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Direct Assessment of Phytochemicals Inherent in Plant Tissues Using Extractive Electrospray Ionization Mass Spectrometry

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Supporting Information

ABSTRACT: An ambient pressure ionization mass spectrometric strategy called internal extractive electrospray ionization mass spectrometry (iEESI-MS) has been developed and applied for direct profiling of labile phytochemicals inherent in various native plant tissues, including leaves, roots, and fruits. By passing the electrospray solvent through the plant tissue, a variety of phytochemicals, such as amino acids, sugars (e.g., glucose, sucrose, polysaccharides, etc.), and alkaloids, were continuously extracted from the sample interior, driven toward the natural/cut electro-spraying tip, and vaporized into gaseous ions for mass spectrometric interrogation. Phytochemical patterns obtained by iEESI−MS permit a rapid differentiation between various species of ginkgo plant and strawberry maturity stages, as well as characterization of physiological/pathologic conditions of chlorophytum comosum. Our experimental results further demonstrate that the established iEESI−MS approach is potentially useful for direct phytochemomics studies with minimal biodegradation, allowing elucidation of plant metabolism with high speed, specificity, and simplicity of analysis.

KEYWORDS: *internal extractive electrospray ionization, mass spectrometry, direct analysis, plant tissues, phytochemicals*

INTRODUCTION

Plants provide oxygen and nutrients for humans and animals and play an essential role in fuel cycle, climate change, and life evolution. Tremendous advances have been achieved in plant sciences and technologies. However, numerous botanic mysteries are yet to be resolved, especially those at the molecular level.[1−3] For example, molecular analysis is required to address the underlying mechanism of fruit maturation[4] and the formation of specific fruit fragrance.[5] The lack of fundamental insights motivates the development of improved analytical strategies to visualize molecular processes running behind the life of plants.

Current analytical techniques routinely applied in plant science include gas chromatography (GC),[6−8] high performance liquid chromatography (HPLC),[6,9,10] capillary electrophoresis (CE),[11,12] and optical spectroscopic methods.[13,14] Because of the high chemical specificity and sensitivity in the analysis of complex matrixes, mass spectrometry (MS) has been increasingly employed to characterize the molecular composition of plants.[15,16,18,19,21,23] Both qualitative and quantitative information on specific metabolites can be acquired using one or a combination of the aforementioned techniques. Furthermore, imaging mass spectrometric methods allow for a 2D, and 3D if required, spatial distribution of targeted compounds in plant tissues with micrometer resolution.[21,23−25] To obviate the necessary sample preparations and observe labile molecules, which are often destroyed during off-line analysis and high-energy desorption procedure, analytical methods capable of direct analysis of phytochemicals inherent in plant tissues are required.

The advent of ambient ionization MS techniques, such as desorption electrospray ionization (DESI),[26] probe electrospray ionization (PESI),[20] and extractive electrospray ionization (EESI),[17] has greatly facilitated rapid molecular characterization of plant materials, without the need for laborious and time-consuming sample preparation. Additionally, leaf spray[22] and similar techniques[27,28] allow electrospray to be generated directly from intact tissue samples, for example, plant leaves.[22] Because in "leaf spray" the analytes are sampled by applying a single solvent droplet or due to its inherent water content, the duration of MS response is relatively short, which could hinder the observation of low-abundance species. The same issue exists in the techniques in which a tiny tissue piece is sampled to MS from the tip of a sharp needle.[27,29]

Herein, we present the application of a novel analytical strategy entitled internal extractive electrospray ionization mass spectrometry (iEESI−MS) for the directly continuous analysis of phytochemicals inherent in plant tissues. In iEESI−MS, solvent is fed through the plant tissue to directly extract endogenous analytes, which are subsequently ionized with the help of a strong electric field. Because of its unique setup, iEESI−MS favors the extraction of "internal" chemicals, which are located in the bulk volume of the plant tissue rather than on the surface. Analytes are extracted with minimal perturbation to the plant integrity, readily detected within seconds. Further-

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more, molecular profile can be continuously collected on the time scale of tens of minutes, depending on the flow rate infused, tissue type, and the sample volume. Phytochemical iEESI–MS patterns obtained in this study were used for a rapid differentiation between various species of the same plant (e.g., ginkgo), as well as characterization of physiological/pathologic conditions (e.g., chlorophytum comosum) and maturity stage (e.g., strawberries).

MATERIALS AND METHODS

Internal Extractive Electrospray Ionization. The iEESI–MS experiments were implemented on a linear ion trap (LTQ) mass spectrometer (Thermo Scientific, U.S.) equipped with a homemade iEESI source. To standardize the sample manipulation, the samples were cut into a fixed shape (e.g., small triangle or cube) and size with a thickness within 3 mm and a side length of ca. 10 mm. The solvent (methanol was used in this study) biased with a high voltage (±4.5 kV) was injected through the bulk tissue, producing a fine mist of charged droplets containing analytes toward the adjacent mass spectrometer inlet. The sample was supported by the ESI capillary (fused silica, i.d., 0.10 mm, o.d., 0.15 mm, Agilent Technologies Co., Ltd., U.S.) inserted into the sample, allowing a distance of ~3 mm between the ESI tip and the sample apex. The apex of the sample was pointed to the ion entrance of the mass spectrometer, with a distance of 4–8 mm (Figure 1). In the current experimental scenario, the flow

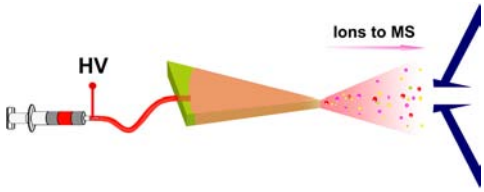

Figure 1. Schematic diagram of the internal extractive electrospray ionization process.

rate of the extraction solvent was set at 1–4 μL/min by a syringe pump (250 μL, Hamiltion, U.S.), and the optimized temperature of the heated capillary was set to be 150 °C. Other parameters were set as default values of the instrument, and no further optimization was performed.

For the comparison purpose, leaf spray experiments were performed in the way described elsewhere.[22] Methanol was chosen because it is a generally good extraction solvent for most phytochemicals. The high voltage was set at ±4.5 kV in the leaf spray experiments under the positive or negative ion detection mode.

For the direct infusion ESI measurements of ginger rhizome extracts, ca. 0.14 g of ginger rhizome tissue was minced and loaded in a 1.5 mL vial, and 1 mL of methanol was added as extraction solvent. After approximately 30 min, the supernatant liquid was collected and stored for further ESI–MS analysis. The extracts were injected at a flow rate of 6 μL/min by the syringe pump without further dilution. The ESI high voltage was set at +4.5 kV, and nitrogen sheath gas was 0.6 MPa for the ESI experiments.

Materials and Reagents. Three sets of ginkgo leaves, such as large green ginkgo leaves (ginkgo 1, full growth), small green ginkgo leaves (ginkgo 2, half growth), and large yellow leaves from golden-leaf ginkgo (ginkgo 3, full growth), comprised 20 leaf samples with similar sizes, respectively. The ginkgo 1 and ginkgo 2 sets were of different leaf sizes, from the same branch of a ginkgo tree about 20 years old. The ginkgo set 3 was obtained from a golden-leaf ginkgo tree of 20 years old. Those two ginkgo trees were grown in the same yard with no essential difference in environmental conditions. In the case of fruit tissues, three sets of strawberries (green unripe strawberries, pink and slightly ripe strawberries, red ripe strawberries) were used. In each maturity category, 13 strawberry samples with similar weight and shape were sampled from the same batch, respectively. For a better reference, the strawberry flesh tissues were cut from the same part of each individual fruit. For the chlorophytum comosum leaves, five sample sets with different physiological conditions were taken: the first set (chlorophytum comosum 1) was diseased with slight yellowness in the leaves (13 individual leave samples were sampled); the second set (chlorophytum comosum 2, without sunlight exposure) was in normal status with healthy, green leaves (13 individual leaves was sampled); the third set (chlorophytum comosum 3) was healthy green leaves exposed to the sunlight (28 °C) for 1 h (7 individual leaves were sampled); the fourth set (chlorophytum comosum 4) was healthy green leaves with 6 h of sunlight exposure (28 °C) (7 individual leaves was sample); and the fifth set (chlorophytum comosum 5) was healthy green leaves with 10 h of sunlight exposure (28 °C) (7 individual leaves was sampled). The five sample sets of chlorophytum comosum were in vivo as a whole plant during the treatment with normal growth conditions. During the sunlight exposure, ca. 5 mL of water was sprayed on the chlorophytum comosum leaves (e.g., the third, fourth, and fifth sets) at intervals of 10 min, preventing the leaves from being frizzled due to water loss caused by the sun heat. Other samples such as garlic bulb, ginger rhizome, and pepper were purchased from the local supermarket. All of the cut plant samples were used directly without further preparations.

Methanol and acetic acid both of Analytical grade were bought from ROE Scientific Inc. (Newark, U.S.). The acetone (Analytical grade) was bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and RDX was purchased from Chem Service, Inc. (PA). The deionized water used for the experiments was provided by the chemistry facilities at ECIT.

Data Analysis. Similar to previous studies,[30,31] the mass spectral fingerprint data were exported for principal component analysis (PCA), which was performed using the Matlab (version 7.8.0, Mathworks, Inc., Natick, MA). The fingerprint mass spectra (MS^1) data were exported into Microsoft Excel and arranged using the m/z values as independent variables, in which the m/z value and its relative signal intensity exported from each sample case were matched, respectively. All of the mass spectral data expressed in relative abundance were further loaded into the Matlab software for PCA analysis. On the basis of the "princomp" function in the "Matlab Toolbox", the arranged spectra fingerprint data in the Excel were fetched for statistical analysis. Hence, the molecular information from different samples was presented in the PCA score plots visually.

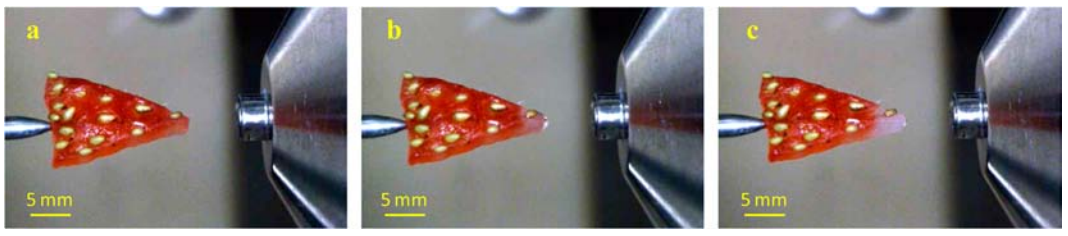

Figure 2. The color change of a ripe strawberry flesh observed during an iEESI process after (a) 0 min; (b) 2.5 min; and (c) ca. 6 min. The fused silica capillary protruding from the metallic tube ca. 10 mm also serves as the sample holder.

RESULTS AND DISCUSSION

Internal Extractive Electrospray Ionization. Internal extractive electrospray ionization mass spectrometry (iEESI–MS) was developed to directly characterize three-dimensional volume of a plant tissue at the molecular level. In an iEESI experiment, solvent (methanol was used in the study) is directly infused through the triangularly shaped tissue sample via a piece of embedded capillary (fused silica) to which high voltage is applied (Figure 1). Note that the sample was standardized in shape and size for a better comparison with leaf spray experiments. However, the triangular shape was not always required in iEESI measurements. Extracted phytochemicals were carried with the solvent pumped through the sample volume toward the apex where a stable electrospray plume was generated in front of MS inlet. The underlying ionization mechanism is believed to be similar to the conventional ESI (see Supporting Information, video).

Figure 2 shows the gradual color change of a strawberry flesh during an iEESI–MS experiment, reflecting internal extraction of chemicals. Notable color bleaching was observed at the apex of the sample (ca. 10 mm^3) after approximately 6 min from the injection of extraction solvent. The observed bleaching effect can be attributed to the loss of characteristic strawberry pigments extracted during the iEESI process (e.g., pelargonidin). The signal at m/z 433 present in the iEESI–MS from red ripe strawberries was identified as the radical-cationic pelargonidin 3-*O*-glucoside (Supporting Information Figure S1) based on tandem MS analysis and literature searching.[32,33] The corresponding signal was not observed from the thoroughly bleached sample as well as from green unripe strawberries. The analysis of extracted ion chromatograms (EIC) corresponding to different analyte species indicates that the analyte-specific iEESI–MS signals become visible after ~0.6 min (data not shown) from the beginning of experiment. The lag time duration in EIC was highly dependent on the flow rate of the extraction solvent, the distance between the capillary tip and the edge of the sample apex, chemical composition of the solvent, as well as the physical properties of the sample matrices, such as density or texture. Analyte signals could normally be recorded over a time period of >15 min without abrupt intensity fluctuations (Supporting Information Figure S2a–e), and the EIC profile of certain analytes followed the exponential decay for >50 min (e.g., gingerol in the ginger rhizome, Supporting Informaton Figure S2f). The high durability of iEESI–MS response is particularly beneficial for the sensitive detection and identification of trace analytes. In a reference leaf spray experiment performed on ginger rhizome sample, a much shorter and less stable EIC profile was generated (Supporting Information Figure S2g), because the single drop of extraction solvent was rapidly evaporated/consumed from the tissue surface.[22]

It is important to note that unlike surface-specific methods, such as DESI and DAPCI, a large share of phytochemicals in iEESI were extracted "internally", that is, from the interior of the bulk plant tissue rather than from the surface. As a demonstration, a 0.5 μL drop of 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) solution (1 ppm in acetone) was spiked onto the surface of chlorophytum comosum leaf sample and allowed to dry at room temperature. Afterward, the spiked leaf sample was investigated by iEESI–MS using the mixture of acetic acid and methanol (1:9, v:v) as the extraction solvent in the negative ion detection mode. Targeted iEESI–MS/MS analysis of the precursor ions isolated at m/z 281 did not reveal the ion fragments characteristic of authentic deprotonated RDX anions (Supporting Information Figure S3).[34,35] While leaf spray coupled with proper solvent is undoubtedly able to extract internal phytochemicals, the solvent need to penetrate through sample surface, thus picking up compounds, that is, RDX, deposited on the sample surface (as shown in Supporting Information Figure S3).

Direct MS Detection of Phytochemicals Inside Plant Tissues. Garlic bulb and ginger rhizome were analyzed by iEESI–MS in positive ion detection mode (Figure 3). Major

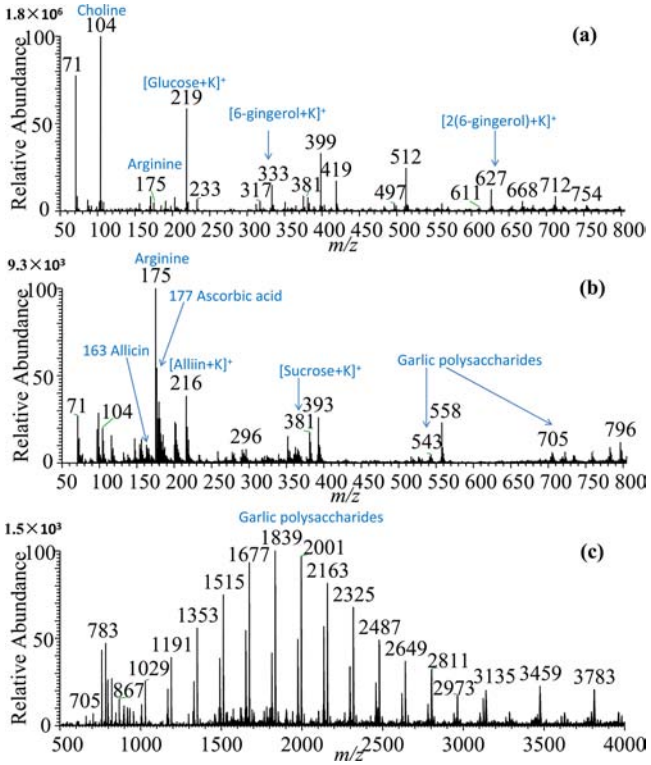

Figure 3. iEESI–MS spectra of (a) ginger rhizome, and (b,c) garlic bulb acquired in the positive ion detection mode using methanol as the extraction solvent.

peaks in the mass spectrum of ginger rhizome (Figure 3a) were identified as choline (m/z 104), cationized glucose (m/z 219 [glucose + K]$^+$), sucrose m/z 381 ([sucrose + K]$^+$), and 6-gingerol (m/z 317 [6-gingerol + Na]$^+$, m/z 333 [6-gingerol + K]$^+$, m/z 611 [2(6-gingerol) + Na]$^+$, and m/z 627 [2(6-gingerol) + K]$^+$), based on MS/MS experiments and literature.[36] Most of the signals were detected as [M + K]$^+$ or [2M + K]$^+$ ions, probably due to the naturally high potassium concentration in ginger rhizome body.[37–39] Other characteristic signals at low intensity were tentatively assigned as potassium adducts of ginger phytochemicals, such as 8-gingerol (m/z 361 [8-gingerol + K]$^+$), 10-gingerol (m/z 389 [10-gingerol + K]$^+$), 6-shogaol (m/z 305 [6-shogaol + K]$^+$), and 8-shogaol (m/z 327 [8-shogaol + Na]$^+$).[36]

The iEESI–MS analysis of a cut garlic bulb revealed the presence of signature organosulfur compounds (e.g., m/z 178 [alliin + H]$^+$, m/z 200 [alliin + Na]$^+$, m/z 216 [alliin + K]$^+$, m/z 163 [allicin + H]$^+$, m/z 180 [allicin + NH_4]$^+$, m/z 185 [allicin + Na]$^+$, etc.), ascorbic acid (m/z 177 [M + H]$^+$), choline (m/z 104), arginine (m/z 175 [arginine + H]$^+$), and sucrose (m/z

381 [sucrose + K]$^+$) (Figure 3b) based on MS/MS analysis and literature.[22,40,41] Furthermore, the dominant high mass peaks, such as m/z 1515, 1677, 1839, 2001, 2001, 2163, 2325, etc. (Figure 3c), are characterized with a step mass shift of 162 Da, which is consistent with the fingerprinting fructan monomer (glucose residue, m/z 162), as reported in previous works.[42,43] Therefore, the aforementioned peaks are tentatively assigned to endogenous garlic polysaccharides. More interestingly, the degree of polymerization (DP 9−14) of garlic polysaccharides in the current study, while compared to the ones previously reported (1010−2811 Da (DP 6−17)),[42,43] can be used as a potential marker for garlic origin discrimination. The latter observation demonstrates the capability of iEESI−MS to detect large phytochemicals, which often remain undetected in desorption-based ambient MS approaches. To further explore the potential of the iEESI−MS technique for the detection of phytochemicals, more plant tissues including green/red peppers were investigated. As shown in Supporting Information Figure S4, various plant constituents, such as sugars and amino acids, were observed at low concentration levels.

Characterization of Labile Metabolites Inside Plant Tissues. Conventionally, tedious sample preparations such as grinding, extraction, separation, and preconcentration are required prior to the analysis of raw plant materials. These sample pretreatment steps are time-consuming and can potentially lead to the degradation of bioactive phytochemicals (e.g., amino acid and biomacromolecules), chemical contamination, and material losses.[3,44,45] For example, alliin in garlic is known to readily convert into allicin by allinase when external mechanical force is applied to garlic bulbs, making it difficult to detect and quantify intact alliin from raw garlic tissues. It has been reported that the conversion of alliin to allicin is rapid and can be completed within 10 s at 23 °C.[46,47] The observation of high-abundance alliin signal from the raw garlic tissue in iEESI−MS (Figure 3b) emphasized the advantage of direct sampling, for example, the extraction of analytes in its native condition and subsequent detection in a timely fashion. Additionally, the almost ignorable detection of allicin in intact garlic bulb double-confirmed the aforementioned statement, and further assured the potential of iEESI−MS in the field of direct phytochemical profiling of plant tissues (data not shown). Using the easy-degrading alliin as a practical molecular indicator, the detailed interaction between the ESI solvent and plant tissue matrixes, and how and where is the inherent extraction of endogenous phytochemicals in plant cells, can be elaborated and will be shown in another publication.

Many factors including the intrinsic concentrations of phytochemicals, the extraction parameters (choice of solvent, density/porosity of tissue sample, and affinity of analytes to the tissue), and the ionization process (competence and ion suppression) may affect the signal abundances shown in the mass spectra. For a better understanding of the effect of the above-mentioned factors, direct infusion ESI measurements of ginger rhizome extracts and the collected solution of iEESI spray (spray carefully collected instead of being directed to MS inlet) were performed for comparison purposes, all using methanol as both the extraction and the spraying solvent. Consequently, the resulted Mass spectra of ginger rhizome, in either positive (shown in Figure 4) or negative (data not shown) ion detection mode, showed a high similarity between the three mass spectral patterns. The ESI−MS pattern was richer in low-intensity peaks such as m/z 654, 682, which was probably caused by the higher efficiency of bulk chemical

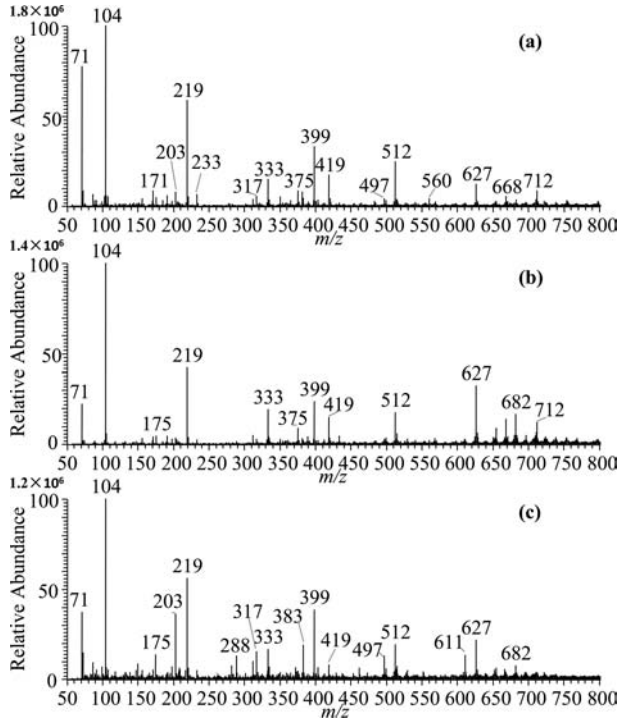

Figure 4. Spectra of ginger rhizome obtained by (a) iEESI−MS of raw sample, (b) direct infusion ESI−MS of ginger extracts, and (c) direct infusion ESI−MS of material collected from the iEESI spray of ginger rhizome in the positive ion detection mode.

extraction from finely minced ginger rhizome (~0.14 g) as compared to the real-time extraction in iEESI. These results also confirm that the iEESI−MS method is capable of direct profiling of phytochemicals inside the tissues, producing similar results obtained by ESI−MS after sample extraction.

Differentiation of Ginkgo Species by iEESI−MS Profiling. Three different species of ginkgo leaves were fingerprinted by iEESI−MS analysis for differentiation purpose. As shown in Figure 5, rich fingerprints were recorded in a wide mass range (m/z 50−1000) in positive ion detection mode for all of the samples tested. In Figure 5a,b corresponding to the ginkgo leaves from the same tree, but with different leaf size (gingko 1, 2), the dominant peaks at m/z 104, 116, 138, 175, 233, 381, 458, 872 were detected, while the peaks at m/z 104, 116, 154, 175, 233, 251, 317, 381 were abundant for the golden-leaf ginkgo leaves (gingko 3, Figure 5c). The differentiation between green and yellow gingko leaves can be most easily seen by comparing the relative intensity of peaks at m/z 458 and 872, which are highly abundant in green leaves and are not present in yellow leaves. Ginkgotoxin (MW 183) was detected as protonated species (m/z 184) from all three sample sets. The relative signal intensity of ginkgotoxin in the green ginkgo leaves (ginkgo 1, 2) was higher than that in the golden-leaf ginkgo leaves (ginkgo 3). On the other hand, proline (MW 115), detected as the peak at m/z 116, was more abundant in the mass spectra of golden-leaf ginkgo leaf (Figure 5c). The molecular difference between the ginkgo leave samples was then visualized using PCA analysis (Figure 5d). While closely related green ginkgo leaves (ginkgo 1, 2) were converged in by PCA, the golden-leaf ginkgo leaves (ginkgo 3) were well separated in the PCA score plot.

Differential Analysis of Strawberry Maturity. Maturation of fruits is a genetically controlled process[4] associated with

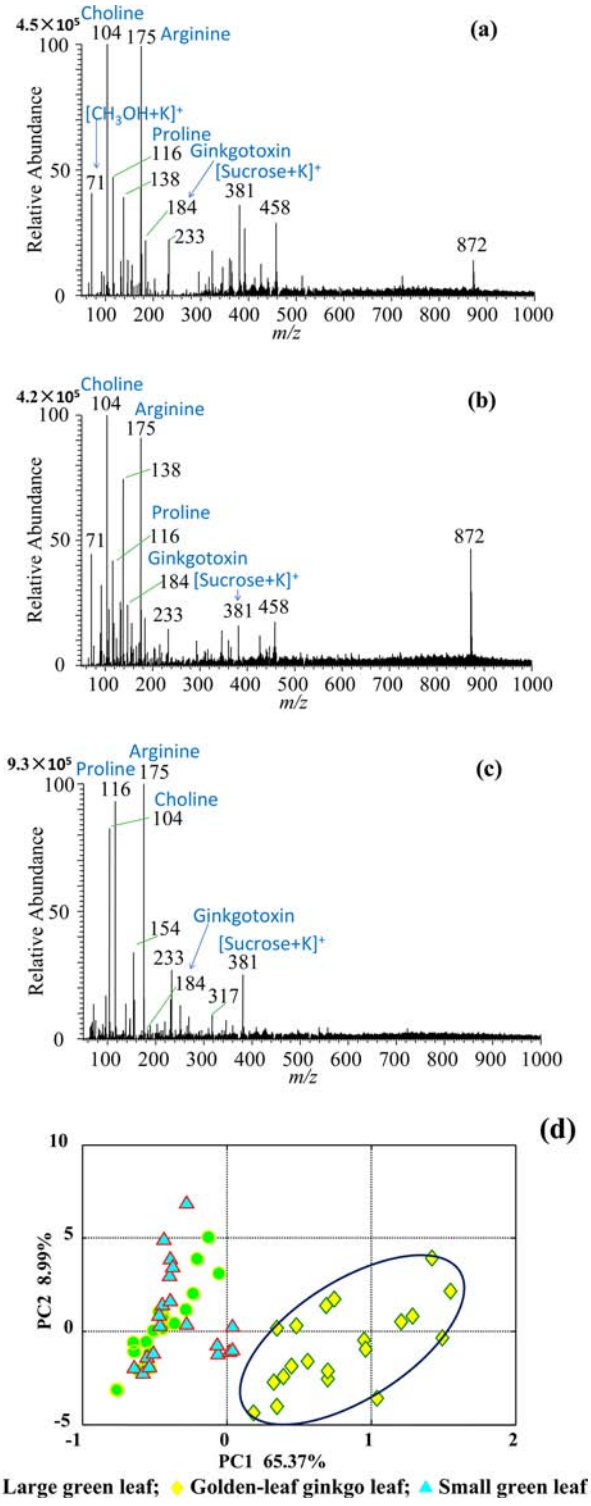

Figure 5. Differentiation of gingko species by iEESI–MS: (a) mass spectra of the small green ginkgo leaf (ginkgo 1); (b) mass spectra of the large green ginkgo leaf (ginkgo 2); (c) mass spectra of the golden-leaf ginkgo leaf (ginkgo 3); and (d) PCA score plot of iEESI–MS fingerprints of the three ginkgo sets. The ellipse is shown to guide the eyes.

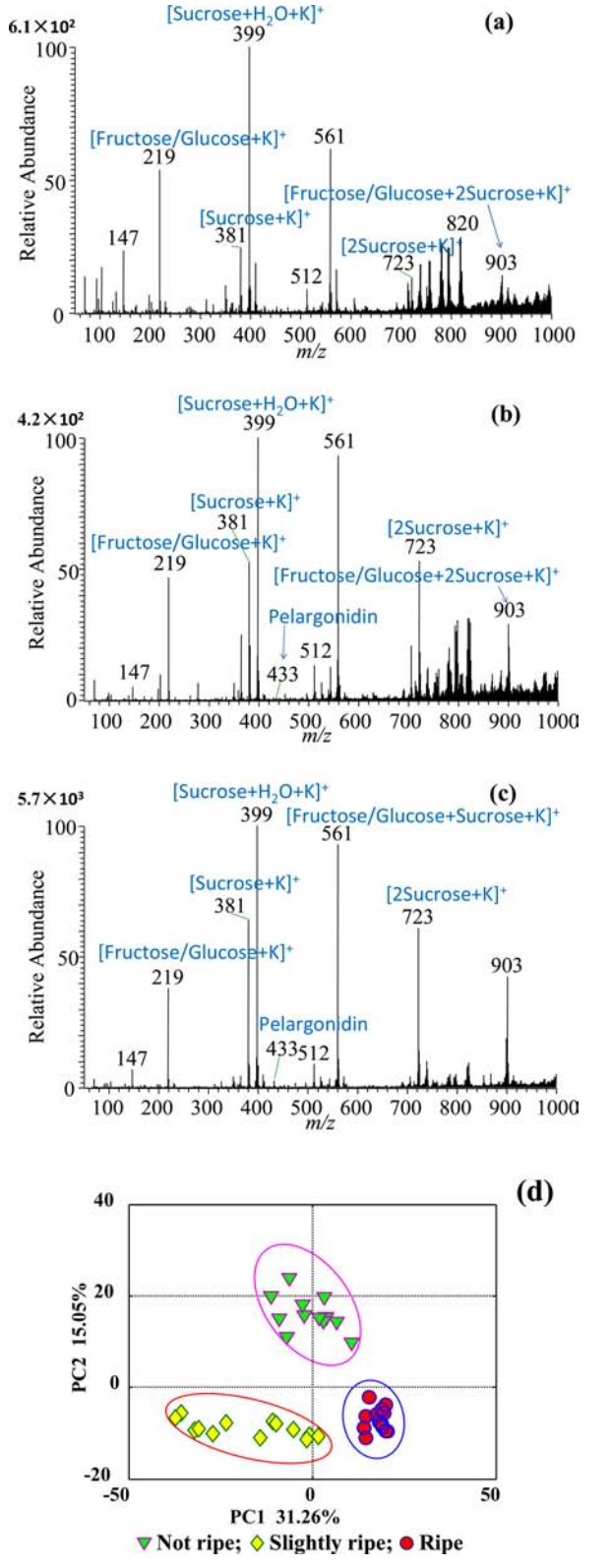

Figure 6. iEESI–MS analysis of strawberries of different maturity stages: (a) unripe strawberry; (b) slightly ripe strawberry; (c) ripe strawberry; and (d) PCA score plot of iEESI–MS fingerprints of the three maturity sets of strawberries. Note that the ellipses are only to guide the eyes.

characteristic phytochemical changes. The iEESI–MS fingerprints of strawberries at different maturity stages revealed distinct characteristic spectral patterns (Figure 6a–c). The peaks at m/z 219 ([fructose/glucose + K]$^+$), 381 ([sucrose + K]$^+$), 399 ([sucrose + H_2O + K]$^+$/[2fructose/glucose + K]$^+$), 561 ([fructose/glucose + sucrose + K]$^+$), 723 ([2sucrose + K]$^+$), and 903 ([fructose/glucose + 2surcose + K]$^+$)[48] were found in strawberries at every maturity stage, although with

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significant variations in relative peak intensities. However, the absolute abundances of peaks at m/z 561, 723, and 903 were increased from the unripe strawberry (m/z 561, $3.51 \times 10^2 \pm 16.9\%$ cps; m/z 723, $107 \times 10^2 \pm 24.1\%$ cps; m/z 903, $7.83 \times 10^1 \pm 22.2\%$ cps) to the ripe strawberry (m/z 561, $5.56 \times 10^2 \pm 16.8\%$ cps; m/z 723, $3.71 \times 10^2 \pm 16.7\%$ cps; m/z 903, $1.67 \times 10^2 \pm 19.9\%$ cps), based on the same signal integration time. The spectral differences were clearly visualized by PCA analysis (Figure 6d). PCA loading results indicate that all of the monosaccharide peaks are the main contributors to the differentiation of the maturity stages (data not shown), consistent with the aforementioned observation and the factor that sugar content continuously grows with fruit ripen process. These peaks can therefore be proposed as a maturity signature of strawberries.

Strawberries turn from green to red color when they reach the maximal maturity stage. Pelargonidin plays an important role in forming the red color of the ripe strawberries,[33] and its content increases during the maturation process. In this study, the pelargonidin 3-*O*-glucoside, a bioactive antioxidant, was detected as a radical cation at m/z 433 and identified on the basis of MS/MS analysis and literature[32,33] (Supporting Information Figure S1). The peak at m/z 433 (pelargonidin 3-*O*-glucoside) was found in the spectra (Figure 6b,c) with a different relative abundance, growing with the maturity stage. For example, pelargonidin 3-*O*-glucoside was clearly visible in the spectra of the ripe strawberry (shown in Figure 6c), while it was not detectable in the unripe strawberry (shown in Figure 6a).

Differentiation of Chlorophytum Comosum Tissue Samples under Different Physiological Conditions. The physiological condition of a plant is a mirror of its metabolism. Under specific physiological stresses, such as disease or strong sunlight exposure, metabolic activity may be altered, triggering the production of specific phytochemicals.[49] In this study, five sets of chlorophytum comosum leaves, including normal (healthy, nonexposed to sunlight), diseased, and three sets exposed to the sunlight for different durations (1, 6, and 10 h), were tested using iEESI–MS. Substantial differences between the diseased and the normal chlorophytum comosum was detected at the molecular level. From the comparison between the mass spectra recorded from the diseased (Figure 7a) and normal healthy (Figure 7b) sample, the signals at m/z 138, 154, 204, 429, 706, 818 were attributed to the metabolic changes in the diseased chlorophytum comosum. Identification of these signals (e.g., via tandem MS experiments) was left beyond the scope of this article. The mass spectra acquired from leaves exposed to sunlight showed higher similarity to the nonexposed healthy leaves (Figure 7b, normal without sunlight exposure; Supporting Information Figure S5a–c, 1, 6, and 10 h of sunlight exposure, respectively). The major markers of sunlight exposure were found to be the peaks at m/z 154 and 818. Interestingly, the signal abundance of peak at m/z 818 was inversely proportional to the time of sunlight exposure (Figure 7b and Supporting Information Figure S5), while the signal abundance of the peak at m/z 154 had positive time correlation. The differences between the collected data sets were visualized by PCA plots (Figure 7c). All five physiological conditions of chlorophytum comosum leaves, including normal, diseased, and sunlight treated, were successfully differentiated.

The relatively high standard deviation of absolute signal intensity (up to ~25%) for the samples within the same physiological group is partly contributed by the heterogeneity

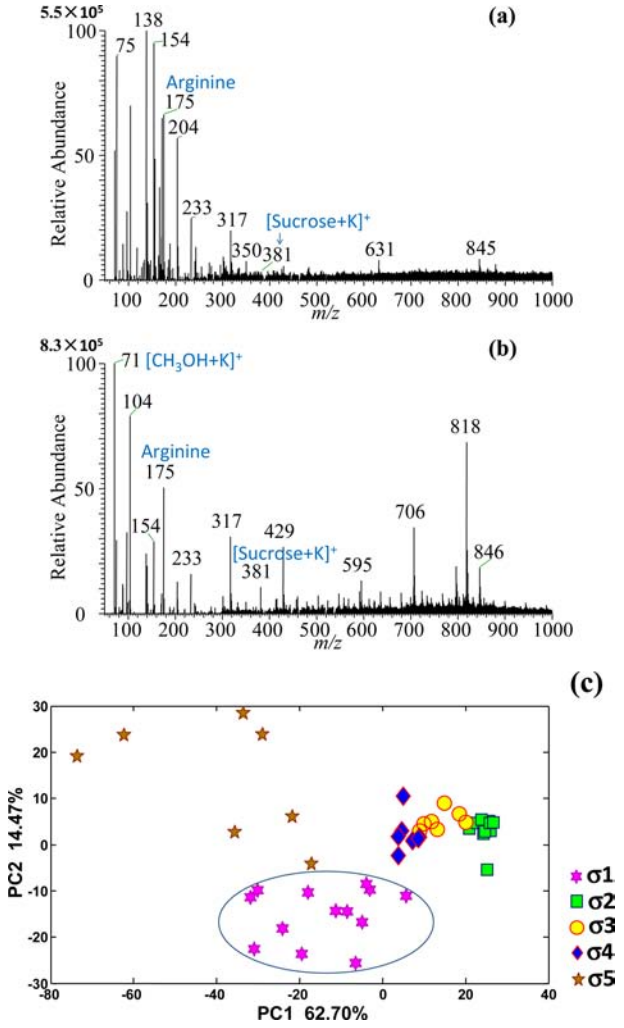

Figure 7. iEESI–MS analysis of the chlorophytum comosum leave samples: (a) the diseased chlorophytum comosum leaf; (b) the normal leaf (without sunlight exposure); and (c) PCA score plot iEESI–MS fingerprints of the chlorophytum comosum with different physiological condition, (σ1) diseased leaves, (σ2) normal healthy leaves (0 h sunlight exposure), (σ3) leaves after 1 h sunlight exposure, (σ4) leaves after 6 h sunlight exposure, and (σ5) leaves after 10 h sunlight exposure.

of plant tissues, but can also be related to experimental procedure. Better quantification capacity of the method should be achieved with an improved standardized protocol for the preparation and mounting of triangular tissue sample.

Comprehensive analysis of plant metabolism enables a better mechanistic understanding of life processes at the molecular level. As demonstrated in the current study, internal extractive electrospray ionization mass spectrometry (iEESI–MS) allows the direct analysis of endogenous phytochemicals inside plant tissues, for example, leaves and fruits, and their response to different internal/external stimulus. It has been demonstrated that different plant species, as well as the state of disease and maturity status, can be readily classified on the basis of iEESI–MS fingerprint molecules, such as ginkgotoxin and proline in gingko leaves, as well as pelargonidin 3-*O*-glucoside and monosaccharides in strawberries. Owing to the high simplicity, versatility and speed of analysis, the introduced method can potentially be employed for phytochemical analysis in

agricultural development, drug discovery, fragrance industry, and natural product research.

■ ASSOCIATED CONTENT

Supporting Information

Video showing stable electrospray and color bleaching during iEESI measurements on strawberry sample. MS/MS iEESI mass spectra of the pelargonidin 3-*O*-glucoside. Analysis of ginger rhizome by iEESI−MS and leaf spray. Detection of RDX using both iEESI−MS and leaf spray-MS. iEESI−MS spectra of red and green pepper. iEESI−MS spectra of the chlorophytum comosum leaves with different sunlight exposure durations. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

CID, collision-induced dissociation; DESI, desorption electrospray ionization; DP, degree of polymerization; EESI, extractive electrospray ionization; ESI, electrospray ionization; HPLC, high performance liquid chromatography; ID, inner diameter; iEESI, internal extractive electrospray ionization; LTQ, linear trap quadruple; MS, mass spectrometry; OD, outer diameter; PCA, principal component analysis; PESI, probe electrospray ionization

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