Anal. Chem. **2007,** *79,* 1447−1455

Differentiation of Maturity and Quality of Fruit Using Noninvasive Extractive Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry

Huanwen Chen,[†,‡] Yanping Sun,[§] Arno Wortmann,[†] Haiwei Gu,[§] and Renato Zenobi*[,†]

Chemistry Department and Applied Biosciences, ETH Zurich, CH-8093 Zürich, Switzerland, College of Chemistry, Jilin University, Changchun 130023, People's Republic of China, and Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

Maturity is an essential factor that determines storage-life and final quality of most fruits and vegetables. Maturity monitoring is thus of paramount importance for postharvest handling and fruit quality regulation. Ideal analytical procedures for maturity investigation require high sensitivity, specificity, and high throughput and should be noninvasive. For the purpose of maturity differentiation, extractive electrospray ionization quadrupole time-of-flight mass spectrometry (EESI-QTOF-MS) is developed for rapid fingerprinting of compounds released from various fruits. Ripening stages of bananas, grapes, and strawberries are successfully differentiated by performing principal component analysis (PCA) of the mass spectral fingerprints of the fruits. Methodological reproducibility was also evaluated experimentally and in terms of PCA clusters. The data indicate that EESI-QTOF-MS is a useful noninvasive tool for rapid investigation and differentiation of maturity and quality of fruits without sample preparation.

Fruits have been consumed by mankind for millennia. Maturity is the essential factor determining the quality and flavor of the fruit, as well as any foods made from fruits.[1] Although the molecular basis[1−4] for flavor and nutrition of fruits remains somewhat of a mystery, it is clear that the maturity at harvest is the most important factor that determines storage-life and final fruit quality.[5−7] Immature fruits are more prone to shriveling and mechanical damage and are of inferior flavor quality when ripe. Overripe fruits are likely to become soft and mealy with insipid flavor soon after harvest. Fruits picked either too early or too late in the season are more susceptible to postharvest physiological disorders than fruits picked at the proper maturity. For best marketing, fruits are often transported over long distances after harvest. In general, it takes days, weeks, or even months for the fruit to be consumed eventually. During this time, maturity[6,7] is affected by environmental factors including temperature, moisture, storage conditions, storage time, etc. Thus, fast maturity monitoring is of great importance to maximize the profits and minimize the economic loss caused by overripe fruits in stock.

Maturity of fruits is usually evaluated in terms of freshness and/or firmness,[8] color of surface or flesh, flavor, and surface defects.[6,7] In some cases (e.g., peaches, bananas), firmness and color of the surface and flesh are measured comprehensively.[9−14] However, flavor has been considered as the most important parameter and has been used widely.[6,7,15,16] To date, electrochemical methods such as electronic noses,[12,17−20] NMR,[6,21−24] optical

* To whom correspondence should be addressed. Fax: (+)41-44-632-1292. E-mail: zenobi@org.chem.ethz.ch.

[†] ETH Zurich.

[‡] Jilin University.

[§] Purdue University.

(1) Lund, S. T.; Bohlmann, J. *Science* **2006**, *311*, 804−805.
(2) Goff, S. A.; Klee, H. J. *Science* **2006**, *311*, 815−819.
(3) Xie, D. Y.; Sharma, S. B.; Paiva, N. L.; Ferreira, D.; Dixon, R. A. *Science* **2003**, *299*, 396−399.
(4) Coombe, B. G. *Am. J. Enol. Vitic.* **1992**, *43*, 101−110.
(5) Remon, S.; Ferrer, A.; Venturini, M. E.; Oria, R. *J. Sci. Food Agric.* **2006**, *86*, 657−665.
(6) Kader, A. A. *Acta Hortic.* **1999**, *485*, 203−209.
(7) Kader, A. A. *Acta Hortic.* **1996**, *434*, 249−256.
(8) Ozer, N.; Engel, B. A.; Simon, J. E. *Trans. ASAE* **1998**, *41*, 871−876.
(9) Hernandez, Y.; Lobo, M. G.; Gonzalez, M. *Food Chem.* **2006**, *96*, 654−664.
(10) Visai, C.; Vanoli, M. *Sci. Hortic.* **1997**, *70*, 15−24.
(11) Ruiz-Altisent, M.; Lleo, L.; Riquelme, F. *J. Food Eng.* **2006**, *74*, 490−499.
(12) Brezmes, J.; Fructuoso, M. L. L.; Llobet, E.; Vilanova, X.; Recasens, I.; Orts, J.; Saiz, G.; Correig, X. *IEEE Sens. J.* **2005**, *5*, 97−108.
(13) Ledbetter, C. A.; Aung, L. H.; Palmquist, D. E. *J. Hortic. Sci. Biotechnol.* **2002**, *77*, 526−533.
(14) Lavilla, T.; Recasens, I.; Lopez, M. L.; Puy, J. *J. Sci. Food Agric.* **2002**, *82*, 1842−1849.
(15) Reid, L. M.; O'Donnell, C. P.; Downey, G. *J. Agric. Food Chem.* **2004**, *52*, 421−427.
(16) Mehinagic, E.; Royer, G.; Symoneaux, R.; Jourjon, F.; Prost, C. *J. Agric. Food Chem.* **2006**, *54*, 2678−2687.
(17) Marrazzo, W. N.; Heinemann, P. H.; Crassweller, R. E.; LeBlanc, E. *Trans. ASAE* **2005**, *48*, 1995−2002.
(18) Supriyadi, Shimizu, K.; Suzuki, M.; Yoshida, K.; Muto, T.; Fujita, A.; Tomita, N.; Watanabe, N. *Flavour Fragrance J.* **2004**, *19*, 44−50.
(19) Gomez, A. H.; Wang, J.; Hu, G. X.; Pereira, A. G. *Sens. Actuators, B* **2006**, *113*, 347−353.
(20) Saevels, S.; Lammertyn, J.; Berna, A. Z.; Veraverbeke, E. A.; Di Natale, C.; Nicolai, B. M. *Postharvest Biol. Technol.* **2003**, *30*, 3−14.
(21) Roh, M. S.; Bentz, J. A.; Wang, P.; Li, E. C.; Koshioka, M. *J. Hortic. Sci. Biotechnol.* **2004**, *79*, 645−651.
(22) Galed, G.; Fernandez-Valle, M. E.; Martinez, A.; Heras, A. *Magn. Reson. Imaging* **2004**, *22*, 127−137.
(23) Kim, S. M.; Chen, P.; McCarthy, M. J.; Zion, B. *J. Agric. Eng. Res.* **1999**, *74*, 293−301.
(24) Maas, J. L.; Line, M. J. *HortScience* **1995**, *30*, 1090−1096.

10.1021/ac061843x CCC: $37.00
Published on Web 01/06/2007

spectroscopy,[11] including techniques such as IR,[25] NIR,[26−29] fluorescence spectroscopy,[30] and HPLC,[31] solid-phase microextraction (SPME),[15,32] GC,[15] electrophoresis,[33] and GC/MS[34,35] have been employed for maturity measurement. Electrochemical methods show a general weakness in specificity; many compounds present in the environment can give rise to false signals. IR and NIR have relatively low sensitivity and specificity for most fruits. NMR usually requires sample pretreatment, long data acquisition times, and expensive instrumentation, which is of limited practical use. Conventional HPLC and GC/MS provide better specificity and sensitivity, but require tedious sample collection and preparation.

Ideally, analytical procedures for fruit maturity investigation should exhibit high sensitivity, good specificity, and high throughput and be noninvasive.[6−8,11] It is well known that mass spectrometry provides high sensitivity and high specificity. As compared to other methods, mass spectrometry was announced to be the best "electronic nose" for practical monitoring the quality of foods.[36] Traditionally, however, mass spectrometry also requires sample pretreatment.[15,18,31,35,37] The recently established methods of direct analysis real-time (DART)[38,39] and desorption electrospray ionization (DESI) mass spectrometry provide a practical way to rapidly investigate ambient samples. For example, DESI is able to monitor surfaces of various samples including pharmaceutical preparations,[40] luggage,[41] biological samples,[42] or dyes in foods[43] without sample pretreatment. Unfortunately, methanol, a well-known and toxic compound, is usually sprayed onto the sample in DESI mass spectrometry to achieve the proper analytical performance, and thus DESI is not a perfect tool for analysis of food that is intended for consumption due to the possibility for contamination by the toxic chemical reagents used.

By combining the merits of DESI[40,44] and a vapor analysis experiment reported by Fenn et al.,[45] a new technique, extractive electrospray ionization (EESI), was developed to directly ionize various ambient samples, even with complex matrixes, for rapid mass spectrometric analysis.[46] A unique feature of EESI is that sample is introduced by a separate channel, which is electronically grounded and located orthogonally to the ESI channel.[42,46] This design provides good flexibility of EESI for applications to different samples, although it was conceived for liquid sample analysis, and offers excellent tolerance to dirty matrixes.[46] EESI requires no sample separation or pretreatment and allows fast online mass spectrometric analysis. Extractive electrospray ionization mass spectrometry was initially demonstrated with an ion trap mass spectrometer equipped with a homemade EESI source[46] and recently implemented for direct breath analysis in the interface of a commercial QTOF mass spectrometer[47] without any hardware modification. Taking advantage of commercially available QTOF instrumentation, EESI-QTOF-MS is employed in this study for fruit maturity differentiation by fast fingerprinting of compounds released from various fruits. Data obtained by EESI-QTOF-MS were processed by principal component analysis (PCA),[48,49] one of the most widely used multivariate statistical analysis tools in a variety of areas.[9,42,50−52] In the current study, PCA is used to visualize the differentiation of the maturity of the tested fruit samples and the reproducible ability of the new technique.

EXPERIMENTAL SECTION

Reagents and Materials. All chemical reagents such as acetic acid were bought from Fluka (Buchs, Switzerland) with the highest purity available and used directly without any further treatment. Deionized water was provided by ETH chemistry facilities.

Fruits such as bananas, grapes, and strawberries were selected from three different maturity stages. A sample set was about 200 g of each type of fruit. For the bananas, there were 4 sample sets at 4 different stages, and each set was composed of 3−4 individual bananas. Each sample set was measured 13 times. These measurements were repeated 9 times 4 h later, to test the reproducibility of the measurement. For the grapes, 3 sample sets were used. Each sample set was about 50 grapes selected at the same maturity. Each sample was measured 14 times. In the case of strawberries, there were 3 sample sets. From each set, 6 individual samples at the same maturity were chosen, and one measurement was made per sample. All fruits were bought from local stores. All bananas were produced in the same location, as

(25) Bodria, L.; Fiala, M.; Guidetti, R.; Oberti, R. *Trans. ASAE* **2004**, *47*, 815−820.
(26) Saranwong, S.; Sornsrivichai, J.; Kawano, S. *Postharvest Biol. Technol.* **2004**, *31*, 137−145.
(27) Herrera, J.; Guesalaga, A.; Agosin, E. *Meas. Sci. Technol.* **2003**, *14*, 689−697.
(28) Kim, J.; Mowat, A.; Poole, P.; Kasabov, N. *Chemom. Intell. Lab. Syst.* **2000**, *51*, 201−216.
(29) Bellon, V.; Vigneau, J. L.; Leclercq, M. *Appl. Spectrosc.* **1993**, *47*, 1079−1083.
(30) Kolb, C. A.; Wirth, E.; Kaiser, W. M.; Meister, A.; Riederer, M.; Pfundel, E. E. *J. Agric. Food Chem.* **2006**, *54*, 299−305.
(31) Alonso-Salces, R. M.; Ndjoko, K.; Queiroz, E. F.; Ioset, J. R.; Hostettmann, K.; Berrueta, L. A.; Gallo, B.; Vicente, F. *J. Chromatogr., A* **2004**, *1046*, 89−100.
(32) Mazida, M. M.; Salleh, M. M.; Osman, H. *J. Food Compos. Anal.* **2005**, *18*, 427−437.
(33) Jock, S.; Geider, K. *Environ. Microbiol.* **2004**, *6*, 480−490.
(34) Monti, G.; De Napoli, L.; Mainolfi, P.; Barone, R.; Guida, M.; Marino, G.; Amoresano, A. *Anal. Chem.* **2005**, *77*, 2587−2594.
(35) Preston, C.; Richling, E.; Elss, S.; Appel, M.; Heckel, F.; Hartlieb, A.; Schreier, P. *J. Agric. Food Chem.* **2003**, *51*, 8027−8031.
(36) Schaller, E.; Bosset, J. O.; Escher, F. *Chimia* **1999**, *53*, 98−102.
(37) Hayasaka, Y.; Adams, K. S.; Pocock, K. F.; Baldock, G. A.; Waters, E. J.; Hoj, P. B. *J. Agric. Food Chem.* **2001**, *49*, 1830−1839.
(38) Williams, J. P.; Patel, V. J.; Holland, R.; Scrivens, J. H. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1447−1456.
(39) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297−2302.
(40) Chen, H. W.; Talaty, N. N.; Takats, Z.; Cooks, R. G. *Anal. Chem.* **2005**, *77*, 6915−6927.
(41) Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H. W.; Cooks, R. G. *Chem. Commun.* **2005**, 1950−1952.
(42) Gu, H.; Chen, H.; Pan, Z.; Jackson, A. U.; Talaty, N.; Xi, B.; Kissinger, C.; Duda, C.; Mann, D.; Raftery, D.; Cooks, R. G. *Anal. Chem.* **2007**, *79*, 89−97.
(43) Chen, H. W.; Zhang, X.; Luo, M. B. *Chin. J. Anal. Chem.* **2006**, *34*, 464−468.
(44) Takats, Z.; Wiseman, J. M.; Cooks, R. G. *J. Mass Spectrom.* **2005**, *40*, 1261−1275.
(45) Fuerstenau, S.; Kiselev, P.; Fenn, J. *Proc. 47th ASMS Conf. Mass Spectrom. All. Top.*; Dallas, TX, 1999; ThOE 3:00.
(46) Chen, H. W.; Venter, A.; Cooks, R. G. *Chem. Commun.* **2006**, 2042−2044.
(47) Chen, H. W.; Wortwamm, A.; Zhang, W. H.; Zenobi, R. *Angew. Chem., Int. Ed.* **2006**, VIP paper, early view.
(48) Johnson, R. A.; Wichern, D. W. *Applied Multivariates Statistical Analysis*, 4th ed.; Prentice Hall: Upper Saddle River, NJ, 1998.
(49) Yang, K.; Trewn, J. *Multivariate Statistical Methods in Quality Management*; McGraw-Hill Co. Inc.: New York, 2004.
(50) Sandusky, P.; Raftery, D. *Anal. Chem.* **2005**, *77*, 7717−7723.
(51) Vogt, F.; Mizaikoff, B. *Anal. Chem.* **2003**, *75*, 3050−3058.
(52) Chen, Z. P.; Morris, J.; Martin, E. *Anal. Chem.* **2005**, *77*, 6563−6570.

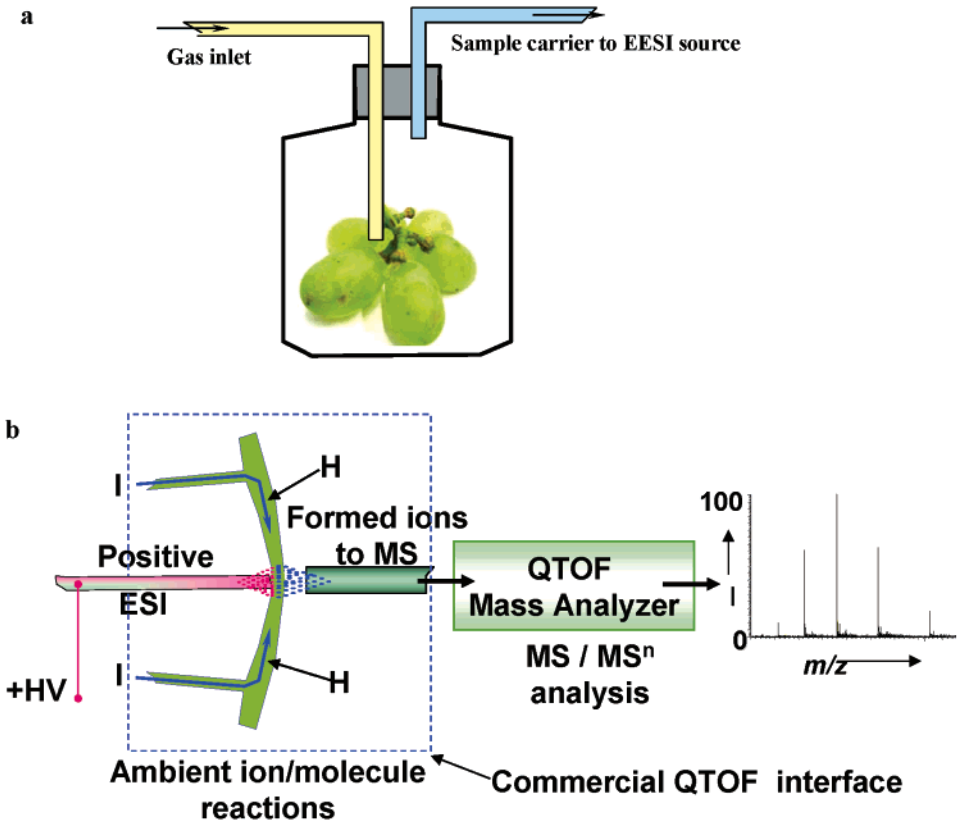

Figure 1. Instrumental setup for experiments: (a) schematic diagram of the sampler for EESI; (b) EESI source implemented in a commercial ESI-QTOF-MS. I, desolvation gas inlet, used for sampling; H, heating region in the desolvation gas outlet. Compounds released from the fruits will be ionized in the region between the ESI spray and the sample stream.

were all of the grapes and strawberries. All of the fruits were used directly without washing or other pretreatment.

EESI-TOF Analysis. To avoid potential chemical contamination, fruits were placed in a clean and dry glass container, which was specially designed so that the compounds released from the fruits inside the container could be directed into the gas inlet of the EESI source (shown in Figure 1). Air of about 45% relative humidity was infused as carrier gas into the container at 200 mL/min, and the sample mixture was introduced into the EESI source through the desolvation gas transfer line, which is part of the original ESI interface and oriented orthogonal to the ESI spray. During this study, ESI was achieved with a 3.5 kV bias voltage, and data were collected using positive ion mode.

Extractive electrospray ionization was implemented in the interface of a commercial ESI-QTOF-MS instrument (QTOF Ultima, Waters Micromass, Manchester, UK) without hardware modification, as described in a previous communication.[47] A 10% (V:V) acetic acid water mixture was infused at 5 μL/min as the electrospray solution. By transfer of protons generated by spraying this acetic acid/water solution, analytes in the sample stream were ionized in the EESI source, and then mass-analyzed by the time-of-flight analyzer. The ESI source was maintained at 80 °C in these experiments. Parent ions were isolated with 1 mass/charge unit width, and collision-induced dissociation (CID) was performed with 10−25 units of collision energy. All of the spectra were collected for 1 min and background subtracted.

Principal Component Analysis (PCA). PCA, as a widely used unsupervised chemometric method, rotates the high dimension space, in which each spectrum is represented by a point, to a 2D or 3D coordinate system on which spectra with similar variation characteristics are classified together. We applied PCA as a tool to clarify the difference between fruits in various maturity states and to test the reproducibility of EESI-QTOF-MS.

Similar to previous studies,[42] PCA was performed using EESI-MS data in TXT format while using data sets that represent the high-resolution data fully. Data were not binned before PCA process. PCA results were obtained on the basis of the mean-centered EESI-QTOF-MS data using the R program (version 2.3.1) and MINITAB 13 (MINITAB Inc., State College, PA). Typically, the first two principal components represent more than 85% of the total variance in this study.

RESULTS AND DISCUSSION

Optimization of EESI. In the typical EESI experiment reported previously,[46] liquid samples were sprayed into the electrospray beam, where micro liquid droplet−droplet extraction/ionization occurs, providing an extraction-based process. In our current work, analytes were introduced into the electrospray beam through the desolvation gas line. This is very similar to the original setup using two separate channels to perform EESI.[46] Using the standard ESI interface of the QTOF-MS instrument, a gaseous sample mixture rather than a liquid solution was directly introduced into the ESI beam as shown in Figure 1, without any hardware modification. However, the physical concept and the operation principle remain the same as that demonstrated using liquid solutions or suspensions.[46]

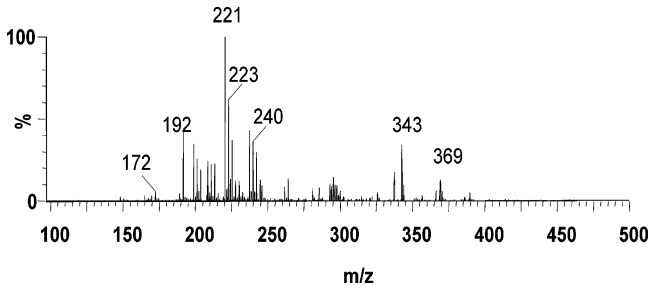

Figure 2. EESI mass spectrum of normal grapes, obtained using HAc/water as spray solution and the EESI source shown in Figure 1b.

No optimization of the EESI configuration was performed because the commercial interface was fixed by the manufacturer. Rather, the influence of pH, of the spray solvent infusion rate, and of the high voltage for the electrospray was investigated. Grapes of normal maturity were used as the sample, which generated mass spectrometric signals as shown in Figure 2. Working conditions were selected by optimizing the signal intensity. Experimentally, it was found that the signal reached a high and stable level under the following conditions: an acetic acid concentration in the range of 5−15% (V:V), an infusion rate in the range of 3−5 μL/min, and a high voltage between 3 and 4 kV. When the infusion rate was lower than 0.5 μL/min and/or the high voltage was less than 1.5 kV, the signal intensity was significantly lower. This was probably caused by insufficient primary ions generated in the electrospray. When the infusion rate was higher than 10 μL/min, the signal also decreased somewhat, and, finally, the signal dropped with further increase of the infusion rate. This was probably due to the inefficient desolvation of the primary ions, which was also observed in DESI.[40,53] The signal was slightly enhanced when the spray voltage was increased above 3 kV. Because the upper limit of the spray voltage in our instrument was 4 kV, higher voltages were not investigated. To facilitate proton generation in the electrospray, acetic acid was added into deionized water to form the spray solution. Signal was very low when pure water was directly used as spray solvent, but it improved appreciably when acetic acid was added into the water. The signal was still increasing but only very slightly when the concentration of acetic acid was higher than 10%. Therefore, we chose an acetic acid concentration of 10%, an infusion rate of 5 μL/min, and an electrospray voltage of 3.5 kV as the working conditions for further experiments.

During the experiments, the ambient air had a relative humidity of about 40−45%, and the gas flow rate was about 200 mL/min. It was found that a higher flow rate could further raise the signal, but it was not tolerated by the sealed QTOF ESI interface. If dry air was used, the number of peaks showing in the mass spectra and the overall intensity decreased considerably. On the other hand, the number of peaks and the signal intensity improved when high moisture air was used. Exhaled breath has been analyzed successfully using this strategy.[47] From the experimental data obtained in the breath analysis[47] and in our current work for fruit analysis, it is clear that typical nonvolatile or semi-volatile compounds such as glucose, urea,[47] and some high molecular weight compounds (shown in Figure 3) were detected using the same setup. Semivolatile compounds cannot be introduced into the vapor−ESI interaction region of the setup proposed by Fenn et al.[45] because the semivolatile compounds have too low a vapor pressure at room temperature. Micro water droplets may pick these up, because compounds such as urea and glucose are easily dissolved in water at room temperature. Experimentally, peaks at high mass range disappeared when a dry nitrogen gas

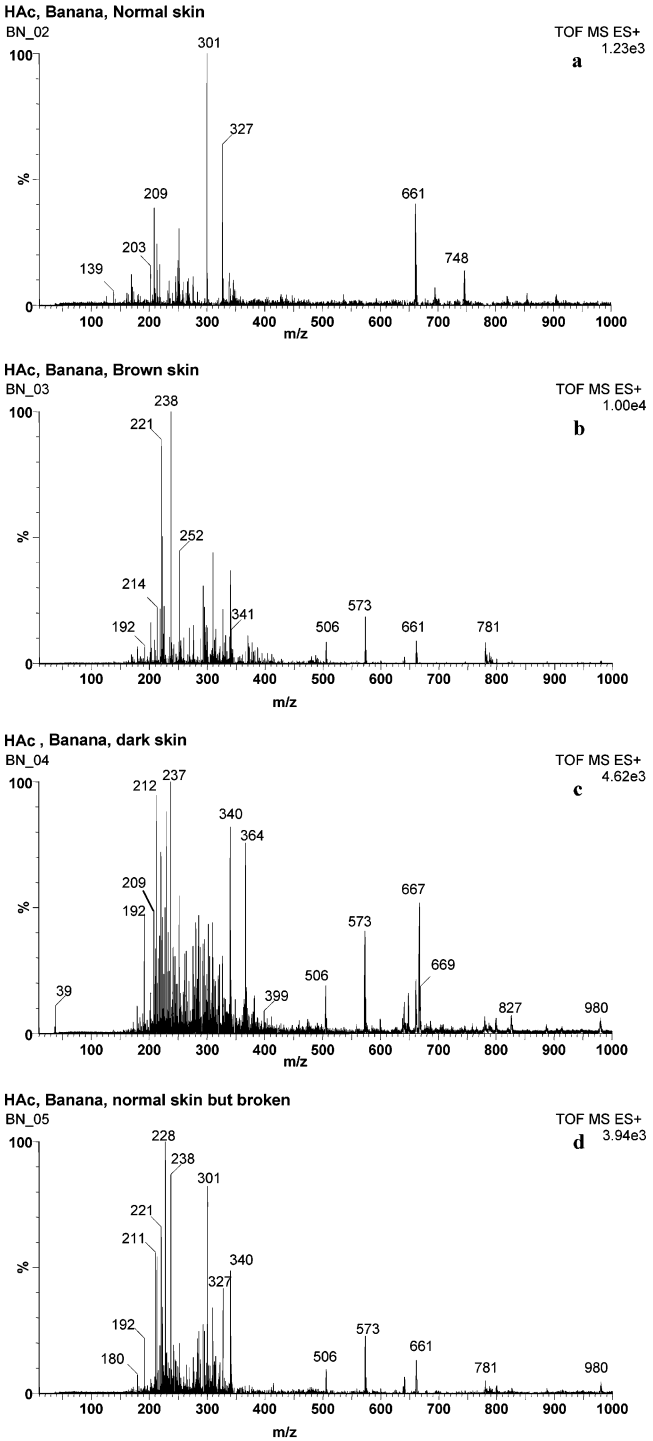

Figure 3. EESI-QTOF-MS spectra of bananas at different maturity and quality stages: (a) bananas of normal maturity with perfect skins; (b) slightly overripe bananas with intact brown skins; (c) strongly overripe bananas with unbroken dark skins; and (d) bananas of normal maturity but broken skins.

(53) Venter, A.; Sojka, P. E.; Cooks, G. *Anal. Chem.*, in press.
(54) Chen, H. W.; Wortmann, A.; Zenobi, R. Manuscript in preparation.

was used as the sampling/carrier gas to impact the fruit surface. This further confirms that semivolatile compounds were picked up by aerosol water droplets, and then ionized by an EESI process. Additionally, experiments carried out using the same method but sampling off the surface of frozen meat[54] were also successful, providing more evidence that neutral compounds were sampled by micro droplets for extractive electrospray ionization. However, for all of the fruit samples studied here, ionization of gas phase surely takes place as well, because fruits release plenty of volatile compounds[1] as flavors. Although the exact mechanism remains to be systematically studied, it is clear that spectral fingerprints detected in EESI from the fruit samples are facilitated by the present of water in the sampling gas flow. The fact that compounds detected in EESI mass spectra are not limited to volatile compounds but include molecules of low vapor pressure is in good agreement with previous studies.[47] To keep the procedure simple and operationally convenient, ambient air (~45% RH) was directly used, and experiments were carried out at room temperature.

Maturity and Quality Differentiation. Generally, fruits can be divided into two groups: (1) fruits that are not capable of continuing their ripening process once removed from the plant, and (2) fruits that can be harvested before they are mature, which will ripen off the plant. Group 1 includes berries, cherries, citrus fruits, grapes, lychees, pineapples, pomegranates, and tamarillos. Group 2 includes apples, apricots, avocados, bananas, cherimoya, guava, kiwis, mangos, nectarines, papayas, passion fruit, pears, peaches, persimmons, plums, quinces, sapodillas, and sapotes. As a demonstration, various fruits from both groups, bananas, grapes, and strawberries, were chosen as typical samples for examination.

(1) Bananas. Bananas were placed in the sampling container, and the compounds released were introduced into the EESI source for ionization, and then mass analyzed by the QTOF mass spectrometer. Mass spectra of different bananas with different quality and at different stages of maturity were collected. It is interesting to see that there are many peaks distributed over a relatively wide mass range (around m/z 50−1000) (shown in Figure 3). Usually, only low molecular weight (typically around 100 Da) species can be detected by techniques such as selected ion flow tube (SIFT)[55−57] and proton-transfer reaction (PTR)[34,58−60] mass spectrometry, probably because these are typically coupled with gas chromatography for sample separation, which is restricted to volatile small molecules. EESI-MS spectra of bananas of different quality stages present very different patterns (as shown in Figure 3a−d). The mass spectrum becomes more and more complex as the bananas are getting riper, showing more and more peaks in the mass range from m/z 120 to 800 (Figure 3a−c). From common-sense experience, very ripe bananas emit a strong fragrance and a characteristic smell. Therefore, it is not surprising that there are more peaks in the mass spectrum of the ripe matured bananas than those of the unripe bananas. The chemicals

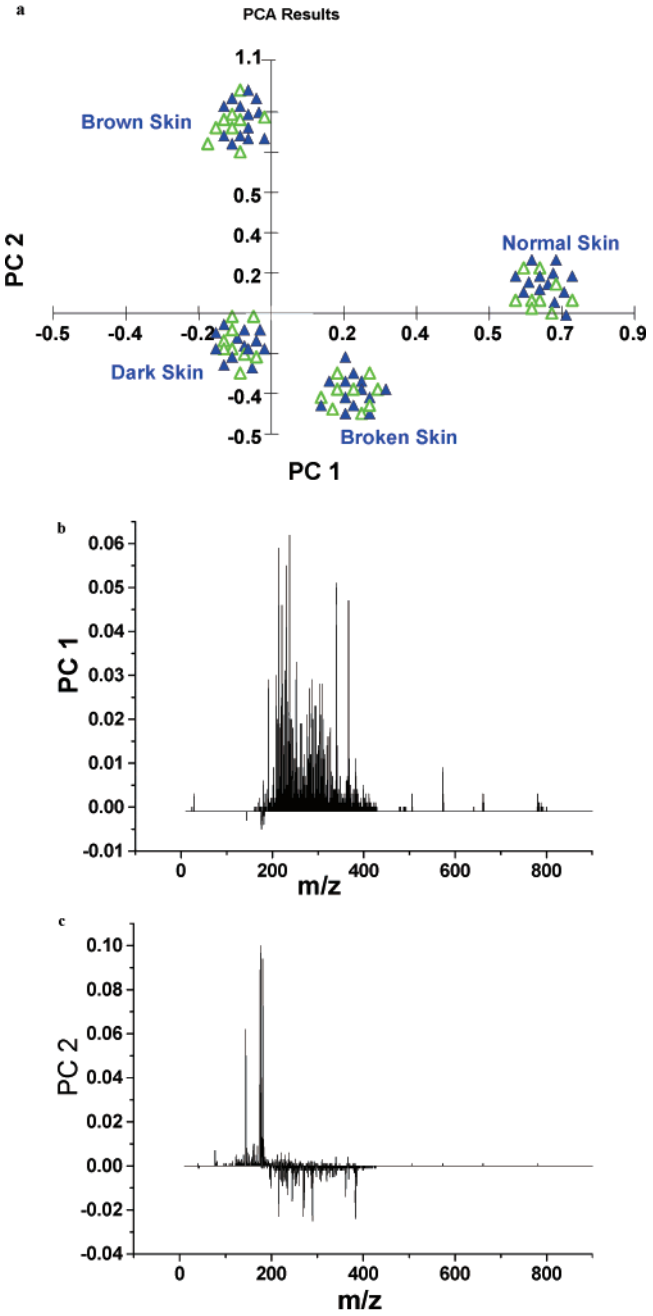

Figure 4. PCA results of bananas at different maturity stages: (a) PCA score plots, unfilled triangles refer to data sets obtained 4 h before the filled ones; (b) differential PC1 components in the loading plot; and (c) differential PC2 components in the loading plot.

released from an unripe banana are clearly distinguishable from those of a ripe one. For example, predominant peaks at m/z 301, 327, 661 in Figure 3a are substantially smaller in Figure 3b; in contrast, peaks at m/z 221, 238, and 573 are almost undetectable in Figure 3a. Similar differences were also observed comparing Figure 3c and d and also in cases where other fruits were examined. β-Damascone (MW 192), a strong aromatic component in fruit,[1,2,4] was detected in the EESI-MS spectra and tentatively identified following MS/MS. The relative abundance of β-damascone is undoubtedly associated with the maturity stages. For example, the intensity of peak at m/z 192 could be useful to differentiate bananas with 4 stages. It was very low in normal

(55) Wang, T. S.; Carroll, W.; Lenny, W.; Boit, P.; Smith, D. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 125−130.
(56) Smith, D.; Spanel, P. *Mass Spectrom. Rev.* **2005**, *24*, 661−700.
(57) Wang, T. S. *Chin. J. Anal. Chem.* **2005**, *33*, 887−893.
(58) Lindinger, C.; Pollien, P.; Ali, S.; Yeretzian, C.; Blank, I.; Mark, T. *Anal. Chem.* **2005**, *77*, 4117−4124.
(59) Blake, R. S.; Whyte, C.; Hughes, C. O.; Ellis, A. M.; Monks, P. S. *Anal. Chem.* **2004**, *76*, 3841−3845.
(60) Pollien, P.; Lindinger, C.; Yeretzian, C.; Blank, I. *Anal. Chem.* **2003**, *75*, 5488−5494.

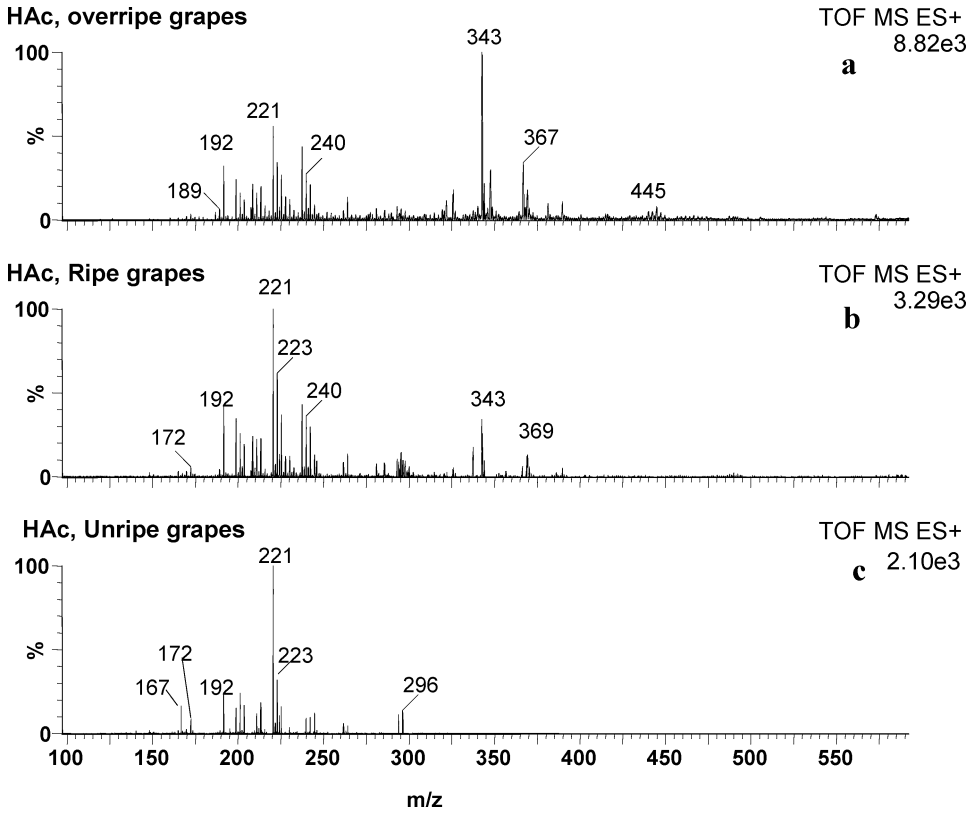

Figure 5. EESI-QTOF mass spectra of grapes at different maturity stages, showing differentiation patterns: (a) overripe grapes; (b) normally ripe grapes; and (c) unripe grapes.

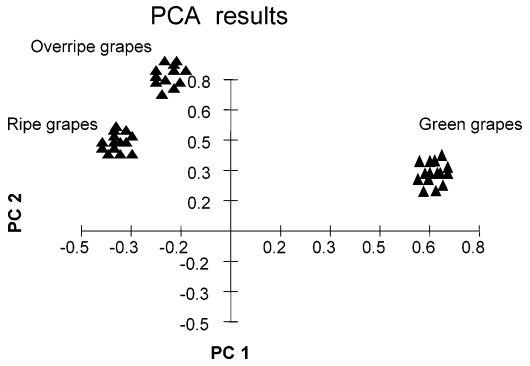

Figure 6. PCA results of grapes at different maturity stages.

bananas with unbroken skin (Figure 3a), it reached the highest abundance in overripe bananas with dark skins (Figure 3c), and medium abundance in the bananas with normal maturity but broken skins (Figure 3d). This indicates the possibility of biosynthesis of β-damascone inside the flesh of the banana. Obviously, the major peaks that differentiate the mass spectra could be useful as potential markers for banana maturity evaluation, especially in cases when entire mass spectral fingerprints are not available.

To further visualize the difference between the spectra obtained from bananas of different maturities, PCA was performed to process the data. As expected, bananas of different maturities are successfully separated in the PCA score plots (shown in Figure 4a). Interestingly, the cluster of bananas with dark skins is located closer to the one with broken skins than to all other clusters, which is in agreement with the experimental data shown in Figure 3c and d. PCA loading plots (Figure 4b,c) are also obtained and show the major peaks differentiating the banana samples. Loading plots of PCA show the differentiating peaks obtained in the EESI-MS. Usually, the differential peaks are good candidates for further considering as biomarkers of maturity or other physiological status of the samples. Structural identification of all of these differential peaks is far beyond the scope of this study. However, the differential peaks, especially those of significant changes in term of sensitivity, could be used alone as a major molecular marker to probe the physiological status of the fruits. In such a case, where a full mass spectrum covering a wide mass range is not necessary, a simple and low cost mass spectrometric sensor[61] could be used instead of a fully outfitted, expensive instrument to monitor the molecular markers, providing a possibility for practical monitoring in industry.

EESI-QTOF-MS is of excellent long-term stability even if a complex sample with a dirty matrix is infused directly. Multiple measurements have been completed to examine the reproducibility in PCA score plots. As can be seen in Figure 4a, the data sets obtained 4 h later are successfully classified and located into the same clusters as the previous data sets. This shows that the EESI is stable and the measurements are repeatable. It also indicates that the bananas' fingerprint does not change a lot over 4 h under the experimental conditions chosen.

(2) Grapes. Three groups of grapes of different maturities, overripe grapes, normally ripe grapes, and unripe grapes, were investigated next. The different grape groups were differentiated

(61) Zhang, C.; Chen, H. W.; Guymon, A. J.; Wu, G.; Cooks, R. G.; Ouyang, Z. *Int. J. Mass Spectrom.* **2006**, *255*−*256*, 1−10.

Figure 7. Typical strawberries for experimental investigation: (a) slightly overripe strawberry; (b) normally ripe strawberry; and (c) green strawberry.

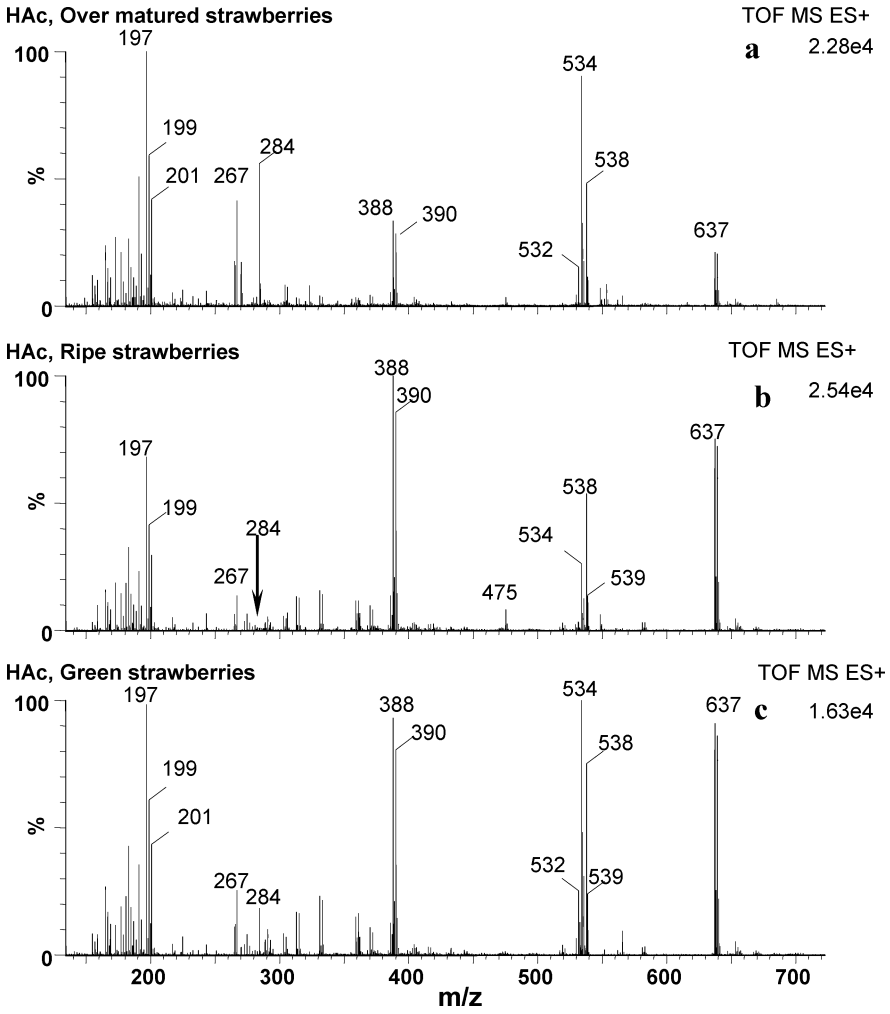

Figure 8. EESI-QTOF mass spectra of strawberries: (a) overripe strawberries; (b) normally ripe strawberries; and (c) green strawberries. Pictures of the typical strawberries used for collecting the mass spectra in this figure are shown in Figure 7a–c, respectively.

very well by the EESI mass spectra as shown in Figure 5a–c. Comparing these spectra with those obtained from bananas (Figure 3), there are fewer peaks detected in the grape samples. This might be because there are either fewer compounds released from grapes than from bananas, or because fewer compounds could be detected by EESI in the grapes samples. However, more peaks were found in the mass spectra of riper grapes. This is consistent with the findings obtained with the bananas samples. More interestingly, grapes provided drastically different mass spectral fingerprints as compared to those recorded for banana samples. The data indicate that fruits could be easily recognized by a mass spectrometer, which in fact has been recognized to be the best "electronic nose" for similar applications.[36,62,63] From the spectra shown in Figure 5, it is clear that peaks at m/z 192 and 221 are commonly found in grapes; however, the relative intensity of peaks at m/z 221 and 343 appears to be associated with the maturity of the grapes examined. Therefore, the intensity ratio of peaks at m/z 221 and 343 could be a useful indicator to differentiate the maturity of grapes. For a close look, isobutyl-3-methoxypyrazine (MW 166), a plant hormone that is known to accumulate in unripe fruits,[1,64] was detected in green grapes as a small peak at m/z 167 and identified by MS/MS. It was nearly undetectable in ripe grapes.

(62) Riccardo Flamini, A. P. *Mass Spectrom. Rev.* **2006**, *25*, 741–774.
(63) Philippe, A.; Guy, F. F. *Mass Spectrom. Rev.* **2006**, *25*, 290–326.
(64) Lacey, M. J.; Allen, M. S.; Harris, E. L. N.; Brown, W. V. *Am. J. Enol. Vitic.* **1991**, *42*, 103–108.

Table 1. Intensity Ratios of Peaks Differentiating the Mass Spectra for Strawberries

	intensity ratio				
	284/637	534/637	284/388	284/197	maturity
spectrum a	3.1	4.5	1.2	0.52	overripe
spectrum b	0.001	0.38	0.0008	0.001	normal
spectrum c	0.19	1.1	0.2	0.2	unripe

The successful discrimination of the different maturity stages of various grapes is also confirmed by the PCA score plots (shown in Figure 6). The PCA loading plots are shown in Figure S1. Multiple measurements from multiple grapes were accomplished, which provided reproducible results in the PCA score plots. Typical spectra from multiple measurements will be discussed later.

(3) Strawberries. It is known that strawberry flavor is one of the most complex flavors in nature. The reason to choose strawberry as a sample, however, was not to profile the components in the flavor. Besides the popularity of strawberries throughout the world, the motivation was to challenge EESI-QTOF-MS for discrimination of small differences among similar, complex samples. Therefore, three typical strawberry groups were selected: (1) overripe strawberries, which actually had begun to rot slightly; a wet area coverage of about 3% of the skin area had emerged on the surface, while other parts of the skin area (ca. 97%) still maintained their original appearance (shown in Figure 7a); (2) ripe strawberries, that is, fruits with fresh and perfect flavor (shown in Figure 7b); and (3) "green" strawberries (not completely green, and still edible; shown in Figure 7c). Apparently, the differences between these strawberries are not distinguishable as easily as those in the previously examined samples. The mass spectra were quite similar when these strawberries were inspected by EESI. However, a close look revealed that the difference between these mass spectral fingerprints is still noticeable (shown in Figure 8a−c). For example, the intensities of peaks at *m/z* 267, 284, 388, 390, 534, and 673 varied in the mass spectra of strawberries of different qualities. To be clearer, the intensity ratios of some interesting peaks are summarized in Table 1. From Table 1, it is self-evident that these strawberries can be separated successfully according to their stages of maturity and quality.

Differentiation of the strawberries can also be done by processing the mass spectral data using PCA. A 3-D graph of the PCA scores (shown in Figure 9) confirms the successful separation of these strawberries in the direction of principal components 1, 2, and 3. The projections of the clusters into the plane of PC1 and PC2 are tight. Inclusion of PC3 gives a clearer classification than the one based only on PC1 or PC2. The PCA loading plots are shown in Figure S2. The 3-D drawing required a relatively long time (~30 min tested with an Acer notebook computer with 256 M memory). This time is strongly dependent on computer calculation speed and the data amount. For high throughput analysis, PCA is still helpful because it can be programmed to run automatically.

Efforts have also been made to identify some peaks by performing MS/MS analysis of several parent ions of interest. For

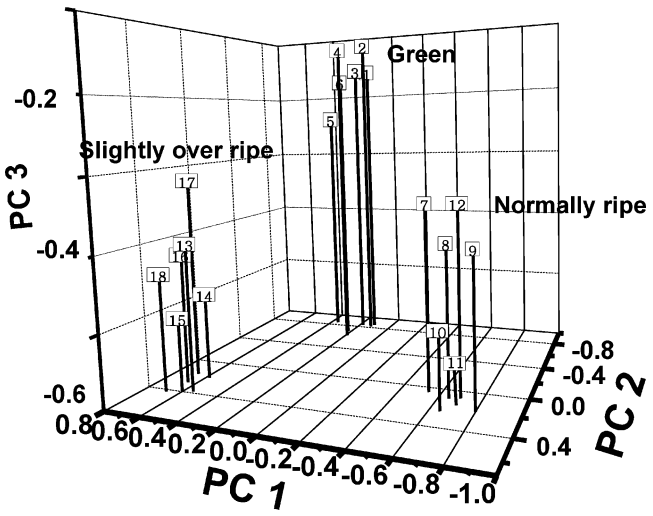

Figure 9. Separation of strawberries of three different maturities.

instance, MS/MS spectra of peaks at *m/z* 267, 284, and 197 in the mass spectra from strawberries (shown in Figure S3a−c) were obtained under the conditions described in the Experimental Section. In Figure S3a, the main fragment of *m/z* 267 is *m/z* 225, due to loss of 42 units, probably ketene. In Figure S1b, the parent ions at *m/z* 284 give fragments of *m/z* 242 and 224 by loss of CH_2=C=O and water successively. The fragment of *m/z* 172 could originate from many processes, for example, by losing C_4H_4 directly from *m/z* 224, or by loss of other species from precursors of higher mass/charge ratios. It is difficult to elucidate the structures of all of the interesting peaks in our QTOF MS, because the tandem MS capability is somewhat limited, even if CID fragments are abundant (shown in Figure S3c). If detailed structural information of the peaks is required, it will be helpful to perform MS^n, for example, using ion trap mass spectrometry. However, as shown in this study, it is not necessary to elucidate the structures to differentiate the maturity and/or quality of fruits.

Reproducibility. Under our experimental conditions, repeatable mass spectra of different fruits such as bananas and grapes have been collected, underscoring that EESI presents good stability and offers reproducible measurements. Similar conclusions can be drawn from the PCA score plots (shown in Figures 4, 6, and 9), in which measurements of different fruits at comparable stages of maturity were classified into very narrow clusters, providing confident differentiation of their maturity. Clearly, reproducible PCA score results can only be obtained from high precision and repeatable measurements. Data obtained in EESI-QTOF-MS are of much higher resolution and mass accuracy (1 ppm) than those obtained in an ion trap mass spectrometer, which facilitates the differentiation of the data sets. A similar conclusion was obtained in a previous NMR study.[65] Mass spectra collected from different measurements on individual grapes and strawberries are shown in Figures S4 and S5, respectively. It can be seen that each individual measurement performed on an individual sample of the same maturity gives a very similar mass spectrum in terms of both the number of peaks and the peak intensities.

Analysis Speed. Traditionally, fruit monitoring requires gas chromatography (GC) or GC/MS as the basic instruments.

(65) Craig, A.; Cloarec, O.; Holmes, E.; Nicholson, J. K.; Lindon, J. C. *Anal. Chem.* **2006**, *78*, 2262−2267.

Volatile compounds are inherently detected when GC is employed for sample separation. Generally, GC needs around 1 h to run a single sample and will take much longer if sample pretreatment such as collection of volatile molecules and/or removal of moisture is necessary. In this study, fruits are directly used without any pretreatment, and no sample collection and separation is required. Therefore, a typical spectrum can be collected within 1 min by using EESI mass spectrometry. As a matter of fact, maturity monitoring challenges most analytical tools in terms of both sensitivity and throughput, but EESI mass spectrometry appears to meet these demands without problems.

CONCLUSIONS

Maturity is an essential factor that determines storage life and final quality of most fruits and vegetables. The storage duration for many fruits with the best flavor is often no longer than a few days. Many analytical tools for maturity monitoring are inadequate in terms of both sensitivity and analysis speed. In this study, EESI-QTOF-MS was developed to fully meet the requirements for fruit maturity monitoring. Successful differentiations of various fruits including bananas, grapes, and strawberries have been demonstrated by providing different spectral fingerprints of the untreated fruit samples in the EESI-QTOF-MS; further discrimination has also been visualized by using cluster pattern recognition based on PCA of the raw data obtained in EESI-QTOF-MS. Because of the good long-term stability of EESI, very reproducible spectra were obtained, and good reproducibility of the measurements was also confirmed by PCA results. Because no sample collection and separation are necessary, EESI-QTOF-MS affords high analysis speed; in a favorable case, a spectral fingerprint can be obtained in a few seconds. Therefore, EESI-QTOF-MS can be used as a tool for maturity differentiation with high sensitivity, high analysis speed, and good reproducibility and without any chemical contamination of the samples.

ACKNOWLEDGMENT

H.C. sincerely acknowledges support from a Wilhelm Simon Fellowship from the CEAC at ETH Zurich.

SUPPORTING INFORMATION AVAILABLE

Five additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review September 30, 2006. Accepted November 27, 2006.

AC061843X