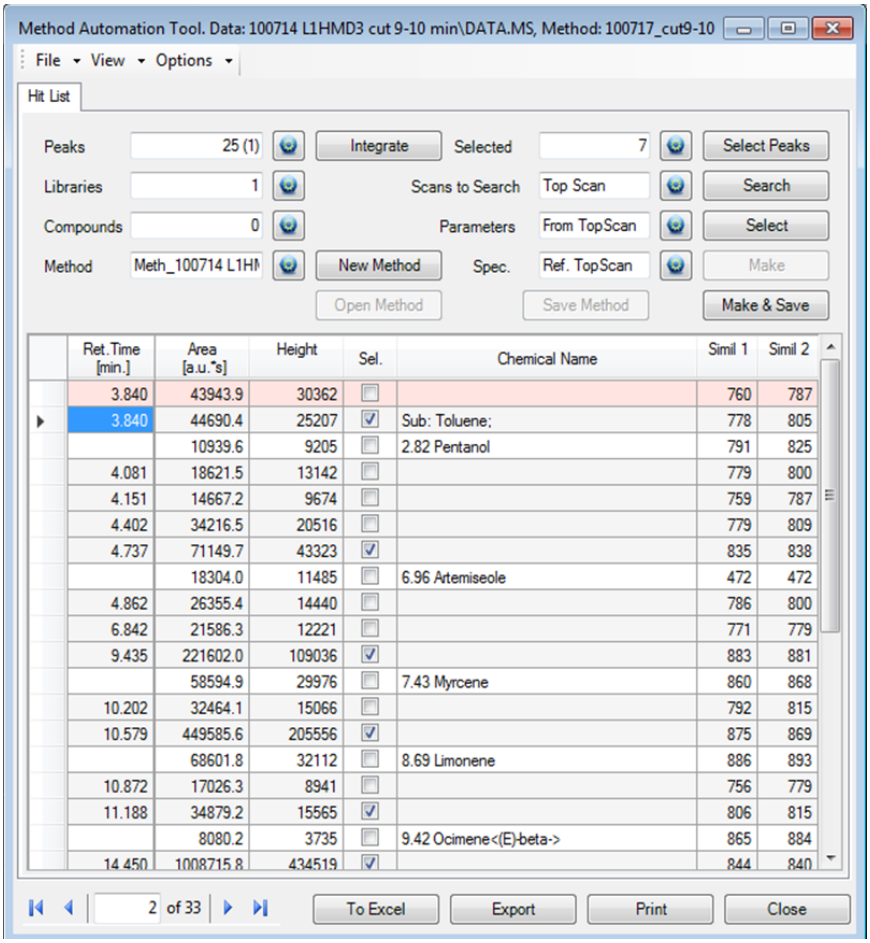

Figure 2-5. Method Automation Window for sample portion 9 after subtraction of toluene. The dialog box reports detection of 25 peaks. The pink line and the line that follows indication MS subtraction, with the highlighted line listing the retention time, peak are and height, as well as similarity factor for toluene after its spectrum was subtracted from the peak.[80]

If, on the other hand, spectra vary, see Figure 2-3, the software searches for 3-5 invariant scans, averages the mass spectra, and compares sample vs. reference compound patterns. Spectra 1-3 correspond to toluene and spectra 4-6 correspond to toluene with a coeluting compound (blue ions). When the acceptance criterion is met, the associated information for that compound, in this case toluene, is added to the database. Then, the software subtracts the average toluene mass spectrum (b) from the TIC (a) signal as shown in Figure 2-4 resulting in residual signal (c). These ion signals are consistent with scans 4-6 in Figure 2-3 after

toluene subtraction. Figure 2-4 (right, top) shows the TIC (black), toluene (blue) and residual (red) peaks. TIC and residual ion traces co-maximize and are shown in the middle. The bottom box illustrates the match for pentanol when the residual (blue) and library spectra (black) are merged. Recall that the mass spectrometer was scanned from 50-350 m/z, which explains the missing sample ions in these figures.

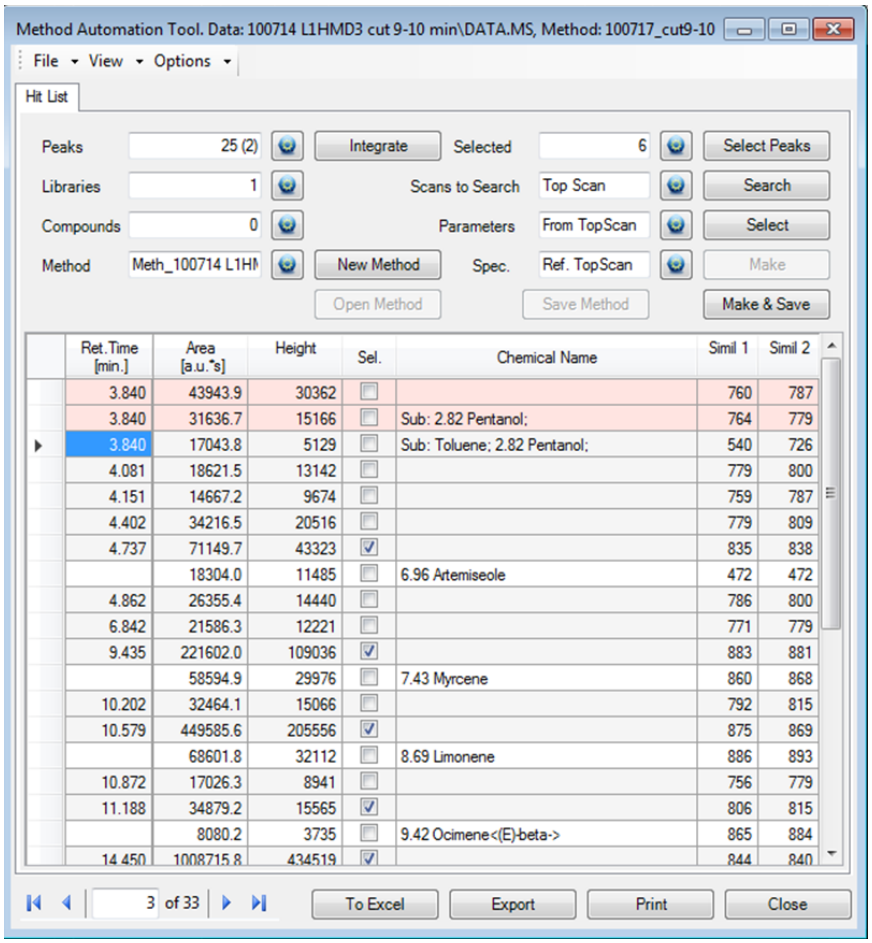

Figure 2-6. Method Automation Window for sample portion 9 after subtraction of toluene and pentanol spectra. The second pink line and the two lines that follow indicate two subtractions have occurred, with the highlighted line reporting retention time, peak area and heights as well as similarity factor of pentanol after its spectrum was subtracted from the peak. The residual spectrum fails to meet the peak acceptance criterion and ends the compound identity search.[80]

The Method Automation Window in Figure 2-5 shows 25 peaks were detected above the user defined peak threshold for sample portion 9. The pink row reports the retention time, peak area/height, and library match similarity values. In this example, the pink row indicates toluene has been subtracted from the TIC in Figure 2-4. Similarly, the two pink rows in Figure 2-6 indicate toluene (b) and pentanol (c) spectra have been subtracted from the TIC (a) resulting in the residual (d) in Figure 2-7. The right-hand side makes evident that the residual TIC (red, top) and each of its contributing ions (middle and bottom) approximate baseline noise.

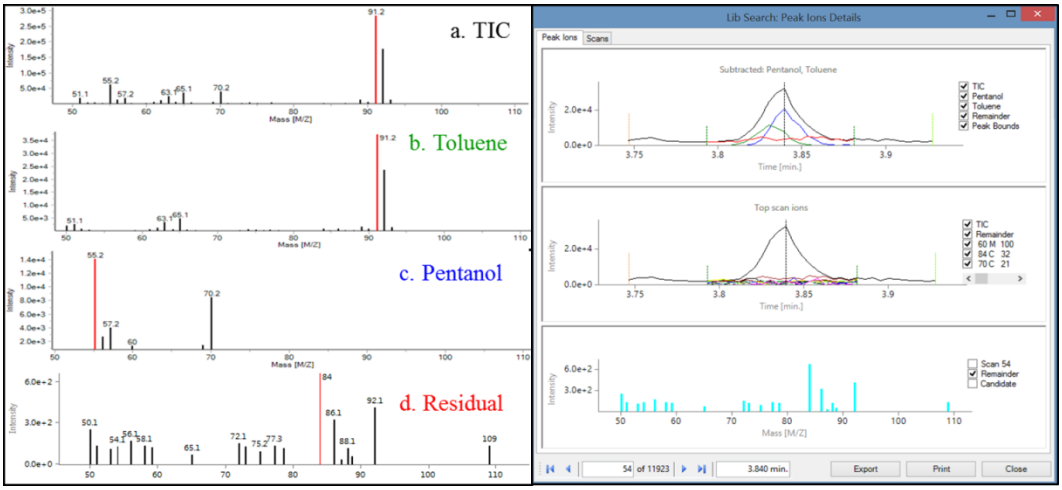

Figure 2-7. MS subtraction of target compounds toluene (b) and pentanol (c) from the TIC (a) peak. The resulting spectrum (d and bottom right-hand box) is ion signal noise, see baseline (red, top box), whose individual ion traces are also shown (middle box).[80]

2.3.2 Target and Nontarget Compound Analysis

More than 350 high elevation metabolites were detected by GC-GC/MS. Of these, 150 were confirmed using reference compounds, with another 104 identified from commercial libraries. In contrast, GC/MS analysis of the same extract detected 285 metabolites. The difference is due to mass on-column. Since 1-min sample

portions are spread over the 2nd dimension column, the 1st column was purposely overloaded, which is impractical in GC/MS since detector saturation is more easily achieved. Reference data confirmed 120 compounds, libraries assigned another 98. Figure 2-8 shows the total and reconstructed ion current (RIC) chromatograms for both high and low elevation teas. Each colored peak in the RIC corresponds to a specific compound in the sample. The software lists these compounds by color in the legend. The RIC chromatogram is the base ion signal of the reconstructed ion after spectral deconvolution of target compounds. When the high elevation database is used to analyze the low elevation tea, 275 target compounds were detected. The balance, 10 metabolites, is unique to the high elevation tea. MS subtraction, nontarget analysis, of the low elevation tea yielded eight unique metabolites. The unique compounds in both teas are of sensory and human health importance, as are many of the common compounds. Although informative, the number of common metabolites in each chemical family: 37 hydrocarbons, 34 oxygenated monoterpenes, 33 oxygenated heterocycles, 17 aliphatic alcohols, 15 monoterpene hydrocarbons, 13 oxygenated sesquiterpenes, 12 aliphatic aldehydes, 12 acids, 11 aliphatic ketones, 10 aliphatic esters, 9 sesquiterpene hydrocarbons, 7 nitrogen and 3 sulfur containing compounds, and 2 oxygenated diterpenes, is less instructive than the differences in concentration of individual compounds.

Table 2-1. Metabolite concentrations (µg/ml) and the relative percent difference (RPD) as determined by MS subtraction and spectral deconvolution algorithms.[80]

Compound	r^2	MS Subtraction	Deconvolution	RPD
Toluene	0.999	6.73	6.72	0.08
Pentanol	0.998	2.94	2.94	0.08
Terpinolene	0.997	3.75	3.60	4.26
trans-Linalool oxide (furanoid)	0.999	4.11	4.06	1.43

To assess quantitative differences between the spectral deconvolution and MS subtraction algorithms, the concentration of toluene, pentanol, terpinolene and trans-linalool oxide (furanoid) was measured. Table 2-1 lists the correlation coefficient (r^2), concentration (µg/ml) and relative percent difference (RPD) of the two algorithms. Excellent agreement was obtained as evident by the RPD, which was < 5% for every compound.

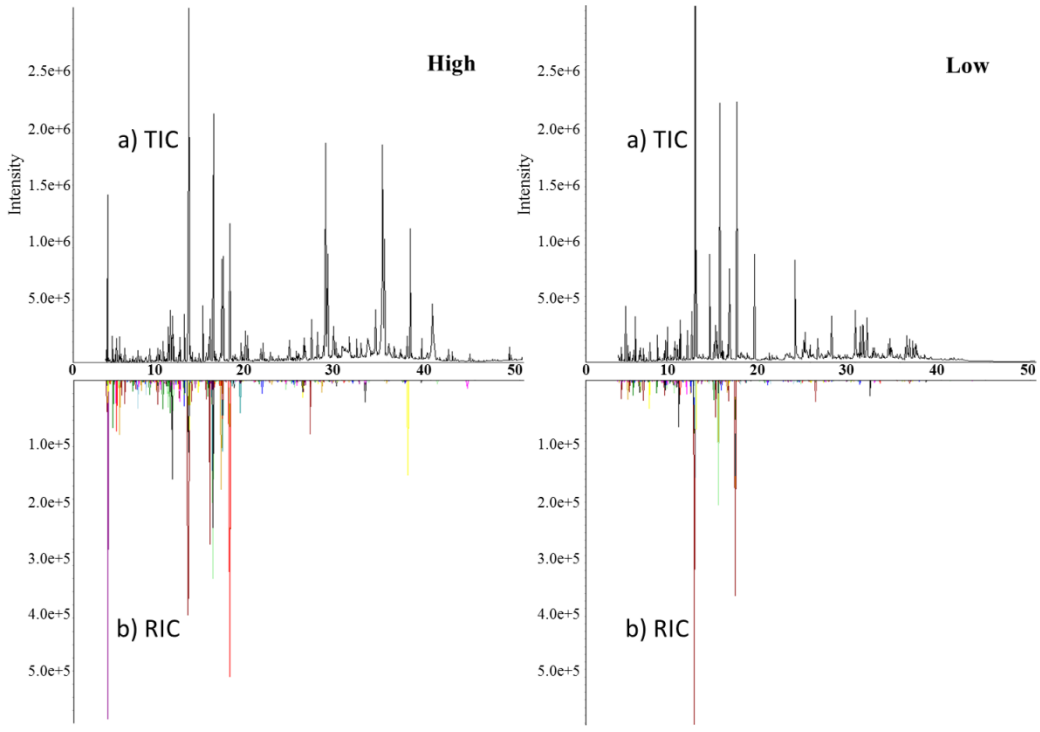

Figure 2-8. GC/MS total and reconstructed ion current (RIC) chromatograms of high and low elevation teas on Rxi-5MS. Each colored peak in the RIC corresponds to a specific compound in the sample. The software lists these compounds by color in the legend.[80]

Part 2: Chemical Profiling of Target/Nontarget Compounds by GC/MS

2.4 Experimental

2.4.1 Sample Collection and Extraction

Tea samples were collected from two counties, Anxi (var. *sinensis*) in Fujian Province and Menghai (var. *assamica*) in Yunnan Province, China in 2014. Tea was collected in spring and summer at both locations. For Fujian, it was May 11-13 and July 31-August 2 and Yunnan, March 18-20 and June 10-12, respectively. The terminal bud plus two leaves from five different plants were collected from four plots each day for three consecutive days. Leaves were minimally processed in the field by microwave to stop enzymatic oxidation [21-22, 83]. The dried leaves were wrapped in plastic and shipped to Tufts University, where they were re-wrapped in aluminum foil and stored in plastic at -20 °C until analyzed. Since no statistical difference was observed between plots [22], samples from the four plots were homogenized to produce replicate samples (n=3).

Aqueous infusions were prepared by brewing 3 g of tea in 30 ml of deionized water at 90 °C, which were allowed to cool to room temperature. 10 ml aliquots were syringe filtered (0.45 µm polytetrafluoroethylene, Fisher Scientific, Pittsburgh, PA) into 10 ml Teflon-sealed vials and stirred with a 0.5 mm thick x 10 mm long polydimethylsiloxane (PDMS) stir bar (Gerstel, Mülheim an der Ruhr, Germany) at 1200 rpm for 1 h. Stir bars were removed from the vials, rinsed with deionized water, dried with a lint-free wipe, and placed into glass desorption tubes for analysis.

2.4.2 GC/MS and GC-GC/MS Analysis

All samples were analyzed using an Agilent (Santa Clara, CA) model 6890/5975 GC/MS equipped with a MultiPurpose Sampler (Gerstel). The thermal desorption unit (TDU, Gerstel) provided splitless transfer of the sample from the stir bar into a CIS inlet (Gerstel). The TDU heated from 40 °C (0.70 min) to 275 °C (3 min) at 600 °C/min under 50 ml/min helium. After 0.1 min the CIS, in solvent vent mode, was heated from -100°C to 275 °C (5 min) at 12 °C/s. The GC column, temperature program and flow rate were 30 m × 250 μm × 0.25 μm RXI-5MS (Restek, Bellefonte, PA), 40 °C (1min) to 280 °C at 5 °C/min, and 1.2 ml/min constant helium, respectively. MS operating conditions were: 70 eV electron impact source, 230 °C ion source, 150 °C quadrupole, and 40 to 350 *m/z* scan range. A standard mix of C_7–C_{30} *n*-alkanes (Sigma-Aldrich, St. Louis, MO) was used to calculate RI. Naphthalene-d_8 (Restek) as the internal standard was used to calculate RPA. A total of 250 reference standards were purchased from: Sigma-Aldrich, Fisher Scientific, Alfa Aesar (Ward Hill, MA), TCI (Tokyo, Japan), Acros Organics (Pittsburgh, PA), and MP Biomedicals (Santa Ana, CA) to confirm metabolite identity.

The Fujian spring tea was analyzed by GC-GC/MS. Operating parameters and heartcutting procedure have been described in detail [21]. Briefly, the first GC (Agilent 6890) housed C1 (30 m × 250 μm × 0.25 μm Rtx-Wax, Restek) and was equipped with a flame ionization detector. The temperature of C1 was

programmed from 40 °C (1 min) to 240 °C at 5 °C/min. C1 was connected to the CIS with a TDU on one end and to a 5-port crosspiece (Gerstel) on the other, operating conditions above. The second oven (Agilent 6890) contained C2 (Rxi-5MS), which was connected to the same crosspiece through a CTS1 freeze trap (Gerstel) on one end and to the MS on the other, see GC/MS operating conditions above. The MCS (Gerstel) supplied countercurrent flow to the crosspiece. A heartcut was made every minute for a total of 40 heartcuts per sample. Each heartcut required a separate injection, which occurred after the preceding heartcut eluted from both columns. Analysis time per sample was 96 h.

2.4.3 Data Analysis Software

The Ion Analytics software (Andover, MA) was used to deconvolve target compounds in the sample. Once found, each compound's mass spectrum was subtracted from the total ion current TIC signal. The residual ion signals were inspected to determine if resulting peak scans were constant (± 20%) or approximated background noise. If constant, the software recorded the retention time, mass spectrum, 3-5 target ions and their relative abundances. Then, the software compared sample data to reference compound data in a database, viz., RI and MS (positive identification), or to commercial libraries and literature (tentative identification). Once assigned, the compound name, CAS#, and RI was added to the MS subtraction method. If neither positive nor tentative identification could be made, a numerical identifier along with the same GC/MS information was uploaded into the MS subtraction method. In contrast, if peaks scans differed

(unresolved peak), the software searched for three invariant scans, averaged their spectra, and then subtracted the average spectrum from the total ion current signal. This process was repeated until the residual signal at each scan approximated background noise. If peak signals failed to meet the user-defined criterion below, no additional information was obtained.

Four parameters were chosen as the compound acceptance criterion for spectral deconvolution. First, the mass spectrum must be constant (≤ 20 % deviation) for at least five consecutive peak scans after spectral deconvolution. Second, the scan-to-scan variance (SSV) must be < 5. The SSV algorithm calculates the relative error by comparing the mass spectrum of each peak scan against one another. The smaller the difference, the closer SSV is to zero, the better the spectral agreement. Third, the Q-value must be ≥ 93. The Q-value measures the total ion ratio deviation of the absolute value of the expected minus observed ion ratios divided by the expected ion ratio times 100 for each ion across the peak. The closer the value is to 100, the higher the certainty between sample and reference, library, and/or literature spectra. Finally, the Q-ratio must be ≤ 20 % deviation. The Q-ratio compares the ratio of the most abundant ion intensity to confirming ion intensities across the peak. These criteria form a single criterion used in the identification of sample components.

2.4.4 Statistical Analysis

Principal component analysis (PCA) was performed using Stata15.[84] A Mann-Whitney test was used to assess statistical significance of the separation in PCA. Metabolites, whose correlation coefficient ($r > 0.75$) and p-value (< 0.05) were considered the strongest contributors to sample differences.

2.5 Results and Discussion

Part 1 of this study demonstrated the use of GC-GC/MS to produce a Yunnan-specific database. Here, spring and summer Fujian tea was analyzed by GC/MS, with metabolites in the database identified by the Ion Analytics software. Analysis of the spring tea by GC-GC/MS was carried out to confirm the identities of both target (database) and nontarget (unique) analytes.

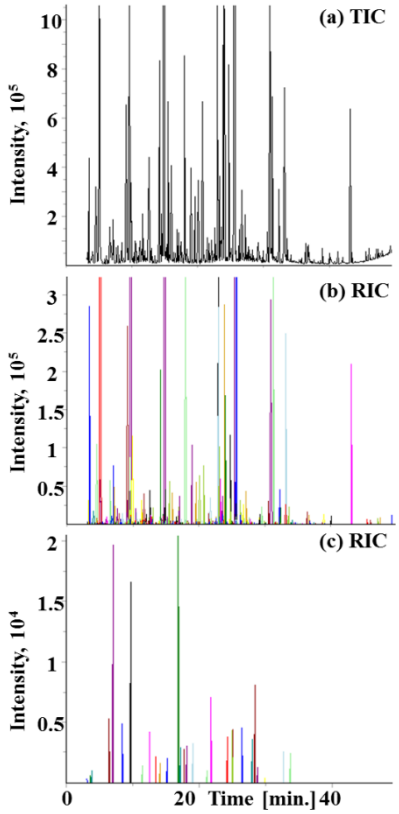

Figure 2-9. Total ion current (TIC) chromatogram of spring tea from Fujian (a) and reconstruction ion current (RIC) chromatograms of target (b) and nontarget (c) compounds.[85]

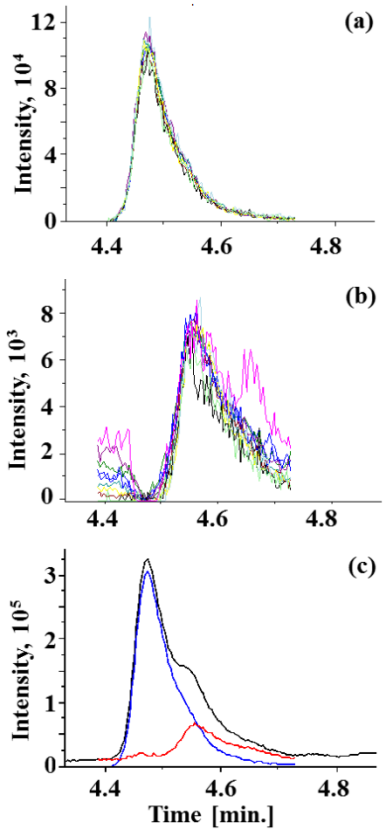

Figure 2-10. Nontarget analysis. MS subtraction of the ion signals (a and b) from the TIC peak (black) yields the blue and red peaks (c), respectively.[85]

2.5.1 Target/Nontarget GC/MS Analysis

Our first objective was to assess the accuracy of the Ion Analytics software to identify target and nontarget compounds by GC/MS. Based on the Yunnan database, spectral deconvolution of spring tea from Fujian yielded 360 target compounds. The following examples are illustrative of the target/nontarget workflow approach. Figure 2-9a-b shows the spring TIC and RIC chromatograms, respectively. Once the target compounds meet the compound identity criterion, the mass spectrum for each compound in the database is subtracted. Then, residual peaks are inspected to evaluate peak scan constancy and/or compound identity. The RIC chromatograms of these peaks are shown in Figure 2-9c. A total

of 39 Fujian-specific compounds were detected, nine of which were confirmed by comparing sample and reference compound RI and spectral data. Another eight were tentatively identified with the remainder issued a numerical identifier.

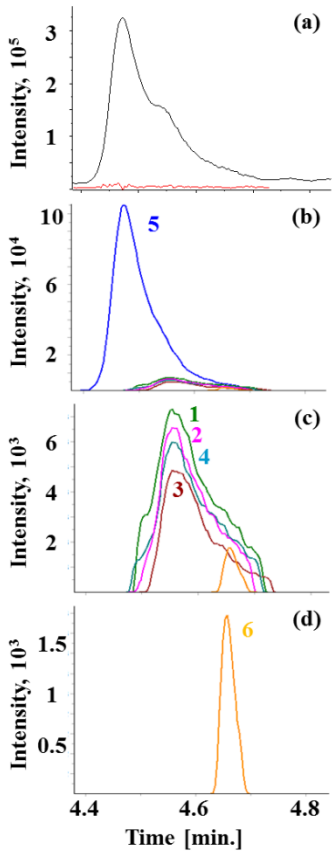

Figure 2-11. Target Analysis. (a) TIC peak from Fig. 2-10 (black). (b) RIC chromatograms of database compounds after spectral deconvolution. (c) RIC peaks after subtraction of the blue peak spectrum. (d) RIC peak after MS subtraction of the peaks at 4.57 min. (a) Background signal (red) after MS subtraction of all target compounds. Note: see Table 2-2 for compound identities.[85]

Similarly, 362 Yunnan compounds were found in Fujian summer tea and another 28 metabolites after MS subtraction. The identities of seven compounds were confirmed, while seven were tentatively identified and the remaining unknown. GC-GC/MS analysis of the spring tea confirmed the presence of 39 compounds found by MS subtraction. Importantly, GC-GC/MS did not reveal any new metabolites (peaks), which GC/MS, the Yunnan database, and MS subtraction

could not find. The Fujian and Yunnan plants produced 383 common metabolites, with 67 and 60 unique to Fujian and Yunnan, respectively.

Figures 2-10 and 2-11 and Table 2-2 make evident that data analysis software that can create and add to a database is critical to identifying target and nontarget compounds. First, the software must be able to automatically inspect GC-GC/MS data files to create the initial database.[80] The objective being to input RI, clean spectra, and other information the analyst deems important. Second, the software must be able to search data files employing database information to identify target compounds. Third, the mass spectrum of each target compound found in the database must be subtracted from the total ion signal. In Figure 2-10, two distinct MS signals (a and b) are found in the TIC (black, c). The first (blue) is clean up to one-half the peak height on the right side of the peak. Comparing the sample mass spectrum against library spectra is straightforward. After subtracting the mass spectrum of the blue peak, the spectra across the red peak are invariant and could be assumed the result of a single compound.

Table 2-2. Metabolite retention windows and indexes on RTX-Wax and RXI-5.[85]

Compound, #	RTX-Wax	RXI-5
Octane, 1	3-4 min	800
Hexanal, 2	7-8 min	800
4-Methyl-3-penten-2-one, 3	8-9 min	800
2-Cyclopenten-1-one, 4	9-10 min	800
5	13-14 min	795
Butanoic acid, 6	20-21 min	803

Note: Fig. 3b-d peak identities.

If, however, database compounds are used to deconvolve the TIC peak (black, a) shown in Figure 2-11, the resulting RICs are shown (b). Subsequent MS subtraction of the blue peak (5) yields the remaining RICs (c). MS subtraction of the four peaks co-maximizing at 4.57 min (1-4), results in the RIC at 4.65 min (6, d). When all compound spectra are subtracted the residual signal (red, a) is equivalent to background noise. To prove these compounds have been correctly assigned GC-GC/MS of the sample was performed. Table 2-2 lists the identity of each compound, its RTX-Wax, polar retention window, and RXI-5, nonpolar RI. Recall, GC-GC/MS separates 1-min sample portions hence, the heartcut window. Known compounds were confirmed by reference standards. Butanoic acid, found at 4.65 min (orange), met the compound identity criterion despite its low signal (< 2000 counts) once the matrix noise (other target compounds) was removed.

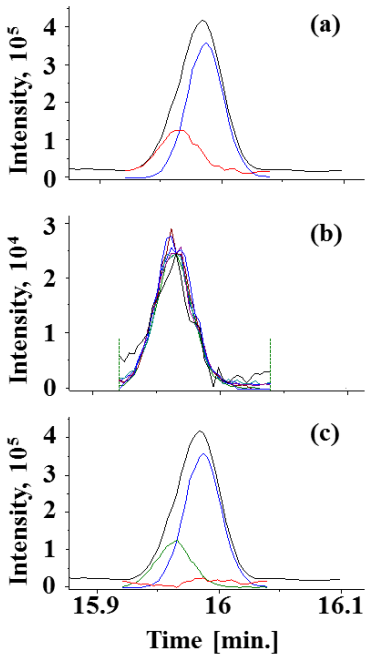

Figure 2-12. Target/Nontarget Analysis. (a) Subtraction of β-cyclocitral mass spectrum (blue) from the TIC (black) produces the residual peak (red). (b) The residual ion signals co-maximize and are invariant across the peak. (c) Subtraction of spectra for β-cyclocitral and unknown (green) results in baseline noise (red).[85]

The example above demonstrates the importance of a high quality database. Another example is Figure 2-12, which shows the RIC trace (blue) of β-cyclocitral after spectral deconvolution (a). Subtraction of β-cyclocitral's mass spectrum from the TIC peak (black) yields the residual spectrum (red). The residual ions co-maximize and are invariant across the peak (b). Since neither reference nor library spectra match the sample spectra, RI and spectra are added and associated with a numerical identifier, which can be compared to new data as it becomes available. Subtraction of spectra for β-cyclocitral and unknown (green) yields the background (red, c). GC-GC/MS confirmed the peak at 15.96 min on the non-polar column was due to a single transfer of analyte from the wax phase.

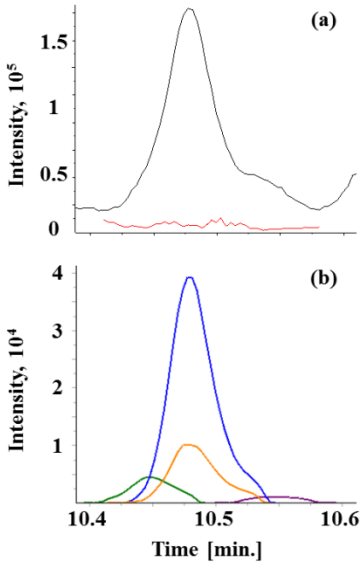

Figure 2-13. Target/Nontarget Analysis. Spectral deconvolution of 2-ethylhexanol (blue, b) and limonene (green, b) from the TIC (black, a) yielded two unknowns. After MS subtraction of these compounds, the identities of 5-ethyl-2(5H)-furanone (orange) and eucalyptol (purple) were determined. MS subtraction of all target compounds equaled background noise (red, a).[85]

The last example illustrates the value of target compound analysis followed by subtraction of each compound's mass spectrum when conducting untargeted

analysis rather than relying on data analysis software to correctly bin spectra or molecular features. For example, Figure. 2-13 shows the TIC peak (black, a). Inspection of each peak scan results in three molecular features with the spectrum at peak maximum dominating the other two in terms of absolute intensity and number of scans. Spectral deconvolution and MS subtraction of database compounds 2-ethylhexanol (blue) and limonene (green) from the TIC yielded two additional peaks (b). Eucalyptol (purple) was confirmed by reference compound, with 5-ethyl-2(5H)-furanone (orange) tentatively identified by comparing MS and RI to commercial library data. Subtraction of all mass spectra approximated background noise (red, a). Similarly, ion binning of Figure 2-10 data yield two molecular features as opposed to six compounds as shown in Figure 2-11 using the target/untargeted approach described herein. By annotating the database and tracking which metabolites are in Yunnan vs. Fujian tea, differences in metabolite chemistry can be determined.

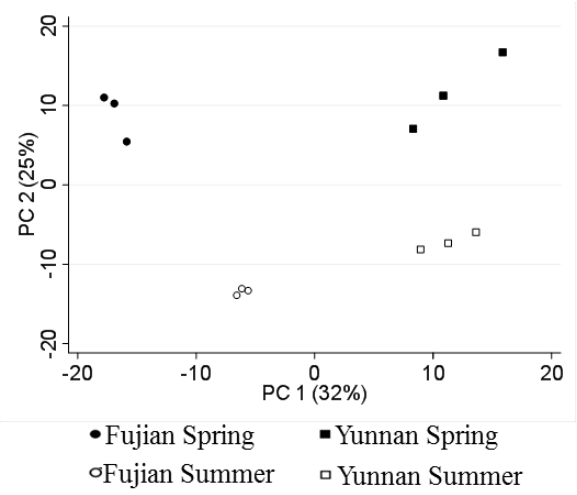

Figure 2-14. PCA score plot of Fujian and Yunnan tea.[85]

2.5.2 Effects of Climate on Tea

Our second objective was to determine if tea plants behave similarly when stressed by the same climate condition and if the finding is independent of location. The relative peak area for each compound by sample location, season, and replicate was analyzed by PCA. Figure 2-14 makes evident that samples differ by location on PC1 and season on PC2. The former includes differences due to farmer practices, subspecies, soil, and climate whereas the latter captures variations due to season. Table 2-3 lists the 10-day average temperature and cumulative rainfall before each harvest.[86] This period was selected based on previous studies, where striking differences in metabolite chemistry were observed five days after the East Asian Monsoon onset.[21-22] Although spring temperatures for both locations were the same, elevational differences between farms yielded 4.5 °C cooler temperatures for Yunnan.[87]

Table 2-3. Cumulative rainfall (mm) and average temperature (°C) 10 days prior to each harvest in Fujian and Yunnan Provinces.[86]

Fujian	Rain	Temp	Yunnan	Rain	Temp
May 1-10	62	22.0 ± 2.3	March 8-17	0	21.8 ± 0.4
July 21-30	140	29.0 ± 1.3	May 31-June 9	98	25.6 ± 1.3

Tables 2-4a and 2-4b list the 109 metabolites that statistically differentiate the tea by location. Positive and negative r values indicate which metabolites were higher in concentration in Yunnan vs. Fujian teas, respectively. The closer the r value is to ± 1, the greater the concentration difference is between samples. Even if the 44 unique metabolites are removed from analysis, the remainder still account for location differences, which means those in common differentiate plant chemistry.

For example, plants grown in Yunnan produce the unique and most of the higher concentration monoterpenes, several of which exhibit floral notes including nerol, (*E*)-β-damascenone, geraniol, *cis*-linalool oxide (furanoid), linalool, and linalool acetate. In contrast, Fujian plants produce more unique and higher concentration metabolites that exhibit fruity notes such as 4-ethylbenzaldehyde, isoamyl alcohol, butyl propanoate, 2-decanone, dihydroactinidiolide, and decanal. Both teas contain compounds that provide health beneficial properties such as analgesic (myrtenol, borneol), anesthetic (α-terpineol, (*E*)-nerolidol), antianxiety (linalool, (*E*)-nerolidol) antibacterial (terpinolene, undecanal), anticancer (terpinen-4-ol, coumarin), anticonvulsant (linalool, octanoic acid), anti-inflammatory (α-phellandrene, (*E*)-anethole), antinociceptive (nerol, 7-methoxycoumarin), and antioxidant (geraniol, cedrol) compounds.[88-94] In these examples, the compounds are either unique or statistically higher in Yunnan vs. Fujian tea.

Independent of location differences, the plants respond similarly to increases in rainfall and temperature from spring to summer conditions (Table 2-3). The more positive the r value on PC2, the higher the metabolite concentration is in spring compared to summer tea. Tables 2-5a and 2-5b list the 52 metabolites that exhibit statistical differences between seasons. Of the spring compounds, (*Z*)-methyl epi-jasmonate and α-ionone are characterized as floral and amyl acetate, γ-nonalactone, methyl hexanoate, 2-heptanone, 3-heptanone, isophorone, 4,6-dimethyl-2-heptanone, and 4-methylbenzaldehyde have fruity notes. On the other hand, summer tea contains higher concentrations of 2-phenoxyethanol, α-cadinol,

caryophyllene oxide, and τ-cadinol are characteristic of woody, herbal and metallic notes.[95] These results are in agreement with farmers' perceptions that spring tea is higher in aromatic quality, since it is more flavorful compared to summer tea.[96-97]

In addition, statistically significant nutraceutical compounds that differentiate spring from summer tea include isoborneol (antiviral, antibacterial), hexanal (antistress, antifungal), carvone (anticonvulsant, analgesic, anticancer, antibacterial), undecanoic acid (antifungal) and 4-methylbenzaldehyde (antiviral).[98-103] Nutraceutical compounds that differentiate summer from spring tea are 2-phenoxyethanol (antiseptic), α-cadinol (antibacterial, antioxidant, anti-inflammatory), α-muurolol (antibacterial, antioxidant), caryophyllene oxide (anticancer, analgesic, anti-inflammatory, antioxidant), τ-cadinol (antibacterial, anticancer, anti-inflammatory), and τ-muurolol (antibacterial, antioxidant).[104-110] Although volatiles are only a small fraction of the total mass, others have shown that the volatile extract has health beneficial effects,[111-112] but no studies have evaluated the seasonal effects on health-related volatile constitutes until now.

2.6 Conclusion

Although software is available to bin ions, differentiating one peak from the next, and track compounds across multiple samples, only Ion Analytics combines deconvolution, MS subtraction, and quantitation in the same program to investigate complex samples analyzed by different vendor instruments. The

target/nontarget approach provides efficient, comprehensive, database annotation and analysis of complex samples. Because the software relies on several data quality metrics to form a single compound identity criterion, statistical analyses leads to the identity of metabolites that drive differences in quality. In this study, the target/nontarget approach provided the means to differentiate samples based on the metabolites from plants grown under different conditions. As functional foods, authentication, safety, and climate studies continue to increase, investigator claims should be based on detailed knowledge of what is being tested

The work in this chapter is based on:
[80] Robbat Jr, A.; Kfoury, N.; Baydakov, E.; Gankin, Y., Optimizing targeted/untargeted metabolomics by automating gas chromatography/mass spectrometry workflows. *J. Chromatogr. A* **2017,** *1505*, 96-105.

[85] Kfoury, N.; Baydakov, E.; Gankin, Y.; Robbat Jr., A. Differentiation of key biomarkers in tea infusions using a target/nontarget gas chromatography/mass spectrometry workflow. *Food Res. Int.* **2018**, *113*, 414-423.

Table 2-4a. PC1 correlations of statistically significant Yunnan metabolites.[85]

Compound	r	p-value	Aroma[95]	Health Property
Myrtenol	0.962	<0.0001	pine, sweet, mint	antibacterial[88] gastroprotective[113] anti-inflammatory, analgesic[89] hypotensive[114]
α-Terpineol	0.950	<0.0001	citrus, terpene, woody	antimicrobial[101] anti-inflammatory, gastroprotective[115] anesthetic[116] antioxidant, hypotensive[114] analgesic[89]
(3*E*)-Methylbutanal oxime*	0.943	<0.0001		
2-Ethyl isovaleraldehyde*	0.939	<0.0001		
Ethyl benzoate*	0.933	<0.0001	fruity, herbal	
Nerol*	0.930	<0.0001	sweet, floral	antinociceptive, anti-inflammatory[117] antibacterial[88] antifungal[118]
Linoleic acid*	0.928	<0.0001		anti-inflammatory[119] chemopreventive[120]
(*E*)-Herboxide	0.928	<0.0001	herbal, woody, minty	
Terpinen-4-ol	0.926	<0.0001	woody, terpene, cooling	antimicrobial[101] anticancer[90] hypotensive[114]
Benzenecarboxylic acid*	0.919	<0.0001	faint balsamic	antibacterial[121]
Terpinolene*	0.916	<0.0001	woody, terpene, lemon	antibacterial[88]
Theaspirane B	0.913	<0.0001	tea, herbal, honey	
Vanillin	0.908	<0.0001	vanilla	analgesic, antidepressant, antimicrobial, antioxidant anti-mutagenic[122]
(*E*)-β-Damascenone	0.902	0.0001	floral, sweet	
150*	0.901	0.0001		
Theaspirane A*	0.891	0.0001	tea, herbal, honey	
Linolenic acid*	0.8833	0.0001		antioxidant, anti-inflammatory, neuroprotective[123]
Linalool	0.876	0.0002	lavender, floral	hypotensive[114] analgesic, anticonvulsant[102] antioxidant[104] antimicrobial, anti-inflammatory[101] antianxiety, anesthetic[91]
Geraniol	0.866	0.0003	floral, rose	antimicrobial, antitumor[101] antioxidant, anti-inflammatory, neuroprotective[124]
Linalool acetate	0.861	0.0003	sweet, green, floral	analgesic[89] antimicrobial, anti-inflammatory[101]

Carvomenthenal*	0.853	0.0004	spicy, herbal	
117	0.853	0.0004		
Pyridine	0.848	0.0005	fishy, sour	
α-Phellandrene*	0.844	0.0006	citrus, terpene, green	antibacterial[88] analgesic, anti-inflammatory[89] antinociceptive[125]
(3*Z*)-Hexenyl acetate	0.840	0.0006	green, sweet, fruity	
α-Terpinene*	0.838	0.0007	citrus, woody, terpene	antibacterial[1] antiviral[20]
Methyl benzoate	0.834	0.0007	cherry, phenolic	
(*Z*)-Herboxide	0.819	0.0011	herbal, woody, minty	
Phenylethyl alcohol	0.819	0.0011	sweet, rose, honey	
158	0.814	0.0013		
Furfural	0.813	0.0013	sweet, bready, caramel	
116*	0.816	0.0013		
Homomenthyl salicylate	0.807	0.0015	mild menthol	
199	0.806	0.0015		
5-Hydroxymethylfurfural*	0.805	0.0016	buttery, caramel, musty	anti-inflammatory, antitumor[126] antioxidant, cardioprotective[127]
n-Tetradecanol	0.803	0.0017	fruity, waxy, coconut	anti-inflammatory, gastroprotective[128]
(2*E*)-Isobutanal oxime*	0.803	0.0017		
γ-Terpinene*	0.796	0.0020	citrus, terpene, sweet	antibacterial[88], antiviral[125]
trans-Linalool oxide (furanoid)	0.793	0.0021	floral	antifungal[129]
cis-Linalool oxide (furanoid)	0.790	0.0022	floral, sweet, woody	antifungal[129]
29	0.788	0.0023		
2-Furanmethanol*	0.773	0.0023	sweet, caramel, burnt	
160*	0.771	0.0033		
157*	0.769	0.0035		
(2*E*,4*E*)-Nonadienal	0.768	0.0035	cucumber, waxy	
Toluene	0.753	0.0047	sweet, paint	
56	0.751	0.0048		
2-Methyl-3-pentanone*	0.751	0.0049	mint	

* Indicates compound is unique to this location

Table 2-4b. PC1 correlations of statistically significant Fujian metabolites.[85]

Compound	r	p-value	Aroma[95]	Health Property
4-Ethylbenzaldehyde*	-0.962	<0.0001	sweet, almond, cherry	
Isoamyl alcohol*	-0.958	<0.0001	alcoholic, banana	
206	-0.958	<0.0001		
58	-0.958	<0.0001		
Coumarin*	-0.956	<0.0001	sweet, hay	antidiabetic[130] anti-inflammatory, antipyretic, anticancer[131]
210*	-0.952	<0.0001		
2,6-Dimethyl-3,7-octadiene-2,6-diol	-0.940	<0.0001	fruity, herbal	
Cedrol	-0.935	<0.0001	sweet, cedar wood	anti-allergy[132] anticancer[133] relaxant[134] antioxidant[104]
Dodecanal	-0.934	<0.0001	citrus, soapy	antibacterial[93]
94*	-0.930	<0.0001		
(2*E*,4*Z*)-Heptadienal	-0.929	<0.0001	fatty, oily, fishy	
Borneol	-0.927	<0.0001	camphor, pine, woody	antibacterial[88] antioxidant[104] anti-inflammatory, analgesic, anesthetic[102]
90	-0.926	<0.0001		
7-Methoxycoumarin*	-0.915	<0.0001	sweet, balsamic	anticancer[135] antinociceptive[136] anti-inflammatory[137]
2-Decanone*	-0.914	<0.0001	floral, fruity	antibacterial[138]
Butyl propanoate	-0.913	<0.0001	sweet, fruity, banana	
Undecanal	-0.904	0.0001	orange, waxy, soapy	antibacterial[138]
161*	-0.903	0.0001		
173	-0.895	0.0001		
2,3,5-Trimethylhexane	-0.891	0.0001		
226*	-0.889	0.0001		
Decanal	-0.879	0.0002	sweet, orange, waxy	antibacterial[138]
221*	-0.878	0.0002		
183	-0.878	0.0002		
p-Acetyltoluene	-0.869	0.0002	sweet, creamy, cherry	
(2*E*,4*E*)-Heptadienal	-0.865	0.0003	fatty, oily, fishy	
52	-0.862	0.0003		
147	-0.860	0.0003		
211*	-0.848	0.0005		
Heptanal	-0.846	0.0005	fruity, green, grassy	antistress[100]

2-Pentylfuran	-0.835	0.0007	fruity, green, earthy	
143	-0.833	0.0008		
γ-Butyrolactone	-0.822	0.0010	creamy, milky, fruity	
Octanoic acid	-0.821	0.0011	fatty, soapy, cheesy	anti-inflammatory, anticonvulsant[139] antitumor[140]
6-Methyl-2-heptanol	-0.820	0.0011	waxy, fatty, citrus	
(4*Z*)-Heptenal	-0.815	0.0012	green, milky, tea	
220*	-0.815	0.0012		
(*E*)-Nerolidol	-0.814	0.0013	floral, woody	antianxiety, anti-malarial, antiparasitic[104] antibacterial[106] anti-inflammatory[105]
(2*Z*)-Octen-1-ol	-0.803	0.0017	sweet, floral	
(*E*)-Anethole*	-0.802	0.0017	sweet, anise	anesthetic[116] antibacterial[88] anti-inflammatory[94] antioxidant[141]
Decanoic acid	-0.798	0.0002	fruity, waxy, soapy	anticonvulsant[142]
(*Z*)-Jasmone	-0.790	0.0022	floral, woody, herbal	antibacterial[143] anticancer[144]
172	-0.789	0.0023		
Dibenzofuran	-0.781	0.0027		
209*	-0.780	0.0028		
214	-0.778	0.0029		
218*	-0.778	0.0029		
103	-0.776	0.0030		
205*	-0.776	0.0030		
4-Ketoisophorone*	-0.774	0.0031	floral, musty, woody	
216*	-0.774	0.0031		
Nonanal	-0.769	0.0035	cucumber, waxy, citrus	antifungal[98]
γ-Octalactone*	-0.768	0.0035	sweet, fruity	
130	-0.767	0.0036		
225*	-0.766	0.0037		
212*	-0.763	0.0039		
2-Ethylfuran*	-0.753	0.0047	sweet, earthy, musty	
Dihydroactinidiolide	-0.751	0.0049	red fruit, woody	
120	-0.751	0.0049		
(*E*)-β-Ionone	-0.751	0.0049	floral, woody, berry	anticancer[145]

* Indicates a compound is unique to this location

Table 2-5a. PC2 correlations of statistically significant spring metabolites.[85]

Compound	r	p-value	Aroma[95]	Health Property
54	0.950	<0.0001		
β-Homocyclocitral	0.947	<0.0001	camphor, cooling	
Amyl acetate	0.941	<0.0001	fruity, banana, sweet	
Isoborneol	0.920	<0.0001	camphor, woody	antiviral, antibacterial[101]
Hexanal	0.909	<0.0001	green, grassy	antistress[100] antifungal[98]
3-Heptanone	0.902	0.0001	green, fruity	
n-Ethylsuccinimide	0.901	0.0001		
2-Octanone	0.899	0.0001	earthy, herbal, woody	
γ-Nonalactone	0.892	0.0001	sweet, coconut	
Methyl hexanoate	0.889	0.0001	fruity, sweet	
2-Heptanone	0.877	0.0001	fruity, herbal, sweet	
Mesitylene	0.869	0.0002		
(*Z*)-Methyl epi-jasmonate	0.855	0.0004	floral, sweet	
2,3-Octanedione	0.853	0.0004	sweet, creamy	
Isophorone	0.842	0.0006	sweet, fruity, cooling	
α-Ionone	0.835	0.0007	sweet, violet, berry	
p-tert-Butylphenol	0.829	0.0009	earthy, leathery	
88	0.828	0.0009		
Pyrethrone	0.828	0.0009		
4,6-Dimethyl-2-heptanone	0.824	0.0010	fruity	
65	0.816	0.0012		
m-tert-Butylphenol	0.814	0.0013		
95	0.811	0.0014		
α-Cyclocitral	0.805	0.0016		
2-Cyclopenten-1-one	0.802	0.0017		
1-Ethylpyrrole	0.793	0.0021	roasted	
174	0.791	0.0022		
Carvone	0.782	0.0027	anise, spearmint	anticancer, antibacterial[101] anticonvulsant, analgesic[102]
Sabina ketone	0.773	0.0032		
Undecanoic acid	0.772	0.0032	waxy, cheesy, fatty	antifungal[99]
110	0.768	0.0035		
6-Methyl-2-heptanone	0.765	0.0038	camphor	

153	0.763	0.0039		
α-Amorphene	0.760	0.0041		
4-Methylbenzaldehyde	0.755	0.0045	fruity, cherry	antiviral[103]

Table 2-5b. PC2 correlations of statistically significant summer metabolites.[85]

Compound	r	p-value	Aroma[95]	Health Property
224	-0.907	<0.0001		
96	-0.899	0.0001		
99	-0.867	0.0003		
113	-0.865	0.0003		
36	-0.845	0.0005		
123	-0.807	0.0015		
2-Phenoxyethanol	-0.801	0.0018	metallic, mild rose	antiseptic[107]
Muurola-4,10(14)-dien-1β-ol	-0.801	0.0018		
α-Cadinol	-0.790	0.0022	herbal, woody	antibacterial, antioxidant[106] anti-inflammatory[108]
α-Muurolol	-0.788	0.0023		antibacterial, antioxidant[106]
217	-0.787	0.0024		
142	-0.786	0.0024		
177	-0.781	0.0027		
Caryophyllene oxide	-0.769	0.0035	woody, cedar	antioxidant[106] anticancer, analgesic[109] anti-inflammatory[108]
epi-α-Cadinol	-0.755	0.0045	herbal	antibacterial[106] anticancer[110] anti-inflammatory[108]
epi-α-Muurolol	-0.754	0.0046		antibacterial[106] antioxidant[104]

Chapter 3. Elevational Effects on Tea Metabolites

3.1 Introduction

Crops grown at different elevations have been shown to differ in quality.[146-148] Tea, for example, has been successfully grown at elevations that range from sea level to 2,700 m above sea level, causing differences in temperature that effect plant growth. At higher elevations, tea plants experience slower shoot growth, a by-product of cooler temperatures, which leads to higher quality teas.[96, 149] Farmers associate aromatic quality with higher elevation teas,[97] since they exhibit sweet, floral, honey-like characteristics compared to green, earthy, hay-like notes in low elevation tea.[149-150] However, reports are inconsistent for the non-volatile catechins and methylxanthines. Some researchers report higher concentrations in high altitude tea whereas others measured higher concentrations in low elevation tea.[96, 151-153]

Review of the medical literature reveals no studies have been conducted based on differences in pre- vs. monsoon or high vs. low elevation teas presumably due to the fact that little is known about sample differences at the molecular level. Although the volatile metabolites represent a small fraction of the total mass, finding indicate that volatile tea extracts have proven health benefits.[111-112] With this in mind, the aim of this is work is to investigate tea quality differences based on elevational effects by collecting tea from the same farm on two different mountains in Yunnan Province, China. GC-GC/MS was used to obtain a comprehensive metabolomic profile of volatile secondary metabolites in tea. In

addition to known sensory compounds, sample portions containing unidentifiable compounds were screened by GC-GC/MS-olfactometry to determine if they were sensory active. Once the library was made, the relative differences in GC/MS peak area for each compound between high and low elevation samples were calculated. In addition, liquid chromatography/mass spectrometry (LC/MS) was used to quantify catechins and some methylxanthines.

3.2 Experimental

3.2.1 Materials

Tea samples were collected in 2013 from two different mountains, (Jinuo, Mengla County, southeast and Bulang, Menghai County, southwest), in Yunnan Province, China. Samples were collected from each mountain at high (1,400 m) and low (600 m) elevations in the first and third (Jinuo only) weeks of May. The high elevation sites were ~5.3 °C cooler than the low elevation sites.[87] On each plant the terminal bud plus two leaves were harvested from five different plants per plot. Samples were collected from four plots each day for three consecutive days. Since no statistical difference between plots was observed in catechin and methylxanthine concentrations in our earlier study,[22] samples from within the plots were homogenized to create each day's samples. No plant was sampled more than once. Leaves were minimally processed in the field by microwave to stop enzymatic oxidation.[21-22] The dried leaves were sealed in plastic bags and shipped to Tufts University, where they were stored at -5 ºC until analyzed.

C_7-C_{30} *n*-alkanes, sodium sulfate, theobromine (TB), paraxanthine (98%), catechol (≥99%), formic acid, methanol, and methylene chloride were purchased from Sigma-Aldrich (St. Louis, MO). Naphthalene-d_8 was purchased from Restek (Bellefonte, PA). Caffeine was purchased from Alfa Aesar (Ward Hill, MA). (-)-Gallocatechin (GC, > 99%) and (-)-catechin gallate (CG, >98%) were purchased from Indofine (Hillsborough Township, NJ, USA). (-)-Epigallocatechin (EGC, 94.6%), (-)-epicatechin (EC, 96.2%), (-)-epigallocatechin gallate (EGCG, 94.0%), (-)-epicatechin gallate (ECG, 96.0%), (+)-catechin (C, 94.9%), and (-)-gallocatechin gallate (GCG, 98.4%) were purchased from ChromaDex (Irvine, CA). 18 MΩ water was obtained from a Hydro Picopure 3 faucet system (Durham, NC). A total of 250 reference standards were purchased from Sigma-Aldrich, Alfa Aesar, TCI (Tokyo, Japan), Acros Organics (Pittsburgh, PA), MP Biomedicals (Santa Ana, CA), and Fisher Scientific (Pittsburgh, PA). Polyvinylidene fluoride syringe filters were purchased from MilliporeSigma (Burlington, MA).

3.2.2 Sample Preparation

For GC/MS analysis, samples were extracted using simultaneous distillation-extraction[21] using 10 g of tea, brewed in 100 mL of deionized water at 90 °C, which was allowed to cool in a sealed container for 30 min. The filtered infusion and 12 mL of methylene chloride were simultaneously distilled for 2 h at 100 °C and 60 °C, respectively. Anhydrous sodium sulfate was used to remove water

from the extract, which was then concentrated to 500 µL under a stream of purified nitrogen.

For LC/MS analysis, sample preparation was adapted from the procedure described by Ahmed et al.[22] 20 mg of each sample was extracted with 1 mL of 80% methanol/water v/v in a 1.5 mL micro-centrifuge tube. Samples were sonicated for 30 min and then centrifuged at 13,000 rpm for 1 min. A 0.45 µm polyvinylidene fluoride syringe filter was used to remove particulates from the supernatant, which was subsequently diluted five-fold for the methylxanthines and catechins and ten-fold for epicatechin with 80% methanol/water solution.

3.2.3 GC-GC/MS and GC/MS Conditions

Representative samples from each mountain at the two elevations were analyzed by automated sequential 2-dimensional GC-GC/MS to produce the metabolite library. Instrument configuration and heartcutting procedure were previously described.[21] Briefly, the first GC (Agilent 6890, Santa Clara, CA) housed C1 (30 m × 250 µm × 0.25 µm Rtx-Wax, Restek) and was equipped with a flame ionization detector. The temperature of C1 was programmed to hold at 40°C for 1 min, then ramped to 240 °C at 5 °C/min. C1 was connected to a CIS inlet (Gerstel, Mülheim an der Ruhr, Germany), operating in splitless mode, on one end and to a 5-port crosspiece (Gerstel) on the other. The second oven contained C2 (30 m × 250 µm × 0.25 µm Rxi-5MS, Restek), which was connected to the crosspiece through a CTS1 freeze trap (Gerstel) on one end and to an Agilent

5975 mass spectrometer on the other. The oven temperature was held at 40 °C for 1 min, and then increased to 280 °C at a rate of 5 °C/min. Both columns operated at 1.2 mL/min constant helium flow. The ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. The MS was scanned from 50 to 350 *m/*, with the electron impact ionization energy at 70 eV. A multipurpose sampler (Gerstel) automatically injected 2 µL of sample, and the MCS (Gerstel) supplied countercurrent flow to the crosspiece. A heartcut was made every minute for a total of 40 heartcuts per sample. Each heartcut required a separate injection that only occurred after each preceding heartcut eluted from both columns. The total analysis time for one sample was 3.5 days.

Three replicate samples from each mountain, elevation and sampling period were analyzed by GC/MS to determine the relative amounts of each analyte in the samples based on a 1 µL injection volume. Concentration differences were calculated as the difference in RPA compared to the internal standard, naphthalene-d_8. A standard mixture of C_7-C_{30} *n*-alkanes was used to calculate the RI for each compound. Reference standards, when available, were used to provide positive confirmation of compound identity.

3.2.4 GC-GC/MS-Olfactometry Conditions

By reversing the two columns, the GC-GC/MS-olfactometry analysis served two purposes. First, the analysis confirmed compound identity by comparing the analyte and reference compound mass spectrum and retention index on the polar

column for positively identified compounds as well as tentatively identified compounds using commercial databases and literature data. Low thermal mass columns, Agilent HP-5MS (30 m × 250 μm × 0.25 μm) and Agilent HP-INNOWax (30 m × 250 μm × 0.25 μm), were connected by a Deans switch (Agilent). The analytical column, HP-INNOWax was connected to an Agilent 5975C MS and Gerstel's olfactory detection port (ODP 3) sniffing port by a 3-way splitter (Agilent). The temperature programs and MS operating conditions were described in section 2.3. Second, tea samples were screened by trained and certified sensory analysts at Tufts University Sensory and Science Center to assess the odor characteristics of the analytes. The method employed was modified from the American Society for Testing and Materials Flavor Profile Method[154] and is a descriptive sensory analysis, based on a 7-point intensity scale, where trained panelists qualify aroma using objective terms based on reference standards.

3.2.5 Data Analysis Software

New data analysis software (Ion Analytics, Andover, MA) was used to automatically inspect GC-GC/MS data to produce an environmental tea database, which could be used with spectral deconvolution to provide target compound analysis by GC/MS.[80] The 40 heartcut data files were analyzed by inspecting each peak in the data file to determine mass spectral constancy across the peak. If constant, the software recorded retention times, mass spectra, 3-5 target ions and their relative abundances for each peak. The software compared the sample data

to reference compound or commercial libraries (e.g. NIST, Wiley, Adams) and literature[76, 78-79] to provide positive or tentative identification. Then, compound name, CAS#, and RI were added to the database. If neither positive nor tentative identification was possible, the same information was uploaded into the database with a numeric identifier as opposed to compound name and CAS#.

If mass spectra varied across the peak, the software searched for 3-5 invariant scans (±20%), averaged their mass spectra, and then subtracted it from the total ion current (TIC) signal. Once subtracted, the residual ion signals were automatically inspected to determine if the resulting peak scans were constant or approximated background noise. If constant, the mass spectrum of the second compound was subjected to the treatment described above, with associated compound information uploaded into the database. If not (unresolved peak), the software repeated the subtraction process until the residual signal approximated background signal. If the resulting signal did not meet the user-defined criteria, see below, no additional information was obtained.

Four parameters were chosen as the compound acceptance criteria. First, the mass spectrum must be constant for at least five consecutive scans, i.e., ≤ 20 % deviation. Second, the SSV must be < 5. The SSV algorithm calculates the relative error by comparing the mass spectrum at each peak scan against one another. The smaller the difference, the closer SSV is to zero, the better the spectral agreement. Third, the Q-value must be ≥ 93. The Q-value measures the

total ion ratio deviation of the absolute value of the expected minus observed ion ratios divided by the expected ion ratio times 100 for each ion across the peak. The closer the value is to 100, the higher the certainty between library and sample spectra. Finally, the Q-ratio must be ≤ 20 % deviation. The Q-ratio compares the ratio of the main ion intensity to confirming ion intensities across the peak. The software assigns a compound name from libraries or numerical identifier when all compound acceptance criteria are met.

3.2.6 LC/UV-MS Conditions

Target compounds were quantified with an Agilent 1260 series LC consisting of a binary pump, an autosampler cooled to 4 °C, a thermostatted column compartment with column-switching valve, a diode array detector (DAD), and an Agilent 6120 quadrupole mass spectrometer with electrospray ionization source. The mobile phase was 0.05% formic acid in water (v/v, solvent A) and 0.05% formic acid in methanol (v/v, solvent B). The injection volume was 1 µL. DAD spectra were acquired from 190 to 500 nm, with eluting compounds monitored at 280 nm. Electrospray parameters were: drying gas flow rate 12 L/min, gas temperature 350 °C, nebulizer pressure 35 psig, capillary voltage 3000 V, and fragmentation voltage 120 V.

Methylxanthines were separated on an Agilent Eclipse Plus C18 reverse phase column (100 × 2.1 mm, 3.5 µm). The flow rate was 0.5 mL/min. The solvent program was 16% B for 7 min, then ramped to 100% over 1 min and then held

constant for 5 min. A 15 min 16% B re-equilibration time established initial operating conditions before the next sample was analyzed. Mass spectra were acquired in positive ion mode from 100 to 220 *m/z*. Catechins were separated on a Phenomenex (Torrance, CA) Synergi Polar-RP column (250 × 4.6 mm, 4 μm). The flow rate was 1.0 mL/min. The solvent program was 40% B for 5.5 min, ramped to 45% B in 1 min, then held isocratic for 6 min, which was then ramped to 100% B in 0.5 min and held constant for 7 min. A 15 min re-equilibration time was established prior to each sample injection. Mass spectra were acquired in negative ion mode using time-based, selected-ion monitoring of four ion groups: group 1, from 0 to 4.90 min, 305, 306, 341, 611, 612 *m/z*; group 2, from 4.90 to 6.65 min, 108, 109, 110, 289, 290, 335, 357, 579 *m/z*; group 3, from 6.65 min to 9.00 min, 108, 109, 110, 169, 457, 458, 459, 493, 503 *m/z*; and group 4, 9.00 to 12.50 min, 441, 442, 477, 487, 509 *m/z*.

3.2.7 LC/MS Quantitation of Catechins and Methylxanthines

5-point calibration curves were produced for methylxanthines (TB and caffeine) from 5 to 340 μg/mL and for catechins (EGC, EC, EGCG, ECG, GC, C, GCG, and CG) from 3 to 495 μg/mL. Paraxanthine and catechol were used as internal standards for methylxanthines and catechins, respectively. The peak areas at *m/z* $[M\text{-}H]^-$ for catechins and *m/z* $[M+H]^+$ for methylxanthines were used to quantify analytes. Concentrations were calculated as follows: $\frac{A_i}{A_{IS}} = m\left(\frac{C_i}{C_{IS}}\right) + b$, where subscripts i and IS refer to the calibrants and internal standards. Calibration curves were acceptable when the correlation coefficient was greater than 0.99.

Target compounds were identified by comparing sample and reference (calibrants) spectra and retention times using Ion Analytics.

3.2.8 Statistical Analysis

All statistics were conducted in R.[155] For GC/MS, the *ropls* R-package[156] was used to perform orthogonal projection to latent structures-discriminant analysis (OPLS-DA) of autoscaled (mean-centered and unit-variance scaled) relative peak areas for each compound to evaluate separation space between high and low elevation teas. The quality of the OPLS-DA model is described by R^2 and Q^2. R^2 measures the degree of fit of data to the model. A 7-fold cross validation was used to produce Q^2, which measures the predictability of the model. The sampling distribution of the estimates was assessed through a bootstrapping technique based on 1000 permutations of the class labels. The p-value was produced by calculating the proportion of models with random permutations of Q^2 greater than the Q^2 value of the model made with actual data. Statistical significance was determined using a cutoff of $\alpha = 0.05$. Metabolites with a variable influence on projection (VIP) > 1.0 and statistically different between groups (Mann-Whitney test, $p < 0.05$) were considered the strongest contributors to differences in tea metabolite chemistry at different elevations. For LC/MS, one-way multivariate analysis of variance (MANOVA) using elevation as the dependent variable and follow-up ANOVAs were made to determine statistically significant differences ($p < 0.05$) in catechin and methylxanthine concentrations at the two elevations.

3.3 Results and Discussion

3.2.1 Total Volatile Metabolomic Profile

A total of 406 compounds were detected by GC-GC/MS. Of these, we confirmed 144 of 259 compounds identified using reference standards. An additional 92 compounds were identified based on their 2-column (5% phenyl-methylpolysiloxane and polyethylene glycol phases) retention index match and mass spectra comparisons with literature and/or commercial libraries. The remaining compounds were identified by RI on one column and/or MS matches. Although some would argue that retention data and corresponding mass spectra are not considered positive identification, the lack of available reference compounds is limiting when conducting total metabolomic investigations.

To assess quantitative differences between elevations, the samples were analyzed by GC/MS, which limited the mass injected to one-half that of GC-GC/MS due to column and/or MS overload. This resulted in the detection of 305 metabolites. Of these, we identified 230 compounds, confirmed 137 of them by reference standards, which means 71 metabolites were assigned a numerical identifier (Table 3-1 and Table 3-2). Approximately half of the metabolites differed in concentration, 85 were higher in concentration at 1,400 m and 78 at 600 m, with 142 exhibiting no change in concentration, i.e., the percent difference was ± 20. Of those that increased in concentration at high elevation, pentacosane represented 11.5% of the total RPA. Pentacosane, a major component of leaf wax, is known to increase in concentration at higher altitudes due to lower

temperatures.[157] For low elevation tea, 2,3-dihydrobenzofuran was 16.4% of the total RPA. This compound is described as green (grassy) and herbal, which is consistent with farmer perceptions of lower quality tea.[158] Of the total detectable metabolites, 262 were common in all samples with the remainder missing in at least one sample. Five metabolites were found only in high elevation teas and, nine in low elevation teas.

3.3.2 Effects of Elevation on Tea Chemistry

To ensure sampling events did not influence our findings, OPLS-DA (volatiles, $p = 0.05$) and MANOVA (non-volatiles, $p = 0.14$) analyses revealed the collection of high and low elevation samples during week 1 had metabolite concentrations that were statistically the same as week 3. OPLS-DA was used to evaluate the difference in volatile metabolite concentrations between teas grown at 1,400 m and 600 m. The model separated the two elevations along the predictive (P1) axis (Figure 3-1), with significant permutation ($p = 0.003$), R^2 (0.939), and Q^2 (0.639) values. VIP analysis determined which metabolites distinguished high from low elevation teas. Table 3-3 lists the 37 the metabolites exhibiting a statistically significant difference between elevations, with 23 vs. 14 compounds higher in concentration in high vs. low elevation teas.

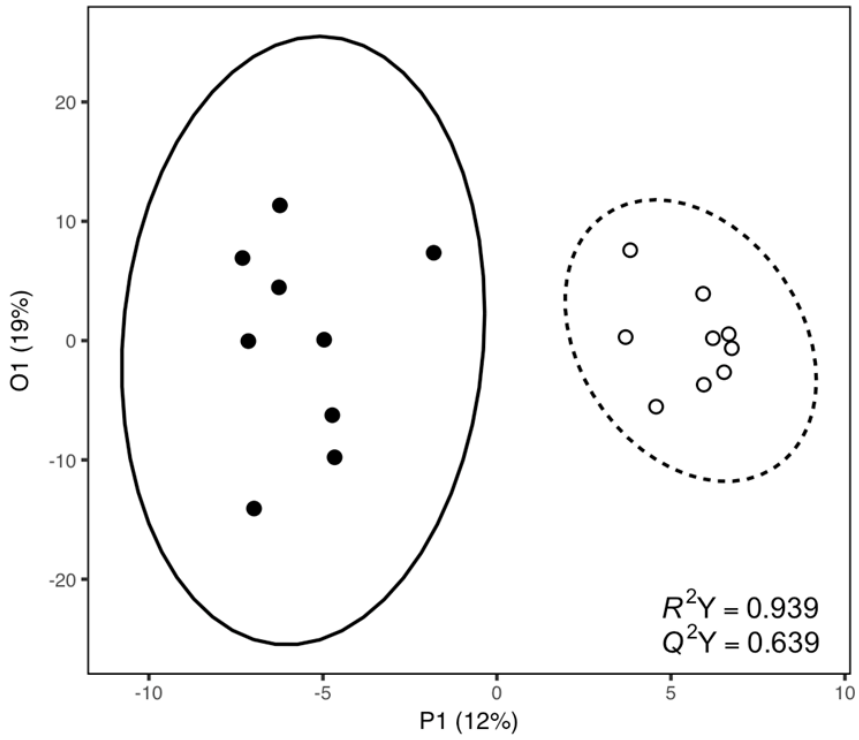

Figure 3-1. OPLS-DA of volatiles from high (filled) and low (unfilled) elevation teas.[159]

Of the high elevation compounds, the relative peak areas of *p*-xylene, 2-cyclohexen-1-ol, benzeneacetonitrile, (*Z*)-jasmone, α-ionene, 2-acetylfuran, and theaspirane are at least twice that of 1-ethyl-1H-pyrrole-2-carboxaldehyde, (2*E*)-hexenol, (*E*)-caryophyllene, (3*Z*)-hexenol, α-calacorene , and 1-ethyl-1H-pyrrole. The former exhibit sweet, floral, honey-like notes associated with high quality tea,[160-163] while the latter possess green, herbal, roasted, woody notes.[161] In comparison, statistics indicate *trans*-linalool oxide (pyranoid), 2,6-dimethyl-3,7-octadien-2,6-diol, (2*E*,4*Z*)-heptadienal, cyclohexanone, isovaleric acid, 2,3-dihydrobenzofuran and dihydroactinidiolide are higher in concentration and differentiate low from high elevation teas. These compounds are typically characterized as cheesy, fatty, fried, fruity, green, herbal, minty, rancid, and woody.[158, 161]

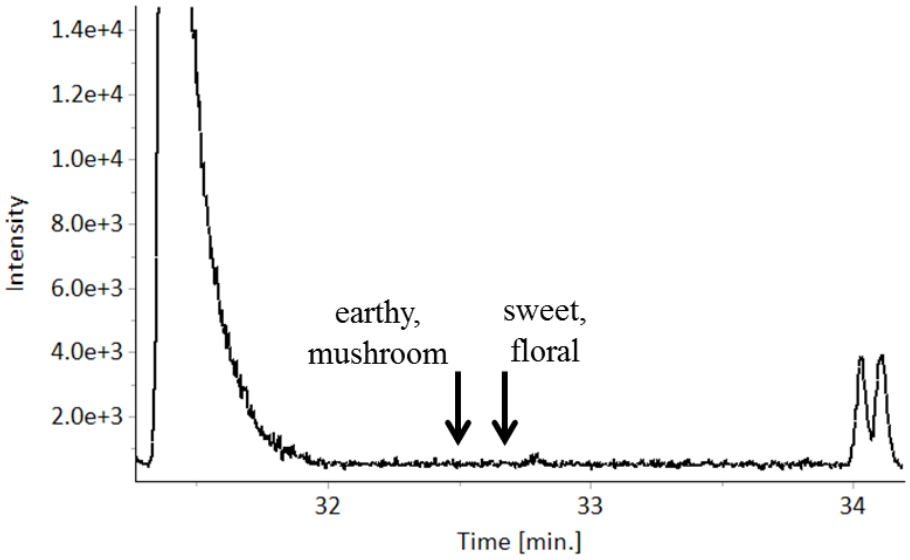

Figure 3-2. GC-GC/MS-olfactometry analysis of heartcut 17-18 min.[159]

Examples of GC-GC/MS-olfactometry analysis of sensory active metabolites are shown in Figures 3-2 and 3-3. For example, the sample portion from 17 to 18 min in Figure 3-2 shows two regions in the TIC chromatogram where odors were detected for compounds whose signals were below the baseline signal. The first was an earthy, mushroom scent and the second, sweet, floral. In Figure 3-3, heartcut 19-20 min shows the TIC and reconstructed ion current chromatogram after spectral deconvolution of compound #47, which elutes at 38.4 min and smells of anise. Compound #52, also shown in the Figure 3-3, coelutes with geraniol at 41.5 min. From a sensory perspective, compound #52 is waxy compared to the floral, rose scent of geraniol. Despite subtracting the mass spectrum of geraniol at each scan across the waxy peak to obtain a clean spectrum of compound #52, assigning an identity was not possible. Nonetheless, evident from the OPLS-DA and GC-GC/MS-olfactometry is the fact that unidentifiable compounds that contributed to differences in high and low elevation teas were not

sensory active. Tea is a complex beverage, containing hundreds of organic compounds, whose flavor, intensity, and balance are due to these and other organics.

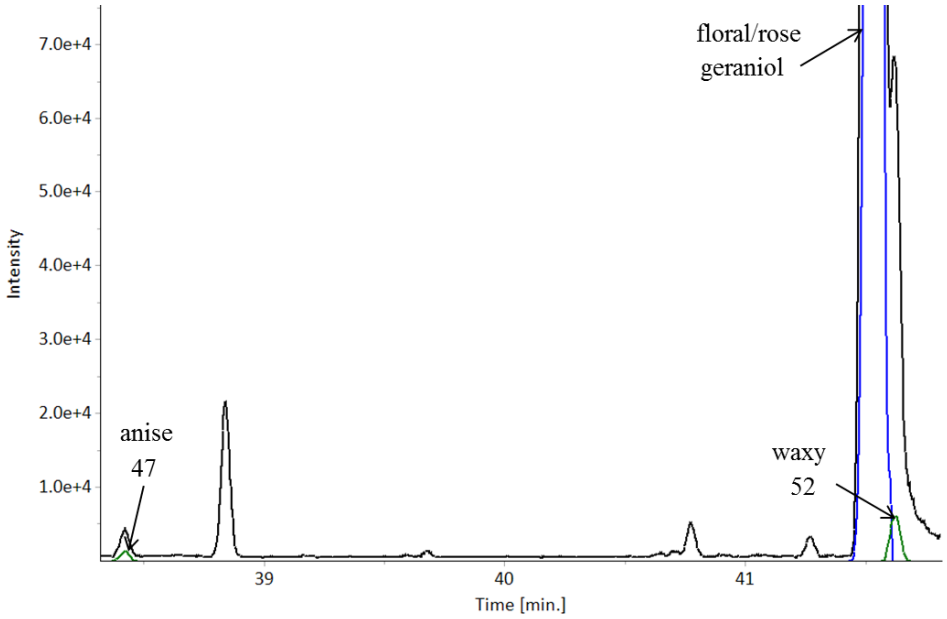

Figure 3-3. GC-GC/MS-olfactometry analysis of heartcut 19-20 min.[159]

A total of 83 volatile metabolites have reported health benefits. Of the 37 statistically important metabolites in Table 3-3 that differentiate high from low elevation tea, six have reported health benefits including (*E*)-caryophyllene (analgesic, antianxiety, antidepressant, anticancer, anti-inflammatory), (3*Z*)-hexenol (antifatigue, antinociceptive, antistress), (*Z*)-jasmone (antibacterial, anticancer), manool (antibacterial, antifungal, anti-inflammatory), α-calacorene (antibacterial, antioxidant), and cadalene (antibacterial, antioxidant)[104, 109, 143-144, 164-169] In addition, 15 compounds were higher in concentration in the 1400 m vs. 600 m samples. γ-cadinene and γ-decalactone were only detected in high elevation teas. None of the 14 compounds that distinguish low elevation tea have

reported health claims. Nonetheless, low elevation tea contains some health beneficial compounds higher in concentration than high elevation teas. It should be pointed out that the remaining health beneficial compound concentrations fall within ± 20% at the two elevations.

As expected, statistical analysis of Jinuo Mountain data revealed a significant elevational effect ($p = 0.005$, $R^2 = 0.912$, and $Q^2 = 0.719$). Metabolites such as 1-ethyl-1H-pyrrole-2-carboxaldehyde, (*Z*)-jasmone, (*E*)-caryophyllene, *trans*-linalool oxide (pyranoid), 2,6-dimethyl-3,7-octadien-2,6-diol, and isovaleric acid are still identified as important differentiators of high and low elevation teas. Several additional metabolites, listed in Table 3-4, become important such as 2-methylpentanal, methyl salicylate, 2,2,6-trimethylcyclohexanone, dehydro-1,8-cineole, hexanoic acid, and hotrienol.[109, 143-144, 161-163, 166-170]

MANOVA analysis of catechins and methylxanthines revealed a significant ($p = 0.0062$) separation between high and low elevation tea (Table 3-5) due to the lower concentrations of ECG, GC, C, and caffeine in the high elevation tea (one-way ANOVA, all $p < 0.05$). No statistical difference was observed for the other analytes. On the one hand, our findings for epicatechin gallate and gallocatechin are in agreement with other investigators.[96, 151-153] On the other, catechin and caffeine were not. Caffeine alone results in a lower flavor profile method analysis bitterness ranking from slight-to-moderate (1½) to slight (1); lower bitterness and astringency are associated with higher quality teas.[96, 151-152] Nonetheless, catechins

in high elevation teas are high enough in concentration to potentially provide the many health benefits associated with them.[13-15]

Table 3-5. Catechin and methylxanthine concentrations in high and low elevation teas.[159]

Compound	High Elevation (mg/g tea leaf ± SD)	Low Elevation (mg/g tea leaf ± SD)	p-value
Theobromine	2.56 ± 0.33	2.86 ± 0.43	0.1181
Caffeine	33.48 ± 3.55	37.44 ± 2.59	0.0157*
Epigallocatechin	9.11 ± 2.63	9.91 ± 2.13	0.4853
Epicatechin	13.12 ± 5.41	15.80 ± 3.67	0.2362
Epigallocatechin gallate	54.18 ± 13.77	51.98 ± 6.75	0.6718
Epicatechin gallate	38.31 ± 6.35	48.18 ± 4.18	0.0013*
Gallocatechin	1.54 ± 0.19	1.89 ± 0.39	0.0267*
Catechin	4.33 ± 1.44	6.25 ± 2.30	0.0497*
Gallocatechin gallate[a]	0.62 ± 0.10	0.61 ± 0.06	0.8441
Catechin gallate	0.28 ± 0.08	0.35 ± 0.13	0.2157

* $p < 0.05$ [a] Estimated due to sub-LOQ levels.

3.4 Conclusion

We demonstrated a 5 °C change in temperature due to elevational differences causes significant plant alterations in tea chemistry. This finding was independent of the mountain from which the teas were grown. High elevation tea contained statistically higher concentrations of volatile compounds whose health beneficial properties include analgesic, antianxiety, antibacterial, anticancer, antidepressant, antifungal, anti-inflammatory, antioxidant, anti-stress, and cardioprotective. Low elevation teas did not contain statistically higher concentrations of any health beneficial compounds. In addition, high elevation teas contained statistically sweeter, floral, honey-like compounds as opposed to low elevation tea, which contained statistically greener, herbal, hay-like, bitter compounds. Given these

results and our previous studies, it is evident that the composition of tea is strikingly different due to growing conditions, which most likely accounts for inconsistencies in the outcomes of clinical trials, whose aims are to investigate the health benefits of tea, since no study includes a detailed metabolomic profile of the sample consumed by participants. Toward this end, we are developing 2-dimensional LC/MS methods with the goal of unraveling the complex metabolomic profile of polyphenolic compounds in tea. This study is part of a larger effort in understanding the complex relationships and feedback loops that occur between human and natural systems.

The material presented in this chapter is based on work supported by the National Science Foundation under grant BCS-1313775: [159]Kfoury, N.; Morimoto, J.; Kern, A.; Scott, E. R.; Orians, C. M.; Ahmed, S.; Griffin, T.; Cash, S. B.; Stepp, J. R.; Xue, D.; Long, C.; Robbat Jr., A., Striking changes in tea metabolites due to elevational effects. *Food Chem.* **2018,** *264*, 334-341.

Table 3-1. Metabolite relative peak areas found in high and low elevation teas.[159]

		Jinuo Mountain												Bulang Mountain						Retention Index	
		May 3-5						May 18-20						May 6-8							
		High Elev.			Low Elev.			High Elev.			Low Elev.			High Elev.			Low Elev.				
No.	Compound	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Sample	Std/Lib
	Monoterpene hydrocarbons																				
1	Cumene[a]	0.002	0.002	0.001	0.001	0.001	0.001		0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001		0.001	0.001	924	924
2	α-Pinene[a]	0.002	0.004	0.004	0.002	0.004	0.002	0.003	0.008	0.003	0.003	0.004	0.006	0.003	0.002	0.004	0.002	0.002	0.004	933	933
3	Camphene[a]	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.002	0.002	0.001	0.002	0.001	0.002	0.002	947	948
4	Myrcene[a]	0.045	0.092	0.088	0.064	0.039	0.060	0.057	0.109	0.071	0.062	0.076	0.109	0.077	0.060	0.071	0.061	0.065	0.068	991	992
5	α-Phellandrene[a]	0.001	0.002	0.003	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.003	0.003	0.001	1005	1006
6	δ-3-Carene[a]	0.001	0.001	0.001	0.001	0.002		0.001	0.004	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	1010	1010
7	α-Terpinene[a]		0.005	0.006	0.003	0.003	0.005	0.003	0.006	0.004	0.005	0.003	0.002	0.004	0.003	0.004	0.006	0.004	0.001	1017	1017
8	Limonene[a]	0.051	0.093	0.096	0.069	0.050	0.066	0.064	0.130	0.079	0.071	0.067	0.101	0.079	0.062	0.072	0.070	0.074	0.084	1028	1029
9	Sylvestrene[b]	0.044	0.079	0.084	0.064	0.047	0.056	0.060	0.121	0.068	0.062	0.061	0.090	0.068	0.053	0.063	0.062	0.064	0.070	1028	1030
10	(*Z*)-β-Ocimene[b]	0.032	0.072	0.073	0.054	0.031	0.055	0.048	0.087	0.064	0.048	0.056	0.065	0.065	0.050	0.063	0.047	0.049	0.053	1038	1038
11	(*E*)-β-Ocimene[b]	0.043	0.115	0.177	0.119	0.060	0.142	0.129	0.231	0.164	0.121	0.144	0.163	0.169	0.129	0.162	0.137	0.141	0.129	1049	1048
12	γ-Terpinene[a]	0.011	0.021	0.023	0.016	0.011	0.014	0.014	0.021	0.016	0.013	0.015	0.023	0.015	0.012	0.014	0.016	0.016	0.014	1059	1060
13	Terpinolene[a]	0.051	0.100	0.096	0.080	0.046	0.088	0.082	0.136	0.099	0.069	0.091	0.112	0.109	0.082	0.097	0.064	0.064	0.112	1090	1090
14	*allo*-Ocimene[a]	0.002	0.003	0.004	0.003	0.003	0.004	0.003	0.004	0.003	0.003	0.004	0.004	0.002	0.002	0.002	0.002	0.002	0.002	1129	1129
15	Bornylene[b]	0.124	0.204	0.204	0.236	0.112	0.245	0.199	0.370	0.305	0.262	0.264	0.349	0.243	0.187	0.208	0.077	0.093	0.101	1229	N/A
	Oxygenated Monoterpenes																				
16	1,8-Dehydro-cineole[b]	0.002	0.002	0.002	0.005	0.003	0.006	0.003	0.004	0.004	0.007	0.009	0.007	0.006	0.005	0.006	0.003	0.001	0.003	990	990
17	(*E*)-Herboxide[a]	0.008	0.016	0.015	0.011	0.007	0.012	0.009	0.020	0.014	0.012	0.015	0.020	0.015	0.011	0.014	0.009	0.011	0.014	991	992
18	(*Z*)-Herboxide[a]	0.003	0.004	0.003	0.004	0.002	0.003	0.003	0.007	0.004	0.004	0.005	0.005	0.004	0.003	0.004	0.002	0.003	0.004	1008	1008
19	Bergamal[a]	0.001	0.002	0.002	0.001	0.001	0.002	0.002	0.002	0.001	0.001	0.002	0.002	0.005	0.004	0.006	0.002	0.002	0.002	1053	1053
20	*cis*-Linalool oxide (furanoid)[a]	0.092	0.106	0.067	0.077	0.051	0.087	0.075	0.124	0.099	0.114	0.100	0.183	0.154	0.118	0.128	0.047	0.051	0.108	1073	1073
21	*trans*-Linalool oxide (furanoid)[a]	0.084	0.076	0.052	0.069	0.041	0.068	0.057	0.095	0.078	0.079	0.131	0.152	0.098	0.075	0.080	0.039	0.042	0.144	1089	1089
22	Linalool[a]	1.710	3.771	3.436	2.784	1.548	2.608	2.815	4.081	3.370	2.326	3.082	3.670	3.102	2.331	3.071	2.127	2.002	2.709	1102	1102
23	Hotrienol[b]	0.087	0.227	0.207	0.451	0.227	0.415	0.287	0.426	0.398	0.399	0.507	0.603	0.490	0.370	0.401	0.159	0.147	0.336	1105	1107
24	*cis-p*-Ment-2-en-1-ol[b]	0.004	0.006	0.005	0.005	0.004	0.006	0.005	0.007	0.005	0.005	0.006	0.006	0.004	0.003	0.004	0.004	0.003	0.004	1121	1118
25	Nerol oxide[b]	0.004	0.009	0.009	0.014	0.008	0.017	0.008	0.015	0.016	0.018	0.018	0.027	0.017	0.013	0.014	0.004	0.004	0.013	1155	1154
26	Borneol[a]	0.041	0.022	0.019	0.006	0.013	0.004	0.010	0.014	0.009	0.017	0.017	0.050	0.033	0.024	0.023	0.029	0.033	0.057	1167	1167

27	*cis*-Linalool oxide (pyranoid)[a]	0.018	0.010	0.014	0.031	0.015	0.022	0.013	0.020	0.022	0.043	0.041	0.036	0.032	0.024	0.029	0.014	0.016	0.055	1172	1172
28	Menthol[a]	0.009	0.009	0.013	0.014	0.008	0.010	0.009	0.014	0.012	0.021	0.019	0.015	0.014	0.009	0.012	0.014	0.012	0.024	1176	1177
29	*trans*-Linalool oxide (pyranoid)[a]	0.046	0.039	0.046	0.090	0.052	0.054	0.042	0.067	0.076	0.142	0.139	0.093	0.069	0.052	0.066	0.064	0.068	0.221	1178	1178
30	Terpinen-4-ol[a]	0.011	0.025	0.025	0.021	0.011	0.018	0.017	0.024	0.022	0.020	0.020	0.031	0.019	0.014	0.018	0.014	0.015	0.016	1181	1181
31	*p*-Cymen-8-ol[b]	0.015	0.019	0.010	0.021	0.017	0.015	0.009	0.017	0.018	0.017	0.019	0.031	0.022	0.017	0.033	0.011	0.011	0.019	1190	1184
32	α-Terpineol[a]	0.560	1.061	1.026	0.803	0.461	0.803	0.745	1.236	0.999	0.893	0.890	1.198	0.943	0.717	0.866	0.621	0.631	0.795	1195	1195
33	Myrtenol[b]	0.006	0.010	0.012	0.019	0.012	0.016	0.011	0.018	0.017	0.018	0.017	0.016	0.051	0.038	0.041	0.006	0.007	0.011	1201	1194
34	Carvomenthenal[b]	0.016	0.018	0.030	0.011	0.019	0.017	0.012	0.018	0.015	0.015	0.012	0.020	0.010	0.008	0.009	0.017	0.016	0.011	1218	1217
35	β-Cyclocitral[a]	0.004	0.006	0.007	0.005	0.004	0.008	0.004	0.009	0.007	0.004	0.005	0.004	0.008	0.005	0.007	0.004	0.004	0.003	1224	1224
36	2-Hydroxy-1,8-cineole[b]	0.019	0.010	0.016	0.006	0.008	0.004	0.004	0.007	0.006	0.014	0.014	0.022	0.011	0.008	0.008	0.012	0.012	0.008	1228	1229
37	Nerol[a]	0.251	0.440	0.458	0.389	0.207	0.342	0.290	0.470	0.377	0.393	0.358	0.649	0.404	0.300	0.362	0.226	0.258	0.281	1232	1233
38	Carvone[a]	0.002	0.001	0.001	0.002	0.002	0.001	0.001	0.002	0.002	0.001	0.001	0.004	0.002	0.002	0.003	0.002	0.001	0.002	1245	1245
39	Geraniol[a]	0.938	1.761	1.822	1.653	0.862	1.424	1.249	2.057	1.716	1.672	1.562	2.577	1.690	1.266	1.485	1.023	1.103	1.261	1259	1259
40	Linalool acetate[a]	0.134	0.284	0.275	0.238	0.128	0.212	0.182	0.302	0.279	0.239	0.232	0.372	0.255	0.202	0.220	0.196	0.186	0.221	1256	1255
41	Geranial[a]	0.015	0.010	0.007	0.008	0.008	0.006	0.008	0.011	0.010	0.015	0.015	0.027	0.008	0.007	0.006		0.005	0.008	1273	1273
42	Geranyl formate[a]	0.015	0.006	0.007	0.006	0.004	0.006	0.006	0.004	0.006	0.005	0.007	0.021	0.010	0.007	0.007	0.008	0.009	0.008	1302	1302
43	(*E*)-β-Damascenone[b]	0.028	0.025	0.035	0.023	0.016	0.025	0.023	0.030	0.040	0.022	0.026	0.058	0.034	0.025	0.029	0.014	0.017	0.039	1390	1386
44	(*Z*)-Jasmone[a]	0.006	0.005	0.006	0.003	0.002	0.003	0.004	0.004	0.006	0.003	0.003	0.004	0.013	0.010	0.013	0.003	0.003	0.002	1404	1404
45	4-(2,4,4-Trimethylcyclohexa-1,5-dienyl)-but-3-en-2one[b]	0.004	0.004	0.007	0.005	0.004	0.005	0.005	0.006	0.006	0.005	0.005	0.006	0.009	0.007	0.007	0.005	0.005	0.006	1420	1423
46	Carvone hydrate[b]	0.010	0.004	0.004	0.003	0.003		0.002	0.004	0.003	0.003	0.002	0.017	0.006	0.005	0.004	0.003	0.003	0.010	1431	1424
47	Geranyl acetone[a]	0.009	0.008	0.009	0.007	0.007	0.008	0.004	0.008	0.010	0.009	0.009	0.048	0.012	0.008	0.007	0.013	0.015	0.005	1455	1454
48	5,6-epoxy-β-Ionone[b]	0.025	0.040	0.048	0.029	0.027	0.056	0.030	0.050	0.027	0.033	0.028	0.030	0.015	0.011	0.019	0.027	0.031	0.018	1490	1482
49	(*E*)-β-Ionone[a]	0.009	0.018	0.018	0.011	0.017	0.019	0.010	0.019	0.011	0.012	0.009	0.013	0.007	0.005	0.007	0.011	0.012	0.009	1491	1491
50	Dihydroactinidiolide[b]	0.041	0.022	0.050	0.034	0.023	0.018	0.018	0.014	0.012	0.039	0.033	0.079	0.012	0.009	0.004	0.057	0.053	0.021	1532	1528
	Sesquiterpene hydrocarbons																				
51	β-Bourbonene[b]	0.002	0.001	0.001	0.001	0.001	0.002	0.001	0.003	0.002	0.001	0.001	0.004	0.004	0.004	0.003	0.003	0.004	0.002	1390	1387
52	(*E*)-Caryophyllene[a]	0.003	0.002	0.002	0.002	0.001	0.001	0.002	0.002	0.002	0.001	0.001	0.002	0.005	0.004	0.005	0.002	0.002	0.001	1422	1421
53	α-Amorphene[b]	0.002	0.003	0.005	0.004	0.005	0.006	0.003	0.004	0.005	0.006	0.005	0.008	0.006	0.005	0.004	0.003	0.003	0.003	1485	1483
54	α-Muurolene[b]	0.001	0.003	0.003	0.003	0.002	0.003	0.002	0.004	0.005	0.003	0.003	0.006	0.008	0.006	0.007	0.003	0.005	0.003	1506	1500
55	δ-Amorphene[b]	0.002	0.002	0.003	0.002	0.002	0.003	0.001	0.002	0.003	0.003	0.002	0.003	0.002	0.001	0.001	0.002	0.002	0.001	1513	1511
56	δ-Cadinene[b]	0.002	0.004	0.003	0.004	0.003	0.003	0.003	0.006	0.006	0.005	0.004	0.006	0.009	0.006	0.009	0.002	0.002	0.004	1529	1531
57	cis-Calamenene[b]	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.007	0.006	0.005	0.006	0.003	0.003	0.002	1528	1528
58	α-Calacorene[b]	0.003	0.006	0.004	0.004	0.003	0.006	0.005	0.008	0.011	0.006	0.005	0.007	0.022	0.016	0.020	0.003	0.002	0.004	1551	1544

59	Cadalene[b]	0.002	0.002	0.001	0.002	0.001	0.002	0.002	0.003	0.005	0.002	0.002		0.012	0.009	0.011	0.001		0.003	1682	1675
	Oxygenated sesquiterpenes																				
60	(*E*)-Nerolidol[a]	0.054	0.051	0.045	0.029	0.026	0.033	0.026	0.038	0.042	0.032	0.027	0.077	0.036	0.028	0.022	0.028	0.029	0.019	1568	1569
61	Caryophyllene oxide[a]	0.003	0.004	0.004	0.002	0.002	0.002	0.002	0.004	0.003	0.003	0.003	0.005	0.005	0.004	0.005	0.003	0.003	0.002	1594	1593
62	Cedrol[b]	0.005	0.004	0.006	0.003	0.004	0.004	0.003	0.006	0.006	0.006	0.007	0.011	0.013	0.009	0.007	0.010	0.010	0.004	1613	1607
63	Humulene epoxide II[b]	0.005	0.004	0.006	0.004	0.003	0.005	0.004	0.004	0.006	0.006	0.006	0.015	0.018	0.014	0.014	0.011	0.012	0.006	1620	1608
64	*epi*-α-Cadinol[b]	0.005	0.010	0.007	0.012	0.006	0.010	0.008	0.015	0.016	0.017	0.014	0.019	0.035	0.027	0.033	0.004	0.005	0.008	1649	1638
65	*epi*-α-Murrolol[b]	0.005	0.008	0.007	0.007	0.005	0.008	0.005	0.011	0.013	0.012	0.010	0.025	0.026	0.019	0.021	0.005	0.005	0.004	1650	1640
66	α-Muurolol[b]	0.001	0.003	0.002	0.003	0.002	0.002	0.002	0.005	0.004	0.004	0.003	0.006	0.007	0.006	0.010	0.001	0.001	0.002	1654	1644
67	α-Cadinol[b]	0.010	0.013	0.012	0.013	0.010	0.015	0.013	0.021	0.027	0.023	0.018	0.036	0.042	0.032	0.035	0.011	0.011	0.010	1662	1652
68	*epi*-α-Bisabolol[b]	0.006	0.006	0.005	0.005	0.003	0.005	0.004	0.006	0.005	0.006	0.004	0.010	0.006	0.006	0.004	0.004	0.004	0.002	1687	1685
69	α-Bisabolol[a]	0.005	0.004	0.005	0.003	0.003	0.004	0.003	0.005	0.006	0.005	0.005	0.009	0.010	0.009	0.008	0.005	0.006	0.004	1690	1691
	Oxygenated diterpenes																				
70	Manool[b]	0.013	0.011	0.020	0.003	0.002	0.002	0.005	0.007	0.008	0.006	0.010	0.011	0.038	0.030	0.017	0.010	0.008	0.008	2070	2057
71	Phytol[b]	0.487	0.343	0.481	0.177	0.491	0.430	0.317	0.825	0.353	0.274	0.268	0.557	0.390	0.284	0.216	0.312	0.414	0.734	2117	2116
	Alcohols																				
72	Pentanol[a]	0.032	0.041	0.065	0.037	0.025	0.064	0.035	0.067	0.102	0.031	0.045	0.067	0.069	0.074	0.075	0.043	0.048	0.046	777	776
73	(2*Z*)-Pentenol[b]	0.012	0.017	0.034	0.023	0.016	0.041	0.016	0.027	0.031	0.012	0.021	0.016	0.010	0.007	0.013	0.015	0.015	0.021	781	774
74	2-Methyl-2-buten-1-ol[b]	0.005	0.003	0.003	0.003	0.004	0.002	0.001	0.002	0.002	0.001	0.002	0.003	0.004	0.003	0.003	0.001	0.001	0.003	783	782
75	3-Methyl-2-buten-1-ol[a]	0.006	0.005	0.007	0.004	0.004	0.005	0.004	0.006	0.009	0.004	0.006	0.008	0.008	0.006	0.006	0.003	0.003	0.007	783	783
76	(3*Z*)-Hexenol[a]	0.004	0.004	0.005	0.002	0.004	0.003	0.003	0.005	0.006	0.003	0.004	0.004	0.005	0.004	0.005	0.003	0.004	0.005	855	856
77	*n*-Hexanol[a]	0.002	0.003	0.002	0.001	0.001	0.003	0.003	0.002	0.005	0.001	0.002	0.002	0.006	0.004	0.007	0.003	0.004	0.006	869	870
78	(2*Z*)-Hexenol[a]	0.005	0.007	0.014	0.004	0.004	0.009	0.007	0.006	0.010	0.010	0.013	0.006	0.014	0.011	0.014	0.008	0.008	0.004	868	868
79	4-Heptanol[a]	0.020	0.022	0.026	0.014	0.014	0.023	0.016	0.023	0.021	0.020	0.033	0.027	0.032	0.024	0.029	0.018	0.019	0.036	891	890
80	2-Butoxyethanol[b]	0.019	0.011	0.019	0.016	0.015	0.021	0.021	0.014	0.017	0.023	0.029	0.023	0.024	0.018	0.027	0.061	0.070	0.040	909	903
81	6-Methyl-2-heptanol[a]	0.003	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.004	0.004	0.003	0.002	0.003	0.003	0.004	963	964
82	1-Octen-3-ol[a]	0.003	0.005	0.006	0.005	0.004	0.007	0.010	0.027	0.015	0.005	0.004	0.013	0.007	0.005	0.009	0.005	0.006	0.007	978	978
83	2-Ethyl-1-hexanol[a]	0.014	0.009	0.013	0.010	0.006	0.014	0.010	0.011	0.011	0.012	0.009	0.016	0.014	0.009	0.015	0.013	0.015	0.012	1029	1028
84	*n*-Dodecanol[a]	0.013	0.007	0.010	0.007	0.005	0.011	0.006	0.010	0.010	0.007	0.008	0.033	0.014	0.010	0.016	0.057	0.068	0.004	1475	1473
85	Fokienol[b]	0.011	0.015	0.012	0.015	0.008	0.015	0.011	0.019	0.016	0.018	0.015	0.022	0.017	0.012	0.012	0.009	0.009	0.005	1602	1596
86	(2*Z*,6*E*)-Farnesol[b]	0.023	0.030	0.028	0.014		0.019	0.015	0.018	0.028	0.022	0.019	0.066	0.023	0.021	0.015	0.023	0.019	0.028	1723	1722
87	*n*-Hexadecanol[a]	0.016	0.018	0.013	0.018	0.020	0.033	0.006	0.015	0.014	0.020		0.113	0.102	0.083	0.052	0.040	0.046	0.050	1883	1883
88	(*E*,*E*)-Geranyl linalool[b]	0.039	0.042	0.058	0.015	0.024	0.045	0.029	0.034	0.058	0.057	0.049	0.077	0.107	0.064	0.062	0.037	0.033	0.062	2034	2034
89	*n*-Octadecanol[b]	0.079	0.062	0.130	0.011	0.025	0.051	0.011	0.026	0.054	0.040	0.030	0.199	0.297	0.232	0.123	0.070	0.085		2086	2083

	Aldehydes																					
90	(2*E*)-Pentenal[b]	0.003	0.003	0.004	0.004	0.002	0.003	0.003	0.005	0.005	0.003	0.004	0.003	0.004	0.002	0.004	0.003	0.003	0.004	769	754
91	Tiglic aldehyde[a]	0.005	0.004	0.008	0.007	0.004	0.010	0.005	0.005	0.006	0.006	0.008	0.005	0.004	0.004	0.003	0.006	0.006	0.008	771	769
92	2-Methylpentanal[b]	0.034	0.047	0.054	0.019	0.023	0.038	0.035	0.055	0.050	0.018	0.025	0.029	0.028	0.029	0.030	0.034	0.036	0.041	772	777
93	3-Methyl-2-butenal[b]	0.005	0.005	0.005	0.004	0.004	0.004	0.004	0.003	0.005	0.003	0.007	0.006	0.006	0.004	0.006	0.005	0.005	0.006	790	781
94	Hexanal[a]	0.010	0.022	0.017	0.009	0.010	0.016	0.010	0.017	0.022	0.014	0.013	0.024	0.018	0.014	0.017	0.016	0.019	0.027	803	803
95	(2*E*)-Hexenal[a]	0.004	0.003	0.005	0.005	0.003	0.002	0.004	0.003	0.004	0.004	0.005	0.008	0.005	0.003	0.005	0.003	0.004	0.003	851	852
96	Heptanal[a]	0.005	0.009	0.006	0.003	0.004	0.005	0.004	0.008	0.006	0.003	0.005	0.007	0.007	0.005	0.007	0.008	0.008	0.005	902	902
97	(2*E*,4Z)-Heptadienal[b]	0.002	0.002	0.011	0.010	0.006	0.019	0.006	0.006	0.008	0.011	0.013	0.010	0.002	0.003	0.002	0.006	0.007	0.009	997	996
98	*n*-Octanal[a]	0.004	0.004	0.006	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.006	0.004	0.002	0.003	0.007	0.006	0.003	1002	1002
99	(2*E*,4*E*)-Heptadienal[a]	0.007	0.007	0.036	0.027	0.019	0.057	0.019	0.023	0.034	0.024	0.024	0.024	0.010	0.007	0.013	0.016	0.017	0.025	1010	1009
100	*n*-Nonanal[a]	0.030	0.025	0.021	0.009	0.013	0.010	0.009	0.016	0.013	0.010	0.012	0.050	0.015	0.011	0.012	0.049	0.049	0.017	1106	1106
101	Safranal[b]	0.009	0.016	0.021	0.030	0.014	0.027	0.019	0.033	0.030	0.030	0.029	0.026	0.089	0.068	0.072	0.012	0.012	0.018	1204	1198
102	*n*-Decanal[a]	0.013	0.014	0.021	0.009	0.007	0.005	0.004	0.008	0.008	0.010	0.011	0.019	0.012	0.008	0.007	0.031	0.035	0.032	1207	1207
	Ketones																					
103	2,4-Pentanedione[b]	0.004	0.004	0.007	0.005	0.003	0.007	0.005	0.008	0.010	0.006	0.007	0.003	0.011	0.007	0.011	0.004	0.006	0.004	787	783
104	4-Methyl-3-penten-2-one[a]	0.064	0.107	0.080	0.050	0.043	0.098	0.061	0.095	0.108	0.056	0.081	0.099	0.087	0.069	0.093	0.047	0.052	0.078	802	803
105	4-Hydroxy-4-methyl-2-pentanone[a]	0.045	0.039	0.039	0.054	0.025	0.046	0.031	0.033	0.041	0.048	0.065	0.095	0.050	0.039	0.050	0.023	0.037	0.044	840	839
106	3-Heptanone[a]	0.004	0.003	0.007	0.002	0.001	0.002		0.012	0.006	0.003	0.005	0.002	0.009	0.007	0.007	0.010	0.010	0.003	885	886
107	Cyclohexanone[a]	0.002	0.003	0.003	0.003	0.003	0.005	0.001	0.002	0.002	0.006	0.009	0.008	0.004	0.003	0.003	0.004	0.002	0.009	893	892
108	1-Octen-3-one[a]	0.004	0.004	0.005	0.006	0.003	0.004	0.004	0.009	0.008	0.006	0.006	0.005	0.006	0.004	0.006	0.004	0.004	0.004	978	978
109	2,3-Octanedione[b]	0.003	0.004	0.006	0.003	0.003	0.004	0.005	0.013	0.006	0.005	0.005	0.009	0.005	0.004	0.006	0.003	0.003	0.005	984	987
110	6-Methyl-5-hepten-2-one[a]	0.005	0.006	0.007	0.006	0.004	0.006	0.005	0.010	0.008	0.007	0.006	0.016	0.010	0.007	0.010	0.006	0.007	0.008	987	987
111	2,2,6-Trimethylcyclohexanone[a]	0.003	0.005	0.005	0.003	0.003	0.005	0.004	0.009	0.005	0.002	0.002	0.003	0.003	0.002	0.004	0.004	0.004	0.004	1034	1035
112	4-Ketoisophorone[a]	0.004	0.003	0.004	0.002	0.002	0.003	0.003	0.005	0.005	0.003	0.003	0.004	0.005	0.004	0.005	0.004	0.004	0.005	1144	1143
113	4-Hydroxy-3-methylacetophenone[b]	0.006	0.004	0.008	0.007	0.005	0.004	0.007	0.008	0.004	0.007	0.007	0.010	0.009	0.006	0.005	0.004	0.005	0.004	1309	1322
114	Hexahydrofarnesyl acetone[b]	0.017	0.010	0.023	0.018	0.009	0.012	0.008	0.015	0.015	0.017	0.015	0.016	0.023	0.017	0.009	0.023	0.020	0.049	1847	1844
	Esters																					
115	(3*Z*)-Hexenyl acetate[a]	0.002	0.002	0.002	0.002	0.001	0.002	0.002	0.005	0.003	0.003	0.003	0.004	0.002	0.002	0.002	0.002	0.002	0.003	1007	1005
116	(3*Z*)-Hexenyl hexenoate[b]	0.003	0.003	0.001	0.001	0.002	0.001	0.001	0.002		0.001	0.002	0.002	0.004	0.002	0.004	0.002	0.001	0.001	1383	1381
117	(*Z*)-Methyl jasmonate[a]	0.011	0.011	0.014	0.008	0.008		0.008	0.010	0.019	0.009	0.008	0.033	0.012	0.009	0.006	0.017	0.018	0.022	1647	1645
118	*cis*-Methyl dihydrojasmonate[a]	0.015	0.017	0.022	0.014	0.011	0.014	0.010	0.016	0.027	0.024	0.021	0.212	0.050	0.035	0.020	0.062	0.060	0.044	1655	1655

119	Isopropyl myristate[a]	0.006	0.004	0.009	0.004	0.004	0.003	0.003	0.006	0.005	0.006	0.005	0.007	0.008	0.006	0.004	0.020	0.019	0.011	1827	1826
120	Methyl palmitate[a]	0.021	0.027	0.050	0.011	0.014	0.014	0.008	0.016	0.029	0.013	0.017	0.010	0.052	0.039	0.022	0.073	0.075	0.042	1926	1925
121	Isopropyl palmitate[a]	0.013	0.008	0.008	0.025	0.059	0.007	0.001	0.004	0.006	0.005	0.004	0.008	0.011	0.008	0.005	0.014	0.015	0.004	2025	2025
122	Methyl linoleate[b]	0.025	0.022	0.040	0.009	0.012	0.019	0.010	0.011	0.037	0.019	0.015	0.015	0.076	0.053	0.029	0.028	0.025	0.028	2099	2101
123	Methyl linolenate[b]	0.014	0.013	0.022	0.006	0.010	0.010	0.008	0.011	0.021	0.010	0.009	0.018	0.036	0.029	0.018	0.021	0.021	0.034	2105	2105
	Hydrocarbons																				
124	Toluene[a]	0.306	0.169	0.272	0.150	0.194	0.082	0.088	0.210	0.244	0.036	0.069	0.350	0.248	0.291	0.261	0.472	0.430	0.466	777	777
125	5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene[b]	0.002	0.003	0.003	0.002	0.002	0.003	0.003	0.006	0.003	0.001	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.002	841	N/A
126	Ethylbenzene[a]	0.002	0.002	0.003	0.001	0.001	0.001	0.001	0.002	0.003	0.001	0.002	0.002	0.004	0.003	0.003	0.003	0.003	0.002	859	859
127	*m*-Xylene[a]	0.006	0.007	0.009	0.002	0.003	0.004	0.003	0.006	0.008	0.002	0.004	0.005	0.011	0.008	0.005	0.004	0.004	0.003	867	867
128	*p*-Xylene[a]	0.006	0.008	0.009	0.002	0.003	0.004	0.003	0.006	0.008	0.002	0.005	0.005	0.011	0.009	0.005	0.004	0.004	0.004	867	867
129	2,6-Dimethyl-1,5-heptadiene[b]	0.004	0.008	0.008	0.008	0.004	0.010	0.006	0.009	0.010	0.009	0.014	0.008	0.027	0.021	0.025	0.007	0.007	0.009	883	882
130	Styrene[b]	0.002	0.002	0.003	0.003	0.002	0.002	0.002	0.007	0.007	0.005	0.008	0.003	0.003	0.003	0.004	0.008	0.009	0.004	890	891
131	*o*-Xylene[a]	0.002	0.002	0.003	0.001	0.001	0.001	0.002	0.003	0.003	0.001	0.002	0.002	0.004	0.003	0.003	0.005	0.005	0.004	892	893
132	Mesitylene[a]	0.002	0.004	0.004	0.002	0.003	0.002	0.002	0.005	0.006	0.002	0.004	0.003	0.008	0.006	0.004	0.006	0.006	0.003	967	967
133	*trans*-2,6-Dimethyl-2,6-octadiene[b]	0.019	0.029	0.037	0.025	0.013	0.030	0.025	0.047	0.037	0.028	0.035	0.044	0.042	0.032	0.045	0.023	0.025	0.035	986	N/A
134	*cis*-2,6-Dimethyl-2,6-octadiene[b]	0.020	0.027	0.035	0.021	0.013	0.028	0.022	0.036	0.032	0.027	0.034	0.033	0.032	0.025	0.033	0.023	0.025	0.033	1001	N/A
135	1,2,4-Trimethylbenzene[b]	0.002	0.003	0.003	0.001	0.001	0.001	0.002	0.003	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.003	0.003	0.002	1022	1021
136	*p*-Cymene[a]	0.023	0.021	0.021	0.026	0.019	0.012	0.020	0.037	0.022	0.019	0.021	0.035	0.018	0.013	0.016	0.010	0.011	0.029	1024	1024
137	Indane[a]	0.001	0.001	0.002	0.001	0.004	0.001	0.002	0.005	0.003	0.003	0.004	0.003	0.002	0.001	0.002	0.002	0.002	0.003	1035	1035
138	*p*-Cymenene[b]	0.002	0.003	0.003	0.002	0.001	0.003	0.002	0.004	0.004	0.002	0.002	0.004	0.003	0.002	0.003	0.003	0.002	0.003	1090	1089
139	2,6-Dimethylcyclohexanol[b]	0.024	0.028	0.036	0.026	0.024	0.038	0.030	0.050	0.028	0.020	0.025	0.028	0.018	0.014	0.023	0.024	0.026	0.023	1109	1110
140	Viridene[b]	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.004	0.004	0.003	0.003	0.003	0.004	0.003	0.004	0.002	0.002	0.002	1155	1163
141	Naphthalene[a]	0.013	0.013	0.024	0.010	0.021	0.013	0.008	0.026	0.022	0.032	0.031	0.020	0.025	0.019	0.020	0.026	0.027	0.017	1186	1186
142	Dodecane[a]	0.005	0.006	0.009	0.003	0.003	0.005	0.003	0.006	0.008	0.005	0.005	0.068	0.014	0.010	0.010	0.017	0.018		1200	1200
143	1-Methylnaphthalene[a]	0.003	0.002	0.005	0.001	0.003	0.002	0.001	0.003	0.003	0.003	0.003	0.004	0.003	0.004	0.003	0.009	0.009	0.002	1311	1312
144	Theaspirane[b]	0.008	0.007	0.005	0.003	0.007	0.006	0.005	0.010	0.008	0.004	0.004	0.005	0.016	0.013	0.024	0.007	0.007	0.012	1319	1315
145	Dehydro-*ar*-ionene[b]	0.004	0.007	0.004	0.001	0.004	0.003	0.003	0.008	0.003	0.002	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.003	1358	1349
146	α-Ionene[b]	0.004	0.007	0.004	0.001	0.004	0.003	0.002	0.010	0.004	0.001	0.002	0.003	0.005	0.004	0.007	0.004	0.004	0.004	1361	1354
147	Tetradecane (C14)[a]	0.017	0.012	0.022	0.007	0.008	0.009	0.005	0.010	0.017	0.008	0.009	0.264	0.026	0.020	0.012	0.044	0.049	0.005	1400	1400
148	1,4-Dimethylnaphthalene[b]	0.003	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.003	0.003	0.002	0.002	0.009	0.009	0.001	1425	1429
149	Cabreuva oxide B[b]	0.003	0.004	0.004	0.006	0.003	0.006	0.005	0.004	0.006	0.005	0.006	0.009	0.004	0.003	0.003	0.002	0.002	0.003	1466	1462

150	Cabreuva oxide D[b]	0.004	0.004	0.005	0.006	0.004	0.008	0.005	0.004	0.007	0.007	0.006	0.010	0.004	0.003	0.004	0.003	0.003	0.003	1483	1479
151	4-Methylbiphenyl[b]	0.003	0.002	0.003	0.001	0.001	0.002		0.002	0.003	0.002	0.001	0.037	0.010	0.007	0.003	0.045	0.045	0.001	1485	1488
152	Pentadecane (C15)[a]	0.019	0.013	0.027	0.009	0.008	0.010	0.003	0.006	0.011	0.012	0.009	0.190	0.035	0.026	0.014	0.056	0.058	0.002	1500	1500
153	Hexadecane (C16)[a]	0.048	0.042	0.077	0.020	0.020	0.028	0.012	0.019	0.048	0.028	0.022	0.244	0.088	0.067	0.038	0.111	0.105	0.005	1600	1600
154	Heptadecane (C17)[a]	0.047	0.025	0.039	0.015	0.017	0.025	0.009	0.018	0.050	0.024	0.018	0.194	0.081	0.061	0.030	0.091	0.090	0.004	1700	1700
155	Anthracene[b]	0.054	0.025	0.034	0.021	0.017	0.013	0.010	0.014	0.022	0.022	0.018	0.028	0.034	0.026	0.017	0.124	0.114	0.008	1790	1789
156	Octadecane (C18)[a]	0.082	0.060	0.216	0.035	0.030	0.058	0.009	0.023	0.110	0.100	0.094	0.178	0.176	0.132	0.074	0.083	0.081	0.003	1800	1800
157	Nonadecane (C19)[a]	0.100	0.095	0.309	0.046	0.050	0.091	0.026	0.030	0.091	0.062	0.067	0.166	0.241	0.178	0.109	0.104	0.105		1900	1900
158	Heneicosane (C21)[a]	0.101	0.111	0.329	0.046	0.065	0.112	0.044	0.043	0.082	0.107	0.089	0.097	0.362	0.256	0.168	0.138	0.147	0.043	2100	2100
159	Docosane (C22)[a]	0.110	0.130	0.288	0.044	0.055	0.121	0.027	0.050	0.157	0.089	0.064	0.043	0.351	0.247	0.162	0.112	0.122	0.053	2200	2200
160	Tricosane (C23)[a]	0.141	0.129	0.253	0.049	0.074	0.143	0.032	0.054	0.073	0.101	0.069	0.101	0.319	0.327	0.205	0.115	0.136	0.127	2300	2300
161	Pentacosane (C25)[a]	0.154	0.168	0.295	0.059	0.134	0.164	0.069	0.137	0.153	0.106	0.118	0.261	0.755	0.610	0.395	0.266	0.290	0.365	2500	2500
	Acids																				
162	Hexanoic acid[a]	0.006	0.003	0.008	0.060	0.014	0.004	0.003	0.005	0.015	0.054	0.041	0.099	0.005	0.003	0.003	0.003	0.004	0.002	997	997
163	Heptanoic acid[a]	0.003	0.005	0.006	0.008	0.005	0.002	0.003	0.004	0.007	0.007	0.008	0.016	0.003	0.002	0.002	0.002	0.003	0.001	1076	1077
164	2-Ethylhexanoic acid[a]	0.008		0.005	0.005	0.004	0.004	0.003	0.002	0.008	0.007	0.007	0.012	0.003	0.002	0.002	0.002	0.002	0.001	1128	1128
165	Octanoic acid[a]	0.006	0.002	0.005	0.016	0.007	0.004	0.004	0.003	0.020	0.017	0.014	0.028	0.006	0.004	0.003	0.003	0.004	0.002	1172	1173
166	Nonanoic acid[a]	0.028	0.009	0.026	0.015	0.012	0.015	0.009	0.011	0.029	0.026	0.026	0.058	0.011	0.007	0.008	0.016	0.027	0.004	1269	1270
167	Geranic acid[a]	0.039	0.008	0.017	0.048	0.011	0.007	0.008		0.017	0.030	0.031	0.104	0.022	0.014	0.006	0.011	0.013	0.006	1353	1352
168	Decanoic acid[a]	0.010	0.002	0.010	0.005	0.003	0.003	0.002	0.004	0.019	0.005	0.005	0.008	0.008	0.005	0.003	0.004	0.007	0.001	1365	1366
169	Dodecanoic acid[a]	0.010	0.002	0.007	0.004	0.005	0.008	0.001	0.006	0.008	0.005	0.004	0.009	0.012	0.007	0.004	0.001	0.002		1561	1562
170	Tetradecanoic acid[a]	0.025	0.007	0.017	0.014	0.012	0.017	0.004	0.008	0.017	0.011	0.011	0.026	0.022	0.015	0.009	0.001	0.003		1758	1759
	Nitrogen/Sulfur Containing																				
171	Pyrrole[a]	0.001	0.002	0.001	0.003	0.003	0.005	0.003	0.005	0.005	0.003	0.002	0.001	0.008	0.006	0.005	0.003	0.004	0.002	770	769
172	1-Ethyl-1H-pyrrole[b]		0.012	0.020		0.010	0.015	0.006	0.008	0.018	0.007	0.001		0.066	0.052	0.031	0.010	0.006	0.003	814	815
173	Dimethyl Sulfoxide[b]	0.049	0.026	0.031	0.040	0.027	0.023	0.015	0.022	0.033	0.007	0.013	0.040	0.023	0.019	0.018		0.014	0.055	837	N/A
174	Methional	0.002	0.002	0.005	0.003	0.003	0.003	0.003	0.005	0.002	0.004	0.005	0.003	0.005	0.004	0.005	0.005	0.004	0.008	905	903
175	Dihydro-3-(2H)-thiophenone[b]	0.004	0.005	0.005	0.006	0.005	0.010	0.004	0.008	0.008	0.006	0.007	0.008	0.005	0.004	0.005	0.004	0.004	0.010	949	954
176	1-Ethyl-1H-pyrrole-2-carboxaldehyde[b]	0.024	0.035	0.025	0.006	0.013	0.012	0.017	0.030	0.016	0.007	0.009	0.013	0.031	0.023	0.023	0.014	0.016	0.020	1050	1050
177	2-Acetylpyrrole[a]	0.094	0.041	0.138	0.063	0.057	0.034	0.046	0.027	0.019	0.031	0.035	0.126	0.058	0.043	0.009	0.069	0.080	0.057	1060	1061
178	Benzeneacetonitrile[a]	0.036	0.042	0.040	0.023	0.020	0.028	0.039	0.053	0.062	0.023	0.022	0.032	0.097	0.073	0.076	0.028	0.030	0.053	1138	1137
179	Benzothiazole[a]		0.007	0.011	0.010	0.005	0.009	0.007	0.008	0.011	0.011	0.011	0.011	0.015	0.011	0.012	0.021	0.022	0.006	1223	1223
180	Indole[a]	0.014	0.085	0.072	0.052	0.059	0.043	0.037	0.066	0.099	0.061	0.048	0.026	0.446	0.338	0.360	0.074	0.076	0.066	1300	1301

	Oxygenated heterocycles																					
181	3-Furaldehyde[b]	0.002	0.003	0.003	0.001	0.002	0.003	0.001	0.002	0.002	0.002	0.003	0.003	0.004	0.004	0.004	0.003	0.002	0.002	815	812
182	Furfural[a]	0.095	0.111	0.118	0.062	0.056	0.123	0.063	0.088	0.079	0.062	0.101	0.115	0.120	0.095	0.098	0.061	0.051	0.097	832	832
183	2-Furanmethanol[a]	0.037	0.081	0.066	0.048	0.066	0.028	0.035	0.062	0.030	0.028	0.037	0.044	0.073	0.056	0.022	0.049	0.050	0.075	853	852
184	2-Acetylfuran[a]	0.020	0.034	0.019	0.014	0.010	0.013	0.013	0.019	0.033	0.016	0.020	0.030	0.046	0.036	0.031	0.014	0.015	0.011	912	912
185	γ-Butyrolactone[b]	0.003	0.002	0.003	0.003	0.002	0.001	0.002	0.002	0.002	0.003	0.004	0.003	0.002	0.002	0.001	0.004	0.003	0.003	914	904
186	2(5H)-Furanone[a]	0.140	0.027	0.098	0.081	0.049	0.011	0.025	0.014	0.014	0.056	0.064	0.142	0.040	0.030	0.008	0.083	0.083	0.079	916	915
187	Benzaldehyde[a]	0.034	0.036	0.060	0.049	0.035	0.071	0.047	0.090	0.049	0.040	0.052	0.070	0.051	0.039	0.055	0.038	0.039	0.092	959	960
188	5-Methylfurfural[a]	0.009	0.010	0.010	0.009	0.005	0.009	0.006	0.011	0.008	0.009	0.011	0.015	0.019	0.015	0.012	0.006	0.006	0.009	963	963
189	2,6,6-trimethyl-6-vinyltetrahydropyran[b]	0.003	0.005	0.006	0.005	0.002	0.003	0.003	0.005	0.006	0.003	0.004	0.007	0.004	0.003	0.003	0.004	0.003	0.004	970	971
190	Phenol[a]	0.039	0.013	0.023	0.021	0.014	0.011	0.012	0.014	0.015	0.026	0.027	0.034	0.023	0.017	0.012	0.017	0.020	0.021	981	981
191	Benzyl alcohol[a]	0.301	0.101	0.168	0.128	0.103	0.067	0.108	0.102	0.177	0.121	0.124	0.161	0.171	0.125	0.066	0.205	0.220	0.240	1037	1038
192	5-Ethyl-2(5H)-furanone[b]	0.007		0.007	0.005	0.005	0.003	0.003	0.001	0.003	0.003	0.003	0.005	0.004	0.003	0.001	0.006	0.006	0.007	1037	N/A
193	Lavender Lactone[b]	0.003	0.002	0.003	0.003	0.002	0.003	0.002	0.003	0.003	0.003	0.004	0.005	0.003	0.002	0.002	0.002	0.002	0.004	1040	1034
194	Benzene acetaldehyde[a]	0.236	0.233	0.401	0.191	0.221	0.294	0.394	0.594	0.265	0.148	0.185	0.271	0.402	0.307	0.394	0.267	0.276	0.769	1044	1044
195	γ-Hexalactone[a]	0.013	0.006	0.011	0.007	0.008	0.003	0.006	0.005	0.009	0.006	0.007	0.012	0.011	0.008	0.006	0.011	0.011	0.010	1055	1055
196	Acetophenone[a]	0.020	0.016	0.033	0.010	0.014	0.019	0.012	0.017	0.016	0.008	0.012	0.016	0.022	0.017	0.025	0.030	0.031	0.013	1066	1065
197	*m*-Cresol[a]	0.003	0.003	0.006	0.003	0.003	0.003	0.004	0.003	0.003	0.002	0.003	0.005	0.004	0.002	0.003	0.004	0.005	0.004	1077	1073
198	α,α-Dimethylbenzenemethanol[a]	0.032	0.018	0.064	0.015	0.020	0.027	0.025	0.027	0.024	0.015	0.022	0.021	0.013	0.009	0.013	0.011	0.012	0.017	1086	1085
199	Maltol[a]	0.016		0.015	0.016	0.010		0.001		0.001	0.008	0.008	0.014	0.003	0.002		0.004	0.005	0.002	1111	1110
200	Phenyl ethyl alcohol[a]	0.048	0.028	0.041	0.038	0.039	0.023	0.043	0.044	0.072	0.042	0.041	0.052	0.101	0.073	0.069	0.060	0.065	0.218	1117	1117
201	Methyl salicylate[a]	0.038	0.036	0.068	0.022	0.024	0.024	0.023	0.038	0.045	0.022	0.022	0.034	0.034	0.026	0.032	0.043	0.046	0.065	1198	1197
202	2,3-Dihydrobenzofuran[b]	0.004	0.150	0.877	0.526	0.598	0.201	0.038	0.004	0.002	0.499	0.081	0.023	0.003	0.002		0.654	0.501	0.007	1228	1219
203	*p*-tert-Butylphenol[a]	0.005	0.009	0.011	0.030	0.010	0.011	0.027	0.022	0.012	0.017	0.016	0.029	0.006	0.005	0.005	0.009	0.010	0.013	1293	1290
204	*m*-tert-Butylphenol[b]	0.005	0.009	0.012	0.030	0.006	0.011	0.027	0.022	0.012	0.017	0.016	0.029	0.006	0.005	0.005	0.009	0.011	0.014	1293	1294
205	5-Pentyl-2(5H)-furanone[b]	0.003	0.002	0.004	0.004	0.004	0.003	0.002	0.004	0.004	0.003	0.003	0.005	0.005	0.004	0.004	0.008	0.004	0.004	1343	1337
206	γ-Nonalactone[a]	0.004	0.002	0.003	0.004	0.002	0.003	0.002	0.004	0.003	0.003	0.004		0.009	0.006	0.006	0.010	0.011	0.010	1366	1365
207	Vanillin[a]	0.016	0.005	0.014	0.017	0.007	0.004	0.005	0.004	0.004	0.008	0.009	0.017	0.005	0.004	0.001	0.008	0.011	0.005	1405	1403
208	Dibenzofuran[a]	0.012	0.005	0.010	0.004	0.003	0.004	0.002	0.004	0.007	0.006	0.006	0.027	0.010	0.008	0.005	0.023	0.022	0.003	1515	1515
209	Benzophenone[b]	0.024	0.022	0.032	0.028	0.017	0.023	0.017	0.022	0.027	0.027	0.023	0.046	0.033	0.024	0.023	0.125	0.113	0.191	1636	1626
210	Benzyl benzoate[b]	0.033	0.022	0.031	0.022	0.019	0.019	0.025	0.026	0.041	0.050	0.042	0.029	0.032	0.023	0.015	0.083	0.078	0.026	1774	1761
211	2-Ethylhexyl salicylate[a]	0.028	0.014	0.016	0.008	0.008	0.007	0.006	0.009	0.014	0.011	0.010	0.013	0.026	0.019	0.012	0.039	0.039	0.025	1814	1813
212	Homomenthyl salicylate[a]	0.018	0.013	0.017	0.004	0.006	0.004	0.005	0.007	0.011	0.006	0.007	0.011	0.013	0.010	0.006	0.031	0.034	0.012	1889	1888

	Unknowns																				
213	1	0.007	0.008	0.011	0.004	0.004	0.006	0.008	0.011	0.006	0.004	0.006	0.005	0.006	0.004	0.009	0.006	0.006	0.007	775	
214	2	0.008	0.005	0.014	0.003	0.005	0.004	0.002	0.003	0.009	0.003	0.006	0.005	0.016	0.013	0.007	0.009	0.009	0.004	809	
215	5	0.006	0.012	0.017	0.008	0.007	0.010	0.011	0.015	0.008	0.009	0.013	0.008	0.027	0.021	0.014	0.012	0.014	0.008	828	
216	7	0.006	0.012	0.008	0.004	0.004	0.004	0.004	0.008	0.006	0.004	0.005	0.008	0.012	0.010	0.006	0.004	0.004	0.007	883	
217	8	0.004	0.031	0.054	0.039	0.026	0.057	0.040	0.058	0.041	0.055	0.073	0.027	0.082	0.064	0.074	0.077	0.073	0.040	916	
218	9		0.032	0.053	0.010	0.004	0.055	0.034	0.066	0.071	0.055	0.067	0.014	0.096	0.076	0.090	0.016	0.018	0.018	918	
219	11	0.010	0.014	0.024	0.024	0.014	0.029	0.015	0.021	0.026	0.029	0.037	0.022	0.038	0.030	0.037	0.014	0.016	0.018	927	
220	13		0.008	0.013	0.012	0.007	0.012	0.011	0.014	0.013	0.012	0.018	0.010	0.017	0.013	0.017	0.015	0.011	0.010	930	
221	14		0.007	0.011	0.007	0.006	0.011	0.007	0.010	0.011	0.010	0.013	0.009	0.016	0.012	0.015	0.010	0.009	0.008	938	
222	15	0.005	0.003	0.004	0.003	0.003	0.005	0.003	0.003	0.003	0.004	0.004	0.006	0.007	0.005	0.006	0.003	0.003	0.005	940	
223	16		0.001	0.026	0.012	0.009	0.029	0.015	0.024	0.029	0.026	0.032	0.015	0.041	0.032	0.039	0.052	0.054	0.012	944	
224	18		0.003	0.089	0.058	0.030	0.101	0.054	0.084	0.095	0.097	0.116	0.058	0.137	0.106	0.131	0.063	0.065	0.048	947	
225	19		0.009	0.015	0.009	0.008	0.016	0.008	0.011	0.017	0.014	0.017	0.011	0.023	0.018	0.021	0.038	0.041	0.010	950	
226	21	0.003	0.003	0.005	0.003	0.002	0.003		0.018	0.011	0.003	0.002	0.009	0.004	0.003	0.005	0.003	0.004	0.003	973	
227	22	0.003	0.002	0.004	0.003	0.002	0.004	0.003	0.012	0.005	0.004	0.004	0.007	0.004	0.003	0.005	0.002	0.002	0.004	984	
228	24	0.038	0.031	0.139	0.067	0.054	0.030	0.073	0.051	0.036	0.096	0.094	0.047	0.146	0.111	0.045	0.098	0.096	0.029	1009	
229	25	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.001	1017	
230	27	0.006	0.003	0.007	0.003	0.003	0.002	0.003		0.004	0.003	0.004	0.005	0.003	0.002	0.002	0.006	0.004	0.003	1052	
231	28	0.006	0.020	0.021	0.016	0.011	0.014	0.013	0.021	0.017	0.013	0.015	0.023	0.015	0.011	0.013	0.015	0.015	0.013	1059	
232	29	0.020		0.012	0.013	0.011	0.015	0.006		0.010	0.086	0.076	0.110	0.003	0.002		0.006	0.005	0.007	1106	
233	30	0.010	0.003	0.009	0.005	0.004		0.003		0.003	0.003	0.003	0.005	0.004	0.002		0.010	0.009	0.004	1009	
234	31	0.016	0.017	0.010	0.004	0.005	0.007	0.011	0.019		0.010	0.007	0.018	0.033	0.023	0.021	0.021	0.023	0.059	1114	
235	32	0.004		0.006	0.003	0.003	0.006	0.005	0.007	0.006	0.005	0.006	0.006	0.004	0.003	0.005	0.005	0.004	0.005	1121	
236	35	0.011	0.028	0.043	0.085	0.051	0.074	0.046	0.070	0.071	0.088	0.079	0.069	0.251	0.183	0.202	0.017	0.017	0.031	1201	
237	36	0.010	0.028	0.043	0.085	0.040	0.073	0.069	0.054	0.046	0.087	0.078	0.068	0.250	0.186	0.203	0.017	0.016	0.030	1201	
238	37	0.005	0.005	0.005	0.006	0.004	0.008	0.007	0.013	0.010	0.009	0.009	0.008	0.008	0.005	0.008	0.004	0.004	0.007	1206	
239	39	0.004	0.009	0.008	0.008	0.007	0.007	0.007	0.010	0.007	0.008	0.008	0.008	0.008	0.006	0.007	0.006	0.006	0.008	1216	
240	40	0.017	0.025	0.069	0.071	0.044	0.033	0.018	0.017	0.014	0.046	0.024	0.048	0.013	0.009	0.006	0.014	0.012	0.015	1218	
241	41	0.026	0.020	0.028	0.021	0.016	0.026	0.017	0.039	0.029	0.029	0.029	0.049	0.037	0.027	0.051	0.015	0.018	0.018	1221	
242	43	0.009		0.015	0.059	0.006	0.005	0.004	0.003	0.003	0.011	0.012	0.021	0.003	0.002	0.002	0.007	0.008	0.009	1240	
243	44	0.003	0.003	0.004	0.003	0.003	0.005	0.002	0.005	0.004	0.004	0.004	0.004	0.005	0.004	0.006	0.002	0.002	0.003	1244	
244	46	0.016	0.015	0.016	0.010	0.009	0.018	0.011	0.023	0.017	0.011	0.011	0.017	0.036	0.028	0.028	0.012	0.014	0.024	1266	
245	47	0.020	0.025	0.023	0.020	0.018	0.031	0.021	0.037	0.029	0.021	0.020	0.037	0.092	0.070	0.080	0.018	0.019	0.041	1275	

246	49	0.015	0.014	0.024	0.017	0.014	0.018	0.014	0.014	0.013	0.017	0.019	0.020	0.015	0.011	0.010	0.018	0.019	0.009	1279		
247	50	0.003	0.004	0.004	0.002	0.003	0.004	0.003	0.005	0.004	0.003	0.002	0.004	0.004	0.003	0.002	0.002	0.003	0.004	1281		
248	51	0.009	0.004	0.006	0.005	0.005	0.007	0.005	0.007	0.006	0.006	0.006	0.017	0.007	0.005	0.005	0.048	0.059	0.006	1355		
249	52	0.001	0.001	0.002	0.002	0.001	0.003	0.001		0.002	0.002	0.002	0.002	0.002	0.001	0.002	0.001	0.001	0.001	1367		
250	54	0.008	0.004	0.007	0.005	0.004	0.005	0.004	0.004	0.007	0.005	0.006		0.004	0.004	0.003	0.074	0.085	0.005	1375		
251	55	0.013	0.015	0.015	0.010	0.011	0.014	0.011	0.054	0.019	0.019	0.014	0.054	0.020	0.015	0.016	0.014	0.013	0.013	1520		
252	56	0.015	0.012	0.009	0.010	0.011	0.012	0.009	0.012	0.011	0.014	0.023	0.060	0.012	0.009	0.012	0.007	0.010	0.017	1526		
253	57	0.020	0.027	0.029	0.019	0.020	0.030	0.020	0.084	0.041	0.032	0.026	0.056	0.030	0.023	0.033	0.019	0.020	0.033	1527		
254	58	0.008	0.006	0.009	0.012	0.006	0.012	0.007	0.009	0.011	0.015	0.012	0.065	0.010	0.008	0.007	0.013	0.012	0.006	1537		
255	59	0.057	0.043	0.047	0.049	0.032	0.034	0.031	0.039	0.022	0.048	0.041	0.074	0.103	0.078	0.043	0.031	0.032	0.055	1564		
256	60	0.032	0.035	0.024	0.020	0.017	0.021	0.019	0.027	0.028	0.020	0.018	0.031	0.016	0.012	0.012	0.013	0.013	0.012	1565		
257	61		0.008	0.008	0.008	0.009	0.014	0.005		0.010	0.019	0.015	0.026	0.017	0.011	0.010	0.028	0.027	0.017	1581		
258	63	0.080	0.033	0.480	0.058	0.018	0.024	0.029	0.036	0.063	0.077	0.066	0.122	0.068	0.051	0.085	0.110	0.108	0.160	1596		
259	64	0.142	0.228	0.155	0.063	0.098	0.111	0.071	0.144	0.128	0.096	0.075		0.125	0.096	0.105	0.348	0.348	0.052	1599		
260	65	0.003	0.006	0.006	0.004	0.004	0.006	0.004	0.008	0.010	0.005	0.005	0.008	0.019	0.014	0.015	0.003	0.003	0.003	1621		
261	66	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.003	0.002	0.001	0.002	0.007	0.005	0.006	0.002	0.002	0.001	1625		
262	68	0.012		0.016	0.032	0.010	0.008	0.007		0.008	0.040	0.031	0.140	0.017	0.013		0.016	0.015	0.005	1636		

Positive[a] or tentative[b] identification made by comparing sample and reference standard (Std) or commercial library (Lib) fragmentation patterns and retention indexes (RI).

Table 3-2. Relative peak areas of unique metabolites in high and low elevation teas[159]

		Jinuo Mountain												Bulang Mountain						Retention Index	
		May 3-5						May 18-20						May 6-8							
		High Elev.			Low Elev.			High Elev.			Low Elev.			High Elev.			Low Elev.				
No.	Compound	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Sample	Std/Lib
	Oxygenated Monoterpenes																				
263	*endo*-Fenchol[a]	0.007	0.003	0.003				0.003	0.005	0.003				0.005	0.003	0.004	0.005	0.005	0.013	1115	1114
	Sesquiterpene hydrocarbons																				
264	γ-Cadinene[b]													0.005	0.004	0.005				1519	1513
	Alcohols																				
265	(3*E*)-Hexenol[a]													0.002	0.001	0.002	0.001	0.001	0.001	851	850
266	(2*E*)-Hexenol[a]	0.002	0.003	0.002				0.003	0.004	0.005				0.004	0.003	0.004	0.002	0.002	0.005	866	865
267	(2*E*)-Octen-1-ol[a]							0.003	0.007	0.005	0.006	0.005	0.005							1069	1068
268	2,6-Dimethyl-3,7-octadiene-2,6-diol[b]				0.172	0.033					0.097	0.134	0.069				0.046	0.056	0.080	1189	1189
	Ketones																				
269	2-Heptanone[a]	0.002		0.004										0.005	0.004	0.005	0.002	0.003	0.002	890	891
	Esters																				
270	2-Ethylhexyl acetate[a]	0.003	0.002	0.003	0.001	0.002	0.002							0.002	0.001	0.002	0.002	0.002		1152	1151
271	Octadecanol acetate[b]							0.013	0.027	0.025	0.027	0.029	0.050							2211	2209
	Hydrocarbons																				
272	1-Ethyl-3-methyl-benzene[b]	0.014	0.017	0.024	0.012	0.012	0.018							0.005	0.004	0.002	0.005	0.006	0.010	961	967
273	10,18-Bisnorabieta-8,11,13-triene[b]				0.004	0.005	0.002				0.002	0.002	0.088				0.014	0.015		2057	N/A
	Acids																				
274	Butanoic acid[a]	0.002	0.002	0.002	0.001		0.001		0.003	0.002	0.004	0.003	0.004	0.005	0.003	0.003				795	794
275	Isovaleric acid[a]	0.003		0.002	0.008	0.002	0.001				0.005	0.007	0.007							841	841
276	2-Methylbutanoic acid[a]	0.003		0.002	0.003	0.002	0.001	0.001		0.003	0.009	0.012	0.024	0.001	0.001	0.001				851	851
	Nitrogen/Sulfur Containing																				
277	3-Ethyl-4-methyl-1H-pyrrole-2,5-dione[b]	0.006	0.007	0.007	0.004	0.004												0.006	0.005	1232	1234
	Oxygenated heterocycles																				
278	2-Cyclohexen-1-ol[b]	0.003	0.007	0.005				0.003	0.006	0.005										891	887
279	2-Cyclohexen-1-one[b]										0.005	0.006	0.002							931	927
280	*cis*-Edulan[b]	0.005	0.004	0.006																1261	1247

281	*p*-Menthane-1,8-diol[b]	0.009	0.005	0.005	0.009	0.004	0.002	0.003	0.007	0.005	0.003	0.004	0.014	0.008	0.005	0.006				1307	N/A
282	γ-Decalactone[a]																0.007	0.007		1468	1468
283	2,5-bis(1,1-dimethylethyl)-Phenol[b]		0.008	0.006	0.008	0.010	0.005				0.006		0.007				0.012	0.013		1518	1512
284	2-Phenoxyethyl isobutyrate[b]																0.009	0.009		1519	N/A
	Unknowns																				
285	3				0.003	0.002														813	
286	4				0.008	0.004	0.018	0.013	0.016	0.026	0.019	0.014	0.040	0.058	0.041	0.043	0.011	0.013	0.074	816	
287	6	0.002		0.003										0.002	0.002					855	
288	10	0.006	0.010	0.006				0.008	0.010	0.005				0.018	0.014	0.017	0.017	0.015	0.012	926	
289	12													0.003	0.002	0.003	0.001	0.001		929	
290	17				0.051	0.029	0.102				0.097	0.116	0.058	0.136	0.106	0.131	0.063	0.065	0.048	947	
291	20				0.008	0.005	0.004	0.002	0.004	0.004	0.004	0.004	0.012	0.006	0.005	0.006	0.001	0.001		967	
292	23													0.004	0.003	0.005	0.001	0.001	0.003	969	
293	26	0.001	0.002	0.014	0.005	0.007	0.005	0.003	0.002	0.002	0.007	0.004	0.004				0.018	0.013		1021	
294	33	0.004	0.007	0.008													0.005	0.007		1155	
295	34	0.009		0.007							0.015	0.017	0.011							1189	
296	38													0.004	0.003	0.005				1221	
297	42	0.019	0.007	0.007				0.004	0.007	0.005				0.009	0.005	0.009	0.012	0.012	0.008	1224	
298	45																0.006	0.009	0.009	1259	
299	48		0.007	0.007	0.029	0.019	0.041	0.019	0.029	0.025	0.038	0.033	0.020	0.039	0.030	0.036				1278	
300	53	0.011	0.006	0.006	0.009	0.003		0.021	0.013	0.005	0.003	0.018	0.033	0.007	0.005	0.003				1372	
301	62	0.007	0.012	0.010	0.011	0.007	0.013	0.010	0.016	0.014	0.019	0.012		0.016	0.011	0.015				1599	
302	67				0.041	0.013					0.054	0.046	0.092							1636	
303	69													0.022	0.008	0.023	0.031	0.026		1661	
304	70	0.002	0.003	0.003	0.004	0.002	0.003	0.003	0.004	0.003	0.005	0.004	0.007							1708	
305	71				0.003	0.004					0.003	0.003	0.009							1897	

Positive[a] or tentative[b] identification made by comparing sample (Exp) and reference standard (Std) or commercial library (Lib) fragmentation patterns and retention indexes (RI).

Note: Compounds considered unique if replicates were non-detectable in at least one sample. For example, γ-Cadinene was found in only the high elevation Bulang Mountain tea sample.

Table 3-3. Statistically important metabolites in high and low elevation tea[159]

No.	Compound	VIP	p-value	% Diff.	Aroma	Health Property
	High Elevation					
176	1-Ethyl-1H-pyrrole-2-carboxaldehyde	2.48	0.0002	51	roasted, smoky	
127	*m*-Xylene	2.26	0.001	49	plastic	
128	*p*-Xylene	2.25	0.001	48	sweet, grain[162]	
278	2-Cyclohexen-1-ol	2.00	0.005	-	caramelized, floral[160]	
178	Benzeneacetonitrile	1.96	0.0008	50	floral[167]	
44	(*Z*)-Jasmone	1.94	0.0002	60	jasmine, floral	antibacterial[143] anticancer[144]
146	α-Ionene	1.93	0.02	48	floral, violet[163]	
266	(2*E*)-Hexenol	1.93	0.005	72	green, leafy, fruity[163]	
216	7	1.89	0.01	37		
52	(*E*)-Caryophyllene	1.88	0.008	47	green, spicy, woody	antianxiety, antidepressant[166] anticancer[109] analgesic[109, 168] anti-inflammatory[168]
184	2-Acetylfuran	1.81	0.01	43	sweet, balsamic	
76	(3*Z*)-Hexenol	1.78	0.02	26	green, grassy	antinociceptive, anti-fatigue[165] anti-stress[169]
260	65	1.65	0.02	53		
287	6	1.62	0.03	-		
70	Manool	1.59	0.03	59		antibacterial, antifungal, anti-inflammatory[164]
213	1	1.56	0.05	30		
125	5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	1.56	0.03	32		
58	α-Calacorene	1.54	0.02	58	woody	antibacterial, antioxidant[104]
172	1-Ethyl-1H-pyrrole	1.51	0.04	75	burnt	
59	Cadalene	1.50	0.03	71		antibacterial, antioxidant[104]

144	Theaspirane	1.35	0.02	44	tea, herbal, honey	
229	25	1.33	0.02	27		
245	47	1.32	0.04	43		
	Low Elevation					
268	2,6-Dimethyl-3,7-octadiene-2,6-diol	2.25	0.001	-	fruity, herbal[163]	
257	66	1.91	0.01	-86		
97	(*2E,4Z*)-Heptadienal	1.67	0.01	-112	fried	
29	*trans*-Linalool oxide (pyranoid)	1.62	0.02	-84	woody, fresh	
107	Cyclohexanone	1.59	0.02	-95	minty	
302	72	1.51	0.01	-		
275	Isovaleric acid	1.27	0.05	-123	cheesy, rancid	
283	2,5-bis(1,1-dimethylethyl)Phenol	1.45	0.03	-157		
50	Dihydroactinidiolide	1.40	0.02	-96	fruity, woody	
232	30	1.40	0.02	-323		
305	76	1.37	0.01	-		
293	27	1.26	0.02	-93		
242	43	1.22	0.008	-199		
202	2,3-Dihydrobenzofuran	1.09	0.02	-154	green, herbal[158]	

Notes:

1. OPLS-DA criteria used to determine compound differences between high and low elevation teas: VIP > 1.0 and p value < 0.05.
2. %Diff. = [(High-Low)/High] x 100.
3. Aroma information was obtained from the Good Scents Company[95] unless otherwise noted

Table 3-4. Statistically important metabolites in Jinuo Mountain high and low elevation tea[159]

No.	Compound	VIP	p-value	% Diff.	Aroma	Health Property
	High Elevation					
278	2-Cyclohexen-1-ol	2.26	0.003	-	caramelized, floral[160]	
266	(2*E*)-Hexenol	2.21	0.003	-	green, leafy, fruity	
288	10	2.20	0.003	-		
176	1-Ethyl-1H-pyrrole-2-carboxaldehyde	2.20	0.002	60	roasted, smoky	
263	*endo*-Fenchol	2.19	0.003	-	camphor, pine, woody	
92	2-Methylpentanal	2.17	0.009	45	green, fruity	
44	(*Z*)-Jasmone	2.05	0.009	42	jasmine, floral	antibacterial[143] anticancer[144]
178	Benzeneacetonitrile	2.04	0.002	46	floral[167]	
127	*m*-Xylene	1.98	0.02	47	plastic	
131	*o*-Xylene	1.97	0.009	44	geranium	
128	*p*-Xylene	1.96	0.02	46	sweet, grain[162]	
135	1,2,4-Trimethylbenzene	1.96	0.04	38	plastic	
213	1	1.93	0.009	43		
145	Dehydro-*ar*-ionene	1.84	0.04	49	licorice	
124	Toluene	1.83	0.01	56	sweet, paint	
297	42	1.83	0.003	-		
146	α-Ionene	1.82	0.04	58	floral, violet[163]	
144	Theaspirane	1.82	0.04	34	tea, herbal, honey	
52	(*E*)-Caryophyllene	1.802	0.009	36	green, spicy, woody	antianxiety, antidepressant[166] anticancer[109] analgesic[109, 168] anti-inflammatory[168]
201	Methyl salicylate	1.78	0.01	41	wintergreen	anti-inflammatory, analgesic[170]
111	2,2,6-Trimethylcyclohexanone	1.74	0.03	48	honey, floral	
259	64	1.74	0.03	39		

125	5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	1.68	0.01	43		
77	*n*-Hexanol	1.63	0.03	44	green, fruity, apple	anti-stress[169]
	Low Elevation					
290	17	2.05	0.003	-		
268	2,6-Dimethyl-3,7-octadiene-2,6-diol	1.84	0.01	-	fruity, herbal[143]	
237	36	1.82	0.04	-72		
275	Isovaleric acid	1.80	0.02	-123	cheesy, rancid	
16	Dehydro-1,8-cineole	1.75	0.009	-113	mint, lemon	
27	*cis*-Linalool oxide (pyranoid)	1.71	0.03	-92	citrus, green	
302	67	1.65	0.01	-		
257	61	1.58	0.02	-98		
299	48	1.56	0.04	-72		
236	35	1.56	0.03	-66		
162	Hexanoic acid	1.55	0.04	-578	fatty, sweaty, cheesy	
29	*trans*-Linalool oxide (pyranoid)	1.53	0.03	-81	woody, floral	
291	21	1.52	0.01	-91		
305	76	1.52	0.01	-		
107	Cyclohexanone	1.49	0.01	-141	minty	
232	29	1.43	0.03	-336		
23	Hotrienol	1.30	0.04	-60	floral, woody	
276	2-Methylbutanoic acid	1.24	0.03	-259	fruity, cheesy, sweaty	
262	68	1.07	0.03	-301		

Notes:

1. OPLS-DA criteria used to determine compound differences between high and low elevation teas: VIP > 1.0 and p value < 0.05.
2. %Diff. = [(High-Low)/High] x 100.
3. Aroma information was obtained from the Good Scents Company[95] unless otherwise noted

Chapter 4. Climate Effects on Tea Quality across Multiple Years

4.1 Introduction

It has long been known that environmental conditions affect tea quality.[171-172] To date, most studies have focused on seasonal[173-183] and elevational[96, 151-153, 184] effects on the non-volatile components. In general, these studies have found that increasing rainfall leads to a decrease in the concentration of non-volatile constituents, whereas changes in elevation had inconsistent results. Although non-volatile constituents are responsible for the taste and are most well-known for contributing to the health beneficial properties of tea, the volatile organics also play an important role in the overall quality of tea. Volatile metabolites contribute to overall flavor and aroma due to their low odor thresholds,[76, 185] as well as the nutritional properties of tea.[111-112]

Despite a total of ~600 compounds reported in the literature,[27, 76, 186-188] only a few studies have investigated aroma compounds as a function of seasonal and elevational variations with respect to tea quality. One study used a flavor index consisting of groups of positive (sweet, flowery) and negative (grassy) aroma compounds to assess Kenyan black tea.[149, 189] The authors reported that tea quality declined at lower elevations or with higher amounts of rainfall. Similarly, Kangra[190] and South Indian[191] black teas contained higher concentrations of aroma compounds in dry vs. rainy seasons. In these studies 40 or fewer compounds were used to classify tea quality. Expanding upon the number of potential sensory nutraceutical metabolites and tracking them over time is critical

to understanding how diverse climate factors affect tea quality, especially since minor compounds often have significant effects.[192]

Only two studies have taken a comprehensive approach to understand how season[21] and elevation[159] affect tea quality. However, these were limited to exploring the effects of a single climate variable and sampling within the same year. To fully understand how climate will affect plant quality, hundreds of VOCs must be identified and tracked across several years of sampling under various environmental conditions. To our knowledge, there has been no research that employs a longitudinal study to explore the effects of more than one climate variable on tea quality. In this work, we explore the effects of season (spring and summer) and elevation (high and low) on tea harvested from two different provinces in China across a three year period.

4.2 Experimental

4.2.1 Materials

Tea samples were collected from two counties, Anxi (var. *sinensis*) in Fujian Province and Menghai (var. *assamica*) in Yunnan Province, China over a three year period from 2014-2016. Table 4-1 lists the dates and elevations for the spring and summer collections in each county. The terminal bud plus two leaves from five different plants were collected from four plots each day for three consecutive days. Leaves were minimally processed in the field by microwave to stop enzymatic oxidation.[21-22, 83] The dried leaves were wrapped in plastic and shipped

to Tufts University, where they were stored in aluminum foil and then plastic at -20 °C until analyzed. Since no statistical difference was observed between plots,[22] these samples were homogenized to produce replicate samples (n=3).

Table 4-1. Harvest dates and elevations for spring and summer harvests in Yunnan and Fujian Provinces.

	Yunnan		**Fujian**	
	1162 m	**1651 m**	**112 m**	**650 m**
2014	March 16-18	March 18-20	May 1-3	May 11-13
	June 8-10	June 10-12	July 28-30	July 21-Aug. 2
2015	March 15-17	March 17-19	May 1-3	May 11-13
	June 15-17	June 18-20	July 27-29	July 30 – Aug.1
2016	March 20-22	March 22-24	May 1-3	May 5-7
	June 20-22	June 22-24	July 24-16	July 27-29

RI was calculated using a standard mix of C_7–C_{30} *n*-alkanes (Sigma-Aldrich, St. Louis, MO). RPA was calculated using naphthalene-d_8 (Restek, Bellefonte, PA) as the internal standard. A total of 250 reference standards were purchased from: Sigma-Aldrich, Fisher Scientific, Alfa Aesar (Ward Hill, MA), TCI (Tokyo, Japan), Acros Organics (Pittsburgh, PA), and MP Biomedicals (Santa Ana, CA).

4.2.2 Sample Preparation

Aqueous infusions were prepared by brewing 3 g of tea in 30 mL of deionized water at 90 °C, which was allowed to cool to room temperature. 10 mL aliquots were filtered (0.45 μm polytetrafluoroethylene syringe filters, Fisher Scientific, Pittsburgh, PA) into 10 mL Teflon-sealed vials and stirred with a 0.5 mm thick × 10 mm long PDMS stir bar (Gerstel, Mülheim an der Ruhr, Germany) at 1200

rpm for 1 h. Stir bars were removed from the vials, rinsed with deionized water, and dried with a lint-free wipe, and placed into glass desorption tubes for analysis.

4.2.3 GC/MS Analysis

GC/MS analyses were performed on an Agilent (Santa Clara, CA) model 6890/5975 equipped with a MultiPurpose Sampler (Gerstel). The TDU (Gerstel) provided splitless transfer of the sample from the stir bar into a CIS inlet (Gerstel). The TDU temperature program and flow rate were 40 °C (0.70 min) to 275 °C (3 min) at 600 °C/min and 50 ml/min helium, respectively. After 0.1 min the CIS, operating in solvent vent mode, was heated from -100°C to 275 °C (5 min) at 12 °C/s. The GC column (30 m × 250 µm × 0.25 µm RXI-5MS, Restek) was heated from 40 °C (1min) to 280 °C at 5 °C/min with 1.2 ml/min of constant helium flow. MS operating conditions were: 70 eV electron impact source, 230 °C ion source, 150 °C quadrupole, and 40 to 350 *m/z* scan range.

4.2.4 Data Analysis Software

The Ion Analytics software (Andover, MA) was used to deconvolve target compounds in the sample. Once found, each compound's mass spectrum was subtracted from the TIC signal. Each resulting peak scan was inspected to determine if the residual ion signals were constant (± 20%) or approximated background noise. If constant, the software recorded the retention time, mass spectrum, 3-5 target ions and their relative abundances. Then, the software compared sample data to reference compound data in a database, viz., RI and MS

(positive identification), or to commercial libraries and literature (tentative identification). Once assigned, the compound name, CAS#, and RI was added to the MS subtraction method. If neither positive nor tentative identification could be made, a numerical identifier along with the same GC/MS information was uploaded manually into the MS subtraction method. In contrast, if peaks scans differed (unresolved peak), the software searched for three invariant scans, averaged their spectra, and then subtracted the average spectrum from the TIC signal. This process was repeated until the residual signal at each scan approximated background noise. If peak signals failed to meet the user-defined criterion below, no additional information was obtained.

Four parameters were chosen as the compound acceptance criterion. First, the mass spectrum must be constant (≤ 20 % deviation) for at least five consecutive peak scans after spectral deconvolution. Second, SSV must be < 5. The SSV algorithm calculates the relative error by comparing the mass spectrum of each peak scan against one another. The smaller the difference, the closer SSV is to zero, and the better the spectral agreement. Third, the Q-value must be ≥ 93. The Q-value measures the total ion ratio deviation of the absolute value of the expected minus observed ion ratios divided by the expected ion ratio times 100 for each ion across the peak. The closer the value is to 100, the higher the certainty between sample and reference, library, and/or literature spectra. Finally, the Q-ratio must be ≤ 20 % deviation. The Q-ratio compares the ratio of the main

ion intensity to confirming ion intensities across the peak. These criteria form a single criterion used in the identification of sample components.

4.2.5 Statistical Analysis

PCA and partial least square-discriminant analysis (PLS-DA) were performed on autoscaled (mean-centered and unit-variance scaled) data using MetaboAnalyst 4.0.[193] Permutational multivariate analysis of variance (PERMANOVA) was conducted using the *adnois* function using 999 permutations in R.[155, 194] PCA was used to determine group differences with confirmation made by PERMANOVA. PLS-DA was used to identify important metabolites contributing to differences in volatile profiles of each group. The quality of the PLS-DA model is described by R^2 and Q^2 values. The p-value was generated by calculating the proportion of the models with random permutations of Q^2 greater than the Q^2 value of the model made with the actual data. Metabolites with a VIP > 1.0 and statistically different between groups (Kruskal Wallis test, $p < 0.05$) were considered the strongest contributors to group differences.

4.3 Results and Discussion

4.3.1 Targeted/Untargeted GC/MS Analysis

In previous studies, GC-GC/MS was used to produce a Yunnan-specific database of ~450 compounds.[21, 80, 159] In this work, GC/MS was used with spectral deconvolution (targeted) and MS subtraction (untargeted) to detect compounds in Yunnan and Fujian tea samples. A total of 506 and 518 metabolites were detected

in Yunnan and Fujian teas, respectively. Of these, 460 metabolites were common to both locations resulting in 46 Yunnan-specific and 58-Fujian-specific compounds detected. Figure 4-1 shows the PCA plot of all the data, revealing a strong separation between Yunnan and Fujian teas (ANOVA, $F = 574.8$, $p < 0.0001$). This result is expected due to differences in farmer practices, subspecies, soil, and climate (Table 4-2) between the two locations. Because these differences confound the ability to distinguish seasons, years, and/or elevations, the samples from each location were treated separately for all further analyses.

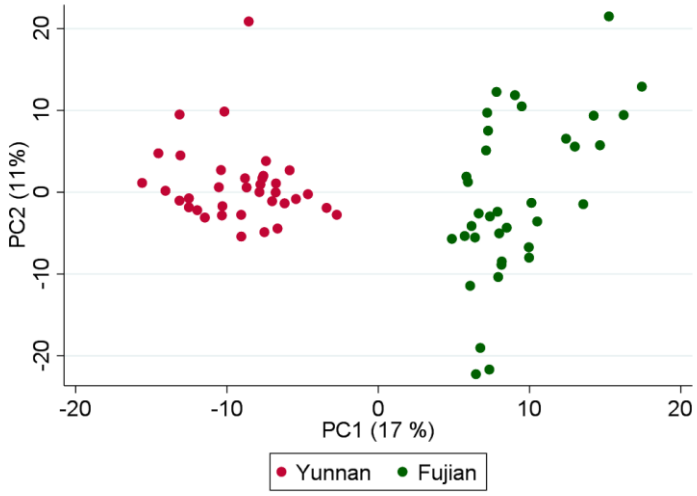

Figure 4-1. PCA score plot of Yunnan and Fujian tea.

4.3.2 Climate Effects on Yunnan Tea

Table 4-2 lists the 10-day cumulative rainfall and average temperature prior to each harvest.[195] This period was selected based on previous studies, where differences in metabolite chemistry were observed five days after the East Asian Monsoon onset.[21-22] Seasonal difference was driven by an increase in rainfall from spring to summer. While there was an increase in temperature, it was much less pronounced in the latter two years. It is important to note that the climate data at

each elevation is based on the latitude/longitude coordinates of each site, which does not take into account the elevation difference. Based on ~500 m difference in elevation the temperature of the high elevation site is ~2.9 °C cooler than that of the low elevation site.[87]

Figure 4-2 shows the score plots of PC1 vs PC2 (a) and PC1 vs PC3 (b). The three axes explain 43% of the sample variation. PCA revealed metabolite profiles separated by elevation (circles vs. triangles) on PC1, season (open vs. closed shapes) on PC2 and year, 2014 from 2015/2016, on PC3. This was confirmed by 3-way PERMANOVA showing a significant main effect of elevation ($F = 34.568$, $df = 1$, $p = 0.001$), season ($F = 11.233$, $df = 1$, $p = 0.001$), and year ($F = 13.045$, $df = 2$, $p = 0.001$).

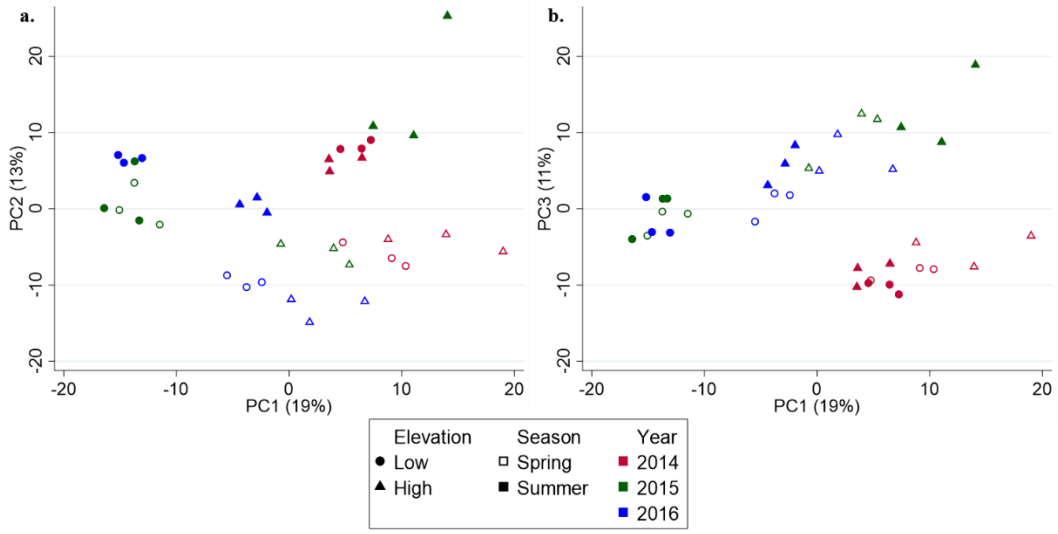

Figure 4-2. PCA score plots of Yunnan tea. a. PC1 vs. PC2. b. PC1 vs. PC3.

Table 4-2. 10-day cumulative rainfall and average temperature prior to each harvest.[195]

Yunnan		10-day period	Rain (mm)	Temp (°C)
2014	1651 m	March 8-17	0.0	22.9±0.8
		May 31-June 9	72.3	28.1±1.1
	1162 m	March 6-15	0.0	22.4±0.7
		May 29 - June 7	40.1	28.5±0.9
2015	1651 m	March 7-16	0.0	23.7±0.4
		June 6-15	69.2	26.7±1.0
	1162 m	March 5-14	0.0	23.6±0.4
		June 4-13	69.4	27.2±1.0
2016	1651 m	March 12-21	0.08	24.7±0.7
		June 12-21	48.3	25.8±0.9
	1162 m	March 10-19	0.06	24.4±0.7
		June 10-19	47.3	25.8±0.9

Fujian		10-day period	Rain (mm)	Temp (°C)
2014	650 m	May 1-10	113.2	18.7±2.4
		July 21-30	92.3	26.6±1.4
	112 m	April 21-30	36.0	20.8±2.0
		July 18-27	100.2	28.6±1.8
2015	650 m	May 1-10	89.1	21.4±2.1
		July 20-29	156.6	24.8±1.4
	112 m	April 21-30	43.3	21.9±2.4
		July 17-26	140.6	26.7±1.4
2016	650 m	April 25-May 4	64.4	21.5±2.0
		July 17-26	9.3	27.2±1.8
	112 m	April 21-30	68.6	22.2±2.1
		July 15-24	10.6	29.0±1.1

The PLS-DA model for elevation showed a strong separation between high and low elevation samples ($R^2 = 0.867$, $Q^2 = 0.659$, $p = 0.001$) and resulted in 138

metabolites with a VIP score > 1.0 and p-value < 0.5 (Table 4-3). Similarly, the PLS-DA model for season showed a significant separation between spring and summer samples (R^2 = 0.884, Q^2 = 0.736, p = 0.001) and resulted in 129 statistically significant metabolites (Table 4-4). The results agree with farmers' perceptions that high elevation and spring teas are higher in aromatic quality since they exhibit sweet, floral, honey-like characteristics compared to green, earthy notes in low elevation and summer teas.[22, 96-97] For example, spring and/or high elevation teas contain significantly higher concentrations of sweet, floral, honey-like compounds such as 2-hydroxy-5-methylacetophenone, isoeugenol, 4-methylbenzaldehyde, norfuraneol, maltol, and 1-nitro-2-phenylethane.[95] In contrast, low elevation and/or summer teas have significantly greater concentrations of 2,6-dimethyl-3,7-octadiene-2,6-diol, octanal, 2-phenoxyethanol, verbenone, and 1-octen-3-ol which are described as herbal, green, fatty, metallic, and earthy.[95]

Unlike our previous work where only compounds significant to high elevation tea had reported health benefits,[159] compounds significant to both elevations have reported health-beneficial properties (Table 4-3). Nutraceutical compounds that differentiate high elevation tea include (*E*)-caryophyllene (anticancer, antidepressant, anti-inflammatory), isoeugenol (antibacterial, antioxidant), *epi*-α-cadinol (antibacterial, anti-inflammatory, anticancer), (*E*)-nerolidol (antianxiety, antimalarial, anticancer), and α-pinene (antiviral, analgesic).[91, 106, 108-110, 125, 166, 196-199] On the other hand, compounds significant to low elevation tea exhibit

antibacterial (verbenone, decanal, undecanal, dodecanal), antifungal (nonanal), and antiseptic (2-phenoxyethanol) properties.[88, 93, 98, 107] In the same regard, compounds significant to both seasons have reported health benefits. Spring compounds include eucalyptol (antibacterial, cardioprotective), menthol (analgesic, decongestant), dimethyl trisulfide (antioxidant, hepatoprotective), and indole (antibacterial, antifungal) and summer compounds include camphor (antibacterial, anti-inflammatory), (*E*)-β-ionone (anticancer, antibacterial), borneol (anti-inflammatory, analgesic), and heptanol (cardioprotective).[88, 102, 108, 114, 143, 145, 199-203] Further studies are needed to determine if these compounds are present in adequate concentrations to provide these purported health benefits.

The PLS-DA model for year showed a strong separation between the yearly samples ($R^2 = 0.854$, $Q^2 = 0.782$, $p = 0.001$) and resulted in 155 statistically significant metabolites (Table 4-5). As evidenced by the climate data and separation on PC3, a vast majority of these metabolites distinguish 2014 from 2015 and 2016. However, it is not clear based on aroma characteristics or health beneficial properties of the compounds that differentiate these years whether one year might be higher quality compared to the others.

In addition, significant interactive effects were seen between year and season ($F = 4.013$, $df = 2$, $p = 0.010$) and year and elevation ($F = 13.293$, $df = 2$, $p = 0.001$), but not season and elevation ($F = 0.775$, $df = 1$, $p = 0.425$). These interactive effects indicate that there is a different effect of season and elevation in at least

one of the three years. As seen in the PCA score plot (Figure 4-2), 2014 samples do not separate by elevation, unlike 2015 and 2016 samples. Compounds such as hexanoic acid, 2-nonanone, decanal, (*E*)-herboxide, 4-methylbenzaldehyde, and 2-methoxy-4-vinylphenol exhibit no change in concentration between elevations in 2014, but are significantly higher in concentration at one elevation or the other in 2015/2016 tea. Also, 2014 and 2016 samples separate similarly in terms of a seasonal separation (PC2) whereas 2015 samples do not follow the same pattern. Several compounds exhibit an opposite change in concentration from spring to summer in 2015 compared to 2014/2016. For example, (*E*)-β-ocimene, methyl salicylate and theaspirane B are greater in concentration in spring in 2014/2016, but were higher in concentration in the summer of 2015. The opposite trend is true for 2,3,3-trimethylpentane, pentyl propanate and ethyl 2-methylbutyrate among others.

4.3.3 Climate Effects on Fujian Tea

Table 4-4 also lists the climate data for Fujian Province. In contrast to Yunnan, the seasonal difference was driven by a temperature increase whereas rainfall patterns were erratic from year to year. Figure 4-3 shows the score plots of PC1 vs PC2 (a) and PC1 vs PC3 (b). The three axes explain 43% of the sample variation. PCA revealed metabolite profiles separated by year on PC2, but no clear seasonal or elevational separation can be seen. The 3-way PERMANOVA confirmed the yearly separation ($F = 7.614$, $df = 2$, $p = 0.001$) and lack of elevational separation ($F = 1.964$, $df = 1$, $p = 0.134$), but revealed a significant seasonal separation ($F =$

3.786, df = 1, p = 0.024) that was not seen in the PCA, likely because yearly differences are much stronger and confound any visible variation seen by season.

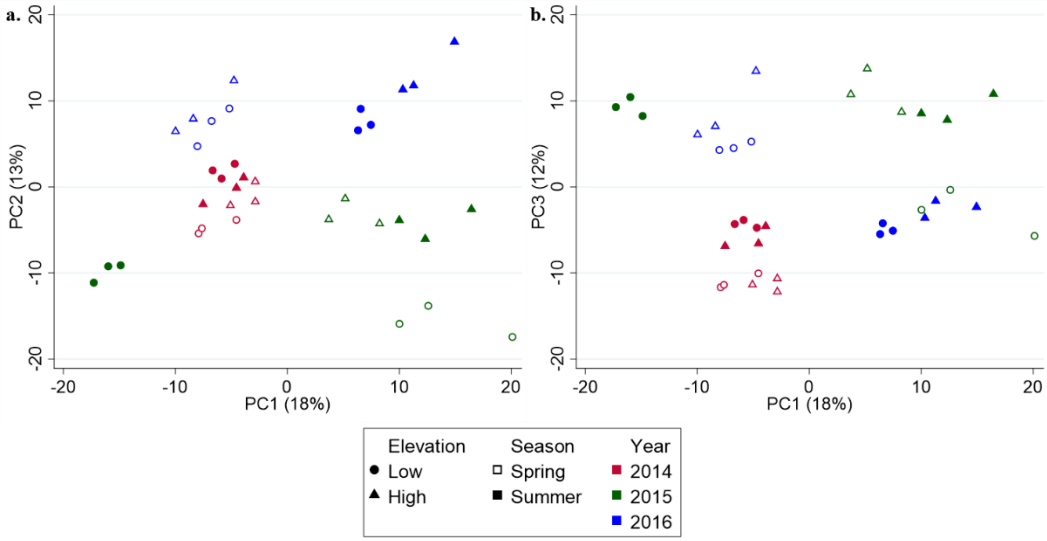

Figure 4-3. PCA score plots of Fujian tea. a. PC1 vs. PC2. b. PC1 vs. PC3.

The PLS-DA model for season showed a strong separation between spring and summer samples ($R^2 = 0.913$, $Q^2 = 0.711$, p = 0.001) and resulted in 101 statistically significant metabolites (Table 4-6). Similar to Yunnan teas, isomenthone, (*E*)-β-ocimene, N-ethylusccinimide, 1-ethyl-1H-pyrrole, and 1-ethyl-1H-pyrrole-2-carboxaldehyde were higher in concentration in spring tea and 2-phenoxyethanol was greater in concentration in summer tea. Also consistent with Yunnan is that the spring tea contains higher concentrations of sweet, floral, honey-like compounds such as 4-ketoisophorone, styrene, (*Z*)-jasmone, allo-ocimene, methyl o-anisate, and 7-methoxycoumarin and that summer tea contains higher concentration of green, earthy, woody compounds including nerol oxide, cis-calamenene, α-copaene, quinolone, α-calacorene, and caryophyllene oxide.

Additionally, both spring and summer teas contain higher concentrations of nutraceutical compounds. Those that differentiate spring from summer include isoborneol (antibacterial, antiviral), coumarin (anti-inflammatory, anticancer), menthone (antibacterial, anti-inflammatory) and perilla aldehyde (antioxidant, antidepressant)[88, 101, 131, 196, 204] and those that differentiate summer from spring include cadalene (antibacterial, antioxidant), caryophyllene oxide (analgesic, anti-inflammatory), methyl anthranilate (antifungal) and quinoline (antimalarial, anticonvulsant, anticancer).[104, 202, 205-206] The changes in concentrations of these compounds are being driven by the increase in temperature from spring to summer.

The PLS-DA model for year showed a strong separation between the yearly samples ($R^2 = 0.901$, $Q^2 = 0.817$, $p = 0.001$) and resulted in 133 metabolites with a VIP score > 1.0 and p-value < 0.5 (Table 4-7). This is likely due to the inconsistent rainfall patterns seen from year to year, which is confirmed by a significant interactive effect between year and season ($F = 4.345$, $df = 2$, $p = 0.004$) but not year and elevation ($F = 1.686$, $df = 2$, $p = 0.144$) or season and elevation ($F = 1.470$, $df = 1$, $p = 0.205$). In 2016, the plants experienced an extremely dry summer, which is opposite of the previous two years. As a result, many metabolites increase/decrease in the opposite manner of 2014 and 2015. For example, safranal (sweet, herbal), norfuraneol (sweet, caramel), cyclohexanone (minty), o-xylene (geranium), isoeugenol (floral, clove), and geranyl acetone (floral, fruity) are higher in concentration in spring tea in 2014 and 2015, but

higher in summer tea in 2016. In the same regard, 2-ethylhexanol (green, oily), camphor (camphor, medicinal), methyl hexanoate (fruity, fatty), biphenyl (floral, green), and 5-ethyl-2(5H)-furnaone (no aroma) are higher in concentration in 2014/2015 summer, but greater in 2016 spring tea. It can be concluded that the concentration of these and other metabolites that behave similarly are driven by changes in rainfall.

4.4 Conclusion

In this work we demonstrated that our targeted/untargeted approach provides efficient and comprehensive analysis of complex samples. We showed that seasonal, elevational and yearly differences cause significant and interactive alterations in tea chemistry. In Yunnan, the cooler temperatures and lower rainfall that occurs in spring and at high elevation, results in higher concentrations of compounds with aromas characteristic of farmers' perceptions of high quality tea. Similarly, the lower temperatures experienced during the Fujian spring season resulted in higher concentrations of metabolites exhibiting aromas in agreement with high quality teas. Compounds found in both seasons and elevations have reported health-beneficial properties and further work is needed to quantify these compounds to determine if they are in high enough concentrations to provide the reported nutraceutical affect. Given the interactive effects between year and season/elevation future studies of seasonal and elevational effects on tea quality should be cautious about drawing conclusions based on only one year of sampling. More studies focused on metabolite responses to yearly variation in

both season and elevation are needed to better understand the complex responses of plants to combined environmental conditions.

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Table 4-3. Statistically important metabolites in high and low elevation Yunnan tea

Compound	VIP	p-value	Aroma[a]	Health Property
High				
165	2.83	0.0001		
222	2.67	0.0001		
52	2.37	0.0003		
7-Methoxycoumarin	2.24	0.0001	sweet, balsamic	antinociceptive[135] anticancer[136] anti-inflammatory[137]
38	2.12	0.0006		
Pyrethrone	2.12	0.0001		
p-Xylene	2.10	0.0001	sweet, grain[162]	
(3*Z*)-Hexenyl isovalerate*	2.08	0.0002	green, fruity	
154	2.01	0.0001		
2,3-Dimethylhexane*	2.01	0.0001		
2,4-Di-tert-butylphenol	1.97	0.0002		antioxidant[207]
Lavender lactone	1.93	0.0003	fruity, minty	
168	1.90	0.002		
Butyl p-toluate	1.89	0.0002		
Methyl o-anisate	1.88	0.0001	floral, fruity	
4-Methylbenzaldehyde*	1.85	0.0006	fruity, cherry	antiviral[103]
Geranic acid	1.82	0.0008	green, woody	
173	1.81	0.0004		
55*	1.79	0.001		
97	1.79	0.002		
(*Z*)-Jasmone	1.78	0.0002	floral, jasmine	antibacterial[143] anticancer[144]
183*	1.77	0.002		
4*	1.77	0.002		
Toluene	1.76	0.002	sweet, paint	
128*	1.76	0.002		
(*E*)-Caryophyllene	1.75	0.003	sweet, clove, woody	anticancer[109] antibacterial[143] antianxiety antidepressant[166] anti-inflammatory[168]
35*	1.74	0.001		
2,2,4-Trimethylhexane*	1.74	0.0006		
4,6-Dimethyl-2-heptanone	1.73	0.001	fruity	
47	1.69	0.0009		
118*	1.68	0.001		
6-Methyl-3,5-heptadiene-2-one	1.68	0.0001	sweet, coconut	
2,4-Dimethyl-1-heptene	1.68	0.004		
186	1.67	0.0001		
Styrene	1.65	0.003	sweet, floral, balsamic	

56*	1.64	0.005		
Bergamal	1.64	0.001	floral, fruity, earthy	
α-Muurolene*	1.62	0.006		
4-Methyl-2-heptanone*	1.61	0.01		
204*	1.60	0.002		
epi-α-Murrolol*	1.58	0.001	herbal, spicy	antibacterial[106] antioxidant[104]
Octane	1.57	0.009	gasoline	
61*	1.57	0.004		
Isoeugenol*	1.57	0.004	floral, clove, woody	anitbacterial[197] antioxidant[198]
193	1.56	0.0001		
(2*E*)-Undecenal	1.56	0.005	fruity, green	antileishmanial[208]
9-Hexadecenoic acid	1.55	0.006		anti-inflammatory[209]
β-Calacorene*	1.55	0.003		
α-Amorphene*	1.54	0.006		
Ethylbenzene	1.54	0.003		
Methyl anthranilate	1.54	0.003	fruity, grape	antifungal[202]
115	1.52	0.005		
189	1.50	0.001		
155	1.50	0.008		
124	1.47	0.002		
Tridecanoic acid*	1.46	0.01	waxy, woody	
2,4,4-Trimethyl-1-pentene	1.46	0.008		
Decane*	1.45	0.004		
Maltol	1.45	0.006	sweet, marshmallow	antianxiety[210] antioxidant[211]
epi-α-Cadinol*	1.44	0.003	herbal	antibacterial[106] anticancer[110] anti-inflammatory[108]
95*	1.44	0.02		
α-Cadinol*	1.42	0.0009	herbal, woody	antibacterial, antioxidant[106] anti-inflammatory [108]
194	1.42	0.01		
2-Hydroxy-5-methylacetophenone*	1.40	0.008	sweet, floral, herbal	
2,6-Dimethyl-2-heptanol	1.40	0.02	floral, woody, herbal	
β-Homocyclocitral*	1.39	0.003	camphor, cooling	
127*	1.38	0.007		
α-Cyclocitral	1.38	0.02		
Crotonic acid	1.38	0.0008	milky	
α-Muurolol	1.37	0.001		antibacterial, antioxidant[106]
Indole*	1.36	0.03	fecal, mothball, floral	antibacterial[143] antifungal[202]
65	1.36	0.03		
Norfuraneol*	1.35	0.02	sweet, caramel	
o-Xylene	1.35	0.009	geranium	
β-Cyclocitral*	1.34	0.01	sweet, herbal, minty	

1-Ethyl-2-methylbenzene	1.34	0.02		
(*E*)-Nerolidol	1.33	0.02	floral, woody	antianxiety, anti-malarial[91] anticancer[110] antibacterial[106] anti-inflammatory[108]
Isoborneol*	1.33	0.009	camphor woody	antiviral, antibacterial[101]
4,4-Dimethyl-2-pentanone	1.32	0.004		
Dodecanamide*	1.32	0.01		
Theaspirane B*	1.32	0.02	tea, herbal, honey	
138*	1.32	0.02		
Dimethyl trisulfide*	1.30	0.02	sulfury, cabbage	antioxidant, hepatoprotective[201]
Theaspirane A*	1.29	0.01	tea, herbal, honey	
Methyl pyruvate*	1.29	0.04		
219	1.29	0.02		
Cadalene*	1.27	0.006		antibacterial, antioxidant[104]
2,3-Dihydrobenzofuran*	1.25	0.01	green, herbal[158]	
1,4-Diacetylbenzene	1.24	0.03		
2-Methoxy-4-vinylphenol*	1.23	0.007	smoky, clove	anti-inflammatory[212]
Pentadecane*	1.22	0.02		
167*	1.22	0.01		
57	1.21	0.005		
cis-Calamenene*	1.19	0.009	herbal, spicy	antimalarial[213] antitumor[214]
Jasmine lactone*	1.18	0.04	jasmine, fruity	
Decanamide	1.18	0.04		
Methyl 4-methyl benzoate	1.17	0.03	sweet, anise, floral	
158*	1.16	0.006		
4-2,6,6-Trimethyl-cyclohexa-1,5-dienyl-but-3-en-2-one*	1.16	0.002		
Methyl benzoate*	1.14	0.005	cherry, phenolic	
4-Methyl-3-penten-2-one*	1.14	0.002	sweet, earthy	
215	1.14	0.02		
Tetradecanol	1.14	0.03	coconut, fruity, waxy	anti-inflammatory, gastroprotective[128]
α-Pinene	1.12	0.01	sweet, pine, camphor	antibacterial[88] analgesic[199] hypotensive[114] antiviral[125]
(4*Z*)-Heptenal*	1.12	0.03	oily, fatty, green	
176	1.11	0.04		
160*	1.11	0.01		
(*Z*)-Methyl jasmonate	1.10	0.009	floral, jasmine	anti-inflammatory, antioxidant, neuroprotective, antistress[215] anticancer[144]
218*	1.08	0.04		
202*	1.06	0.03		
Muurola-4,1014-dien-1-β-ol*	1.04	0.01		
2-Octanone	1.02	0.04	herbal, earthy	
26*	1.01	0.002		

Low				
Verbenone	2.55	0.0001	camphor, menthol	antibacterial[88]
4-Methyldecane	2.51	0.0001		
45	2.15	0.0001		
2-Methyl-1H-pyrrole	2.09	0.0003		
4-Ethylbenzaldehyde	1.96	0.0005	bitter, almond	
2-Phenyl-2-propanol	1.91	0.0002	green, sweet, earthy	
Octanal*	1.73	0.001	green, fatty, citrus	
Nonanal	1.72	0.001	cucumber, waxy	antifungal[98]
90*	1.70	0.01		
Decanal	1.66	0.009	orange, green, waxy	antibacterial[93]
1-Methylpyrrolidinone*	1.65	0.01		
2,6-Dimethyl-3,7-octadiene-2,6-diol*	1.60	0.01	fruity, herbal	
80*	1.59	0.01		
(2*Z*)-Octen-1-ol*	1.53	0.01		
Dodecanal	1.50	0.009	citrus, green, waxy	antibacterial[93]
2-Ethylhexanoic acid*	1.50	0.02		
2-Phenoxyethanol*	1.49	0.03	mild rose, metallic	antiseptic[107]
Isoamyl alcohol	1.44	0.01	alocholic, banana	
Undecanal	1.42	0.004	citrus, waxy, soapy	antibacterial[93]
2,4-Dimethylbenzaldehyde*	1.35	0.02		
Pentyl propanate	1.32	0.03	fruity, apricot	
94	1.32	0.04		
87*	1.17	0.02		
2-Phenylphenol	1.17	0.007		
53	1.06	0.04		

[a]Aroma information obtained from the Good Scents Company[95] unless otherwise noted. *Compound is affected by more than one environmental factor.

Table 4-4. Statistically important metabolites in spring and summer Yunnan teas

Compound	VIP	p-value	Aroma	Health Property
Spring				
139	2.64	0.0001		
137	2.43	0.0001		
133	2.23	0.0001		
2-Methylpentanal	2.21	0.0001	fruity, green	
1-Ethyl-1H-pyrrole-2-carboxaldehyde	2.12	0.0001	roasted, smoky	
Menthone	2.02	0.0006	green, minty	antibacterial[88] anti-inflammatory[196]
1-Ethyl-1H-pyrrole	2.00	0.0001	roasted	
Fluoranthene	1.97	0.0003		
160*	1.94	0.0003		
Butyl butanoate	1.84	0.0002	sweet, fruit	
2-Methoxy-4-vinylphenol*	1.76	0.0005	smoky, clove	anti-inflammatory[212]
Sabina ketone	1.76	0.0004		
142	1.70	0.004		
4-2,6,6-Trimethyl-cyclohexa-1,5-dienyl-but-3-en-2-one*	1.69	0.0004		
180	1.68	0.002		
39	1.61	0.001		
2-Hydroxy-5-methylacetophenone*	1.59	0.003	sweet, floral, herbal	
Isoeugenol*	1.58	0.004	floral, clove, woody	anitbacterial[197] antioxidant[198]
76	1.57	0.0001		
Benzyl nitrile*	1.50	0.0007	floral[167]	
(*Z*)-Herboxide*	1.48	0.02	herbal, woody	
Safranal*	1.47	0.005	sweet, herbal	antinociceptive[216] antimicrobial[217]
41	1.47	0.005		
Dimethyl trisulfide*	1.46	0.006	sulfury, cabbage	antioxidant, hepatoprotective[201]
204*	1.45	0.01		
42	1.43	0.0001		
Benzyl Benzoate	1.42	0.006	herbal, balsamic	antibacterial[218]
Eucalyptol	1.42	0.005	eucalyptus, sweet	antibacterial[88] analgesic[199] antiviral[125] cardioprotective[114]
Phenylethyl acetate	1.42	0.0001	rose, honey	
95*	1.40	0.008		
Benzylideneacetone	1.39	0.01	floral, fruity	
4-Methyl-2-heptanone*	1.38	0.01		
55*	1.37	0.01		
(3*Z*)-Hexenyl acetate*	1.37	0.03	green, sweet, fruity	
1-Nitro-2-phenylethane	1.36	0.001	floral, spice	cardioprotective[219]

Cyclohexanone	1.31	0.03	minty	
118*	1.31	0.02		
56*	1.31	0.007		
2,3-Dimethylhexane*	1.30	0.04		
Decane*	1.29	0.003		
61*	1.28	0.03		
Indole*	1.27	0.03	fecal, mothball, floral	antibacterial[143] antifungal[202]
4-Methylbenzaldehyde*	1.24	0.03	fruity, cherry	antiviral[103]
67	1.24	0.04		
Menthol	1.23	0.04	peppermint, cooling	antibacterial[88] decongestant[200] cardioprotective[114] analgesic[199]
Isomenthone	1.22	0.004	sweet, peppermint	
110	1.21	0.002		
Benzoic acid	1.19	0.01	faint balsamic	antibacterial[121]
Norfuraneol*	1.15	0.007	sweet, caramel	
(*E*)-Herboxide*	1.13	0.04	herbal, woody	
N-Ethylsuccinimide	1.13	0.04		
Ethyl benzoate	1.12	0.03	fruity, herbal	
α-Muurolene*	1.11	0.02		
(*E*)-β-Ocimene*	1.09	0.03	sweet, herbal	antibacterial[88]
5-Methylfurfural	1.07	0.01	sweet, caramel	
4*	1.07	0.03		
Summer				
210	2.63	0.0001		
224	2.59	0.0001		
77	2.42	0.0001		
γ-Octanolactone	2.27	0.0001	sweet, coconut	
172	2.15	0.0001		
3,5,5-Trimethylcyclohex-3-en-1-ol	2.15	0.0001		
2-Methyldecane	2.12	0.0001		
2-Ethylhexanoic acid*	1.98	0.0001		
1-Octen-3-ol	1.92	0.0001	mushroom	
Butyl propanoate	1.92	0.0001	earthy, fruity	
(2*E*,4*E*)-Heptadienal	1.91	0.0004	fatty, oily, fishy	
166	1.88	0.0004		
171	1.83	0.0005		
(2*E*,4*Z*)-Heptadienal	1.83	0.0001	fatty, oily, fishy	
(2*E*)-Heptenal	1.82	0.0002	green, fatty	antimicrobial [98]
(2*E*,4*E*)-Decadienal	1.80	0.0001	fatty, meaty	
19	1.79	0.0006		
58	1.79	0.001		
33	1.77	0.0005		
2,3-Octanedione	1.74	0.002	buttery, broccoli	

2,3-Dihydrobenzofuran*	1.72	0.001	green, herbal[158]	
(2*E*)-Octenal	1.69	0.0002	green, fatty	antimicrobial[98]
218*	1.67	0.0004		
Isoelemicin	1.66	0.0002	spice	-
Butyl acrylate	1.66	0.0001	fruity, spicy	-
1-Octen-3-one	1.66	0.0006	mushroom	-
Heptanal	1.63	0.003	fruity, grassy	antistress[100]
2-Methylbenzaldehyde	1.62	0.006	cherry	antiviral[103]
147	1.60	0.005		
Camphor	1.60	0.008	camphor, medicinal	antibacterial[88] anti-inflammatory[108]
2,2,6-Trimethylcyclohexanone	1.57	0.003	floral, honey	
(2*Z*)-Octen-1-ol*	1.56	0.002		
181	1.55	0.005		
Octadecane*	1.54	0.003		
177	1.54	0.003		
2,6-Dimethyl-3,7-octadiene-2,6-diol*	1.52	0.001	fruity, herbal	
3-Methylacetophenone	1.50	0.01		
γ-Butyrolactone	1.49	0.007	sweet, fatty, oily	
(3*E*,5*E*)-Octadien-2-one	1.47	0.0007	grassy, fruity	
60	1.46	0.001		
Hexanoic acid	1.46	0.003	sweaty, cheesy	
87*	1.44	0.01		
Dihydroactinidiolide	1.44	0.008	fruity, woody	
Hexadecane	1.44	0.003		
Heptanol	1.43	0.001	herbal, musty	cardioprotective[203]
Pentanoic acid	1.40	0.01	sweaty, rancid	
(4*Z*)-Heptenal*	1.40	0.01	oily, fatty, green	
167*	1.39	0.003		
141*	1.38	0.005		
36*	1.37	0.004		
221	1.35	0.007		
(3*Z*)-Hexenyl isovalerate*	1.35	0.02	green, fruity	
(2*E*)-Hexenal	1.34	0.004	green, fruity, fatty	antimicrobial[98]
2-Phenoxyethanol*	1.32	0.002	mild rose, metallic	antiseptic[107]
140*	1.30	0.01		
Nonadecane*	1.30	0.02		
90*	1.29	0.005		
116	1.29	0.01		
127*	1.28	0.02		
α-Ionone	1.26	0.01	woody, violet, berry	
(2*E*)-Octen-1-ol*	1.24	0.04		

4-Vinylanisole	1.22	0.0001	green, herbal, nutty	
156	1.22	0.005		
2-Ethylhexanol	1.22	0.03	green, oily, citrus	
Octanal*	1.18	0.02	green, fatty, citrus	
2-Pentylfuran	1.13	0.03	fruity, green, earthy	
148*	1.12	0.02		
138*	1.11	0.04		
1-Methylpyrrolidinone*	1.10	0.0002		
Pentadecane*	1.09	0.01		
Tetradecane	1.07	0.04		
(*E*)-β-Ionone	1.06	0.03	woody, floral, berry	anticancer[145] antibacterial[143] antibacterial[88] antioxidant[104]
Borneol	1.01	0.0001	camphor, woody	anti-inflammatory, analgesic, anesthetic[102]

[a]Aroma information obtained from the Good Scents Company[95] unless otherwise noted. *Compound is affected by more than one environmental factor.

Table 4-5. Statistically important metabolites in 2014-2016 Yunnan teas

Compound	VIP	p-value	Aroma	Health Property
2014				
22	2.44	0.0001		
Fokienol	2.40	0.0001		
Pyridine	2.33	0.0001	fishy, sour	
Indane	2.30	0.0001		
159	2.28	0.0001		
Benzyl alcohol	2.22	0.0001	floral, cherry	antioxidant[211]
Indene	2.16	0.0001		
2,4-Dimethylheptane	2.03	0.0001		
α-Calacorene	2.02	0.0001	woody	antibacterial, antioxidant[104]
β-Calacorene*	1.98	0.0002		
Muurola-4,1014-dien-1-β-ol*	1.93	0.0001		
Viridene	1.90	0.001		
(3*Z*)-Hexenyl acetate*	1.90	0.0001	green, sweet, fruity	
α-Phellandrene	1.83	0.0001	citrus, terpene, green	analgesic, anti-inflammatory[89] antibacterial[88] analgesic[199]
Methyl benzoate*	1.83	0.0002	cherry, phenolic	
Benzyl acetate	1.80	0.0001	sweet, floral, fruity	antifungal[202]
158*	1.78	0.0001		
44	1.76	0.0001		
1,2,4-Trimethylbenzene	1.75	0.0005	plastic	
Theaspirane A*	1.74	0.0001	tea, herbal, honey	
Carvone	1.73	0.0001	spearmint, anise	anticonvulsant, analgesic[102] antimicrobial, anticancer[220]
185	1.73	0.003		
4-Methyloctane	1.72	0.0009		
169	1.70	0.0001		
Theaspirane B*	1.69	0.0002	tea, herbal, honey	
Heptadecane	1.66	0.0006		
cis-Calamenene*	1.65	0.003	herbal, spicy	antimalarial[213] antitumor[214]
Homomenthyl salicylate	1.65	0.0007	mild menthol	
Terpinolene	1.64	0.0004	woody, terpene	antibacterial[88]
α-Terpinene	1.63	0.001	citrus, woody	antibacterial[88] antiviral[125]
Octadecane*	1.63	0.002		
(*Z*)-Herboxide*	1.59	0.0007	herbal, woody	
Fluorene	1.59	0.0002		
89	1.59	0.0001		
(*E*)-Herboxide*	1.59	0.001	herbal, woody	
epi-α-Cadinol*	1.59	0.003	herbal	antibacterial[106] anticancer[110] anti-inflammatory[108]
(3*Z*)-Hexenyl butanoate	1.59	0.0001	fruity, green	
p-tert-Butylphenol	1.57	0.0001	earthy, leathery	

153	1.56	0.009		
m-tert-Butylphenol	1.54	0.0001		
Acetophenone	1.54	0.0003	floral, almond	
190	1.53	0.03		
21	1.52	0.0001		
18	1.52	0.02		
4-tert-Butylphenylacetone	1.52	0.0001		
30	1.51	0.0008		
cis-Methyl dihydrojasmonate	1.49	0.0001	floral, jasmine	
Cadalene*	1.48	0.009		antibacterial, antioxidant[104]
88	1.48	0.0001		
α-Amorphene*	1.46	0.001		
Limonene	1.45	0.001	lemon, orange	antibacterial[88]cardioprotective[114] anti-inflammatory, analgesic[199]
β-Homocyclocitral*	1.44	0.0008	camphor, cooling	
tert-Pentyl acetate	1.43	0.01		
6-Methyl-5-hepten-2-one	1.41	0.0002	fruity, green, musty	
29	1.41	0.001		
128*	1.40	0.01		
202*	1.38	0.002		
Hotrienol	1.38	0.009	floral, woody, spice	
γ-Terpinene	1.36	0.01	citrus, terpene	antibacterial[88] antiviral[125]
2,2,4-Trimethylhexane*	1.35	0.03		
Isoborneol*	1.35	0.002	camphor, herbal	antiviral, antibacterial[101]
(*E*)-β-Ocimene*	1.34	0.02	sweet, herbal	antibacterial[88]
1,2,3-Trimethylbenzene	1.33	0.0002		
178	1.33	0.0008		
epi-α-Murrolol*	1.32	0.01	herbal, spicy	antibacterial[106] antioxidant[104]
2-Ethylhexyl salicylate	1.31	0.007	floral, sweet	
p-Cymene	1.29	0.007	citrus, terpene, woody	antibacterial[88] hypotensive[114] antiviral[125] analgesic[199]
2-Cyclopenten-1-one	1.28	0.03		anti-inflammatory[221]
Safranal*	1.28	0.04	sweet, herbal	antinociceptive[216] antimicrobial[217]
Geranial	1.25	0.0003	citrus, mint	antibacterial[88] antifungal[118]
103	1.22	0.0005		
2,2,5,5-Tetramethyltetrahydrofuran	1.21	0.02		
111	1.21	0.004		
2-Methylnaphthalene	1.17	0.0001	herbal	
201	1.17	0.0004		
β-Cyclocitral*	1.16	0.005	sweet, herbal	
Geranylacetone	1.15	0.0001	floral, green, earthy	
Bornylene	1.14	0.02		
3-Phenyl-2-butanone	1.14	0.007		

3,4-Diethyl-1,1'-biphenyl	1.13	0.0001		
Nonadecane*	1.12	0.01		
Cumene	1.10	0.005		
2,6-Dimethylcyclohexanol	1.10	0.0003		anesthetic[222]
α-Cadinol	1.09	0.04	herbal, woody	antibacterial, antioxidant[106] anti-inflammatory[108]
(4*Z*)-Heptenal*	1.04	0.04	oily, fatty, green	
Linalool 3,7-oxide	1.02	0.004	floral, woody	
Methyl pyruvate*	1.02	0.002		
1-Methylnaphthalene	1.00	0.0001	camphor, medicinal	
2016				
4-Ethyl-2-methoxyphenol	2.30	0.0001	smoky, phenolic	
82	2.04	0.0001		
Pyranone	2.02	0.0001		
Tetradecanoic acid	1.89	0.0002	coconut, waxy	antimicrobial[223]
2,4-Dimethylbenzaldehyde*	1.84	0.0008	almond, cherry	antiviral[103]
85	1.77	0.0001		
2-Hydroxy-γ-butyrolactone	1.73	0.002		
4-Methyl-3-penten-2-one*	1.59	0.004	sweet, earthy	
80*	1.57	0.01		
2-Hydroxy-2-cyclopenten-1-one	1.56	0.0002	maple, caramel	
26*	1.55	0.0008		
Furfural	1.55	0.01	sweet, bready	
83	1.54	0.005		
70	1.54	0.0003		
2(5H)-Furanone	1.51	0.001	buttery	
1,2-Cyclopentanedione	1.49	0.0003		
p-Acetyltoluene	1.46	0.003	sweet, creamy	
140*	1.43	0.001		
Dodecane	1.42	0.01		
218*	1.39	0.03		
Dodecanoic acid	1.09	0.03	coconut, fatty	cardioprotective[224]antibacterial, anti-inflammatory[225]
(2*E*)-Octen-1-ol*	1.02	0.02	green, fatty	
174	1.87	0.0001		
175	1.80	0.0001		
Phorone	1.68	0.0002		
γ-Nonalactone	1.39	0.0002	sweet, coconut	
Benzeneacetaldehyde	1.06	0.0003	floral, honey	
2015/2016				
Catechol	1.82	0.0006		antioxidant, anti-inflammatory[226]
Pentadecanoic acid	1.66	0.001	waxy	
γ-Heptalactone	1.60	0.01	sweet, nutty	

20	1.58	0.001		
93	1.49	0.01		
141*	1.48	0.003		
Tridecane	1.39	0.01		
94	1.37	0.0001		
Octadecanoic acid	1.36	0.01		antimicrobial[223]
Tetradecanamide	1.35	0.0001		
Tridecanoic acid*	1.35	0.009	waxy, woody	
187	1.30	0.0001		
23	1.24	0.006		
Myrtenol	1.23	0.007	pine, sweet, minty	antibacterial[88] hypotensive[114] analgesic, anti-inflammatory[89]
113	1.22	0.005		
75	1.17	0.01		
220	1.14	0.0001		
Dodecanamide*	1.12	0.0006		
183*	1.06	0.01		
Hexadecanoic acid	1.04	0.04	slight waxy	antimicrobial[223]
2014/2015				
Isopropyl myristate	1.84	0.0001		
48	1.82	0.0001		
2-Methylbutanoic acid	1.73	0.0006	cheesy, fruity	
35*	1.59	0.004		
(*Z*)-Methyl *epi*-jasmonate	1.56	0.003	sweet, floral	
(*Z*)-β-Ocimene	1.55	0.0001	herbal	antibacterial[88]
Benzyl nitrile*	1.43	0.009	floral[167]	
148*	1.37	0.001		
δ-Decalactone	1.25	0.01	coconut, peach	
Butanoic acid	1.23	0.001	cheesy, sweaty	
Methyl salicylate	1.23	0.01	wintergreen	anti-inflammatory, analgesic[170]
Jasmine lactone*	1.20	0.003	jasmine, fruity	
4-2,4,4-Trimethylcyclohexa-1,5-dienylbut-3-en-2-one	1.20	0.003		
36*	1.19	0.04		
Isovaleric acid	1.11	0.001	cheesy, fruity	
Salicylaldehyde	1.04	0.01	wintergreen	
p-Cymenene	1.04	0.03	spicy, medicinal	
2015				
134	1.14	0.0002		
170	1.02	0.0002		
2014/2016				
Benzothiazole	1.07	0.009	sulfury, rubbery	

[a]Aroma information obtained from the Good Scents Company[95] unless otherwise noted. *Compound is affected by more than one environmental factor.

Table 4-6. Statistically important metabolites in spring and summer Fujian teas

Compound	VIP	p-value	Aroma	Health Property
Spring				
N-Ethylsuccinimide	2.78	0.0001		
2-Ethylfuran	2.35	0.0001	sweet, earthy, musty	
4-*keto*-Isophorone	1.76	0.0001	floral, woody	
Isomenthone	1.71	0.0001	sweet, peppermint	
Isoborneol	2.85	0.0001	camphor, herbal	antiviral, antibacterial[101]
18	2.70	0.0001		
153	2.65	0.0001		
6-Methyl-2-heptanone	2.59	0.0001	camphoraceous	
3-Heptanone	2.44	0.0001	green, fatty, fruity	
Coumarin	2.36	0.0001	sweet, hay	antidiabetic[130] anti-inflammatory, antipyretic, anticancer[131]
Pyrethrone	2.21	0.0001		
89	2.21	0.0001		
168*	2.19	0.0001		
202	2.16	0.0001		
163	2.16	0.0004		
Butyl acetate*	2.10	0.0004	sweet, fruity	
54	1.91	0.002		
Geranic acid	1.90	0.002	green, woody	
Styrene	1.89	0.0009	sweet, floral, balsamic	
2,6-Dimethylcyclohexanol	1.88	0.0008	roasted, phenolic	anesthetic[222]
(*E*)-β-Ocimene	1.85	0.0005	sweet, herbal	antibacterial[88]
(3*E*)-Methylbutanal oxime	1.83	0.002		
2,2,6-Trimethylcyclohexanone	1.82	0.005	floral, honey	
Menthone	1.78	0.0005	green, minty	antibacterial[88] anti-inflammatory[196]
7-Methoxycoumarin	1.75	0.001	sweet, balsamic	anticancer[135] antinociceptive[136] anti-inflammatory[137]
160	1.67	0.0008		
Ethylbenzene	1.66	0.004		
102	1.65	0.0008		
115	1.63	0.008		
Methyl o-anisate	1.63	0.005	floral, fruity	
Fluoranthene	1.62	0.005		
1-Ethyl-1H-pyrrole	1.62	0.0008	roasted	
201	1.58	0.01		
(*Z*)-Jasmone	1.54	0.0008	floral, jasmine	antibacterial[143] anticancer[144]
76	1.53	0.006		
2-Heptanone	1.51	0.007	fruity, herbal, sweet	
α-Amorphene	1.49	0.03		

214	1.49	0.009		
α-Cyclocitral	1.48	0.006		
allo-Ocimene	1.48	0.009	sweet, floral, peppery	
Perilla aldehyde	1.48	0.01	fruity, grassy	anti-inflammatory, antioxidant, antidepressant[204]
1-Ethyl-1H-pyrrole-2-carboxaldehyde	1.47	0.01	roasted, smoky	
γ-Terpinene	1.43	0.02	citrus, terpene, sweet	antibacterial[88] antiviral[125]
65	1.28	0.005		
211*	1.27	0.003		
cis-Methyl dihydrojasmonate	1.27	0.03	floral, jasmine, green	
Tetradecane	1.24	0.02		
β-Homocyclocitral	1.10	0.03	camphor, cooling, woody	
Summer				
Cubebol	2.34	0.0001	spicy, minty	
Spathulenol	2.25	0.0001	earthy, herbal	antiproliferative, anti-inflammatory, antimicrobial, antioxidant[227]
beta-Cubebene	2.17	0.0001	fruity, citrus	
194	1.47	0.009		
2-Hydroxy-2-cyclopenten-1-one	2.54	0.0001	maple, caramel	
Nerol oxide	2.39	0.0001	green, herbal	
Methyl anthranilate	2.39	0.0001	fruity, grape	antifungal[202]
Muurola-4,1014-dien-1-β-ol	2.18	0.004		
Cadalene	2.08	0.0008		antibacterial, antioxidant[104]
cis-Calamenene	2.03	0.0009	herbal, spicy	antimalarial[213] antitumor[214]
217	2.03	0.0005		
Bornylene	1.98	0.04		
α-Muurolol	1.97	0.0001		antibacterial, antioxidant[106]
β-Calacorene	1.95	0.001		
alpha-Copaene	1.90	0.0004	woody, spice	
Caryophyllene oxide	1.90	0.002	woody, spice	anticancer, analgesic, anti-inflammatory[205]
Pentanal	1.89	0.001	fruity, fermented	
α-Calacorene	1.89	0.005	woody	antibacterial, antioxidant[104]
epi-α-Cadinol	1.89	0.01	herbal	antibacterial[106] anticancer[110] anti-inflammatory[108]
36*	1.88	0.0009		
epi-α-Murrolol	1.87	0.02	herbal, spicy	antibacterial[106] antioxidant[104]
176	1.85	0.003		
176	1.84	0.003		
Quinoline	1.84	0.001	musty, earthy	antimalarial, anticancer, antibacterial, anticonvulsant, antifungal, analgesic anti-inflammatory[206]
epi-Cubebol	1.83	0.0001		

224	1.83	0.002		
40	1.82	0.003		
192	1.82	0.0008		
Benzyl nitrile	1.80	0.001	floral[167]	
96	1.79	0.0008		
Aniline	1.77	0.0003		
Hotrienol	1.76	0.04	floral, woody, spice	
167	1.73	0.0008		
111	1.65	0.01		
4-Ethylbenzaldehyde	1.53	0.02	bitter, almond	
17	1.48	0.01		
31*	1.48	0.01		
154	1.48	0.02		
Benzophenone	1.45	0.01	fruity, floral, metallic	
2-Phenoxyethanol	1.45	0.009	mild rose, metallic	antiseptic[107]
2-Ethylhexyl salicylate	1.44	0.01	floral, sweet	
(*E*)-β-Damascenone	1.44	0.02	floral, sweet, fruity	
79	1.39	0.04		
181	1.39	0.03		
Benzeneacetaldehyde	1.38	0.02	floral, honey	
99	1.33	0.004		
219	1.33	0.04		
Dimethyl sulfoxide*	1.29	0.0004	garlic, bitter	antioxidant, analgesic, neuroprotective, cardioprotective, anti-inflammatory[228]
1,2-Cyclopentanedione*	1.28	0.02		
6	1.27	0.04		
123	1.26	0.03		
23*	1.10	0.03		
230	1.05	0.03		

[a]Aroma information obtained from the Good Scents Company[95] unless otherwise noted. *Compound is affected by more than one environmental factor.

Table 4-7. Statistically important metabolites in 2014-2016 Fujian teas

Compound	VIP	p-value	Aroma	Health Property
2016				
71	2.70	0.0001		
2-Ethylhexanoic acid	2.42	0.0001		
140	2.33	0.0001		
10	2.31	0.0001		
86	2.30	0.0001		
Ethyl 2-methyl butyrate	2.28	0.0001	sweet, fruity	
3-Methylacetophenone	2.23	0.0001		
2-Hydroxy-γ-butyrolactone	2.23	0.0001		
4,4-Dimethyl-2-pentanone	2.18	0.0001		
(*E*)-Isobutyraldehyde oxime	2.07	0.0006		
Camphor	2.05	0.0001	camphor, medicinal	antibacterial[88] anti-inflammatory[108]
1,2-Cyclopentanedione*	2.04	0.0001		
84	2.01	0.0003		
1-Methylpyrrolidinone	2.01	0.0001		
117	2.00	0.0001		
3-Methylpyridine	1.99	0.0003	green, earthy, nutty	
6	1.96	0.002		
20	1.95	0.0001		
Neral	1.93	0.0001	sweet, lemon	antibacterial[88] antifungal[118]
Heptanol	1.86	0.0002	green, herbal, musty	cardioprotective[203]
23*	1.81	0.004		
Cyclohexanone	1.75	0.0002	minty	
Decane	1.75	0.002		
114	1.70	0.0001		
25H-Furanone	1.68	0.004	buttery	
Eucalyptol	1.66	0.009	eucalyptus, sweet	antibacterial[88] analgesic[199] cardioprotective[114] antiviral[125]
Butyl acrylate	1.62	0.0001	fruity, spicy	
Geranylacetone	1.62	0.0006	floral, green, earthy	
5-Methylfurfural	1.61	0.0001	sweet, caramel	
Octanol	1.61	0.0004	fruity, green, earthy	anesthetic[229]
(3*Z*)-Hexenyl isovalerate	1.61	0.0002	green, fruity	
4-Methyl-3-penten-2-one	1.61	0.003	sweet, earthy	
α-Pinene	1.56	0.0004	pine, camphor	antibacterial[88] hypotensive[114] antiviral[125] analgesic[199]
Tridecane	1.56	0.01		
Hexyl acetate	1.55	0.009	sweet, fruity	
o-Guaiacol	1.55	0.0001	phenolic, smoky	
148	1.53	0.003		
Propanoic acid	1.52	0.01	cheesy, pungent	

2-Cyclopentene-1,4-dione	1.48	0.01		
Dimethyl sulfoxide*	1.48	0.007	garlic, bitter	antioxidant, neuroprotective, cardioprotective, analgesic, anti-inflammatory[228]
Vanillin	1.47	0.0005	vanilla	antimicrobial, antioxidant, antimutagenic, analgesic, antidepressant[122]
p-Cymene	1.47	0.02	citrus, terpene, woody	antibacterial[88] hypotensive[114] antiviral[125] antioxidant[211]
Nerol	1.47	0.02	sweet, floral	antifungal[118] antinociceptive, anti-inflammatory[117]
70	1.46	0.003		
2-Nonanone	1.42	0.02	green, earthy, soapy	antimicrobial[230]
39	1.37	0.03		
6-Methyl-2-heptanol	1.35	0.005	waxy, fatty, citrus	
226	1.33	0.03		
1-Hydroxy-2-propanone	1.33	0.02	sweet, caramel	
203	1.33	0.0004		
Benzothiazole	1.31	0.001	sulfury, rubbery	
Limonene	1.30	0.006	lemon, orange	antibacterial[88] cardioprotective[114] anti-inflammatory, analgesic[199]
(2*Z*)-Octen-1-ol	1.29	0.0003		
Norfuraneol	1.29	0.03	sweet, caramel	
Butyl acetate*	1.23	0.005	sweet, fruity	
2-Methoxy-4-vinylphenol	1.20	0.04	smoky, clove	anti-inflammatory[212]
Isophorone	1.18	0.02	sweet, woody, cooling	
2-Ethylhexanol	1.14	0.006	green, oily, citrus	
5-Ethyl-2(5H)-furanone	1.08	0.01	spice	
75	1.04	0.02		
174	1.66	0.0001		
175	1.61	0.0001		
Nonanol	1.48	0.0001	fatty, orange, floral	anesthetic[229]
Octanal	1.38	0.0001	green, fatty, citrus	
Nonanal	1.35	0.0001	cucumber, waxy	antifungal[98]
Butyl butanoate	1.18	0.0001	sweet, fruit	
2015/2016				
2,2,4-Trimethylhexane	2.00	0.0002		
Terpinen-4-ol	1.96	0.0001	woody, terpene, cooling	hypotensive[114] antibacterial[88] anticancer[90] antiviral[125]
91	1.95	0.0001		
47	1.94	0.0001		
35	1.90	0.0002		
206	1.85	0.002		
36*	1.78	0.004		
Linalool acetate	1.78	0.0002	sweet, green, floral	analgesic[199] antibacterial[88]

Pentanol	1.77	0.0004	balsamic, sweet	
2-Phenyl-2-propanol	1.77	0.0001	green, sweet, earthy	
200	1.74	0.0009		
31*	1.72	0.001		
26	1.46	0.0001		
2,4-Di-tert-butylphenol	1.43	0.0008		antioxidant[207]
α-Terpineol	1.42	0.0046	citurs, terpeney, woody	hypotensive[114] gastroprotective[115] analgesic[199] antibacterial[88] antiviral[125]
72	1.41	0.001		
2,3-Dimethylhexane	1.40	0.0001		
67	1.35	0.004		
Sabina ketone	1.34	0.0001		
204	1.30	0.0001		
Methyl isobutyl ketone	1.28	0.0004	herbal, fruity	
o-Xylene	1.26	0.003	geranium	
(2*E*)-Undecenal	1.25	0.002	fruity, green	antileishmanial[208]
Geraniol	1.24	0.006	floral, rose	antimicrobial, neuroprotective anti-inflammatory, antioxidant[124]
Isoamyl alcohol	1.24	0.0002	alocholic, banana	
184	1.24	0.01		
2-Ethyl-3,5-dimethylpyrazine	1.21	0.003	roasted, coffee	
Tetradecanamide	1.21	0.02		
2,4-Dimethyl-1-heptene	1.20	0.003		
41	1.16	0.0005		
Butyl p-toluate	1.13	0.006		
48	1.12	0.002		
56	1.09	0.0005		
97	1.04	0.0004		
2014				
4-Phenyl-3-buten-2-one	2.52	0.0001	fruity, spice	
m-tert-Butylphenol	2.52	0.0001		
p-tert-Butylphenol	2.41	0.0001	earthy, leathery	
142	2.29	0.0001		
Indane	2.29	0.0001		
Fokienol	2.28	0.0001		
1-Nitro-2-phenylethane	2.08	0.0001	floral, spice	cardioprotective[219]
(*E*)-Anethole	2.02	0.0001	sweet, anise	antibacterial[88] anti-inflammatory[94] antioxidant[141]
Butyl propanoate	2.01	0.0001	sweet, earthy, fruity	
(*Z*)-Methyl jasmonate	1.88	0.0003	floral, jasmine	anticancer[144] anti-inflammatory, antioxidant, neuroprotective, antistress[215]
2-Butoxyethanol	1.86	0.0002		
δ-Decalactone	1.86	0.0001	coconut, peach	

(*Z*)-Methyl *epi*-jasmonate	1.82	0.0002	sweet, floral	
168*	1.79	0.003		
230	1.73	0.0002		
Jasmine lactone	1.66	0.0001	jasmine, fruity	
131	1.59	0.003		
211*	1.48	0.003		
Benzyl acetate	1.38	0.0001	sweet, floral, fruity	antifungal[202]
Viridene	1.36	0.0001		
Indene	1.33	0.002		
Benzyl alcohol	1.21	0.002	floral, cherry	antioxidant[211]
Indole	1.15	0.004	fecal, mothball, floral	antibacterial[143] antifungal[202]
191	1.14	0.0001		
γ-Octanolactone	1.13	0.02	sweet, coconut, waxy	
2014/2016				
130	1.24	0.0002		
Menthol	1.18	0.0001	peppermint, cooling	antibacterial[88] decongestant[200] cardioprotective[114] analgesic[199]
3,4-Diethyl-1,1'-biphenyl	1.14	0.0002		
2014/2015				
2,3,5-Trimethylhexane	2.12	0.0001		
180	1.22	0.006		
80	1.17	0.01		
2015				
Methyl 4-methyl benzoate	1.04	0.0001	sweet, anise, floral	
28	1.02	0.0001		

[a]Aroma information obtained from the Good Scents Company[95] unless otherwise noted. *Compound is affected by more than one environmental factor.

Chapter 5. Direct Contact Sorptive Extraction: A Robust Method for Sampling Plant Volatiles in the Field

5.1 Introduction

Plants produce volatile organic compounds (VOCs) that have a wide range of structure, function, and volatility.[8] Plant VOCs change in response to a variety of abiotic and biotic factors, including precipitation, temperature, humidity, herbivory, and pathogen attack,[21, 231-232] which have diverse physiological and ecological effects.[233-234] Most studies use plants grown in greenhouse or laboratory conditions often producing volatile profiles different from plants grown in their natural habitat.[235-236] Minimizing artifacts from unnatural growing conditions is especially important when investigating plant responses to ecological stimuli.

In this study we report on the development of a field-practical, direct-contact sample collection method for the analysis of plant VOCs. The rationale being that current *in situ* sampling methods, based on static headspace (SHS) or dynamic headspace (DHS), enclose a plant or plant parts in a glass or plastic chamber are problematic. First, changes in temperature, humidity and light due to chamber materials occur. For example, polyester and glass chambers increase the temperature by as much as 5.2ºC and 7.5ºC, respectively, leading to changes in VOC composition and emission rates.[8, 235, 237] Humidity inside the chamber is also

higher effecting stomatal closure, which controls the emission rate of some but not all plant VOCs.[238-239] Glass and plastic chambers block up to 40% and 76% UVB light, respectively, causing significant differences in volatile composition and concentration.[237, 240-241] Second, despite lower cost and ease of portability and disposal, sampling chambers made of polyethylene terephthalate (PET), polyacetate, or nylon often leach into the sample potentially masking compounds of interest.[242-243] Third, adsorption onto or diffusion through chamber materials results in loss of analyte. For example, poor recovery was obtained for *Z*-jasmone, geraniol, nerolidol and vanillin due to adsorption and diffusion effects through these materials.[237, 244] Additional problems associated with headspace sampling includes analyte breakthrough and the inability to collect multiple samples easily. Despite breakthrough losses, investigators often use high flow rates to reduce sampling times. For example, α-pinene, myrcene, β-myrcene, and sabinene are common plant metabolites; these and others easily pass through Tenax at flow rates above 500 ml/min.[237, 244-246]

Our objective is to develop a field-practical sample collection method for large-scale studies. Direct-contact sorptive extraction (DCSE) uses a PDMS coated magnetic stir bar (Twister) attached to the plant by a magnet enabling collection of VOCs from both direct-contact and the surroundings. Unlike currently employed sample collection techniques, replication is not limited by equipment, time or weather. While many have used Twisters suspended above the plant or in the headspace of an enclosed sampling chamber,[247-250] VOC sampling by direct-

contact has received relatively little attention.[251-254] For example, only two studies have been published in which PDMS tape was used to directly sample VOCs from plants under highly controlled conditions.[255-256] Direct sampling methods have not resulted in universal acceptance for *in situ* plant VOC collection nor have results been compared to traditional purge and trap methods. Here we compare sampling results using tea (*Camellia sinensis*) plants as our model system in the context of selectivity, sensitivity, and precision. Volatiles released from tea are well known to alter tea quality and mediate the behavior of various pests and their predators.[21, 149, 257-259] Field tests were conducted to identify changes in VOC response to the plant hormone methyl jasmonate (MeJA) and by the lepidopteran herbivore *Ectropis obliqua*, which have been studied in growth chamber experiments.[258-260]

5.2 Experimental

5.2.1 Tea Plants

Plants used in the laboratory study were purchased from Logee's Nursery (Danielson, CT). Sunshine Professional potting mix was purchased from SunGro Horticulture (Agawame, MA). Plants were repotted into 1 L pots with potting mix and fertilized with Scott's Azalea, Camellia, & Rhododendron food (16-2-3 N-P-K) at a rate of 1.23 ml of granules per pot. Plants were housed in a growth chamber under a full-spectrum grow light (ProLume MH1000/U, 16 h daylight). A Plexiglas water container was placed between the plants and light source to reduce heat from the grow light. Soil moisture was controlled by watering plants

with 350 ml tap water twice a week. Field experiments were conducted on mature, clonally propagated tea plants (cultivar Longjing #43) at the Tea Research Institute garden, Chinese Agricultural Academy of Sciences (Hangzhou, Zhejiang Province, China).

5.2.2 Chemicals and Materials

The internal standard, naphthalene-d_8 purchased from Restek (Bellefonte, PA), was used to calculate relative peak area. Methyl jasmonate, Triton-X, ethanol, C_7-C_{30} *n*-alkanes, and, TWEEN were purchased from Sigma Aldrich (St. Louis, MO). A total of 250 reference compounds were purchased from Sigma Aldrich, MP Biomedicals (Santa Ana, CA), Fisher Scientific (Pittsburgh, PA), Alfa Aesar (Ward Hill, MA), Acros Organics (Pittsburgh, PA), and TCI (Nihonbashi-honco, Japan). Twisters and Tenax TA sorbent tubes were purchased from Gerstel Inc. (Linthicum, MD).

5.2.3 Sampling Methods

For direct-contact, Twisters were placed on the bottom of each tea leaf, the side of maximum VOC release (data not shown). The Twisters were held in place with a neodymium magnet (4 mm dia. x 1 mm) on the top side of the leaf. No discoloration or indentation was observed on the leaves after sampling. For dynamic headspace, two leaves and an expanding bud were enclosed in a PET drink cup by putting the shoot through a hole cut on the cup lid. The cups were purchased from a local store and used as is. The Tenax tube was placed in a hole

drilled in the bottom of the cup and sealed with polytetrafluoroethylene tape. The outflow end of the sorbent tube was attached to an in-house vacuum line. Ambient air served as the carrier gas and was controlled using a RMA-26 flow meter (Dwyer, Michigan City, IN) set to 0.75 L/min.

5.2.4 Direct-Contact Sorptive Extraction and Dynamic Headspace

One tea plant was sprayed to runoff with 1 mM MeJA in 10% ethanol and 0.125% Triton-X, and the second, the control plant, was sprayed with ethanol/surfactant 24 h before sample collection. Plants were kept in separate rooms in the greenhouse to avoid cross contamination by the control plant after hormone treatment. Plants were taken to the growth chamber where they were sampled in triplicate for 1 h. A 15 min purge of the DHS chamber using ambient air was made between sampling events. Sorbent tubes were conditioned at 280 °C (Twister) and 300 °C (Tenax) using the Gerstel tube conditioner and then analyzed by GC/MS to ensure if peaks were present they could be attributed to each sorbent's phase or column bleed. If not, tubes were reconditioned and reanalyzed. Method blanks for both sorbents were collected by placing them in the center of the growth chamber without plants. If the RPA of compounds found in the method blanks were ≥ those from the samples, they were not recorded. DHS breakthrough was determined by placing two Tenax tubes in series. If plant VOCs were detected on the second tube, breakthrough occurred.

5.2.5 Field Trial

Experiments were performed in the field at the Tea Research Institute from July 2-10. The objective was to compare VOC emission from treated (*Ectropis obliqua* or MeJA) and untreated plants. Four replicates of each were collected and analyzed. For treated samples, two tea shoots (~ 4 – 5 leaves each) and two second and third instar larvae of *E. obliqua* (Lepidoptera) were put inside a breathable nylon mesh bag. The larvae were placed on the leaves at 1:00 pm and fed until 4:30 pm. If one or both shoots had no visible damage, larvae were allowed to continue feeding on the undamaged shoot(s) until 6:30 pm. At 6:00 pm, ~ 6.8 m of a 1 m wide row of tea plants was sprayed with 1.8 L (0.26 L/m^2) of 1 mM MeJA, 10% ethanol and 0.03% TWEEN solution or the control solution, which was 10% ethanol and 0.03% TWEEN. For all sampling events, Twisters were placed on the 2nd leaf from the top of the shoots at 10:30 am on the following day. For MeJA and the control, leaves were selected at approximate, even spacing from one another. For the *E. obliqua* treatment, only leaves that had received herbivory were used. Twisters were left for 7 days, collected and sealed in vials for transport to Tufts for analysis.

5.2.6 Gas Chromatography/Mass Spectrometry

A TDU (Gerstel GmbH, Müllheim an der Ruhr, Germany) was used to provide splitless transfer of the sample from the sorbent tubes into a CIS inlet (Gerstel), held at -100°C. The TDU was heated from 40 °C (0.70 min) to 275 °C (3 min) at 600 °C/min under 50 ml/min helium gas flow. After 0.1 min the CIS was heated

to 275 °C at 12 °C/s and held for 5 min. Analyses were performed on an Agilent 6890/5975 GC/MS (Santa Clara, CA) equipped with a MultiPurpose Sampler (Gerstel) for automated injection. Samples were separated on a Restek 30 m x 250 µm x 0.25 µm RXI-5MS column. The oven temperature was held at 40 °C for 1 min and then increased to 280 °C at a rate of 5 °C/min with a constant flow of helium at 1.2 ml/min. The ion source and quadrupole temperatures were set at 230 °C and 150 °C respectively, and the MS scanned at 70 eV between 40 and 350 *m/z*. A standard mixture of C_7–C_{30} *n*-alkanes was used to calculate the RI for each compound.

5.2.7 Data Analysis Software

Automated sequential GC-GC/MS was used to create a comprehensive database of 450 secondary metabolites in tea.[21] To date, ~ 200 compounds have been confirmed by matching their RI and MS fragmentation pattern to commercially obtained standards. Another 150 have been tentatively identified by comparing library data (NIST, Adams, literature[76-79]) to tea compounds. The balance of compounds in the database is numerically labeled.

Ion Analytics (Andover, MA) software was used to identify metabolites in the DCSE and DHS samples by comparing RI and MS data against the database and to obtain their peak areas.[41, 80] Since none of our previous work focused on potential metabolites in tea due to insect and hormone treatment and the fact that on-site sampling was based on intact leaves, 43 new compounds were detected.

Compound identity was based on the following set of conditions. First, peak scans must be constant for five or more consecutive scans (differences ≤ 20%). Second, the SSV (relative error) must be < 5. The SSV calculates relative error by comparing the mass spectrum at each peak scan against another. The smaller the difference, the closer the SSV is to zero, the better the MS agreement. Third, the Q-value must be ≥ 93. The Q-value is an integer between 1 and 100; it measures the total ratio deviation of the absolute value of the expected minus observed ion ratios divided by the expected ion ratio times 100 for each ion across the peak. The closer the value is to 100, the higher the certainty between database and sample spectra. Finally, the Q-ratio compares the ratio of the main ion intensity to confirming ion intensities across the peak; it also must be ≤ 20%. When all criteria are met, the software assigns a compound name or numerical identifier to the peak from the database.

5.2.8 Statistical Analysis

All statistical analysis was conducted in R.[155] PCA was conducted on auto-scaled and centered data using the *prcomp* function. To assess statistical significance of separation in PCA, ANOVA of principal component scores was conducted using the *Anova* function from the *car* package.[261]

5.3 Results and Discussion

5.3.1 Comparison of DHS and DCSE Data

Findings revealed significant differences in the chemical profile produced by each technique. For example, the total number of metabolites for both the control and MeJA treated plants was 213 for DHS and 251 for DCSE (Table 5-1). Given the number of analytes and background noise from Tenax (Figures 5-1a and b), compound identity and differences in relative peak area were established using the spectral deconvolution software. Since researchers often use unfiltered air in the field when sampling by DHS,[262-264] the difference in total ion current chromatograms between the Tenax adsorbent blank after conditioning and from the growth chamber is striking. When compared to DCSE (Figure 5-1c and d), DHS background signals are more complex (yielding unresolved mixtures). The adsorbent blanks revealed siloxane peaks from the DB5 column and polydimethylsiloxane peaks from the stir bar. However, ~50 peaks appear in the method blank chromatogram for DHS. Of these, 25 compounds were plant VOCs, with another 12 due to the plastic cup.[242-243] In contrast, the DCSE method blank revealed 14 plant VOC peaks. We considered background compounds as matrix interferents and eliminated them from the data. For example, many metabolites, including furfural , benzaldehyde, phenol , benzene acetaldehyde, acetophenone and *n*-nonanol, found by DCSE were not reported by DHS, since their Tenax background signals were higher than in tea.[265-266]

Table 5-2 lists the unique compounds detected by each method, whose S/N ≥ 10/1. Compounds such as 2,6-dimethyl-3,7-octadiene-2,6-diol, homomenthyl salicylate, octadecanol acetate, hydrocarbons $C_{17\text{-}19}$ and fatty acids $C_{12\text{-}13}$ were collected by DCSE but not DHS due to their low volatility (< 0.1 Pa) and/or high concentration in the waxy part of the leaf.[267-268] Also collected by DCSE were high volatility organics, whose vapor pressures are > 1.3 kPa. Examples of these include pentanal, 4-methyl-2-pentanone, dimethyl disulfide, 2-ethylhexene, 2-hexanone, hexanal, and butyl acetate. In our breakthrough experiment, 36 compounds passed through both DHS tubes, with 9 trapped only on the second tube (Table 5-2). Note: these 36 compounds are volatile, were detected by DCSE and should have been trapped by DHS if not for the high flow rate often used by investigators in the field.

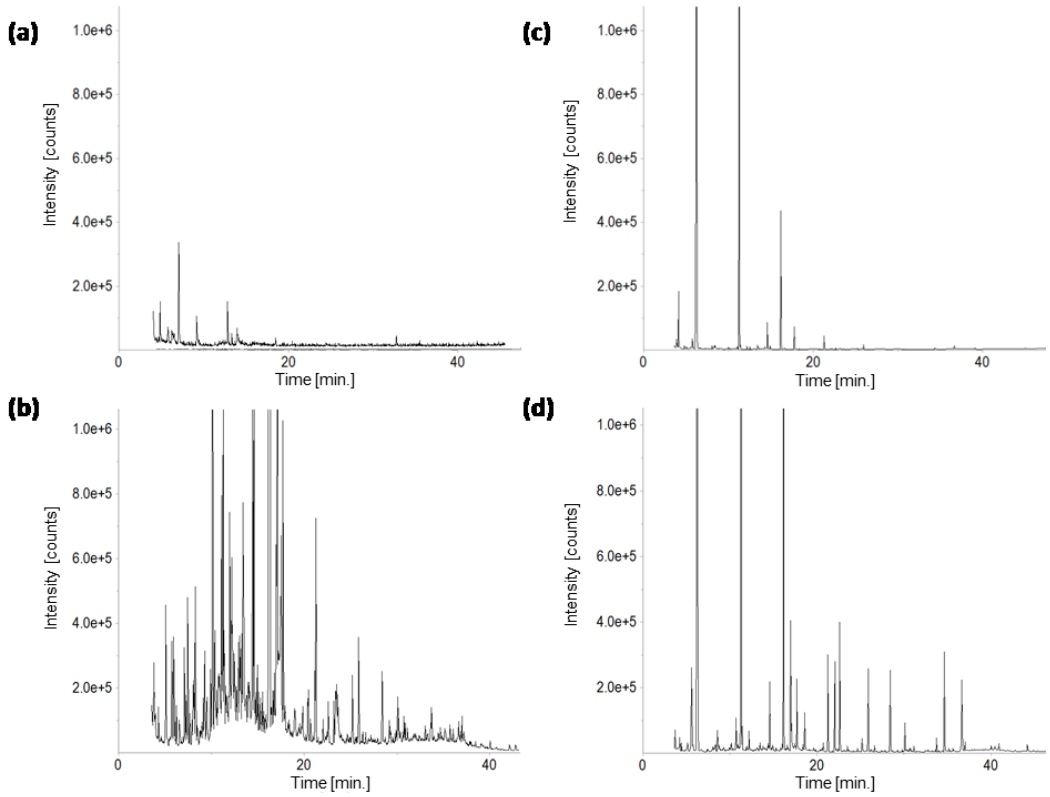

Figure 5-1. Adsorbent (a, c) and method (b, d) blanks for Tenax and PDMS (Twister) show much higher background for DHS from unfiltered air and the PET headspace chamber.[269]

DHS successfully trapped 2,4-pentanedione and γ-hexalactone, whose solubility in water is high, log $K_{ow} \leq 0.34$, but not PDMS. Of the 11 terpenes trapped only by DHS, bergamal, cuminaldehyde, (*E*)-β-ionone, *cis*-calamenene, α-calacorene, cedrol, and α-cadinol concentrations were the same ($p > 0.05$) in both control and treated plants presumably due to mechanical damage when covering leaves or increases in chamber temperature.[270-272]

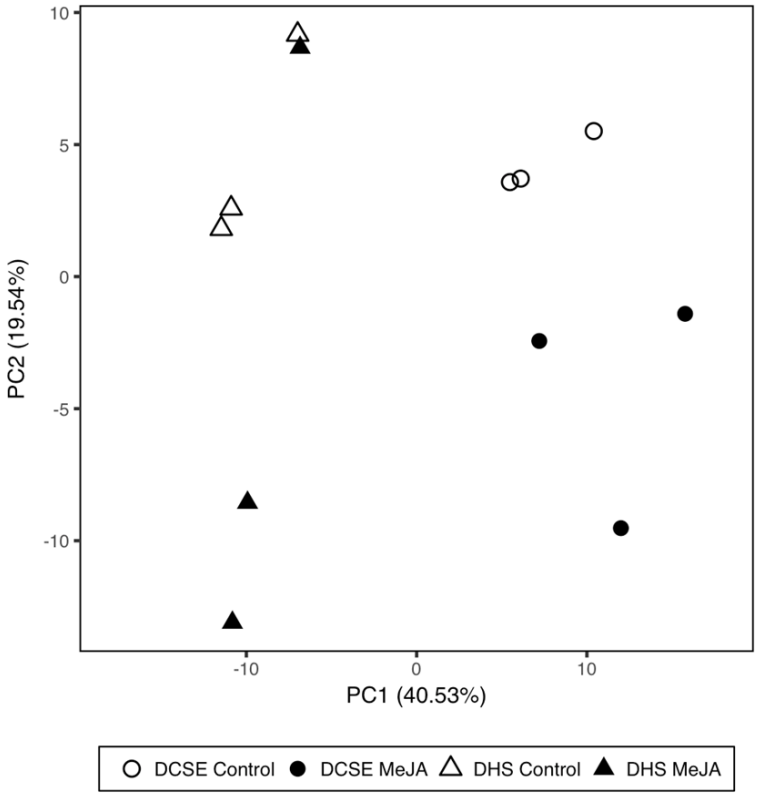

Figure 5-2. PCA of VOC profiles from control (white) and MeJA treated (black) tea plants grown in greenhouse collected by DCSE (circles) and DHS (triangles).[269]

When the data in Table 5-1 was analyzed by PCA, 80% of the variation is explained by four components. PC1 explains 41% of the variation (Figure 5-2), which is associated with differences in the sampling methods (ANOVA, $F = 118.27$, $p < 0.001$). No statistical difference was observed between the control and MeJA treatments (ANOVA, $F = 1.98$, $p = 0.193$). The relative peak areas of the control and treated plant metabolites are strongly correlated to PC1 scores, positively for DCSE ($r > 0.9$, $p \leq 0.05$) and negatively for DHS ($r < -0.9$, $p \leq 0.05$). The list of compounds in Table 5-3 confirms metabolite volatility and uniqueness drive the variation between sampling methods.

Differences in metabolite treatment and control chemistry are associated with PC2. As expected, differences in the PC scores were significant along this axis (ANOVA, $F = 6.17$, $p = 0.035$), but differences due to sampling methods were not (ANOVA, $F = 0.003$, $p = 0.958$). Metabolites associated with control plants are positively correlated with PC2 whereas treated metabolites are negatively correlated. Only treated plant metabolites, namely, benzyl alcohol, (2*E*)-hexenyl acetate, (3*Z*)-hexenyl butanoate, and (3*Z*)-hexenyl isovalerate were highly correlated with PC2 ($r < -0.9$, $p \leq 0.05$). These compounds increase in concentration in response to MeJA.[258-259] Table 5-1 lists both common and unique MeJA induced metabolites. (*Z*)-3-methyl-butyl aldoxime, (*Z*)-2-methyl-butyl aldoxime , (*E*)-2-methyl-butyl aldoxime , (*E*)-3-methyl-butyl aldoxime, (*E*)-β-ocimene , *cis*-linalool oxide (furanoid), *cis*-linalool oxide (pyranoid), *trans*-linalool oxide (pyranoid), δ-cadinene, and (*E*)-nerolidol are examples of MeJA induced VOC emissions in other plants.[192, 258-259, 273]

5.3.2 Field Trial

DCSE was used in the field to sample control, *E. obliqua* and MeJA treated tea plants. Although 125 metabolites were detected in all three treatments, their peak areas differed greatly (Table 5-4). We detected 13 unique compounds produced by *E. obliqua* and MeJA treatments missing from the control plants, whose concentrations differed greatly. Only MeJA treated plants produced 2,5-bis(1,1-dimethylethyl)-phenol whereas only *E. obliqua* treated plants produced 2-methyl-

2-buten-1-ol, *p*-cymene , ɣ-decalactone, and epi-α-cadinol. Each treatment also produced three compounds in common with the control but not each other.

PCA was performed to evaluate differences in VOC treatment profiles. Four principle components capture 80% of the variation; the first two account for ~ 60%. Figure 5-3 illustrates treatment differences compared to the control. Treatments are well separated in the score plot of the first two PC axes. Control plants are separated from herbivory treatments along PC1, which was strongly, positively correlated ($r > 0.95$, $p \leq 0.05$) with 1-ethyl-3-methyl-benzene, 1-ethyl-2-methyl-benzene, benzene acetaldehyde, isophorone, menthol, 1-methylnapthalene, and dibenzofuran (in the direction of control plants). In addition, the compounds most negatively correlated ($r < -0.85$, $p \leq 0.05$) with PC1 are 2-ethylhexene, 3,5-dimethyl-2-hexene, phenol, and (2*E*)-hexenyl benzoate (in the direction of treated plants). MeJA and *E. obliqua* herbivory treatments are best separated along PC2, which is most strongly, negatively correlated (Pearson's $r < -0.85$, $p \leq 0.05$) with butanoic acid, (2*E*)-hexenal, phenyl ethyl alcohol, (*E*)-caryophyllene, and octadecane (in the direction of MeJA treatment). ANOVA using both PC1 and PC2 values as the response variable shows significant differences among treatments (PC1: $F = 12.58$, $df = 2$, $p = 0.003$; PC2: $F = 6.79$, $df = 2$, $p = 0.019$).

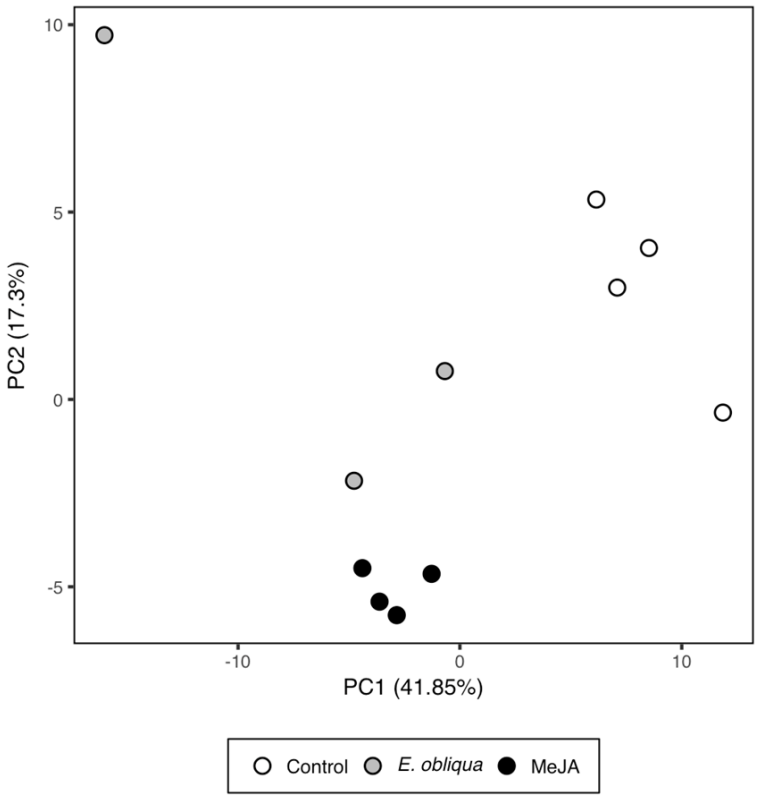

Figure 5-3. PCA of VOCs collected by DCSE at the Tea Research Institute in Hangzhou, China. Tea plants were treated with a control spray (white), MeJA (black), or *E. obliqua* larvae (gray).[269]

The PCA results are in agreement with other studies in which plant VOCs are induced by plant hormones and herbivores (Table 5-5). For example, benzaldehyde, benzene acetaldehyde, δ-valeryllactone, nonanal, decanal, and benzothiazole were also found in greater concentration in control plants compared to herbivore or hormone treated plants.[256, 259-260] In comparison, γ-terpinene[274] was reported in higher concentration in herbivore-treated plants, whereas (2*E*)-hexenal, and (*E*)-caryophyllene[258, 260] were all found to have higher concentrations in MeJA treated plants. In addition, Cai *et al*. reported differences between VOCs from MeJA treated and *E. obliqua* treated potted tea plants in growth chamber experiments.[258] In this study, the plant exhibited similar response for some VOCs

but not others, which may be due to differences in sampling techniques and/or growing conditions.

5.4 Conclusion

DCSE is a robust alternative to DHS for *in situ* sampling of plant VOCs. It is straight-forward to set up. It easily scales to large sample sizes and is more sensitive and less prone to matrix interferents than DHS. DCSE captures a wider range of volatile compounds and can be used to distinguish the effects of herbivory in the field, especially in remote or difficult to reach areas such as a forest canopy, or habitats with rugged terrain. Although reliable in adverse weather conditions, loss of sample can occur. Care must be taken to ensure tight sorbent/leaf attachment without damaging the leaf. While PDMS Twisters are selective, mixed phase ethylene glycol-silicon (EG-silicon) Twisters can be used to collect more polar organics than PDMS alone. However, EG-silicon Twisters will sorb water, which can lead to loss of analyte when purging water prior to analysis. Ethylene glycol phases are also unstable at temperatures that exceed 220 °C. DCSE provides significant advantages when studying important chemical ecology questions related to herbivory attacks on plants or extreme changes in climate conditions.

The material presented in this chapter is based on work supported by the National Science Foundation under grant BCS-1313775: [169]Kfoury, N.; Scott, E.; Orians, C.; Robbat, A., Direct Contact Sorptive Extraction: A Robust Method for Sampling Plant Volatiles in the Field. *J. Agric. Food Chem.* **2017,** *65* (38), 8501-8509.

Table 5-1. Tea metabolite relative peak areas determined by DHS and DCSE sampling.[269]

No.	Compound	DHS						DCSE						ID
		Control			Methyl jasmonate			Control			Methyl jasmonate			
1	Acetaldehyde	0.442	0.198	0.140	0.269	0.157	0.186	0.198	0.126	0.161	0.156	0.093	0.122	T
2	Ethanol	0.133	0.185	0.100	0.149	0.147	0.123	0.590	0.441	0.968	0.448	0.459	0.874	Std.
3	Methyl vinyl ketone	0.141	0.064	0.051	0.468	0.767	0.053	0.188	0.184	0.242	0.260	0.221	0.327	Std.
4	2-Butanone	0.133	0.109	0.072	0.281	0.304	0.080	0.498	0.420	0.519	1.618	1.034	2.211	T
5	2-Methylfuran		0.002		0.076	0.129	0.001	0.184	0.176	0.186	0.399	0.156	0.201	T
6	2-Butanol	0.026		0.028	0.157	0.116	0.013				0.263	0.268	0.436	T
7	Isobutyronitrile				0.207	0.118					0.081	0.048	0.101	T
8	Tetrahydrofuran	0.010	0.010	0.005	0.016	0.016	0.009	0.423	0.168	0.181	0.260	0.126	0.256	T
9	Isovaleraldehyde	0.102	0.097		0.223	0.217	0.031	0.209	0.178	0.103	0.196	0.205	0.557	Std.
10	Benzene	0.487	0.283	0.219	0.214	0.262	0.268	0.737	0.375	0.463	0.620	0.462	0.433	Std.
11	1-Butanol	0.061		0.025	0.056		0.015	3.145		0.213		0.290	0.398	T
12	3-Methylhexane										0.033	0.031	0.032	T
13	Pentanal	0.068	0.050	0.037	0.045	0.051	0.039	0.290	0.139	0.199	0.265	0.154	0.453	T
14	Heptane							0.062	0.049	0.067	0.068	0.051	0.060	Std.
15	Hydroxyacetone	0.433	0.348	0.031	0.413	0.438	0.542	4.987	7.462	5.051	14.500	5.793	8.210	Std.
16	Methyl methacrylate							0.096	0.130	0.055	0.101	0.421	0.255	T
17	2,4,4-Trimethyl-1-pentene		0.034		0.034	0.026		0.112			0.136			T
18	2-Ethoxyethanol	0.033	0.021		0.017	0.011		0.250	0.224	0.272	0.382	0.195	0.523	T
19	2-Methylbutanenitrile	0.028	0.016		7.697	4.506	0.030	0.067	0.076	0.043	2.002	1.795	4.933	T
20	4-Methyl-2-pentanone							0.035	0.012		0.018	0.015		T
21	3-Methylbutanenitrile				15.543	9.033	0.044				6.540	4.413	9.951	T
22	Isoamyl alcohol				0.115	0.069					0.133	0.084	0.254	Std.
23	Dimethyldisulfide							0.060	0.090	0.045	0.144	0.047	0.053	T
24	Propanoic acid	0.367	0.231	0.185	0.250	0.154	0.107	0.737	0.494	0.795	0.632	0.369	0.381	Std.

25	2-Propenoic acid	0.109	0.035	0.016	0.083	0.124	0.005			0.175				T
26	(2*E*)-Pentenal										0.042	0.036	0.054	T
27	*see table 5-4*													
28	Pyrrole				0.030	0.023	0.006	0.122	0.078	0.091	0.137	0.084	0.161	T
29	*see table 5-4*													
30	Pentanol							0.043	0.029	0.038	0.056	0.039	0.052	Std.
31	Toluene	2.377	2.654	0.434	1.556	2.157	0.602	0.685	0.528	0.861	0.701	0.657	0.753	Std.
32	(*E*)-2-Methylpropyl aldoxime				0.082	0.126					0.221	0.100	0.395	T
33	2,4-Pentanedione	0.020	0.017	0.008										
34	Unknown 1										0.117	0.051	0.126	
35	2-Ethylhexene										0.059	0.046		T
36	*see table 5-4*													
37	3-Methyl-2-butenal	0.012	0.018	0.007	0.052	0.058	0.007	0.064	0.059	0.087	0.047	0.033	0.052	T
38	*see table 5-4*													
39	2-Hexanone							0.044	0.028	0.034	0.038	0.032	0.048	Std.
40	Cyclopentanone	0.049	0.040	0.039	0.036	0.041	0.031	0.491	0.305	0.297	0.310	0.270	0.363	T
41	Octane	0.074	0.078	0.046				0.089	0.073	0.118	0.108	0.071	0.082	Std.
42	4-Methyl-3-penten-2-one	0.028	0.030		0.030	0.044		0.032	0.040	0.040	0.053	0.037	0.049	Std.
43	Hexanal							0.324	0.205	0.245	0.243	0.189	0.233	Std.
44	Unknown 2				0.005	0.011	0.015					0.069	0.164	
45	Butanoic acid	0.194	0.114	0.081				0.930	0.323	0.584	0.639	0.386	0.373	Std.
46	Butyl acetate							0.028	0.023	0.024	0.025	0.021	0.020	T
47	*see table 5-4*													
48	3-Furaldehyde	0.005	0.006	0.006	0.011	0.014	0.013	0.123	0.071	0.058	0.177	0.057	0.101	T
49	*see table 5-4*													
50	1-Ethyl-1*H*-pyrrole	0.011	0.007	0.004	0.013	0.007	0.004	0.018			0.085			T
51	2,4-Dimethylheptane							0.093	0.079	0.118	0.083	0.080	0.118	T

52	Unknown 3										0.044	0.015	0.043	
53	Furfural							0.936	0.827	0.777	1.217	0.546	0.851	Std.
54	Unknown 4	1.070	0.618	0.168	0.658	0.653	0.211							
55	(2*E*)-Hexenal	0.034	0.036	0.029	0.052	0.100	0.022				0.019	0.032	0.022	Std.
56	(3*Z*)-Hexenol	0.156	0.109	0.025	1.114	0.616	0.033				0.159	0.428	0.281	Std.
57	2-Methylbutanoic acid	0.008	0.006	0.002		0.011	0.006	0.030	0.050	0.119				Std.
58	Ethylbenzene	0.639	0.828	0.119	0.399	0.734	0.154	0.085	0.092	0.055	0.086	0.108	0.052	Std.
59	(*Z*)-3-Methylbutyl aldoxime				3.262	1.891					4.161	0.588	4.910	T
60	Unknown 5	0.083	0.088	0.033	0.493	0.851	0.053				0.546	0.273	0.243	
61	2-Furanmethanol	0.008	0.009	0.008	0.011	0.026	0.006	0.404	0.503	0.428	0.693	0.420	0.205	Std.
62	*m*-Xylene	1.544	2.117	0.220	1.071	1.940	0.294	0.166	0.222	0.149	0.185	0.277	0.145	Std.
63	*p*-Xylene	1.544	2.119	0.220	1.071	1.941	0.294	0.166	0.221	0.147	0.183	0.278	0.145	Std.
64	(2*E*)-Hexenol	0.016	0.010								0.073	0.057	0.068	Std.
65	(*Z*)-2-Methylbutyl aldoxime				1.131	0.795					2.330	1.491	3.950	T
66	*n*-Hexanol	0.029	0.023	0.008	0.427	0.549	0.031	0.037	0.024	0.033	1.083	0.628	1.500	Std.
67	Isoamyl acetate	0.138	0.063	0.011	0.229	0.163	0.029	0.215	0.119		0.361	0.342	0.559	Std.
68	(*E*)-2-Methylbutyl aldoxime				0.312	0.270					0.393	0.255	0.541	T
69	(2*Z*)-Hexenol				0.011	0.009					0.053	0.067	0.055	Std.
70	2,6-Dimethyl-1,5-heptadiene				0.014	0.020					0.086	0.047	0.056	T
71	3-Heptanone	0.074	0.085	0.024	0.117	0.162	0.025	0.047	0.025	0.027	0.039	0.059	0.049	Std.
72	(*E*)-3-Methylbutyl aldoxime				2.253	1.564					2.738	2.115	3.199	T
73	2-Heptanone	0.079	0.062	0.024	0.014	0.042	0.028	0.178	0.061	0.048	0.088	0.070	0.083	Std.
74	Styrene	0.500	0.807	0.153	0.572	1.183	0.067	0.491	0.574	0.347	0.403	0.501	0.381	T
75	2-Methylcyclopentanone	0.016	0.358	0.235	0.479	0.289	0.305	0.324	0.206	0.284	0.280	0.178	0.218	T
76	Unknown 6	0.749	0.360	0.230	0.480	0.295	0.313	0.309	0.207	0.278	0.288	0.157	0.229	
77	*o*-Xylene	0.516	0.725	0.083	0.329	0.580	0.112	0.076	0.099	0.068	0.073	0.116	0.035	Std.

78	Cyclohexanone	0.442	0.198	0.140	0.269	0.157	0.186	0.198	0.126	0.161	0.156	0.093	0.122	Std.
79	*see table 5-4*													
80	Nonane	0.596	0.343	0.143	0.257	0.492	0.135	0.215	0.178	0.149	0.294	0.148		Std.
81	Heptanal	0.157	0.128	0.077	0.163	0.243	0.090	0.231	0.166	0.185	0.223	0.176	0.253	Std.
82	1-Nitropentane	0.161	0.126	0.084	0.899	0.751	0.096	0.434	0.231	0.263	0.742	0.435	0.921	T
83	2-Acetylfuran	0.020	0.014	0.014	0.020	0.018	0.015	0.127	0.126	0.110	0.179	0.105	0.134	Std.
84	γ-Butyrolactone	0.041	0.029	0.019	0.033	0.038	0.023	0.219	0.214	0.181	0.276	0.183	0.188	T
85	2(5*H*)-Furanone	0.119	0.097	0.077	0.089	0.087	0.103	0.712	0.702	0.544	1.135	0.449	0.586	Std.
86	Unknown 7	0.016	0.012					0.126	0.134	0.106	0.290	0.092	0.133	
87	Cumene	0.066	0.077	0.014	0.040	0.059	0.018	0.029	0.036	0.024	0.025	0.028		Std.
88	1,2-Cyclopentanedione	0.047	0.032	0.098	0.115		0.059	0.841	0.787	0.666	1.119	0.531	0.729	T
89	α-Pinene	0.547	1.247	0.065	0.296	0.683	0.093	0.584	3.619	4.259	0.530	3.423	3.249	Std.
90	Unknown 8	0.011	0.006	0.004	0.068	0.064	0.005	0.018	0.016		0.028	0.014		
91	*N,N*-Diethylformamide	0.157	0.094	0.060	0.085	0.083	0.084	0.108	0.055	0.058	0.069	0.066	0.068	T
92	Camphene				0.021	0.032		0.027	0.096	0.109	0.035	0.083	0.075	Std.
93	2-Ethylhexanal	0.150	0.544	0.035	0.142	0.098	0.034			0.268				Std.
94	*see table 5-4*													
95	Benzaldehyde							0.556	0.494	0.522	0.546	0.490	0.598	Std.
96	*see table 5-4*													
97	5-Methylfurfural	0.017	0.010	0.021	0.012	0.013	0.015	0.117	0.077	0.059	0.141	0.048	0.141	Std.
98	1-Ethyl-3-methylbenzene	0.391	0.390	0.096	0.296	0.489	0.117	0.128	0.211	0.135	0.119	0.152	0.105	T
99	1,2,3-Trimethylbenzene	0.371	0.432	0.056	0.213	0.298	0.062	0.059	0.103	0.034	0.052	0.087	0.038	T
100	Dimethyl trisulfide							0.045	0.129	0.048	0.149	0.053	0.098	T
101	β-Pinene	0.726	1.332	0.044	0.392	0.714	0.067	0.733	3.424	4.093	0.678	3.045	3.120	Std.
102	1-Octen-3-one	0.029	0.018	0.010	0.022	0.023	0.011	0.047	0.030	0.039	0.035	0.027	0.034	T
103	1-Ethyl-2-methylbenzene	0.293	0.354	0.046	0.182	0.304	0.060	0.041	0.100	0.035	0.048	0.075		T

104	2-Methylbutyl acrylate							0.051	0.041		0.054	0.107	0.792	T
105	1-Octen-3-ol				0.032	0.376	0.030	0.089	0.081	0.133	0.105	0.075	0.136	Std.
106	α-Methylstyrene	0.066	0.042	0.017	0.042	0.032	0.016	0.255	0.286	0.282	0.306	0.202	0.308	Std.
107	2,3-Octanedione	0.025	0.023	0.015			0.015	0.020	0.022	0.028	0.025	0.015	0.027	T
108	6-Methyl-5-hepten-2-one	0.079	0.065	0.022	1.111	0.668	0.021	0.215	0.121	0.123	0.148	0.147	0.425	Std.
109	Dehydroxylinalool 3,7-oxide	0.041	0.028	0.013	0.013	0.296	0.511	0.078	0.054	0.056	0.071	0.064	0.183	T
110	Phenol							0.299	0.255	0.248	0.509	0.275	0.321	Std.
111	*see table 5-4*													
112	Myrcene	0.148	0.448		0.561	1.103		0.136	0.589	0.604	0.143	0.489	0.486	Std.
113	Mesitylene	1.138	1.361	0.160	0.735	1.295	0.204	0.208	0.394	0.175	0.189	0.321	0.124	Std.
114	*see table 5-4*													
115	Hexanoic acid							0.693	0.384	0.307	0.693	0.687	0.424	Std.
116	Decane							0.459	0.395	0.386	0.500	0.299	0.342	Std.
117	*n*-Octanal	0.283	0.222	0.144	0.313	0.519	0.156	0.424	0.317	0.335	0.423	0.256	0.452	Std.
118	α-Phellandrene	0.071	0.175		0.086	0.156		0.020	0.041	0.046	0.018	0.035	0.034	Std.
119	(3*Z*)-Hexenyl acetate	1.528	0.140	0.010	12.873	7.084	0.015	0.306			8.645	17.814	10.563	Std.
120	*see table 5-4*													
121	δ-3-Carene	0.423	1.199	0.023	0.234	0.635	0.034	0.281	1.188	1.355	0.239	0.966	0.977	Std.
122	Benzyl chloride	0.189	0.442	0.008			0.013	0.067	0.074		0.337	0.038	0.034	T
123	Hexyl acetate	0.132	0.054	0.021	0.300	0.299					0.057	0.078	0.072	Std.
124	α-Terpinene	0.015	0.046		0.025	0.044		0.279	1.186		0.238	0.972	0.977	Std.
125	(2*E*)-Hexenyl acetate	0.059			0.403	0.475					0.183	0.330	0.379	T
126	4-Cyanocyclohexene							0.596	0.062	0.282	0.118	0.191	0.159	T
127	1,2,4-Trimethylbenzene	0.377	0.383	0.044	0.186	0.277	0.057	0.072	0.123	0.067	0.066	0.097	0.051	T
128	*p*-Cymene	0.928	1.645	0.101	0.665	1.607	0.131	0.214	0.545	0.594	0.186	0.402	0.418	Std.
129	Limonene	1.600	3.480	0.141	1.476	4.187	0.151	0.861	2.661	4.381	0.757	1.970	3.128	Std.

130	Sylvestrene	2.758	7.242	0.138	2.559	7.563	0.155	1.695	6.058	7.869	1.431	4.557	5.527	T
131	2-Ethyl-1-hexanol	2.374	4.880	0.783	5.479	4.821	0.472	0.805	0.482	0.559	0.699	0.851	0.545	Std.
132	Indane	0.110	0.154	0.017	0.080	0.171	0.021	0.033	0.058	0.030	0.028	0.045	0.025	Std.
133	Unknown 9	0.108	0.154	0.016	0.073	0.160	0.021	0.024	0.049		0.021	0.042		
134	(*Z*)-β-Ocimene				0.051	0.025								T
135	Benzyl alcohol	0.269	0.215	0.104	0.369	0.505	0.101	0.212	0.234	0.275	0.318	0.254	0.421	Std.
136	Lavender lactone	0.027	0.026	0.010	0.099	0.202	0.008	0.017	0.013		0.017	0.033	0.058	T
137	*N,N*-Dimethylbenzylamine							0.097	0.021		0.141	0.018		T
138	*see table 5-4*													
139	Benzene acetaldehyde							0.049	0.033	0.036	0.076	0.069	0.121	Std.
140	(*E*)-β-Ocimene				0.687	0.355					0.163	0.163	1.157	T
141	δ-Valeryllactone	0.065	0.047	0.047	0.069	0.133	0.047	0.186	0.159	0.125	0.220	0.160	0.191	T
142	1-Methyl-3-propylbenzene	0.244	0.299	0.038	0.162	0.331	0.046	0.057	0.128	0.053	0.047	0.091	0.042	T
143	γ-Hexalactone	0.129	0.076	0.034	0.096	0.101	0.027							Std.
144	Bergamal	0.015	0.011	0.005	0.032	0.026	0.005							Std.
145	2-Ethyl-1,4-dimethylbenzene	0.240	0.258	0.034	0.147	0.236	0.040	0.048	0.101		0.042	0.081		T
146	γ-Terpinene	0.016	0.026		0.031	0.050		0.022	0.060	0.141	0.019	0.044	0.106	Std.
147	4-Methyldecane							0.049	0.050	0.044	0.040	0.035	0.032	T
148	*n*-Octanol	0.069	0.051	0.014	0.085	0.136	0.046	0.038	0.022	0.025	0.033	0.020	0.026	Std.
149	2-Methyldecane							0.079	0.094	0.050	0.058	0.055	0.038	T
150	Acetophenone							0.519	0.537	0.509	0.580	0.829	0.689	Std.
151	3-Methyldecane							0.080	0.092	0.074	0.081	0.067	0.061	T
152	*see table 5-4*													
153	*cis*-Linalool oxide (furanoid)				0.126	0.137					0.129	0.193	0.158	Std.
154	Heptanoic acid							0.249	0.185	0.096	0.268	0.308	0.245	Std.
155	Unknown 10				0.045	0.054						0.045	0.210	

156	*trans*-Linalool oxide (furanoid)				0.235	0.173			0.072	0.224	0.605	0.816	0.518	Std.
157	Terpinolene					0.094	0.130	0.060	0.132	0.111	0.223	0.361	0.228	Std.
158	*p*-Cymenene	0.145	0.201	0.012	0.160	0.391	0.015	0.055	0.111	0.122	0.044	0.085	0.088	T
159	2-Phenyl-2-propanol	0.101	0.066	0.023	0.088	0.106	0.027	0.124	0.195	0.181	0.106	0.142	0.166	Std.
160	Fenchone	0.399	0.510	0.015	0.274	0.329	0.024	0.212	0.498	0.533	0.260	0.461	0.433	T
161	Undecane							0.466	0.420	0.493	0.415	0.294	0.330	Std.
162	Linalool	0.048	0.045		2.631	1.467	0.014	0.037	0.054	0.054	1.498	3.997	2.017	Std.
163	*n*-Nonanal	1.348	1.080	0.720	1.405	2.095	0.720	1.817	1.159	1.564	1.576	1.351	1.820	Std.
164	Maltol	0.025		0.057	0.013			0.226	0.165	0.129	0.170	0.098	0.087	Std.
165	*endo*-Fenchol	0.118	0.174	0.017				0.108	0.179	0.204	0.097	0.106	0.128	Std.
166	Isodurene							0.063	0.117	0.066	0.048	0.083	0.050	T
167	Phenyl ethyl alcohol	0.083	0.074	0.023	0.038	0.073	0.026	0.018	0.034		0.176	0.195		Std.
168	*see table 5-4*													
169	*allo*-Ocimene				0.105	0.060					0.016	0.041	0.053	Std.
170	4-Acetyl-1-methylcyclohexene	0.064	0.111	0.013		0.070	0.014		0.024			0.016	0.035	T
171	2-Ethyl hexanoic acid	0.192	0.157	0.052	0.146	0.089	0.043	0.088	0.068	0.044	0.091	0.275	0.105	Std.
172	*trans*-Pinocarveol	0.056	0.126	0.002	0.039	0.103	0.004	0.034	0.128	0.165	0.046	0.110	0.250	T
173	Benzeneacetonitrile	0.024	0.123	0.008	9.192	16.804	0.035				5.105	8.808	35.184	Std.
174	*see table 5-4*													
175	ε-Caprolactone			0.021			0.020	0.108	0.078		0.127	0.157		T
176	Viridene	0.047	0.058	0.004	0.035	0.059	0.003	0.016	0.044	0.038				T
177	Camphor	0.340	0.471	0.020	0.200	0.323	0.029	0.160	0.432	0.518	0.134	0.277	0.366	Std.
178	Nerol oxide	0.047	0.069	0.066				0.039	0.077	0.092	0.032	0.055	0.059	T
179	*trans*-Pinocamphone	0.121	0.176		0.100	0.181	0.018	0.063	0.142	0.168	0.111	0.095	0.120	T
180	2-Ethylhexyl acetate	0.033	0.028	0.015	0.007	0.044	0.055	0.052	0.051	0.051	0.041	0.036	0.048	Std.
181	Borneol	0.394	0.765	0.039	0.268	0.585	0.043	0.186	0.704	0.948	0.170	0.440	0.648	Std.

182	*cis*-Linalool oxide (pyanoid)				0.029	0.022					0.064	0.062	0.052	Std.
183	*n*-Nonanol							0.088	0.070	0.063	0.089	0.063	0.081	Std.
184	Menthol	0.188	0.150	0.035	0.119	0.144	0.043	0.074	0.102	0.105	0.066	0.068	0.083	Std.
185	*cis*-Pinocamphone	0.213	0.288	0.026	0.148	0.249	0.032	0.148	0.260	0.294	0.116	0.165	0.281	T
186	*trans*-Linalool oxide (pyranoid)				0.095	0.078	0.005				0.152	0.188	0.197	Std.
187	Terpinen-4-ol	0.086	0.159		0.120	0.320		0.111	0.312	0.320	0.085	0.196	0.246	Std.
188	Naphthalene	17.686	12.338	7.626	12.629	12.193	9.008	6.281	4.963	6.712	5.594	3.656	4.966	Std.
189	(3*Z*)-Hexenyl butanoate	0.043			0.953	0.742	0.019	0.039	0.051	0.045	0.254	0.562	0.650	T
190	2,6-Dimethyl-3,7-octadiene-2,6-diol										0.236	0.502	0.575	T
191	*p*-Cymen-8-ol				0.012	0.087								T
192	Cryptone	0.156	0.400	0.015	0.016	0.158	0.017		0.067	0.097				T
193	Octanoic acid							0.539	0.347	0.232	0.489	0.513	0.469	Std.
194	α-Terpineol	0.100	0.196	0.009	0.126	0.427	0.010	0.133	0.405	0.437	0.109	0.251	0.289	Std.
195	Methyl salicylate	0.347	0.387	0.273	0.233	0.511	0.049	0.113	0.170	0.120	0.166	0.211	0.247	Std.
196	Dodecane							0.179	0.218	0.253	0.159	0.150	0.210	Std.
197	Myrtenol				0.084	0.268	0.010	0.084	0.124	0.132	0.062	0.062	0.093	T
198	*n*-Decanal	0.592	0.452	0.385	1.112	2.307	0.390	1.013	0.641	0.640	0.850	0.719	0.909	Std.
199	Levoverbenone	0.053	0.104	0.010	0.043	0.118	0.012	0.016	0.074	0.101		0.044	0.069	T
200	Benzenecarboxylic acid							0.096	0.112		0.154	0.127	0.098	T
201	*see table 5-4*													
202	Rose ether							0.095	0.094	0.091	0.074	0.067	0.078	T
203	Methenamine							0.440	0.398	0.364	0.992	0.071	0.515	T
204	*see table 5-4*													
205	(3*Z*)-Hexenyl valerate	0.058			1.627	1.098	0.007				0.647	0.880	1.769	T
206	*o*-Methylthymol	0.164	0.373		0.112	0.455	0.007		0.270	0.339	0.000	0.162	0.223	T
207	2-Methoxy-*p*-cymene	0.164	0.376	0.006	0.114	0.459	0.012	0.073	0.285	0.371	0.053	0.177	0.257	T

208	*see table 5-4*													
209	(3*Z*)-Hexenyl isovalerate	0.061			0.364	0.342	0.004				0.175	0.296	0.252	T
210	Unknown 11	0.123	0.103	0.071	0.047	0.079	0.075	0.032	0.044	0.049	0.035	0.026	0.038	
211	Cuminaldehyde	0.053	0.098		0.026	0.044								T
212	Carvone	0.085	0.076	0.012	0.066	0.564	0.011	0.037	0.040	0.043	0.036	0.030	0.049	Std.
213	3-Phenoxypropanol							0.157	0.142	0.115	0.115	0.067	0.076	T
214	*see table 5-4*													
215	Linalool acetate				0.013	0.033	0.003		0.032	0.023		0.015	0.029	Std.
216	Geraniol	0.054			0.121	0.077					0.055	0.045		Std.
217	*see table 5-4*													
218	Caprolactam	0.615	0.271	0.236	0.262	0.219	0.157	1.808	1.103	1.630	2.151	1.365	1.667	Std.
219	Geranial				0.249	0.439						0.058	0.240	Std.
220	4-Ethylguaicol	0.054	0.216		0.032	0.378		0.049	0.207	0.188	0.047	0.112	0.182	T
221	Nonanoic acid							0.673	0.624	0.367	0.687	0.035	0.790	Std.
222	Tridecane	0.274	0.358	0.203	0.594	0.459	0.065	0.174	0.159	0.236	0.115	0.135	0.189	Std.
223	Indole				0.514	0.582	0.007				0.169	0.174	3.314	Std.
224	Geranyl formate				0.073	0.081								Std.
225	*N,N*-Dibutylformamide	0.132	0.096	0.071	0.131	0.057	0.104	1.133	0.477	0.378	0.811	0.575	0.542	T
226	Undecanal	0.066	0.065	0.042	0.118	0.246	0.042	0.152	0.110	0.133	0.117	0.091	0.117	Std.
227	1-Methylnaphthalene	0.056	0.067	0.018	0.047	0.090	0.016	0.029	0.056	0.031	0.029	0.038	0.029	Std.
228	*see table 5-4*													
229	*see table 5-4*													
230	*see table 5-4*													
231	*see table 5-4*													
232	*see table 5-4*													
233	γ-Nonalactone	0.058		0.040				0.086	0.065	0.042	0.056	0.029	0.074	Std.

234	(3*Z*)-Hexenyl hexenoate	0.078	0.017	0.012	1.400	0.996	0.017	0.035	0.034	0.023	0.212	0.278	1.487	T
235	Decanoic acid	0.563	0.314	0.303	0.489	0.561	0.254	0.537	0.473	0.195	0.576	0.527	0.464	Std.
236	*see table 5-4*													
237	*see table 5-4*													
238	*see table 5-4*													
239	Tetradecane	0.264	0.198	0.149	0.278	0.293	0.073	0.238	0.156	0.203	0.168	0.131	0.182	Std.
240	(*Z*)-Jasmone	0.009	0.007	0.003	0.020	0.021	0.004	0.038	0.103	0.154	0.037	0.031	0.023	Std.
241	Vanillin							0.071	0.079	0.045	0.132	0.047	0.043	Std.
242	Dodecanal				0.098	0.160	0.029	0.113	0.079	0.125	0.094	0.088	0.117	Std.
243	Longifolene	0.104	0.290	0.016	0.072	0.318	0.020	0.053	0.220	0.350	0.042	0.138	0.234	T
244	2-Ethylhexyl pentanoate	0.177	0.080	0.136	0.282	0.073	0.031					0.039		T
245	(*E*)-Caryophyllene	0.014	0.008	0.006	0.016	0.016	0.005	0.016	0.013		0.016	0.014	0.021	Std.
246	*see table 5-4*													
247	*see table 5-4*													
248	Geranyl acetone	0.055	0.032	0.019	0.808	0.149	0.018	0.207	0.120	0.075	0.145	0.106	0.158	Std.
249	*see table 5-4*													
250	*n*-Dodecanol							0.744	0.551	0.492	1.022	0.457	0.677	Std.
251	α-Amorphene				0.028	0.028			0.017	0.057	0.023			T
252	*see table 5-4*													
253	(*E*)-β-Ionone	0.013	0.006		0.011	0.012								Std.
254	Pentadecane							0.494	0.290	0.393	0.480	0.293	0.369	Std.
255	α-Muurolene				0.048	0.039						0.013	0.044	T
256	Butylated hydroxytoluene	0.161	0.086	0.151	0.065	0.042	0.095	0.094	0.059	0.038	0.091	0.039	0.031	T
257	Unknown 12				3.650	1.491					3.662	5.053	20.771	
258	*see table 5-4*													
259	*see table 5-4*													

260	(*E,E*)-α-Farnesene				3.635	1.478		0.048	0.056	0.031	3.658	5.058	20.786	T
261	*see table 5-4*													
262	2,4-*di*-*tert*-Butylphenol	0.956	0.281	0.104	0.532	0.012	0.089	0.050	0.031	0.036	0.048	0.272	0.052	T
263	δ-Cadinene				0.031	0.028					0.038	0.040	0.026	T
264	*cis*-Calamenene	0.195	0.115	0.094	0.213	0.051	0.050							T
265	*see table 5-4*													
266	α-Calacorene	0.009	0.006	0.004	0.036	0.017	0.003							T
267	(*E*)-Nerolidol				0.136	0.100					0.168	0.384	2.121	Std.
268	Dodecanoic acid							1.238	0.706	0.890	0.771	0.546	0.573	Std.
260	*see table 5-4*													
270	*see table 5-4*													
271	*see table 5-4*													
272	Hexadecane	0.248	0.137	0.122	0.161	0.162	0.073	0.263	0.128	0.165	0.221	0.162	0.168	Std.
273	Unknown 13					0.098	0.143				0.163	0.226	0.151	
274	Cedrol	0.021	0.012	0.009	0.020	0.019	0.008							T
275	Unknown 14	0.628	0.332	0.251	0.451	0.561	0.247	1.773	1.407	2.678	1.483	1.007	1.187	
276	Benzophenone										0.132	0.060	0.046	T
277	*see table 5-4*													
278	*epi*-α-Murrolol				0.036	0.034								T
279	(*Z*)-Methyl jasmonate				0.245	0.172	0.022				0.037	0.028	0.418	Std.
280	(*Z*)-Methyl dihydrojasmonate	0.058	0.040		0.069	0.054	0.028	0.044	0.025		0.035	0.014	0.040	Std.
281	α-Cadinol	0.028	0.017	0.020	0.043	0.033								T
282	*see table 5-4*													
283	Tridecanoic acid							0.086	0.044		0.048	0.040		T
284	*n*-Tetradecanol	0.098	0.074	0.078	0.120	0.077	0.061	0.369	0.306	0.232	0.598	0.210	0.387	T
285	2,2',5,5'-Tetramethyl-1,1'-biphenyl	0.050	0.045	0.021	0.045	0.019	0.020	0.445	0.049		0.525	0.094	0.248	T

286	Heptadecane							0.270	0.083	0.066	0.320	0.132	0.129	Std.
287	2-Ethylhexyl benzoate	0.426	0.065	0.059	0.093	0.069	0.044				0.682	0.417	0.235	T
288	Tetradecanoic acid	0.490	0.458	0.472	0.541	0.476	0.391	1.612	1.332	0.612	2.449	0.962	0.789	Std.
289	Octadecane	0.149	0.069	0.094	0.099	0.083	0.058	0.297	0.113	0.102	0.291	0.155	0.182	Std.
290	2-Ethylhexyl salicylate							0.045	0.039	0.043	0.047	0.026	0.076	Std.
291	Isopropyl myristate	0.039	0.022	0.016	0.047	0.031	0.016	0.037	0.027	0.029	0.036	0.021	0.029	Std.
292	Unknown 14	2.246	2.202	1.251	2.087	0.116	1.237					0.181		
293	Unknown 15	0.708	0.383	0.230	0.472	0.335	0.137				0.167	0.121	0.333	
294	Pentadecanoic acid	0.022	0.079	0.151	0.085	0.113	0.137	0.664	0.678	0.249	1.155	0.332	0.333	T
295	*n*-Hexadecanol							0.725	0.490	0.252	0.724	0.418	0.458	Std.
296	Homomenthyl salicylate							0.023	0.016	0.023	0.027	0.014	0.021	Std.
297	Nonadecane							0.211	0.081	0.081	0.224	0.130	0.122	Std.
298	Methyl palmitate	0.075	0.047	0.060	0.054	0.054	0.048	0.279	0.113	0.117	0.318	0.175	0.172	Std.
299	n-Hexadecanoic acid	10.535	8.771	8.266	7.313	6.448	8.002	43.236	19.303	9.332	50.647	13.998	12.820	Std.
300	Isopropyl palmitate	0.103	0.094	0.088	0.117	0.035	0.044	0.078	0.039	0.104	0.075	0.071	0.080	Std.
301	*n*-Octadecanol	0.273	0.202	0.153	0.252	0.200	0.112	1.758	2.238	2.675	2.470	0.678	0.640	T
302	Heneicosane	0.096	0.076	0.137	0.086	0.066	0.062	0.518	0.368	0.431	0.610	0.334	0.449	Std.
303	Docosane	0.109	0.057	0.146	0.058	0.051	0.078	0.406	0.273	0.168	0.758	0.212	0.241	Std.
304	Octadecanol acetate							0.072	0.057	0.035	0.160	0.038	0.054	T
305	Tricosane	0.121	0.055	0.114	0.054	0.047	0.071	0.456	0.216	0.185	0.558	0.221	0.264	Std.
306	Pentacosane	0.173	0.074	0.136	0.082	0.070	0.085	0.517	0.292	0.274	0.863	0.304	0.315	Std.
307	Squalene	0.026	0.013	0.025	0.007	0.010	0.022	0.668	0.207		0.788	0.138	0.091	T

Notes:

1) Table 5-1 and Table 5-4 compound numbers are listed by retention time.
2) Positively (STD) or tentatively (T) identified by comparing sample and reference standard or commercial library data

Table 5-2. Average relative peak areas of the unique compounds found by DCSE and DHS.[269]

No.[a]	Compound	Vapor Pressure (Pa)[b]	log K_{ow}[b]	DCSE	DHS1[c]	DHS2[c]
12	3-Methylhexane	8386	3.71	0.032		0.033
13	Pentanal	3466	1.44	0.250	0.048	0.049
14	Heptane	6026	4.66	0.059		0.038
16	Methyl methacrylate	3866	1.35	0.094		
20	4-Methyl-2-pentanone	2133	1.25	0.024		
23	Dimethyl disulfide	3826	1.77	0.081		
26	(2*E*)-Pentenal	1533	1.25	0.044		0.013
30	Pentanol	373	1.41	0.036		
33	2,4-Pentanedione	894	0.34		0.015	
34	Unknown 1			0.098		
35	2-Ethylhexene	2746	4.52	0.052		0.016
39	2-Hexanone	1467	1.44	0.035		0.014
43	Hexanal	1333	1.97	0.258		0.085
46	Butyl acetate	1333	1.77	0.025		0.013
51	2,4-Dimethylheptane	1373	5.17	0.097		0.041
52	Unknown 3			0.034		
53	Furfural	267	0.73	0.847		0.067
54	Unknown 4				0.618	0.020
58	Ethylbenzene	1333	3.15	0.479	0.080	0.066
60	Unknown 5			0.267	0.354	0.024
63	*p*-Xylene	1200	3.15	1.198	0.190	0.162
67	Isoamyl acetate	533	2.12	0.106	0.319	0.03
71	3-Heptanone	533	1.97	0.081	0.041	0.029
76	Unknown 6			0.405	0.245	0.039
77	*o*-Xylene	933	3.12	0.391	0.078	0.079
78	Cyclohexanone	667	0.81	0.232	0.143	0.026
81	Heptanal	533	2.50	0.143	0.206	0.045
87	Cumene	600	3.66	0.046	0.028	0.016
95	Benzaldehyde	133	1.48	0.524		
98	1-Ethyl-3-methylbenzene	400	3.67	0.297	0.142	0.150
100	Dimethyl trisulfide	147	2.93	0.074		
103	1-Ethyl-2-methylbenzene	347	3.67	0.207	0.060	0.053
104	Butyl acrylate	533	2.39	0.046		
110	Phenol	53	1.46	0.267		
113	Mesitylene	307	3.60	0.816	0.235	0.148
115	Hexanoic acid	27	1.84	0.462		
116	Decane	213	6.07	0.413		
126	4-Cyanocyclohexene	39	1.30	0.313		
127	1,2,4-Trimethylbenzene	253	3.60	0.221	0.079	0.071
129	Limonene	200	4.45	1.839	2.293	0.205

131	2-Ethyl-1-hexanol	27	2.82	3.135	0.657	0.259
134	(*Z*)-β-Ocimene	213	4.26		0.038	
137	*N,N*-Dimethyl benzenemethanamine	120	1.98	0.080		
143	γ-Hexalactone	24	0.26		0.075	
144	Bergamal	67	2.69		0.021	
145	2-Ethyl-1,4-dimethylbenzene	200	4.13	0.159	0.068	0.032
147	4-Methyldecane	120	6.42	0.048		
149	2-Methyldecane	107	6.42	0.074		0.047
150	Acetophenone	33	1.66	0.522		
151	3-Methyldecane	107	6.42	0.082		
154	Heptanoic acid	13	2.37	0.178		
159	2-Phenyl-2-propanol	27	1.73	0.069	0.152	0.016
161	Undecane	80	6.6	0.460		
166	Isodurene	67	4.06	0.082		
173	Benzeneacetaldehyde	53	1.78	0.039		
183	*n*-Nonanol	4.4	3.53	0.073		
190	2,6-Dimethyl-3,7-octadiene-2,6-diol	0.047	1.62	0.438		
191	*p*-Cymen-8-ol	25	2.53		0.050	
193	Octanoic acid	6.5	2.90	0.373		
196	Dodecane	27	7.13	0.217		
200	Benzenecarboxylic acid	0.4	1.87	0.104		
202	Rose ether	0.6	1.16	0.093		
203	Methenamine	12	2.17	0.401		
211	Cuminaldehyde	7.8	2.73		0.035	
213	3-Phenoxypropanol	0.18	1.63	0.086		
221	Nonanoic acid	2.9	3.43	0.555		
224	Geranyl formate	9.1	3.73		0.077	
241	Vanillin	0.06	1.18	0.065		
250	Dodecanol	0.11	5.13	0.600		
253	(*E*)-β-Ionone	1.7	3.85		0.015	
254	Pentadecane	2	8.73	0.392		
264	*cis*-Calamenene	0.91	6.25		0.105	
266	α-Calacorene	0.55	5.47		0.019	
268	Dodecanoic acid	0.19	5.03	0.945		
274	Cedrol	0.13	3.53		0.016	
276	Benzophenone	0.12	3.18	0.079		
278	*epi*-α-Murrolol	0.011	3.52		0.035	
281	α-Cadinol	0.011	3.52		0.038	
283	Tridecanoic acid	0.09	5.56	0.065		
286	Heptadecane	0.43	9.79	0.14		
290	2-Ethylhexylsalicylate	0.0011	5.93	0.05		
295	*n*-Hexadecanol	0.0012	7.25	0.489		
296	Homomenthyl salicylate	0.0055	5.95	0.021		

297	Nonadecane	0.09	10.85	0.125		
304	Octadecanol acetate	0.0081	9.21	0.055		

[a] Compounds are numbered and identified according to Table 5-1. [b] Information obtained from the public databases PubChem, ChemSpider, and FooDB.[92, 275-276] [c] DHS1 and 2 represent the order of Tenax tubes for the breakthrough analysis. If compounds were detected on both tubes then both RPAs were reported.

Table 5-3. PCA Correlations of DHS and DCSE.[269]

No.[a]	Compound	r	p-value
PC1			
1	Acetaldehyde	0.920	0.0002
14	Heptane	0.965	0.000007
28	Pyrrole	0.950	0.00003
30	Pentanol	0.985	0.000002
39	2-Hexanone	0.966	0.000007
40	Cyclopentanone	0.926	0.0001
43	Hexanal	0.947	0.00004
46	Butyl acetate	0.951	0.00003
48	3-Furaldehyde	0.927	0.0001
51	2,4-Dimethylheptane	0.923	0.0001
53	Furfural	0.973	0.000004
83	2-Acetylfuran	0.980	0.000002
84	γ-Butyrolactone	0.966	0.000007
85	2(5*H*)-Furanone	0.938	0.00008
86	Unknown 7	0.911	0.0002
95	Benzaldehyde	0.969	0.000007
97	5-Methylfurfural	0.934	0.00009
106	α-Methylstyrene	0.945	0.00004
110	Phenol	0.977	0.000003
115	Hexanoic acid	0.933	0.00009
116	Decane	0.965	0.000007
147	4-Methyldecane	0.914	0.0002
150	Acetophenone	0.927	0.0001
151	3-Methyldecane	0.935	0.00009
154	Heptanoic acid	0.935	0.00009
161	Undecane	0.920	0.0002
183	*n*-Nonanol	0.983	0.000002
193	Octanoic acid	0.955	0.00002
202	Rose ether	0.924	0.0001
241	Vanillin	0.900	0.0004
250	*n*-Dodecanol	0.977	0.000003
254	Pentadecane	0.966	0.000007
274	Cedrol	-0.902	0.0004
284	*n*Ttetradecanol	0.919	0.0002
290	2-Ethylhexyl salicylate	0.919	0.0002
295	*n*-Hexadecanol	0.948	0.00004

296	Homomenthyl salicylate	0.965	0.000007
297	Nonadecane	0.935	0.00009
302	Heneicosane	0.974	0.000004
PC2			
125	(2*E*)-Hexenyl acetate	-0.941	0.0006
135	Benzyl alcohol	-0.952	0.0005
189	(3*Z*)-Hexenyl butanoate	-0.907	0.003
209	(3*Z*)-Hexenyl isovalerate	-0.905	0.003

[a] Compounds are numbered and identified according to Table 5-1.

Table 5-4. Herbivore, hormone and control metabolite peak areas.[269]

		E. obliqua			MeJA				Control				
No.	**Compound**	**1**	**2**	**3**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**ID**
10	Benzene	65935.7	51056.4		16025.1	21105.4	19580.7	21981.9	23227.6	16373.2		16275.4	Std.
26	(2*E*)-Pentenal	2549.2	6658.5	15295.1	1997.5	1413.8	1252.8	1265.6	1026.7	1786.9	1459.2	1321.7	T
27	Pyridine	4482.7	39631.8	43290.8	23652.4	31628.1	35949.6	15006.7	49999.6	35725.0	39901.9	44040.6	T
29	2-Methyl-2-buten-1-ol		6990.0	3401.1									T
31	Toluene	44749.8	68605.4	42980.9	35388.6	36614.9	39675.0	37923.7	91450.0	61221.7	81647.0	116003.7	Std.
33	3-Methyleneheptane	36055.8	30688.6	10139.1	22378.8	29819.1	29668.8	22508.5					T
36	Tiglic aldehyde		57125.1	66260.2	63866.7	82522.9	67259.3	72281.4	68608.1	78934.2	62185.3	62087.0	T
37	3-Methyl-2-butenal		55768.6	65545.5	63866.7	82768.4	65710.1	72281.4	65876.2	77250.8	59985.5	61823.4	T
38	(3*Z*)-Octene	17137.6	38929.6	20176.0	119487.0	27334.9	23238.1	23409.5					T
42	4-Methyl-3-penten-2-one		27269.6	13175.3	16364.2	19960.7	19104.5	21350.3	35228.7	20037.6	17945.8	19577.9	Std.
43	Hexanal	25715.9	33852.6	19656.6	26933.0	32455.4	26892.5	26129.0	14117.3	13586.4	15973.7	26819.5	Std.
45	Butanoic acid		39438.8	37935.4	40651.1	37678.3	48771.1	43072.7		39268.2	20048.2	43755.2	Std.
47	3,5-Dimethyl-2-hexene	10013.0	8145.6	2938.6	6170.6	9041.1	8286.9	6873.0					T
48	3-Furaldehyde		8558.7	13810.9	7555.3	7692.0	7337.4	7640.7	14273.3	10897.3	9914.2	9896.3	T
49	Isovaleric acid		69038.6	8903.1	25573.6	29098.5	27205.3	36060.5					Std.
53	Furfural	5135.0	84763.5	169151.6	69478.0	64413.9	48480.2	82000.1	114159.9	102827.6	127296.5	75810.6	Std.
55	(2*E*)-Hexenal		2576.3	2145.3	2963.7	3222.0	3794.0	3150.6					Std.
56	(3*Z*)-Hexenol							22324.7	9284.8	9090.1	22610.3		Std.
58	Ethylbenzene		10722.7	10528.0	11845.3	12831.7	11745.4	11311.4	15869.3	14392.7	14111.7	42258.8	Std.
61	2-Furanmethanol		4912.7	32906.5	15419.3	12374.9	10722.4	13849.3	30668.9	20779.9	26553.0	23000.8	Std.
62	*m*-Xylene		13920.8	14525.8	13404.8	15894.1	15566.4	13349.5	22433.0	18769.3	18618.9	23084.5	Std.
63	*p*-Xylene		14194.6	15986.3	16300.9	17034.9	16123.4	16174.1	22844.8	19089.5	20336.4	28122.5	Std.
66	*n*-Hexanol	1553.8	5904.6	5410.0	4950.3	7000.7	5586.6	4485.2	4456.2	4511.9	4771.8	5368.4	Std.

70	2,6-Dimethyl-1,5-heptadiene		6417.8	4430.3	6435.4	4604.1	6001.5	5204.4	7373.5	6901.0	6265.1	8629.3	T
71	3-Heptanone		6792.2	5071.3	5407.6	7104.5	6644.3	4910.0	8872.4	6651.5	8079.3	5090.1	Std.
73	2-Heptanone		14524.3	2731.2	5853.7	6817.6	6780.1	4484.5					Std.
74	Styrene	1027.1	24781.9	23011.3	18622.2	20613.0	21258.2	23513.9	32345.0	22998.0	26268.0	24417.0	T
77	*o*-Xylene		6634.8	6466.1	6558.7	7170.8	6550.4	6711.6	8614.3	8387.6	8438.8	10855.4	Std.
78	Cyclohexanone		5214.6	5704.6	5146.4	5201.5	4461.9	5213.5	8752.8	9645.4	7600.3	8227.8	Std.
79	Pentanoic acid	22878.9	50604.7	62298.2	101295.4	114551.8	25881.2	87768.5	9364.8	89363.2	14421.9	174210.7	T
81	Heptanal	2711.7	26699.1	14907.0	22607.3	26491.8	24078.1	20356.2	19277.6	16990.8	23038.3	29747.3	Std.
83	2-Acetylfuran				3574.6	3791.3	2938.5	4520.5	6449.3	4402.0	5619.1	5345.6	Std.
84	γ-Butyrolactone		7644.7	8262.7	4035.2	3576.8	3630.1	3905.9	7690.9	6632.4	6553.1	7801.0	T
85	2(5*H*)-Furanone	8173.0	43430.2	55273.6	28018.7	27906.0	23316.2	31986.6	48938.0	43415.9	42246.7	39507.7	Std.
89	α-Pinene	2444.3	4508.6	4771.4	3258.5	2717.7	3035.0	4376.4	4619.9	3869.6	6008.2	4067.4	Std.
94	6-Methyl-heptan-2-ol		2754.3	3175.2	3972.4	3164.0	3549.1	3236.7	4419.2	4208.2	3349.7	4097.6	Std.
95	Benzaldehyde	4660.3	61402.6	71209.5	78067.3	61735.1	56419.3	68934.4	93607.8	89201.8	90802.6	102189.8	Std.
96	1-Ethyl-4-methyl-benzene		2484.9	7222.6	6907.2	7308.3	7264.3	7869.1	12547.5	10496.3	12057.8	12655.3	T
97	5-Methylfurfural		1821.5	5453.0	5032.3	4293.3	5056.4	6771.7	5347.1	4977.3	7413.5	5661.3	Std.
98	1-Ethyl-3-methyl-benzene		2080.8	2498.9	2319.5	2168.3	2177.9	2261.2	3525.9	3254.6	3388.4	4305.9	T
102	1-Octen-3-one	8038.9	4920.0	3428.7	5917.9	3726.7	4874.3	3714.1	5044.7	4009.7	4142.9	4830.8	T
105	1-Octen-3-ol		17002.9	19450.9	20022.6	20264.8	28361.2	14440.8	4854.6		3646.5		Std.
107	2,3-Octandione		5303.4	2420.1	4474.8	4671.5	6705.0	3231.6	7199.5	8462.1			T
108	6-Methyl-5-hepten-2-one		8028.7	18344.8	21325.1	13048.2	15715.7	23986.7	8409.3	8857.5	11450.5	18384.8	Std.
109	Dehydroxylinalool 3,7-oxide		3696.6	3918.9	4167.5	3626.7							T
110	Phenol	516234.7	641203.1	275131.7	313452.9	381501.5	257174.4	399714.3	22012.6	18685.1	30660.4	10710.4	Std.
111	1-Ethyl-2-methyl-benzene		3417.8	4685.2	3541.3	4255.2	3729.4	5187.9	8141.1	7848.7	8284.0	8168.2	T
113	Mesitylene	6037.6	17496.4	10157.1	8640.6	9363.4	9846.0	8704.1	12854.3	11568.1	12694.0	10960.5	Std.
114	(*E*)-Herboxide	3088.9	15960.0	5506.7	9689.8	9326.3	13278.1	6386.0	5578.3	4867.8	4585.4	6837.9	Std.
115	Hexanoic acid	62405.9	30763.3	69918.0	115484.2	92804.9	131078.9	181940.2	9191.1	81250.8	30594.9	208652.5	Std.

117	*n*-Octanal		21450.3	15954.2	24041.0	25521.9	18827.7	15232.9	20102.7	21599.0	22472.0	32675.3	Std.
119	(3*Z*)-Hexenyl acetate		9144.7	5802.2	5245.0	5032.8	6486.9	113097.1	5874.8	5159.1	6436.5	11449.4	Std.
120	(*Z*)-Herboxide		3020.5	4050.1	1954.7	3026.1	2906.3	3412.9	3590.8	3194.0	3705.7	5759.4	Std.
121	δ-3-Carene		1462.5	1000.9	1063.3	1013.6	1045.6	1018.6	1180.5	1023.3	1987.3	2524.2	Std.
127	1,2,4-Trimethylbenzene		1653.9	1686.1	2310.4	1862.1	1935.0	2341.1	3225.6	3259.7	3378.5	3162.1	T
128	*p*-Cymene		44642.5	26732.1	2597.0	4562.3	9586.9	6413.0	6739.5	8938.5	7878.1	8601.0	Std.
129	Limonene		343533.6	21949.0	2894.5	8058.6	74077.8	4855.1	8356.9	11227.0	116822.1	41297.0	Std.
131	2-Ethyl-1-hexanol	161075.3	1633137.7	1622233.1	1840608.8	2363978.8	6261332.2	1348758.3	198408.4	128285.5	123077.3	95935.1	Std.
132	Indane		1653.5	1632.0	2072.8	2260.9	2118.2	1832.8	2141.7	2183.1	2079.6	2818.8	Std.
135	Benzyl alcohol	3008.3	7751.9	8080.5	5516.3	5224.5	5441.6	6841.6	7451.2	14021.1	11849.9	9372.1	Std.
136	Lavender lactone		1857.1	3526.3	2233.9	1959.8	3346.8	3081.8	3252.3	3555.2	4955.6	7840.6	T
138	1-Methyl-2-pyrrolidinone	12664.6	94227.8	86352.7	63406.0	109671.6	91240.8	80203.3	114850.8	97125.4	113678.3	129531.4	Std.
139	Benzene acetaldehyde		2989.9	5058.8	3551.3	2846.8	3861.3	4365.8	10821.8	9867.0	9771.8	13446.2	Std.
141	δ-Valeryllactone	10078.5	14253.0	15426.7	11907.3	13572.0	12870.5	11503.0	19383.3	16531.4	20755.5	20934.6	T
143	γ-Hexalactone	4760.2	9712.3	18293.1	20594.5	20918.7	13218.9	11845.9					Std.
146	γ-Terpinene		19236.8	5364.7			4098.3	1147.2					Std.
150	Acetophenone	44490.1	465384.7	392530.7	390297.9	389736.7	578368.7	399364.6	602667.4	380792.4	399635.5	335936.4	Std.
152	*m*-Cresol	6465.3	11975.1	4637.2	3407.9	5171.5	5741.5	4321.9	9282.2	9293.9	11103.9	8596.1	Std.
154	Heptanoic acid	84233.5	26677.2	7947.3	22983.9	37115.6	21426.2	23341.6	2483.5	29965.4	20687.0	21065.1	Std.
158	*p*-Cymenene		1606.0	1118.5									T
159	2-Phenyl-2-propanol	4673.0	70299.5	26472.1	8800.5	14743.9	13458.2	20017.2	29732.4	18342.1	13409.1	23031.3	Std.
161	*n*-Undecane	37621.0	39047.4	18959.6	45385.5	41460.5	39819.2	38637.4	19611.4	25849.8	44402.5	22811.5	Std.
163	*n*-Nonanal	2155.9	64038.2	49280.4	68797.4	77973.7	65984.1	57851.1	122833.1	73380.7	99283.3	170593.1	Std.
167	Phenyl ethyl alcohol		2117.3	3059.7	3213.1	3966.4	3800.4	3207.1					Std.
168	Isophorone		14674.9	19244.4	18851.4	16789.8	19239.4	18652.2	35893.8	34304.7	41374.2	39871.3	Std.
171	2-Ethylhexanoic acid	13160.5	17870.3	6248.2	25521.7	29638.9	34376.4	16281.5					Std.

173	Benzeneacetonitrile	6083.6	7875.4	7025.9	5676.3	6072.3	9358.3	10049.2		9514.4	8556.8	21022.6	Std.
174	4-*keto*-Isophorone		2103.4	2526.8	2396.3	2120.6	2609.8	2279.5	2419.8	3974.3	4789.3	4761.4	Std.
180	2-Ethylhexyl acetate		13045.7	7529.0					10667.9	8073.8	8781.3	9027.3	Std.
184	Menthol		6750.2	6775.4	4998.1	5565.6	7130.6	6226.8	9077.8	11442.5	11281.6	16183.8	Std.
188	Naphthalene	15102.0	55676.8	61543.8	66515.0	58118.1	62846.3	58853.7	81552.3	85179.8	84639.5	78226.3	Std.
193	Octanoic acid	66872.6	57911.3	19303.1	128939.4	200317.6	42415.4	97905.5	8012.3	147450.3	48486.3	125497.6	Std.
194	α-Terpineol		14528.5	14372.2	4634.3	7163.0	6732.3	8047.6	2507.7	9420.5	3188.5	4874.4	Std.
195	Methyl salicylate	3244.7	4027.9	2706.6	6300.2	6307.3	7941.9	18886.4	1601.2	9789.9	12200.0	53459.6	Std.
196	Dodecane	7386.3	18201.0	11812.7	37677.3	24677.4	33984.4	31433.8	23958.3	42834.3	91327.9	38109.1	Std.
198	*n*-Decanal	2704.5	24012.6	19129.9	31826.8	22468.8	19355.9	23645.5	40854.3	39705.6	54814.9	97392.8	Std.
201	2,3-Dihydrobenzofuran		4810.1	5176.4						13064.9	4680.3	4393.1	T
203	Methenamine	224967.2	348608.6	116701.2	126542.3	216298.9	70760.4	229984.2	286459.0	74625.9	171312.3	56718.4	T
204	Benzothiazole	18637.8	18682.9	25347.2	24801.1	23668.9	26555.0	25649.5	37097.3	41584.6	45257.4	40940.7	Std.
208	Quinoline	330184.7	328802.5	267103.8	300735.7	319768.0	279532.4	341979.4	570968.0	319293.9	392563.5	299395.5	Std.
212	Carvone		4371.0	6462.9	2417.0	4141.5	4113.2	2466.5	5681.7	5677.2	6964.4	7544.3	Std.
214	2,4-Dichlorobenzaldehyde	95329.4	145895.2	133318.6	118587.0	134491.9	111009.2	139356.9	212656.7	203801.0	221397.5	166676.0	T
217	Isoquinoline	170632.9	148109.7	100777.7	126368.4	150203.3	109866.1	136606.6	228053.4	137518.1	141591.8	97696.9	T
221	Nonanoic acid	26451.7	45317.9	14796.1	78045.6	85804.9	28346.8	62199.9	12046.6	81572.8	50809.1	91355.2	Std.
222	Tridecane	2846.7	64323.2	38032.5	219654.7	70412.7	98540.4	107585.5	73041.2	235009.8	617883.7	248084.5	Std.
223	Indole	3697.1	5666.2	5503.3	8193.9	8031.0	8414.9	7425.6	7385.2	5339.0	7140.0	5406.0	Std.
227	1-Methylnaphthalene	16359.3	25142.5	27304.6	27451.1	23665.2	29453.9	29046.0	36956.1	41103.4	40393.2	40009.9	Std.
228	1-Methylisoquinoline	70144.2	72787.2	50324.1	60807.7	70654.9	52393.5	70788.5	103675.9	63201.0	66714.0	45421.4	T
229	2-Methylnaphthalene	11381.7	9619.7	12788.3	10831.3	8752.5	11850.4	11810.9	15918.5	16163.5	15684.0	20172.7	T
230	2,6-Dichloroacetophenone	10960.2	14053.7	13864.3	16576.6	14567.2	18258.2	17688.5	36389.2	35706.4	39799.6	28469.3	T
231	1(3*H*)-Isobenzofuranone	46110.4	37274.4	63223.5	42105.7	67387.9	54562.0	47825.2	78924.3	88539.8	94528.2	71627.1	T
232	5-Methylquinoline	39453.9	35531.9	21347.0	27590.3	33693.1	25805.1	33800.8	59980.9	25384.0	31576.0	22318.0	T

234	(3*Z*)-Hexenyl hexenoate	1209.6	3562.2	3135.0	4328.0	4286.0	5088.8	5235.8	4615.0	4158.6	4570.5	9398.7	T
235	Decanoic acid	11908.7	20022.7	3657.2	25663.4	32369.8	10804.6	22041.8		30857.0	22741.5	50553.8	Std.
236	Biphenyl	29473.2	30065.5	35790.5	36161.1	34231.7	38265.9	37771.9	55458.8	60251.3	59989.0	58180.8	T
237	1,4-Dimethylnaphthalene	10402.2	11949.9	14915.1	12713.3	12459.0	15415.3	14832.5	19918.4	22365.7	23515.2	24469.6	T
238	β-Bourbonene		5386.3	6169.4					7884.7	8807.3	11917.0	7987.8	T
239	Tetradecane (C14)		48302.6	21396.0	60466.1	55088.3	53079.8	63975.8	40661.4	119576.8	131596.9	142358.8	Std.
240	(*Z*)-Jasmone	2465.9	3984.2	4839.4	5813.5	5563.8	5256.0	5229.2	3160.3	5440.1	3963.8	5685.0	Std.
245	(*E*)-Caryophyllene	7545.0	23117.1	21912.6	27884.2	32446.7	28762.1	30905.9	22862.5	25137.4	24503.2	26460.1	Std.
246	1,3-Dimethylnaphthalene	13433.7	15174.9	17838.9	14742.0	14506.3	16922.6	17371.8	25150.0	25041.7	26906.9	27272.0	T
247	Coumarin	31998.4	49210.6	92371.0	44027.1	43823.3	59936.8	80092.1	23881.5	106357.6	91461.2	67182.6	T
248	Geranyl acetone	3905.3	15089.0	2474.8	16138.4	14893.8	16532.3	18037.6	20865.7	23080.2	18774.3	50054.4	Std.
249	γ-Decalactone	11331.9	10737.4										Std.
250	*n*-Dodecanol	31461.9	44230.0	24312.4	43352.2	49869.7	38546.3	55543.8	43002.1	41099.0	49397.6	79277.0	Std.
251	α-Amorphene	2108.7	5283.0	4730.2	5349.2	4418.0	5830.2	5388.2	5489.3	6335.9	6875.7	11908.8	T
252	4-Methylbiphenyl	10217.3	9741.9	13546.5	11099.2	11869.5	12888.2	13037.3	17357.9	18939.3	20916.8	21688.8	T
254	Pentadecane		189706.9	30981.8	141067.3	131902.4	132259.7	239798.5	74999.8	283560.3	249610.3	291720.5	Std.
255	α-Muurolene	1993.3	5340.8	8169.6	6326.5	6458.1	8028.9	10968.3	7291.1	9689.1	7623.1	11216.2	T
258	2,5-*bis*(1,1-Dimethylethyl)phenol				18129.8	16370.8	14921.6	13792.4					T
259	γ-Cadinene		3189.3	4755.9	5150.5	5563.0	7442.8	6031.1	5193.0	4936.3	5161.3	5569.5	T
260	(*E,E*)-α-Farnesene	4576.2	4052.2	4454.1	4544.6	6517.0	11041.5	10770.9	8577.8	6535.9	16508.7	305145.2	T
261	Dibenzofuran	30746.5	57645.4	75745.9	62805.4	67092.2	70587.2	69928.4	98197.8	107944.4	117714.8	120878.9	Std.
263	δ-Cadinene		4694.1	4679.4	4036.6	3946.3	4566.0	6319.5	5014.2	5562.6	4573.5	6595.6	T
264	*cis*-Calamenene	8666.8	20990.7	20759.0	20279.9	16663.1	19612.3	34954.8	26762.0	31063.1	35571.4	29570.6	T
265	Dihydroactiniolide	8162.9	12454.0	19239.8	15983.6	19920.8	16527.7	19445.2	23215.0	25156.4	18634.5	23944.9	T
266	α-Calacorene	2686.8	3791.8	4290.7	3445.5	4305.0	5186.4	8881.2	6474.5	6269.4	6471.7	5288.2	T

268	Dodecanoic acid	540319.4	715349.3	20008.6	1045949.1	901777.3	314890.9	540107.5					Std.
269	Fluorene	44308.1	37736.9	51242.9	39863.4	49687.1	45679.9	17774.1	55969.7	63259.9	73276.3	71401.9	T
270	(2*E*)-Hexenyl benzoate	19209.0	18785.3	11562.1	15015.1	13816.4	14416.8	20593.8					T
271	Fokienol	31685.1	28927.3	38854.5	24297.6	32815.9	32344.6	35339.4	43153.9	39390.8	45297.9	48789.8	T
272	Hexadecane (C16)		805627.3		314642.5	275558.5	523289.7	823531.3	131944.2	239372.7	194200.1	265831.6	Std.
274	Cedrol	104714.5	144081.6	137765.4	117149.3	134605.1	172058.2	170432.8	144586.0	147956.2	157510.7	173552.6	T
277	*epi*-α-Cadinol	2520.9	3741.6	4704.5									T
279	(*Z*)-Methyl jasmonate	20047.9	110068.3	71407.2	72841.0	88200.3	121085.2	174867.1	72310.0	62808.2	69634.7	67605.8	Std.
281	α-Cadinol	9188.4	36068.0	26898.9	17735.2	28895.9	32864.0	29588.9	31118.3	16825.5	25153.4	33481.9	T
282	Cadalene	6179.3	4041.4	8369.5	3994.0	6025.1	4039.5	7688.9	5597.5		5970.8	5117.5	T
286	Heptadecane (C17)	19799.6	326286.5	199269.2	204941.1	302365.1	557261.6	417479.8	104007.6	153478.5	161468.0	164873.5	Std.
289	Octadecane (C18)	51090.3	151036.3	140262.9	124337.4	169215.0	231030.7	157959.2	59261.6	88665.3	86568.1	103305.1	Std.
291	Isopropyl myristate	2123.1	2631.9	1388.2	4374.1	3582.3	3177.7	3497.3	1322.1	1788.0	2687.6	2981.6	Std.
295	*n*-Hexadecanol	309821.6	342583.3	67463.7	431270.4	384634.8	345837.8	358047.8	37034.0	59379.4	41960.5	53319.6	Std.
296	Methyl palmitate	22548.7	15850.5	5499.2	22634.0	31896.0	9516.8	18607.0	10759.1	24494.8	25353.5	30351.9	Std.
297	Nonadecane (C19)				110993.3	141309.9	134508.3	120929.0	47791.8	85624.1	71436.0	90244.3	Std.
300	Isopropyl palmitate	25540.0	26953.2	23795.4	46197.8	33635.3	39984.6	12746.7	6799.8	12863.4	11907.0	16538.6	Std.
301	*n*-Octadecanol	15464.3	13438.0	11316.0	14510.1	13111.2	15005.4	13265.9	13603.5	9995.3	11221.0	8477.9	T
302	Heneicosane	31528.2	26401.3	21763.9	41243.2	52629.2	25823.6	30929.8	21541.3	26836.1	28573.4	33886.3	Std.
303	Docosane	49485.4	50597.0	7810.1	55935.6	65727.5	52731.6	41555.2	8945.7	8702.3	15062.5	15660.0	Std.
305	Tricosane	19932.5	29910.2	6229.3	12842.5	16367.2	8775.4	8208.5	11800.3	17196.0	18550.7	15271.1	Std.
306	Pentacosane	18094.2	41201.0	3422.7	6808.7	5978.0	1057.4	4235.1	11163.9	14337.0	15898.3	15739.1	Std.

Note: Positively (STD) or tentatively (T) identified by comparing sample and reference standard or commercial library data

Table 5-5. PCA Correlations for Field Trial[159]

No.[a]	Compound	r	p-value
PC1			
35	2-Ethylhexene	-0.911	0.0007
47	3,5-Dimethyl-2-hexene	-0.901	0.001
62	*m*-Xylene	0.924	0.0004
63	*p*-Xylene	0.928	0.0004
70	2,6-Dimethyl-1,5-heptadiene	0.838	0.005
77	*o*-Xylene	0.924	0.0004
78	Cyclohexanone	0.937	0.0003
95	Benzaldehyde	0.936	0.0003
96	1-Ethyl-4-methylbenzene	0.944	0.0002
98	1-Ethyl-3-methylbenzene	0.978	0.000007
103	1-Ethyl-2-methylbenzene	0.981	0.000005
110	Phenol	-0.861	0.003
120	(*Z*)-Herboxide	0.861	0.003
127	1,2,4-Trimethylbenzene	0.944	0.0002
136	Lavender lactone	0.885	0.002
138	1-Methyl-2-pyrrolidinone	0.861	0.003
139	Benzene acetaldehyde	0.960	0.00007
141	δ-Valeryllactone	0.900	0.001
163	*n*-Nonanal	0.862	0.003
168	Isophorone	0.981	0.000005
174	4-*keto*-Isophorone	0.929	0.0004
184	Menthol	0.959	0.00007
188	Naphthalene	0.926	0.0004
198	*n*-Decanal	0.847	0.005
204	Benzothiazole	0.909	0.0008
212	Carvone	0.888	0.002
227	1-Methylnaphthalene	0.964	0.00005
230	2,6-Dichloroacetophenone	0.839	0.005
236	Biphenyl	0.905	0.0009
237	1,4-Dimethylnaphthalene	0.940	0.0002
246	1,3-Dimethylnaphthalene	0.927	0.0004
252	4-Methylbiphenyl	0.917	0.0006
261	Dibenzofuran	0.987	0.000002
265	Dihydroactiniolide	0.871	0.003
270	(2*E*)-Hexenyl benzoate	-0.870	0.003

PC2			
45	Butanoic acid	-0.846	0.0317
55	(2*E*)-Hexenal	-0.874	0.0317
167	Phenyl ethyl alcohol	-0.845	0.0317
245	(*E*)-Caryophyllene	-0.846	0.0317
289	Octadecane (C18)	-0.878	0.0317

[a] Compounds are numbered and identified according to Table 5-4.

Chapter 6. Conclusion and Future Work

This dissertation demonstrates that the combination of automated sequential GC-GC/MS, to produce targeted metabolite databases, and GC/MS with spectral deconvolution and MS subtraction, to track metabolites across samples, is a powerful, efficient, and comprehensive approach towards understanding climate effects on tea quality. This targeted/untargeted approach (Chapter 2) was used to assess elevational (Chapter 3) and seasonal effects on tea chemistry and quality across a three year period (Chapter 4) and determine *in situ* effects of herbivory on tea chemistry (Chapter 5). No other analytical tool is capable of providing this level of detail for such a vast array of secondary metabolites in tea.

Neither chemical nor sensory analysis alone is sufficient to understand the complex linkage between secondary metabolite chemistry and product quality. While the work presented in this dissertation describes metabolomic profiling of volatiles present in tea, it does not provide the means to determine which compounds or mixture of compounds is responsible for imparting the aroma of tea. While aroma characteristics of compounds are presented, it is unknown whether they are present at concentrations above their odor threshold.[277] In order to link the chemical information and sensory characteristics, experiments should be performed on a GC with dual detection by MS and Olfactometry (GC-O).[278] GC-O uses a trained sensory panelist on the end of a sniffing port to detect and evaluate volatile metabolites (known and unknown) eluting from the column.

Column effluent can be split 30:60 between the MS and sniffing port to compare mass spectral information with odor characteristics.

In the same regard, this work does not provide the means to determine which compounds or mixtures of compounds are responsible for the nutraceutical properties of tea. Work is needed to quantify compounds with reported health-beneficial properties to determine if they are present in concentrations high enough to impart the reported affect. The results of this and the GC-O work will enable researchers to make clear statements about how each climate parameter effects the sensory and nutritional quality of tea.

While GC/MS is ideal for detecting low boiling, thermally stable metabolites, LC/MS is better for the detection of high molecular weight, thermally unstable organics such as methylxanthines, catechins, other polyphenols and amino acids, which are key contributors to the taste of tea, as well as the stimulant and health benefits. Preliminary work determined the concentration of eight catechins and three methylxanthines decrease from spring to summer and from low to high elevation.[22, 159] Despite well-established methods for the analysis of tea polyphenols, few comprehensive studies of non-volatiles in tea by LC/MS exist.[26, 279-280] Towards this end it would be beneficial for the future of this work to include metabolomic profiling of polyphenolics and other non-volatiles in tea using automated sequential, multidimensional liquid chromatography/mass spectrometry (LC-LC/MS) to build a non-volatile tea database. This database can

then be used in the same manner as the volatile tea database to employ a targeted/untargeted approach for routine LC/MS analyses. The information gained from this and other information our interdisciplinary team has assembled would provide advice to farmers on how best to address the expected changes, both small and extreme, in climate and the knowledge to understand the complex relationships and feedback loops between human and natural systems.

References

1. Kang, Y.; Khan, S.; Ma, X., Climate change impacts on crop yield, crop water productivity and food security - A review. *Prog. Nat. Sci.* **2009,** *19*, 1665-1674.

2. Kurukulasuriya, P.; Rosenthal, S. *Climate change and agriculture: a review of impacts and adaptations*; World Bank: Washington, DC, 2013.

3. IPCC, *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aaspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, 2014.

4. USGCRP *Climate Science Special Report: Fourth National Climate Assessment, Volume 1*; U.S. Global Change Research Program: Washington, DC, 2017; p 470.

5. Vermeulen, S. J.; Campbell, B. M.; Ingram, J. S. I., Climate Change and Food Systems. *Ann. Rev. Environ. Resourc.* **2012,** *37*, 195-222.

6. Wheeler, T.; von Braun, J., Climate Change Impacts on Global Food Security. *Science* **2013,** *341* (6145), 508.

7. Myers, S. S.; Zanobetti, A.; Kloog, I.; Huybers, P.; Leakey, A. D. B.; Bloom, A. J., et al., Increasing CO2 threatens human nutrition. *Nature* **2014,** *510*, 139.

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

9. Peñuelas, J.; Staudt, M., BVOCs and global change. *Trends Plant Sci.* **2010,** *15* (3), 133-144.

10. Han, W. *Climate Change and its Impact on Tea Economy Counteract Strategies in China*; Intergovernmental Group on Tea of the FAO: Colombo, Sri Lanka, 2012.

11. Challinor, A. J.; Wheeler, T. R.; Craufurd, P. Q.; Ferro, C. A. T.; Stephenson, D. B., Adaptation of crops to climate change through genotypic responses to mean and extreme temperatures. *Agric. Ecosyst. Environ.* **2007,** *119* (1), 190-204.

12. Chen, M.-L., Tea and Health - An Overview. In *Tea Bioactivity and Therapeutic Potential*, Zhen, Y.-s., Ed. Taylor & Francis: New York, NY, 2002.

13. Sharangi, A. B., Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) - A review. *Food Res. Int.* **2009,** *42*, 529-535.

14. Vuong, Q. V., Epidemiological Evidence Linking Tea Consumption to Human Health: A Review. *Crit. Rev. Food Sci. Nutr.* **2014,** *54* (4), 523-536.

15. Hayat, K.; Iqbal, H.; Malik, U.; Bilal, U.; Mushtaq, S., Tea and Its Consumption: Benefits and Risks. *Crit. Rev. Food Sci. Nutr.* **2015,** *55* (7), 939-954.

16. Yang, C. S.; Hong, J., Prevention of Chronic Diseases by Tea: Possible Mechanisms and Human Relevance. *Annu. Rev. Nutr.* **2013,** *33* (1), 161-181.

17. Chang, K., World tea production and trade: Current and future development. Food and Agriculture Organization of the United Nations: Rome, 2015.

18. Zhai, P.; Zhang, X.; Wan, H.; Pan, X., Trends in Total Precipitation and Frequency of Daily Precipitation Extremes over China. *J. Clim.* **2005,** *18* (7), 1096-1108.

19. Boehm, R.; Cash, S.; Anderson, B.; Ahmed, S.; Griffin, T.; Robbat, A., et al., Association between Empirically Estimated Monsoon Dynamics and Other Weather Factors and Historical Tea Yields in China: Results from a Yield Response Model. *Climate* **2016,** *4* (2), 20.

20. Wang, J.; Huang, J.; Rozell, S., Climate Change and China's Agricultural Sector: An Overview of Impacts, Adaptation and Mitigation. Change, I.-I. P. o. C., Ed. Agriculture and Trade: International Centre for Trade and Sustainable Development, Geneva, Switzerland and International Food & Agricultural Trade Policy Council, Washington, DC, USA.

21. Kowalsick, A.; Kfoury, N.; Robbat Jr, A.; Ahmed, S.; Orians, C.; Griffin, T., et al., Metabolite profiling of *Camellia sinensis* by automated sequential, multidimensional gas chromatography/mass spectrometry reveals strong monsoon effects on tea constituents. *J Chromatogr. A* **2014,** *1370*, 230-239.

22. Ahmed, S.; Stepp, J. R.; Orians, C.; Griffin, T.; Matyas, C.; Robbat Jr, A., et al., Effects of Extreme Climate Events on Tea (*Camellia sinensis*) Functional Quality Validate Indigenous Farmer Knowledge and Sensory Preferences in Tropical China. *PloS One* **2014,** *9* (10), e109126.

23. Braicu, C.; Ladomery, M. R.; Chedea, V. S.; Irimie, A.; Berindan-Neagoe, I., The relationship between the structure and biological actions of green tea catechins. *Food Chem.* **2013,** *141* (3), 3282-3289.

24. Chen, X.-Q.; Hu, T.; Han, Y.; Huang, W.; Yuan, H.-B.; Zhang, Y.-T., et al., Preventive Effects of Catechins on Cardiovascular Disease. *Molecules* **2016,** *21* (12).

25. Chen, Z.-m.; Lin, Z., Tea and human health: biomedical functions of tea active components and current issues. *J. Zhejiang. Univ. Sci. B* **2015,** *16* (2), 87-102.

26. Kalili, K. M.; de Villiers, A., Off-line comprehensive two-dimensional hydrophilic interaction×reversed phase liquid chromatographic analysis of green tea phenolics. *J. Sep. Sci.* **2010,** *33* (6-7), 853-863.

27. Yang, Z.; Baldermann, S.; Watanabe, N., Recent studies of the volatile compounds in tea. *Food Res. Int.* **2013,** *53* (2), 585-599.

28. Davis, J. M.; Giddings, J. C., Statistical theory of component overlap in multicomponent chromatograms. *Anal. Chem.* **1983,** *55* (3), 418-424.

29. Giddings, J. C., Sample dimensionality: A predictor of order-disorder in component peak distribution in multidimensional separation. *J Chromatogr. A* **1995,** *703* (1), 3-15.

30. Beens, J. Chromedia.

31. Giddings, J. C., Two-dimensional separations: concept and promise. *Anal. Chem.* **1984,** *56* (12), 1258A-1270A.

32. Giddings, J. C., Concepts and comparisons in multidimensional separation. *J. High Resolut. Chromatogr.* **1987,** *10* (5), 319-323.

33. Mac Namara, K.; Leardi, R.; Hoffman, A., Developments in 2D GC with Heartcutting. *LCGC North America* **2004**, 82-91.

34. Górecki, T.; Harynuk, J.; Panić, O., The evolution of comprehensive two-dimensional gas chromatography (GC×GC). *J. Sep. Sci.* **2004,** *27* (5-6), 359-379.

35. Prebihalo, S. E.; Berrier, K. L.; Freye, C. E.; Bahaghighat, H. D.; Moore, N. R.; Pinkerton, D. K., et al., Multidimensional Gas Chromatography: Advances in Instrumentation, Chemometrics, and Applications. *Anal. Chem.* **2018,** *90* (1), 505-532.

36. Seeley, J. V., Recent advances in flow-controlled multidimensional gas chromatography. *J Chromatogr. A* **2012,** *1255*, 24-37.

37. Seeley, J. V.; Seeley, S. K., Multidimensional Gas Chromatography: Fundamental Advances and New Applications. *Anal. Chem.* **2013,** *85* (2), 557-578.

38. Shi, X.; Wang, S.; Yang, Q.; Lu, X.; Xu, G., Comprehensive two-dimensional chromatography for analyzing complex samples: recent new advances. *Anal. Methods* **2014,** *6* (18), 7112-7123.

39. Robbat Jr, A.; Kowalsick, A., Using Automated Sequential Two-dimensional Gas Chromatography/Mass Spectrometry to produce a Library of Essential Oil Compounds and Track their presence in Gin, Based on Spectral Deconvolution Software. In *Flavor, Frangrance, and Odor Analysis*, Marsili, R., Ed. CRC Press: Boca Raton, 2011; pp 183-206.

40. Antle, P. M.; Zeigler, C. D.; Wilton, N. M.; Robbat Jr, A., A More Accurate Analysis of Alkylated PAH and PASH and its Implications in Environmental Forensics. *Int. J. Environ. Anal. Chem.* **2014,** *94* (4), 332-347.

41. Robbat, A.; Kowalsick, A.; Howell, J., Tracking juniper berry content in oils and distillates by spectral deconvolution of gas chromatography/mass spectrometry data. *J Chromatogr. A* **2011,** *1218* (32), 5531-5541.

42. Rasmussen, G. T.; Isenhour, T. L., The Evaluation of Mass Spectral Search Algorithms. *J. Chem. Inf. Mod.* **1979,** *19* (3), 179-186.

43. Stein, S. E.; Scott, D. R., Optimization and testing of mass spectral library search algorithms for compound identification. *Journal of the American Society of Mass Spectrometry* **1994,** *5* (9), 859-886.

44. McLafferty, F. W.; Zhang, M.; Stauffer, D. B.; Loh, S. Y., Comparison of algorithms and databases for matching unknown mass spectra. *J. Am. Soc. Mass Spectrom.* **1998,** *9* (1), 92-95.

45. Stein, S. E., Mass Spectral Reference Libraries: An Ever-Expanding Resource for Chemical Identification. *Anal. Chem.* **2012,** *84* (17), 7274-7282.

46. Sparkman, D., Informatics and Mass Spectral Databases in the Evaluation of Environmental Mass Spectral Data. In *Comprehensive Environmental Mass Spectrometry*, Lebedev, A., Ed. ILM Publications: Saint Albans, 2012; pp 89-121.

47. Koo, I.; Kim, S.; Zhang, X., Comparative analysis of mass spectral matching-based compound identification in gas chromatography–mass spectrometry. *J Chromatogr. A* **2013,** *1298*, 132-138.

48. Samokhin., A.; Sotnexova, K.; Lashin, V.; Revelsky, I., Evaluation of mass spectral library search algorithms implemented in commercial software. *J. Mass Spectrom.* **2015,** *50*, 820-825.

49. Koo, I.; Zhang, X.; Kim, S., Wavelet- and Fourier-Transform-Based Spectrum Similarity Approaches to Compound Identification in Gas Chromatography/Mass Spectrometry. *Anal. Chem.* **2011,** *83* (14), 5631-5638.

50. Kim, S.; Koo, I.; Jeong, J.; Wu, S.; Shi, X.; Zhang, X., Compound Identification Using Partial and Semipartial Correlations for Gas Chromatography–Mass Spectrometry Data. *Anal. Chem.* **2012,** *84* (15), 6477-6487.

51. Li, C.; Han, J.; Huang, Q.; Li, B.; Zhang, Z.; Guo, C., An effective two-stage spectral library search approach based on lifting wavelet decomposition for complicated mass spectra. *Chemometr. Intell. Lab Syst.* **2014,** *132*, 75-81.

52. Koo, I.; Kim, S.; Shi, B.; Lorkiewicz, P.; Song, M.; McClain, C., et al., EIder: A compound identification tool for gas chromatography mass spectrometry data. *J Chromatogr. A* **2016,** *1448*, 107-114.

53. Chapman, J. R., Trends in automatic data processing. *Int. J. Mass Spectrom.* **1982,** *45*, 207-218.

54. Likic, V. A., Extraction of pure components from overlapped signals in gas chromatography-mass spectrometry (GC-MS). *BioData Min.* **2009,** *2* (6).

55. Putri, S. P.; Yamamoto, S.; Tsugawa, H.; Fukusaki, E., Current metabolomics: Techonological advances. *J. Biosci. Bioeng.* **2013,** *116* (1), 9-16.

56. Du, X.; Zeisel, S. H., Spectral Deconvolution for Gas Chromatography Mass Spectrometry-Based Metabolomics: Curremt Status and Future Perspectives. *Comput. Struct. Biotechnol. J.* **2013,** *4* (5), 1-10.

57. Yi, L.; Dong, N.; Yun, Y.; Deng, B.; Ren, D.; Liu, S., et al., Chemometric methods in data processing of mass spectrometry-based metabolomics: A review. *Anal. Chim. Acta* **2016,** *914*, 17-34.

58. Ni, Y.; Qiu, Y.; Jiang, W.; Suttlemyre, K.; Su, M.; Zhang, W., et al., ADAP-GC 2.0:Deconvolution of Coeluting Metabolites from GC/TOF-MS Data for Metabolomics Studies. *Anal. Chem.* **2012,** *84*, 6619-6629.

59. Johnson, M. L.; Pipes, L.; Veldhuis, P. P.; Farhy, L. S.; Boyd, D. G.; Evans, W. S., AutoDecon, a deconvolution algorithm for identification and characterization of leuteinizing hormone secretory bursts: Description and validation using synthetic data. *Anal. Biochem.* **2008,** *381*, 8-17.

60. Stein, S. E., An Integrated Method for Spectrum Extraction and Compound Identification from Gas Chromatography/Mass Spectrometry Data. *J. Am. Soc. Mass Spectrom.* **1999,** *10*, 770-781.

61. Hiller, K.; Hangebrauk, J.; Jager, C.; Spura, J.; Schreilber, K.; Schomburg, D., MataboliteDetector: Comprehensive Analysis Tool for Targetd and Nontargeted GC/MS Based Metabolome Analysis. *Anal. Chem.* **2009,** *81*, 3429-3439.

62. Xia, J.; Mandal, R.; Sinelnikov, I. V.; Boroadhurst, D.; Wishart, D. S., MetaboAnalyst 2.0-a comprehensive server for metabolomic data analysis. *Nucleic Acids Res.* **2012,** *40*, W127-133.

63. Carroll, A. J.; Badger, M. R.; Millar, A. H., The MetabolomeExpress Project: enabling web-based processing, analysis and transparent dissemination of GC/MS metabolomics datasets. *BMC Bioinformatics* **2010,** *11*, 376.

64. Lommen, A., MetAlign: Interface-Driven, Versatile Metabolomics Tool for Hyphenated Full-Scan Mass Spectrometry Data Preprocessing. *Anal. Chem.* **2009,** *81*, 3079-3086.

65. Strohalm, M.; Kavan, D.; Novak, P.; Volny, M.; Havlicek, V., mMass 3: A Cross-Platform Software Environment for Precise Analysis of Mass Spectrometric Data. *Analytical Chemistry* **2010,** *82*, 4648-4651.

66. Pluskal, T.; Castillo, S.; Villar-Briones, A.; Oresic, M., MZmine 2: Modular framework processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* **2010,** *11*, 395.

67. Wenig, P.; Odermatt, J., OpenChrom: a cross-platform open source software for the mass spectrometric analysis of chromatographic data. *BMC Bioinformatics* **2010,** *11*, 405.

68. O'Callaghan, S.; De Souza, D. P.; Isaac, A.; Wang, Q.; Hodkinson, L.; Olshansky, M., et al., PyMS: a Python toolkit for processing of gas chromatography-mass spectrometry (GC-MS) data. Application and comparative study of selected tools. *BMC Bioinformatics* **2012,** *13*, 115.

69. Smits, M. M.; Carleer, R.; Colpaert, J. V., PYQUAN: A rapid workflow around the AMDIS deconvolution software for high throughput analysis of pyrolysis GC/MS data. *J. Anal. Appl. Pyrolysis* **2016,** *118*, 335-342.

70. Styczynski, M. P.; Moxley, J. F.; Tong, L. V.; Walther, J. L.; Jensen, K. L.; Stephanopoulos, G. N., Systematic Identification of Conserved Metabolites in GC/MS Data for Metabolomics and Biomarker Discovery. *Anal. Chem.* **2007,** *79*, 966-973.

71. Luedemann, A.; Strassburg, K.; Erban, A.; Kopka, J., TagFinder for the quantitative analysis of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling experiments. *Bioinformatics* **2008,** *24* (5), 732-737.

72. Skogerson, K.; Wohlgemuth, G.; Barupal, D. K.; Fiehn, O., The volatile compound BinBase mass spectral database. *BMC Bioinformatics* **2011,** *12*, 321.

73. ASTM, Standard Specification for Analytical Data Interchange Protocol for Chromatographic Data. In *E1947-98*, ASTM International: West Conshohocken, PA, 2014.

74. ASTM, Standard Specificaiton for Analytical Data Interchange Protocol for Mass Spectrometric Data. In *E2077-00*, ASTM International: West Conshohocken, PA, 2016.

75. ASTM, Standard Guide for Analytical Data Interchange Protocol for Chromatographic Data. In *E1948-98*, ASTM International: West Conshohocken, PA, 2014.

76. Pripdeevech, P.; Wongpornchai, S., Odor and Flavor Volatiles of Different Types of Tea. In *Tea in Health and Disease Prevention*, 1 ed.; Preedy, V. R., Ed. Elsevier: Chiang Rai/Chiang Mai, 2013; pp 307-322.

77. Zhu, M.; Li, E.; He, H., Determination of Volatile Chemical Constitutes in Tea by Simultaneous Distillation Extraction, Vacuum Hydrodistillation and Thermal Desorption. *Chromatographia* **2008,** *68* (7-8), 603-610.

78. Lv, H.-P.; Zhong, Q.-S.; Lin, Z.; Wang, L.; Tan, J.-F.; Guo, L., Aroma characterisation of Pu-erh tea using headspace-solid phase microextraction combined with GC/MS and GC–olfactometry. *Food Chem.* **2012,** *130* (4), 1074-1081.

79. Babushok, V. I.; Linstrom, P. J.; Zenkevich, I. G., Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *Journal of Physical and Chemical Reference Data* **2011,** *40* (4).

80. Robbat Jr, A.; Kfoury, N.; Baydakov, E.; Gankin, Y., Optimizing targeted/untargeted metabolomics by automating gas chrromatography/mass spectrometry workflows. *J Chromatogr. A* **2017,** *1505*, 96-105.

81. Robbat Jr, A.; Wilton, N. M., A new spectral deconvolution-Selection ion monitoring method for the analysis of alkylated polycyclic aromatic hydrocarbons in complex mixtures. *Talanta* **2014,** *125*, 114-124.

82. Antle, P. M.; Zeigler, C.; Gankin, Y.; Robbat Jr, A., New Spectral Deconvolution Algorithms for the Analysis of Polycyclic Aromatic Hydrocarbons and Sulfur Heterocycles by Comprehensive Two-Dimensional Gas Chromatography-Quadrupole Mass Spectrometery. *Anal. Chem.* **2013,** *85* (21), 10369-10376.

83. Gulati, A.; Rawat, R.; Singh, B.; Ravindranath, S. D., Application of Microwave Energy in the Manufacture of Enhanced-Quality Green Tea. *J. Agric. Food Chem.* **2003,** *51* (16), 4764-4768.

84. StataCorp *Stata Statistical Software: Release 15*, StataCorp LLC: College Station, TX, 2017.

85. Kfoury, N.; Baydakov, E.; Gankin, Y.; Robbat, A., Differentiation of key biomarkers in tea infusions using a target/nontarget gas chromatography/mass spectrometry workflow. *Food Res. Int.* **2018,** (113), 414-423.

86. WorldWeatherOnline World Weather Online. https://www.worldweatheronline.com/ (accessed August 28, 2017).

87. NOAA Environmental Temperature Lapse Rate. http://www.spc.noaa.gov/exper/soundings/help/lapse.html.

88. Iscan, G., Antibacterial and Anticandidal Activities of Common Essential Oil Constituents. *Rec. Nat. Prod.* **2017,** *11* (4), 374-388.

89. Salakhutdinov Nariman, F.; Volcho Konstantin, P.; Yarovaya Olga, I., Monoterpenes as a renewable source of biologically active compounds. *Pure Appl. Chem.* **2017,** *89* (8), 1105.

90. Nakayama, K.; Murata, S.; Ito, H.; Iwasaki, K.; Villareal, M. O.; Zheng, Y.-W., et al., Terpinen-4-ol inhibits colorectal cancer growth via reactive oxygen species. *Oncol. Lett.* **2017,** *14* (2), 2015-2024.

91. Russo, E. B., Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br. J. Pharmacol.* **2011,** *163* (7), 1344-1364.

92. Kim, S.; Thiessen, P. A.; Bolton, E. E.; Chen, J.; Fu, G.; Gindulyte, A., et al., PubChem Substance and Compound databases. *Nucleic Acids Res.* **2016,** *44* (D1), D1202-D1213.

93. Fujita, K.-i.; Chavasiri, W.; Kubo, I., Anti-Salmonella Activity of Volatile Compounds of Vietnam Coriander. *Phytother. Res.* **2015,** *29* (7), 1081-1087.

94. Kim, K. Y.; Lee, H. S.; Seol, G. H., Anti-inflammatory effects of trans-anethole in a mouse model of chronic obstructive pulmonary disease. *Biomed. Pharmacother.* **2017,** *91*, 925-930.

95. Perflavory, The Good Scents Company. Oak Creek, WI, 2015.

96. Han, W.-Y.; Huang, J.-G.; Li, X.; Li, Z.-X.; Ahammed, G. J.; Yan, P., et al., Altitudinal effects on the quality of green tea in east China: a climate change perspective. *Eur. Food Res. Technol.* **2017,** *243*, 323-330.

97. Ahmed, S.; Stepp, J. R., Green Tea: The Plants, Processing, Manufacturing and Production. In *Tea in Health and Disease Prevention*, Preedy, V., Ed. Academic Press: London, UK, 2012.

98. Battinelli, L.; Daniele, C.; Cristiani, M.; Bisignano, G.; Saija, A.; Mazzanti, G., In vitro antifungal and anti-elastase activity of some aliphatic aldehydes from *Olea europaea* L. fruit. *Phytomedicine* **2006,** *13*, 558-563.

99. Das, S. K.; Adhya, S.; Banerhee, A. B., Effect of undecanoic acid on germination of microconidia of wild and undecanois acid resistant mutant of *Trichophyton Rubrum*. *Mycopathologia* **1977,** *61* (2), 121-123.

100. Kako, H.; Kobayashi, Y.; Yokogoshi, H., Dopamine release from rat pheochromocytoma (PC12) cells and rat brain striata induced by a series of straigh carbon chain aldehydes with variations in carbon chain length and functional groups. *Eur. J. Pharmacol.* **2012,** *691*, 86-92.

101. Koziol, A.; Stryjewska, A.; Librowski, T.; Salat, K.; Gawal, M.; Moniczewski, A., et al., An Overview of the Pharmacological Properties and Potential Applications of Natural Monoterpenes. *Mini-Rev. Med. Chem.* **2014,** *14*, 1156-1168.

102. Oz, M.; Lozon, Y.; Sultan, A.; Yang, K.-H. S.; Galadari, S., Effects of monoterpenes on ion channels of excitable cells. *Pharmacol. Ther.* **2015,** *152*, 83-97.

103. Yang, J.-Y.; Kim, M.-G.; Park, J.-H.; Hong, S.-T.; Lee, H.-S., Evaluation of benzaldehyde derivatives from *Morinda officinalis* as anti-mite agents with dual function as acaricide and mite indicator. *Sci. Rep.* **2014,** *4*, 7149.

104. Rossi, D.; Guerrini, A.; Maietti, S.; Bruni, R.; Paganetto, G.; Poli, F., et al., Chemical fingerprinting and bioactivity of Amazonian Ecuador *Croton lechleri* Mull. Arg. (Euphorbiaceae) stem bark essential oil: A new functional food ingredient? *Food Chem.* **2011,** *126* (3), 837-848.

105. Tung, Y. T.; Chua, M. T.; Wang, S. Y.; Chang, S. T., Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (Cinnamomum osmophloeum) twigs. *Bioresour. Technol.* **2008,** *99* (9), 3908-3913.

106. Guerrini, A.; Sacchetti, G.; Grandini, A.; Spagnoletti, A.; Asanza, M.; Scalvenzi, L., Cytotoxic Effect and TLC Bioautography-Guided Approach to Detect Health Properties of Amazonian Hedyosmum sprucei Essential Oil. *Evid.-based Complement Altern. Med.* **2016**, 8.

107. Buhrer, C.; Bahr, S.; Siebert, J.; Wettstein, R.; Geffers, C.; Obladen, M., Use of 2% 2-phenoxyethanol and 0.1% octenidine as antiseptic in premature newborn infants of 23-26 weeks gestation. *J. Hosp. Infect.* **2002,** *51*, 305-307.

108. Tung, Y. T.; Yen, P. L.; Lin, C. Y.; Chang, S. T., Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (Cinnamomum osmophloeum) leaves. *Pharm. Biol.* **2010,** *48* (10), 1130-1136.

109. Fidyt, K.; Fiedorowicz, A.; Strzadala, L.; Szumny, A., beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med.* **2016,** *5* (10), 3007-3017.

110. Sun, S.; Du, G. J.; Qi, L. W.; Williams, S.; Wang, C. Z.; Yuan, C. S., Hydrophobic constituents and their potential anticancer activities from Devil's Club (Oplopanax horridus Miq.). *J. Ethnopharmacol.* **2010,** *132* (1), 280-285.

111. Takahashi, A.; Watanabe, T.; Fujita, T.; Hasegawa, T.; Saito, M.; Suganuma, M., Green tea aroma fraction reduces β-amyloid peptide-induced toxicity in Caenhorhabditis elegans transfected with human β-amyloid minigene. *Biosci. Biotechnol. Biochem.* **2014,** *78* (7), 1206-1211.

112. Li, Y.-S.; Kawasaki, Y.; Tomita, I.; Kawai, K., Antioxidant properties of green tea aroma in mice. *J. Clin. Biochem. Nutr.* **2017,** *61* (1), 14-17.

113. Viana, A. F. S. C.; da Silva, F. V.; Fernandes, H. d. B.; Oliveira, I. S.; Braga, M. A.; Nunes, P. I. G., et al., Gastroprotective effect of (-)-myrtenol against ethanol-induced acute gastric lesions: possible mechanisms. *J. Pharm. Pharmacol.* **2016,** *68* (8), 1085-1092.

114. Santos, M. R. V.; Moreira, F. V.; Fraga, B. P.; Souza, D. P. d.; Bonjardim, L. R.; Quintans-Junior, L. J., Cardiovascular effects of monoterpenes: a review. *Rev. Bras. Farmacogn.* **2011,** *21*, 764-771.

115. Souza, R. H. L.; Cardoso, M. S. P.; Menezes, C. T.; Silva, J. P.; De Sousa, D. P.; Batista, J. S., Gastroprotective activity of α-terpineol in two experimental models of gastric ulcer in rats. *J. Pharm. Sci.* **2011,** *19* (4), 277-281.

116. Ghelardini, C.; Galeotti, N.; Mazzanti, G., Local Anaesthetic Activity of Monoterpenes and Phenylpropanes of Essential Oils. *Planta Med* **2001,** *67* (06), 564-566.

117. González-Ramírez, A. E.; González-Trujano, M. E.; Orozco-Suárez, S. A.; Alvarado-Vásquez, N.; López-Muñoz, F. J., Nerol alleviates pathologic markers in the oxazolone-induced colitis model. *Eur. J. Pharmacol.* **2016,** *776*, 81-89.

118. Miron, D.; Battisti, F.; Silva, F. K.; Lana, A. D.; Pippi, B.; Casanova, B., et al., Antifungal activity and mechanism of action of monoterpenes against dermatophytes and yeasts. *Rev. Bras. Farmacogn.* **2014,** *24* (6), 660-667.

119. Hur, S. J.; Park, Y., Effect of conjugated linoleic acid on bone formation and rheumatoid arthritis. *Eur. J. Pharmacol.* **2007,** *568* (1), 16-24.

120. Moon, H.-S., Biological effects of conjugated linoleic acid on obesity-related cancers. *Chem. Biol. Interact.* **2014,** *224*, 189-195.

121. Brul, S.; Coote, P., Preservative agents in foods: Mode of action and microbial resistance mechanisms. *Int. J. Food Microbiol.* **1999,** *50* (1), 1-17.

122. Abuhamdah, S.; Talji, D.; Abuirmeileh, N.; Bahnassi, A.; Salahat, I.; Abuirmeileh, A., Behavioral and Neurochemical Alterations Induced by Vanillin in a Mouse Model of Alzheimer's Disease. *Int. J. Pharmacol.* **2017,** *13*, 573-582.

123. Behl, T.; Kotwani, A., Omega-3 fatty acids in prevention of diabetic retinopathy. *J. Pharm. Pharmacol.* **2017,** *69* (8), 946-954.

124. Pavan, B.; Dalpiaz, A.; Marani, L.; Beggiato, S.; Ferraro, L.; Canistro, D., et al., Geraniol Pharmacokinetics, Bioavailability and Its Multiple Effects on the Liver Antioxidant and Xenobiotic-Metabolizing Enzymes. *Front. Pharmacol.* **2018,** *9* (18).

125. Astani, A.; Reichling, J.; Schnitzler, P., Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. *Phytother. Res.* **2010,** *24* (5), 673-679.

126. Wölkart, G.; Schrammel, A.; Koyani, C. N.; Scherübel, S.; Zorn-Pauly, K.; Malle, E., et al., Cardioprotective effects of 5-hydroxymethylfurfural mediated by inhibition of L-type Ca2+ currents. *Br. J. Pharmacol.* **2017,** *174* (20), 3640-3653.

127. Kim, H. K.; Choi, Y.-W.; Lee, E. N.; Park, J. K.; Kim, S.-G.; Park, D.-J., et al., 5-Hydroxymethylfurfural from Black Garlic Extract Prevents TNFα-induced Monocytic Cell Adhesion to HUVECs by Suppression of Vascular Cell Adhesion Molecule-1 Expression, Reactive Oxygen Species Generation and NF-κB Activation. *Phytother. Res.* **2011,** *25* (7), 965-974.

128. Kim, G.; Kim, J.-E.; Kang, M.-J.; Jang, A.-R.; Kim, Y. R.; Kim, S., et al., Inhibitory effect of 1-tetradecanol on Helicobacter pylori-induced production of interleukin-8 and vascular endothelial growth factor in gastric epithelial cells. *Mol. Med. Rep.* **2017,** *16* (6), 9573-9578.

129. Zhang, Z.-Z.; Li, Y.-B.; Qi, L.; Wan, X.-C., Antifungal Activities of Major Tea Leaf Volatile Constituents toward Colletorichum camelliae Massea. *J. Agric. Food Chem.* **2006,** *54* (11), 3936-3940.

130. Vahid, H.; Rakhshandeh, H.; Ghorbani, A., Antidiabetic properties of Capparis spinosa L. and its components. *Biomed. Pharmacother.* **2017,** *92*, 293-302.

131. Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D., The Pharmacology, Metabolism, Analysis, and Applications of Coumarin and Coumarin-Related Compounds. *Drug. Metab. Rev.* **1990,** *22*, 503-529.

132. Chakraborty, S.; Kar, N.; Kumari, L.; De, A.; Bera, T., Inhibitory effect of a new orally active cedrol-loaded nanostructured lipid carrier on compound 48/80-induced mast cell degranulation and anaphylactic shock in mice. *Int. J. Nanomedicine* **2017,** *12*, 4849-4868.

133. Zhang, S.-Y.; Li, X.-B.; Hou, S.-G.; Sun, Y.; Shi, Y.-R.; Lin, S.-S., Cedrol induces autophagy and apoptotic cell death in A549 non-small cell lung carcinoma cells through the P13/Akt signaling pathway, the loss of mitochondrial transmembran potential and the generation of ROS. *Int. J. Mol. Med.* **2016,** *38* (1), 291-299.

134. Dayawansa, S.; Umeno, K.; Takakura, H.; Hori, E.; Tabuchi, E.; Nagashima, Y., et al., Autonomic responses during inhalation of natural fragrance of "Cedrol" in humans. *Auton. Neurosci.* **2003,** *108* (1), 79-86.

135. Cheriyan, B.; Kadhirvelu, P.; Nadipelly, J.; Shanmugasundaram, J.; Sayeli, V.; Subramanian, V., Anti-nociceptive effect of 7-methoxy coumarin from Eupatorium Triplinerve vahl (Asteraceae). *Pharmacogn. Mag.* **2017,** *13* (49), 81-84.

136. Mousavi, S. H.; Davari, A.-S.; Iranshahi, M.; Sabouri-Rad, S.; Tayarani Najaran, Z., Comparative analysis of the cytotoxic effect of 7-prenyloxycoumarin compounds and herniarin on MCF-7 cell line. *Avicenna J. Phytomed.* **2015,** *5* (6), 520-530.

137. Silván, A. M.; Abad, M. J.; Bermejo, P.; Sollhuber, M.; Villar, A., Antiinflammatory Activity of Coumarins from Santolina oblongifolia. *J. Nat. Prod.* **1996,** *59* (12), 1183-1185.

138. Zheng, M.; Shi, J.; Shi, J.; Wang, Q.; Li, Y., Antimicrobial effects of volatiles produced by two antagonistic Bacillus strains on the anthracnose pathogen in postharvest mangos. *Biol. Control* **2013,** *65* (2), 200-206.

139. Alaribe Chinwe, S. A.; Anyakora, C.; Ota, D.; De Waard, M.; Coker, H. A. B., GC-MS analysis, anti-inflammatory and anti-seizure effects of n-octanoic acid from special breed palm kernel nut oil. *Planta Med* **2014,** *80* (10), PD118.

140. Nahab, F. B.; Handforth, A.; Brown, T.; Shin, C.; Quesada, A.; Dong, C., et al., Octanoic Acid Suppresses Harmaline-Induced Tremor in Mouse Model of Essential Tremor. *Neurotherapeutics* **2012,** *9* (3), 635-638.

141. Donati, M.; Mondin, A.; Chen, Z.; Miranda, F. M.; do Nascimento, B. B.; Schirato, G., et al., Radical scavenging and antimicrobial activities of Croton zehntneri, Pterodon emarginatus and Schinopsis brasiliensis essential oils and their major constituents: estragole, trans-anethole, β-caryophyllene and myrcene. *Nat. Prod. Res.* **2015,** *29* (10), 939-946.

142. Chang, P.; Terbach, N.; Plant, N.; Chen, P. E.; Walker, M. C.; Williams, R. S. B., Seizure control by ketogenic diet-associated medium chain fatty acids. *Neuropharmacology* **2013,** *69*, 105-114.

143. Muroi, H.; Kubo, I., Combination Effects of Antibacterial Compounds in Green Tea Flavor Against *Streptococcus-mutans*. *J. Agric. Food Chem.* **1993,** *41* (7), 1102-1105.

144. Tong, Q. S.; Jiang, G. S.; Zheng, L. D.; Tang, S. T.; Cai, J. B.; Liu, Y., et al., Natural jasmonates of different structures suppress the growth of human neuroblastoma cell line SH-SY5Y and its mechanisms. *Acta Pharmacol. Sin.* **2008,** *29* (7), 861-869.

145. Furtado Kelly, S.; de Oliveira Andrade, F.; Campos, A.; Rosim Mariana, P.; Vargas-Mendez, E.; Henriques, A., et al., β-ionone modulates the expression of miRNAs and genes involved in the metastatic phenotype of microdissected persistent preneoplastic lesions in rats submitted to hepatocarcinogenesis. *Mol. Carcinog.* **2016,** *56* (1), 184-196.

146. Trad, M.; Gaaliche, B.; Renard, C. M. G. C.; Mars, M., Inter- and intra-tree variability in quality of figs. Influence of altitude, leaf area and fruit position in the canopy. *Sci. Hort.* **2013,** *162*, 49-54.

147. Issaoui, M.; Flamini, G.; Brahmi, F.; Dabbou, S.; Hassine, K. B.; Taamali, A., et al., Effect of the growing area conditions on differentiation between Chemlali and Chétoui olive oils. *Food Chem.* **2010,** *119* (1), 220-225.

148. Yue, T.-X.; Chi, M.; Song, C.-Z.; Liu, M.-Y.; Meng, J.-F.; Zhang, Z.-W., et al., Aroma Characterization of Cabernet Sauvignon Wine from the Plateau of Yunnan (China) with Different Altitudes Using SPME-GC/MS. *Int. J. Food Prop.* **2015,** *18* (7), 1584-1596.

149. Owuor, P. O.; Obaga, S. O.; Othieno, C. O., The Effects of Altitude on the Chemical Composition of Black Tea. *J. Sci. Food Agric.* **1990,** *50* (1), 9-17.

150. Mahanta, P. K.; Baruah, S.; Owour, P. O.; Murai, T., Flavour Volatiles of Assam CTC Black Teas Manufactured from Different Plucking Standards and Orthodox Teas Manufactured from Different Altitudes of Darjeeling. *J. Sci. Food Agric.* **1988,** *45* (4), 317-324.

151. Chen, G.-H.; Yang, C.-Y.; Lee, S.-J.; Wu, C.-C.; Tzen, J. T. C., Catechin content and the degree of its galloylation in oolong tea are inversely correlated with cultivation altitude. *J. Food Drug Anal.* **2014,** *22*, 303-309.

152. Zhang, C.; Suen, C. L.-C.; Yang, C.; Quek, S. Y., Antioxidant capacity and major polyphenol composition of teas as affected by geographical location, plantation elevation and leaf grade. *Food Chem.* **2018,** *244*, 109-119.

153. Chen, Y.; Jiang, Y.; Duan, J.; Shi, J.; Xue, S.; Kakuda, Y., Variation in catechin contents in relation to quality of 'Huang Zhi Xiang' Oolong tea (*Camellia sinensis*) at various growing altitudes and seasons. *Food Chem.* **2010,** *119* (2), 648-652.

154. ASTM, Manual on Descriptive Anlysis Testing for Sensory Evaluation. In *The Flavor Profile Method*, American Society of Testing Materials: Philadelphia , PA, 1992; Vol. MNL 13.

155. R *R: A language and environment for statistical computing*, R Foundation for Statistical Computing: Vienna, Austria, 2014.

156. Thèvenot, E. A.; Roux, A.; Xu, Y.; Exan, E.; Junot, C., Analysis of the Humnan Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. *J. Proteome Res.* **2015,** *14* (8), 3322-3335.

157. Shepherd, T.; Griffiths, D. W., The effects of stress on plant cuticular waxes. *New Phytol.* **2006,** *171*, 469-499.

158. Bellincontro, A.; Matarese, F.; D'Onofrio, C.; Accordini, D.; Tosi, E.; Mencarelli, F., Management of postharvest grape withering to optimise the aroma of the final wine: A case study on Amarone. *Food Chem.* **2016,** *213*, 378-387.

159. Kfoury, N.; Morimoto, J.; Kern, A.; Scott, E. R.; Orians, C. M.; Ahmed, S., et al., Striking changes in tea metabolites due to elevational effects. *Food Chem.* **2018,** *264*, 334-341.

160. Vazquez-Cruz, M. A.; Jimenez-Garcia, S. N.; Torres-Pacheco, I.; Guzman-Maldonado, S. H.; Guevara-Gonzalez, R. G.; Miranda-Lopez, R., Effect of Maturity

Stage and Storage on Flavor Compounds and Sensory Description of Berrycactus (*Myrtillocactus geometrizans*). *J. Food Sci.* **2012,** *77* (4), C366-C373.

161. Acree, T.; Arn, H., Flavornet and human odor space. Cornell University: Geneva, NY, 2004.

162. Joshi, R.; Gulati, A., Fractionation and identification of minor and aroma-active constituents in Kangra orthodox black tea. *Food Chem.* **2015,** *167*, 290-298.

163. Whitener, M. E. B.; Stanstrup, J.; Panzeri, V.; Carlin, S.; Divol, B.; Du Toit, M., et al., Untangling the wine metabolome by combining untargeted SPME-GCxGC-TOF-MS and sensory analysis to profile Sauvignon blanc co-fermented with seven different yeasts. *Metabolomics* **2016,** *12* (3), 25.

164. Olivier, D. K.; Van Wyk, B. E., The major diterpenoids of the genus Arctopus (Apiaceae) with notes on their chemotaxonomic and medicinal significance. *S. Afr. J. Bot.* **2013,** *85*, 94-98.

165. Aou, S. J.; Mizuno, M.; Matsunaga, Y.; Kubo, K.; Li, X. L.; Hatanaka, A., Green odor reduces pain sensation and fatigue-like responses without affecting sensorimotor function. *Chem. Senses* **2005,** *30*, I262-i263.

166. Bahi, A.; Al Mansouri, S.; Al Memari, E.; Al Ameri, M.; Nurulain, S. M.; Ojha, S., beta-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. *Physiol. Behav.* **2014,** *135*, 119-124.

167. Odeh, I.; Abu-Lafi, S.; Al-Najjar, I., Determination of Unifloral Honey Volatiles from Centaurea iberica and Zizyphus spinachristi by Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. *Acta Chromatogr.* **2014,** *26* (3), 485-493.

168. Klauke, A. L.; Racz, I.; Pradier, B.; Markert, A.; Zimmer, A. M.; Gertsch, J., et al., The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. *Eur. Neuropsychopharmacol.* **2014,** *24* (4), 608-620.

169. Kako, H.; Fukumoto, S.; Kobayashi, Y.; Yokogoshi, H., Effects of direct exposure of green odour components on dopamine release from rat brain striatal slices and PC12 cells. *Brain Res. Bull.* **2008,** *75*, 706-712.

170. Higashi, Y.; Kiuchi, T.; Furuta, K., Efficacy and safety profile of topical methyl salicylate and menthol patch in adult patients with mild to moderate muscle strain: A randomized, double-blind, parallel-group, placebo-controlled, multicenter study. *Clin. Ther.* **2010,** *32* (1), 34-43.

171. Carr, M. K. V., The Climatic Requirments of the Tea Plant: A Review. *Exp. Agric.* **1972,** *8*, 1-14.

172. Hilton, P. J., Effects of Season and Nitrogen Fertiliser upon the Flavanol Composition and Tea Making Quality of Fresh Shoots of Tea (*Camellia sinensis* L.) in Central Africa. *J. Sci. Food Agric.* **1973,** *24*, 819-826.

173. Lee, J.-E.; Lee, B.-J.; Chung, J.-O.; Hwang, J.-A.; Lee, S.-J.; Lee, C.-H., et al., Geographical and Climatic Dependencies of Green Tea (Camellia sinensis) Metabolites: A 1H NMR-Based Metabolomics Study. *J. Agric. Food Chem.* **2010,** *58* (19), 10582-10589.

174. Lee, J.-E.; Lee, B.-J.; Chung, J.-O.; Kim, H.-N.; Kim, E.-H.; Jung, S., et al., Metabolomic unveiling of a diverse range of green tea (Camellia sinensis) metabolites dependent on geography. *Food Chem.* **2015,** *174*, 452-459.

175. Amit, L.; R., P. N.; Amod, K., Quality evaluation of black CTC teas based upon seasonal variations. *Int. J. Food Sci. Technol.* **2014,** *49* (2), 493-500.

176. Yao, L.; Caffin, N.; D'Arcy, B.; Jiang, Y.; Shi, J.; Singanusong, R., et al., Seasonal Variations of Phenolic Compounds in Australia-Grown Tea (Camellia sinensis). *J. Agric. Food Chem.* **2005,** *53* (16), 6477-6483.

177. Wang, L. Y.; Wei, K.; Jiang, Y. W.; Cheng, H.; Zhou, J.; He, W., et al., Seasonal climate effects on flavanols and purine alkaloids of tea (*Camellia sinensis* L.). *Eur. Food Res. Technol.* **2011,** *233* (6), 1049-1055.

178. Jayasekera, S.; Kaur, L.; Molan, A.-L.; Garg, M. L.; Moughan, P. J., Effects of season and plantation on phenolic content of unfermented and fermented Sri Lankan tea. *Food Chem.* **2014,** *152*, 546-551.

179. Jayasekera, S.; Molan, A. L.; Garg, M.; Moughan, P. J., Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea. *Food Chem.* **2011,** *125* (2), 536-541.

180. Malec, L. S., Seasonal variations in theaflavin, thearubigin and caffeine contents of argentinian black teas. *J. Sci. Food Agric.* **1988,** *45* (2), 185-190.

181. Xu, W.; Song, Q.; Li, D.; Wan, X., Discrimination of the Production Season of Chinese Green Tea by Chemical Analysis in Combination with Supervised Pattern Recognition. *J. Agric. Food Chem.* **2012,** *60* (28), 7064-7070.

182. Erturk, Y.; Ercisli, S.; Engul, M.; Eser, Z.; Haznedar, A.; Turan, M., Seasonal variation of total phenolic, antioxidant activity and minerals in fresh tea shoots (Camellia sinensis var. sinensis). *Pak. J. Pharm. Sci.* **2010,** *23* (1), 69-74.

183. Sharma, V.; Joshi, R.; Gulati, A., Seasonal clonal variations and effects of stresses on quality chemicals and prephenate dehydratase enzyme activity in tea (*Camellia sinensis*). *Eur. Food Res. Technol.* **2011,** *232* (2), 307-317.

184. Ohno, A.; Oka, K.; Sakuma, C.; Okuda, H.; Fukuhara, K., Characterization of Tea Cultivated at Four Different Altitudes Using 1H NMR Analysis Coupled with Multivariate Statistics. *J. Agric. Food Chem.* **2011,** *59* (10), 5181-5187.

185. Clifford, M. N.; Crozier, A., In *Phytochemicals in Teas and Tisanes and their Bioavailability*, Crozier, A.; Ashihara, H.; Baberan, T., Eds. Blackwell Publishing Ltd: West Sussex, 2012; pp 45-98.

186. Engelhardt, U. H., Chemistry of tea. In *Comprehensive Natural Products II*, Liu, H.-W.; Manders, L., Eds. Elsevier: Oxford, 2010; pp 999-1032.

187. Chaturedula, V. S. P.; Prakash, I., The aroma, taste, color and bioactive onstituents of tea. *J. Med. Plant Res.* **2011,** *5* (11), 2110-2124.

188. Yamanishi, T.; Kobayashi, A.; Sato, H.; Ohmura, A.; Nakamura, H., Flavor of Black Tea. *Agric. Biol Chem.* **1965,** *29* (11), 1016-1020.

189. Owuor, P. O., Changes in quality parameters of commercial black seedling tea due to the time of the year in the Eastern Highlands of Kenya. *Food Chem.* **1992,** *45* (2), 119-124.

190. Ashu, G.; D, R. S., Seasonal Variations in Quality of Kangra Tea (Camellia sinensis(L) O Kuntze) in Himachal Pradesh. *J. Sci. Food Agric.* **1996,** *71* (2), 231-236.

191. Muthumani, T.; Verma, D. P.; Venkatesan, S.; Kumar, R. S., Infuence of seasons on quality of south Indian black teas. *J. Nat. Prod. Plant Resour.* **2013,** *3* (1), 30-39.

192. McCormick, A. C.; Gershenzon, J.; Unsicker, S. B., Little peaks with big effects: establishing the role of minor plant volatiles in plant–insect interactions. *Plant Cell Environ.* **2014,** *37* (8), 1836-1844.

193. Chong, J.; Soufan, O.; Li, C.; Caraus, I.; Li, S.; Bourque, G., et al., MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res.* **2018**, gky310-gky310.

194. Oksanen, J.; Blanchet, F., Guillaume; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D., et al. *vegan: Community Ecology Package*, https://cran.r-project.org, https://github.com/vegandevs/vegan, 2018.

195. NASA, Precipitation Measurement Missions. NASA.

196. Shan, M. Q.; Qian, Y.; Yu, S.; Guo, S. C.; Zhang, L.; Ding, A. W., et al., Anti-inflammatory effect of volatile oil from *Schizonepeta tenuifolia* on carrageenin-induced pleurisy in rats and its application to study of appropriate harvesting time coupled with multi-attribute comprehensive index method. *J. Ethnopharmacol.* **2016,** *194*, 580-586.

197. Resende, D. B.; Martins, H. H. d. A.; Souza, T. B. d.; Carvalho, D. T.; Piccoli, R. H.; Schwan, R. F., et al., Synthesis and in vitro evaluation of peracetyl and deacetyl glycosides of eugenol, isoeugenol and dihydroeugenol acting against food-contaminating bacteria. *Food Chem.* **2017,** *237*, 1025-1029.

198. Marteau, C.; Guitard, R.; Penverne, C.; Favier, D.; Nardello-Rataj, V.; Aubry, J.-M., Boosting effect of ortho-propenyl substituent on the antioxidant activity of natural phenols. *Food Chem.* **2016,** *196*, 418-427.

199. Guimarães, A. G.; Quintans, J. S. S.; Quintans-Júnior, L. J., Monoterpenes with Analgesic Activity—A Systematic Review. *Phytother. Res.* **2013,** *27* (1), 1-15.

200. Eccles, R., Menthol: Effects on nasal sensation of airflow and the drive to breathe. *Curr. Allergy Asthma Rep.* **2003,** *3* (3), 210-214.

201. Morales-López, J.; Centeno-Álvarez, M.; Nieto-Camacho, A.; López, M. G.; Pérez-Hernández, E.; Pérez-Hernández, N., et al., Evaluation of antioxidant and hepatoprotective effects of white cabbage essential oil. *Pharm. Biol.* **2017,** *55* (1), 233-241.

202. Nidiry, E. S.; Babu, C. S. B., Antifungal activity of tuberose absolute and some of its constituents. *Phytother. Res.* **2005,** *19* (5), 447-449.

203. Tse, G.; Yeo, J. M.; Tse, V.; Kwan, J.; Sun, B., Gap junction inhibition by heptanol increases ventricular arrhythmogenicity by reducing conduction velocity without affecting repolarization properties or myocardial refractoriness in Langendorff-perfused mouse hearts. *Mol. Med. Rep.* **2016,** *14* (5), 4069-4074.

204. Song, Y.; Sun, R.; Ji, Z.; Li, X.; Fu, Q.; Ma, S., Perilla aldehyde attenuates CUMS-induced depressive-like behaviors via regulating TXNIP/TRX/NLRP3 pathway in rats. *Life Sci.* **2018,** *206*, 117-124.

205. Nguyen, L. T.; Myslivečková, Z.; Szotáková, B.; Špičáková, A.; Lněničková, K.; Ambrož, M., et al., The inhibitory effects of β-caryophyllene, β-caryophyllene oxide and α-humulene on the activities of the main drug-metabolizing enzymes in rat and human liver in vitro. *Chem. Biol. Interact.* **2017,** *278*, 123-128.

206. Anjali; Pathak, D.; Singh, D., Quinoline: A Diverse Therapeutic Agent. *Int. J. Pharm. Sci. Res.* **2016,** *7*, 1-13.

207. Choi, S. J.; Kim, J. K.; Kim, H. K.; Harris, K.; Kim, C.-J.; Park, G. G., et al., 2,4-Di-tert-butylphenol from Sweet Potato Protects Against Oxidative Stress in PC12 Cells and in Mice. *J. Med. Food* **2013,** *16* (11), 977-983.

208. Donega, M. A.; Mello, S. C.; Moraes, R. M.; Jain, S. K.; Tekwani, B. L.; Cantrell, C. L., Pharmacological Activities of Cilantro's Aliphatic Aldehydes against Leishmania donovani. *Planta Med* **2014,** *80* (18), 1706-1711.

209. Astudillo, A. M.; Meana, C.; Guijas, C.; Pereira, L.; Lebrero, P.; Balboa, M. A., et al., Occurance and biological activity of palmitoleic acid isomers in phagocytic cells. *J. Lipid Res.* **2018,** *59*, 237-249.

210. Kumar, D.; Kumar, S., Isolation and Characterization of Bioactive Phenolic Compounds from Abies Pindrow Aerial Parts. *Pharm. Chem. J.* **2017,** *51* (3), 205-210.

211. Shiratsuchi, H.; Chang, S.; Wei, A.; El-Ghorab, A. H.; Shibamoto, T., Biological activities of low-molecular weight compounds found in foods and plants. *J. Food Drug Anal.* **2012,** *20*, 359-365.

212. Jeong, J. B.; Hong, S. C.; Jeong, H. J.; Koo, J. S., Anti-inflammatory effect of 2-methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation, and acetylation of histone H3. *Arch. Pharm. Res.* **2011,** *34* (12), 2109-2116.

213. Afoulous, S.; Ferhout, H.; Raoelison, E. G.; Valentin, A.; Moukarzel, B.; Couderc, F., et al., Helichrysum gymnocephalum Essential Oil: Chemical Composition and Cytotoxic, Antimalarial and Antioxidant Activities, Attribution of the Activity Origin by Correlations. *Molecules* **2011,** *16* (10), 8273-8291.

214. Takei, M.; Umeyama, A.; Arihara, S., T-cadinol and calamenene induce dendritic cells from human monocytes and drive Th1 polarization. *Eur. J. Pharmacol.* **2006,** *537* (1-3), 190-199.

215. Adebesin, A.; Ajayi Abayomi, M.; Olonode Elizabeth, O.; Omorogbe, O.; Umukoro, S., Methyl Jasmonate Ameliorates Unpredictable Chronic Mild Stress-Induced Behavioral and Biochemical Alterations in Mouse Brain. *Drug Dev. Res.* **2017,** *78* (8), 381-389.

216. Boskabady, M. H.; Farkhondeh, T., Antiinflammatory, Antioxidant, and Immunomodulatory Effects of Crocus sativus L. and its Main Constituents. *Phytother. Res.* **2016,** *30* (7), 1072-1094.

217. Carradori, S.; Chimenti, P.; Fazzari, M.; Granese, A.; Angiolella, L., Antimicrobial activity, synergism and inhibition of germ tube formation by Crocus sativus-derived compounds against Candida spp. *J. Enzyme Inhib. Med. Chem.* **2016,** *31*, 189-193.

218. Nieto-Bobadilla, M. S.; Siepmann, F.; Djouina, M.; Dubuquoy, L.; Tesse, N.; Willart, J. F., et al., Controlled delivery of a new broad spectrum antibacterial agent against colitis: In vitro and in vivo performance. *Eur. J. Pharm. Biopharm.* **2015,** *96*, 152-161.

219. Vasconcelos, T. B. d.; Ribeiro-Filho, H. V.; Lahlou, S.; Pereira, J. G. d. C.; Oliveira, P. S. L. d.; Magalhães, P. J. C., Vasodilator effects and putative guanylyl cyclase stimulation by 2-nitro-1-phenylethanone and 2-nitro-2-phenyl-propane-1,3-diol on rat aorta. *Eur. J. Pharmacol.* **2018,** *830*, 105-114.

220. Moro, I. J.; Gondo, G. D. G. A.; Pierri, E. G.; Pietro, R. C. L. R.; Soares, C. P.; De Sousa, D. P., et al., Evaluation of antimicrobial, cytotoxic and chemopreventive activies of carvone and its derivatives. *Braz. J. Pharm. Sci.* **2017,** *53* (4), e00076.

221. Ianaro, A.; Ialenti, A.; Maffia, P.; Di Meglio, P.; Di Rosa, M.; Santoro, M. G., Anti-Inflammatory Activity of 15-Deoxy-$\Delta^{12,14}$-PGJ_2 and 2-Cyclopenten-1-one: Role of the Heat Shock Response. *Mol. Pharmacol.* **2003,** *64* (1), 85-93.

222. Chowdhury, L.; Croft, C. J.; Goel, S.; Zaman, N.; Tai, A. C.-S.; Walch, E. M., et al., Differential Potency of 2,6-Dimethylcyclohexanol Isomers for Positive Modulation of $GABA_A$ Receptor Currents. *J. Pharmacol. Exp. Ther.* **2016,** *357* (3), 570-579.

223. Kima, J.; Seo, J.; Bae, M.; Yoo, J.; Bang, M.; Cho, S., et al., Antimicrobial constituents from Allium hookeri Root. *Nat. Prod. Commun.* **2016,** *11* (2), 237-238.

224. Alves Naiane Ferraz, B.; Queiroz Thyago, M.; Almeida Travassos, R.; Magnani, M.; Andrade Braga, V., Acute Treatment with Lauric Acid Reduces Blood Pressure and Oxidative Stress in Spontaneously Hypertensive Rats. *Basic Clin. Pharmcol. Toxicol.* **2016,** *120* (4), 348-353.

225. Wang, J.; Lu, J.; Xie, X.; Xiong, J.; Huang, N.; Wei, H., et al., Blend of organic acids and medium chain fatty acids prevents the inflammatory response and intestinal barrier dysfunction in mice challenged with enterohemorrhagic Escherichia coli O157:H7. *Int. J. Immunopharmacol.* **2018,** *58*, 64-71.

226. Park, K. H.; Lee, M. W., Anti-oxidative, anti-inflammatory and whitening effects of phenolic compounds from Bambusae Caulis in Liquamen. *Nat. Prod. Res.* **2012,** *26* (18), 1687-1691.

227. do Nascimento, K. F.; Moreira, F. M. F.; Alencar Santos, J.; Kassuya, C. A. L.; Croda, J. H. R.; Cardoso, C. A. L., et al., Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of Psidium guineense Sw. and spathulenol. *J. Ethnopharmacol.* **2018,** *210*, 351-358.

228. Santos, N. C.; Figueira-Coelho, J.; Martins-Silva, J.; Saldanha, C., Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. *Biochem. Pharmacol.* **2003,** *65* (7), 1035-1041.

229. Horishita, T.; Harris, R. A., *n*-Alcohols Inhibit Voltage-Gated Na^+ Channels Expressed in *Xenopus* Oocytes. *J. Pharmacol. Exp. Ther.* **2008,** *326* (1), 270-277.

230. Popova, A. A.; Koksharova, O. A.; Lipasova, V. A.; Zaitseva, J. V.; Katkova-Zhukotskaya, O. A.; Eremina, S. I., et al., Inhibitory and Toxic Effects of Volatiles Emitted by Strains of Pseudomonas and Serratia on Growth and Survival of Selected Microorganisms, Caenorhabditis elegans, and Drosophila melanogaster. *Biomed. Res. Int.* **2014,** *2014*, 11.

231. Dong, F.; Yang, Z.; Baldermann, S.; Sato, Y.; Asai, T.; Watanabe, N., Herbivore-induced volatiles form tea (Camellia sinensis) plants and their involvement in intraplant communication and changes in endogenous nonvolatile metabolites. *J. Agric. Food Chem.* **2011,** *59* (24), 13131-13135.

232. Hare, J. D., Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annu. Rev. Entomol.* **2011,** *56* (1), 161-180.

233. Karban, R.; Yang, L. H.; Edwards, K. F., Volatile communication between plants that affects herbivory: a meta-analysis. *Ecol. Lett.* **2014,** *17* (1), 44-52.

234. Kessler, A., Inducible plant defences and the environmental context. *Funct. Ecol.* **2016,** *30* (11), 1738-1739.

235. Kigathi, R. N.; Unsicker, S. B.; Reichelt, M.; Kesselmeier, J.; Gershenzon, J.; Weisser, W. W., Emission of Volatile Organic Compounds After Herbivory from *Trifolium pratense* (L.) Under Laboratory and Field Conditions. *J. Chem. Ecol.* **2009,** *35* (11), 1335-1348.

236. Beck, J. J., Addressing the Complexity and Diversity of Agricultural Plant Volatiles: A Call for the Integration of Laboratory- and Field-Based Analyses. *J. Agric. Food Chem.* **2012,** *60* (5), 1153-1157.

237. Stewart-Jones, A.; Poppy, G. M., Comparison of Glass Vessels and Plastic Bags for Enclosing Living Plant Parts for Headspace Analysis. *J. Chem. Ecol.* **2006,** *32*, 845-864.

238. Assmann, S. M.; Snyder, J. A.; Lee, Y.-R. J., ABA-deficient (aba1) and ABA-insensitive (abi1-1. abi2-1) mutants of *Arabidopsis* have a wild-type stomatal response to humidity. *Plant Cell Environ.* **2001,** *23* (4), 387-398.

239. Niinemets, U.; Reichstein, M., Controls on the emission of plant volatiles through stomata: A sensitivity analysis. *J. Geophys. Res.* **2003,** *108* (D7), 4211.

240. Ferreira, M. I.; Uliana, M. R.; Costa, S. M.; Magro, M.; Vianello, F.; Ming, L. C., et al., Exclusion of solar UV radiation increases the yield of curcuminoid in Curcuma longa L. *Ind. Crops Prod.* **2016,** *89*, 188-194.

241. Timkovsky, J.; Gankema, P.; Pierik, R.; Holzinger, R., A plant chamber system with downstream reaction chamber to study the effects of pollution on biogenic emissions. *Environ. Sci. Process Impacts* **2014,** *16* (10), 2301-2312.

242. Nerin, C.; Asensio, E., Migration of organic compounds from a multilayer plastic-paper material intended for food packaging. *Anal. Bioanal. Chem.* **2007,** *389* (2), 589-596.

243. Arvanitoyannis, I. S.; Bosnea, L., Migration of Substances from Food Packaging Materials to Foods. *Crit. Rev. Food Sci. Nutr.* **2010,** *44* (2), 63-76.

244. Raguso, R. A.; Pellmyr, O., Dynamic headspace analysis of floral volatiles: a comparison of methods. *Oikos* **1998,** *81* (2), 238-254.

245. Simon, V.; Riba, M.-L.; Waldhart, A.; Torres, L., Breakthrough volume of monoterpenes on Tenax TA: influence of temperature and concentration for α–pinene. *J Chromatogr. A* **1995,** *704* (2), 465-471.

246. Janson, R. W., Monoterpene emissions from Scots pine and Norwegian spruce. *J. Geophys. Res. Atmos.* **1993,** *98* (D2), 2839-2850.

247. Bicchi, C.; Cordero, C.; Iori, C.; Rubiolo, P., Headspace Sorptive Extraction (HSSE) in the Headspace Analysis of Aromatic and Medicinal Plants. *J. High Resolut. Chromatogr.* **2000,** *23* (9), 539-546.

248. Cheung, W. H. K.; Pasamantes, A.; Peirano, D. J.; Zhao, W.; Grafton-Cardwell, E. E.; Kapaun, T., et al., Volatile organic compound (VOC) profiling of citrus tristeza virus infection in sweet orange citrus varietals using thermal desorption gas chromatography time of flight mass spectrometry (TD-GC/TOF-MS). *Metabolomics* **2015,** *11*, 1514-1525.

249. Demyttenaere, J. C. R.; Morina, R. M.; De Kimpe, N.; Sandra, P., Use of headspace solid-phase microextraction and headspace sorptive extraction for the detection of the volatile metabolites produced by toxigenic *Fusarium* species. *J Chromatogr. A* **2004,** *1027*, 147-154.

250. Splivallo, R.; Bossi, S.; Maffei, M.; Bonfante, P., Discrimination of truffle fruiting body versus mycelial aromas by stir bar sorptive extraction. *Phytochemistry* **2007,** *68*, 2584-2598.

251. Bicchi, C.; Cordero, C.; Liberto, E.; Rubiolo, P.; Sgorbini, B.; Sandra, P., Sorptive tape extraction in the analyss of the volatile fraction emitted from biological solid matrices. *J Chromatogr. A* **2007,** *1148* (2), 137-144.

252. Sgorbini, B.; Ruosi, M. R.; Cordero, C.; Liberto, E.; Rubiolo, P.; Bicchi, C., Quantitative determination of some volatile suspected allgerns in cosmetic creams spread on skin by direct contact sorptive tape extraction-gas chromatography-mass spectrometry. *J Chromatogr. A* **2010,** *1217* (16), 2599-2605.

253. Soini, H. A.; Linville, S. U.; Wiesler, D.; Posto, A. L.; Williams, D. R.; Novoty, M. V., Investigation of Scents on Cheeks and Foreheads of Large Felines in Connection to the Facial Marking Behavior. *J. Chem. Ecol.* **2012,** *38* (2), 145-156.

254. Soini, H. A.; Bruce, K. E.; Klouckova, I.; Brereton, R. G.; Penn, D. J.; Novotny, M. V., In Situ Surface Sampling of Biological Objects and Preconcentration of Their Volatiles for Chromatographic Analysis. *Anal. Chem.* **2006,** *78* (20), 7161-7168.

255. Boggia, L.; Sgorbini, B.; Bertea, C. M.; Cagliero, C.; Bicchi, C.; Maffei, M. E., et al., Direct Contact-Sorptive Tape Extraction coupled with Gas Chromatography- Mass Spectrometry to reveal volatile topographical dynamics of lima bean (*Phaseolus lunatus* L.) upon herbivory by *Spodoptera littoralis* Boisd. . *BMC Plant Biol.* **2015,** *15* (102), 1-13.

256. Errard, A.; Ulrichs, C.; Kuhne, S.; Mewis, I.; Drungowski, M.; Schreiner, M., et al., Single- versus Multiple-Pest Infestation Affects Differently the Biochemistry of Tomato (*Solanum lycopersicum* 'Alisa Craig'). *J. Agric. Food Chem.* **2015,** *63* (46), 10103-10111.

257. Jang, J.; Yang, Y. C.; Zhang, G. H.; Chen, H.; Lu, J. L.; Du, J. Y., et al., Effect if Ultra-Violet B on Release of Volatiles in Tea Leaf. *Int. J. Food Prop.* **2012,** *13* (3), 608-617.

258. Cai, X.-M.; Sun, X.-L.; Dong, W.-X.; Wang, G.-C.; Chen, Z.-M., Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants. *Chemoecology* **2013,** *24* (1), 1-14.

259. Shi, J.; Wang, L.; Ma, C.-y.; Lv, H.-p.; Chen, Z.-M.; Lin, Z., Aroma changes of black tea prepared from methyl jasmonate treated tea plants. *J. Zhejiang Univ. Sci. B* **2014,** *15* (4), 313-321.

260. Shi, J.; Ma, C.-y.; Qi, D.; Lv, H.-p.; Yang, T.; Peng, Q., et al., Transcriptional responses and flavor volatiles biosynthesis in methyl jasmonate-treated tea leaves. *BMC Plant Biol.* **2015,** *15* (1), 233.

261. Fox, J.; Weisberg, S., *An R Companion to Applied Regression*. Second ed.; Sage: Thousand Oaks, CA, 2011.

262. Kessler, A.; Baldwin, I. T., Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *Plant J.* **2004,** *38* (4), 639-649.

263. Uesugi, A.; Morrell, K.; Poelman, E. H.; Raaijmakers, C. E.; Kessler, A., Modification of plant-induced responses by an insect ecosystem engineer influences the colonization behaviour of subsequent shelter-users. *J. Ecol.* **2016,** *104* (4), 1096-1105.

264. Cozzolino, S.; Fineschi, S.; Litto, M.; Scopece, G.; Trunschke, J.; Schiestl, F. P., Herbivory Increases Fruit Set in *Silene latifolia*: A Consequence of Induced Pollinator-Attracting Floral Volatiles? *J. Chem. Ecol.* **2015,** *41* (7), 622-630.

265. MacLeod, G.; Ames, J. M., Comparative Assessment of the Artefact Background on Thermal Desorption of Tenax GC and Tenax TA. *J. Chromatogr.* **1986,** *355*, 393-398.

266. Martin, J. A.; Kwak, J.; Harshman, S. W.; Chan, K.; Fan, M.; Geier, B. A., et al., Field sampling demonstration of portable thermal desorption collection and analysis instrumentation. *Int. J. Environ. Anal. Chem.* **2016,** *96* (4), 299-319.

267. Eglinton, G.; Hamilton, R. J., Leaf Epicuticular Waxes. *Science* **1967,** *156* (3780), 1322-1335.

268. Buschhaus, C.; Jetter, R., Composition and Physiological Function of the Wax Layers Coating Arabidospsis Leaves: β−Amyrin Negatively Affects the Intracuticular Water Barrier. *Plant Physiol.* **2012,** *160*, 1120-1129.

269. Kfoury, N.; Scott, E.; Orians, C.; Robbat, A., Direct Contact Sorptive Extraction: A Robust Method for Sampling Plant Volatiles in the Field. *J. Agric. Food Chem.* **2017,** *65* (38), 8501-8509.

270. Pazouki, L.; Kanagendran, A.; Li, S.; Kannaste, A.; Memari, H. R.; Bichele, R., et al., Mono- and sesquiterpenes release from tomato (*Solanum lycopersicum*) leaves upon mild and severe heat stress and through recovery: From gene expression to emission responses. *Environ. Exper. Bot.* **2016,** *132*, 1-15.

271. Smith, L.; Beck, J. J., Duration of emission of volatile organic compounds from mechanically damaged plant leaves. *J. Plant Physiol.* **2015,** *188*, 19-28.

272. Farre-Armengol, G.; Filella, I.; Llusia, J.; Niinemets, U.; Penuelas, J., Changes in floral bouquets from compound-specific responses to increasing temperatures. *Global Change Biol.* **2014,** *20*, 3660-3669.

273. Sufang, Z.; Jianing, W.; Zhen, Z.; Le, K., Rhythms of volatiles release from health and insect damaged *Phaseolus vulgaris*. *Plant Signal Behav.* **2013,** *8* (10), e25759.

274. Cai, X.-M.; Sun, X.-L.; Dong, W.-X.; Wang, G.-C.; Chen, Z.-M., Variability and stability of tea weevil-induced volatile emissions from tea plants with different weevil densities photoperiod and ifestation duration. *Insect Sci.* **2012,** *19* (4), 507-517.

275. Wishart, D. S., FooDB. The Metabolomics Innovation Centre, 2016.

276. Pence, H. E.; Williams, A., ChemSpider: An Online Chemical Information Resource. *J. Chem. Educ.* **2010,** *87* (11), 1123-1124.

277. *Sensory-Directed Flavor Analysis*. CRC Press: Boca Raton, FL, 2007.

278. Delahunty, C. M.; Eyres, G.; Dufour, J. P., Gas chromatography-olfactometry. *J. Sep. Sci.* **2006,** *29* (14), 2107-2125.

279. Sheng, N.; Zheng, H.; Xiao, Y.; Wang, Z.; Li, M.; Zhang, J., Chiral separation and chemical profile of Dengzhan Shengmai by integrating comprehensive with multiple heart-cutting two-dimensional liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *J Chromatogr. A* **2017,** *1517*, 97-107.

280. Scoparo, C. T.; de Souza, L. M.; Dartora, N.; Sassaki, G. L.; Gorin, P. A. J.; Iacomini, M., Analysis of Camellia sinensis green and black teas via ultra high performance liquid chromatography assisted by liquid–liquid partition and two-dimensional liquid chromatography (size exclusion×reversed phase). *J Chromatogr. A* **2012,** *1222*, 29-37.