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SPECIAL FEATURE: TUTORIAL

Neutral desorption sampling coupled to extractive electrospray ionization mass spectrometry for rapid differentiation of biosamples by metabolomic fingerprinting

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It is of increasing interest and practical importance to develop convenient methods based on mass spectrometry for high-throughput analyses of biological samples. This is usually difficult because of the complex matrix and ion suppression effects. Generation of ions at ambient conditions is a promising solution to these problems because the sample is easily accessible and the ion suppression effect is reduced significantly. A new method for rapid on-line detection of metabolic markers in complex biological samples is described here. It combines atmospheric pressure desorption sampling by a gentle stream of air or nitrogen with extractive electrospray ionization (EESI) and mass spectrometric analysis. The resulting mass spectral fingerprints are shown to be able to detect spoilage of meat even in the frozen (−20 °C) state and the contamination of spinach by *E. coli*, and to identify metabolites and contaminants on human skin within seconds, in an on-line and high-throughput fashion. Typical molecular markers are identified using MS/MS data and by comparison with reference compounds. Differences between closely related samples are easily visualized by using principal component analysis (PCA) of the mass spectra data. The detection limit achieved is 10 fg/cm^2 (S/N = 3) for histamine on the surface of frozen meat. The technique reported here shows potential for more advanced applications in multiple disciplines, including food regulation, homeland security, *in vivo* metabolomics, and clinical diagnosis. Copyright © 2007 John Wiley & Sons, Ltd.

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KEYWORDS: neutral desorption sampling; extractive electrospray ionization; biological samples; mass spectrometry; *in vivo* analysis; skin analysis; frozen meat analysis; spinach; *E. coli*; differential metabolomics; principal component analysis

INTRODUCTION

Because of its intrinsic high sensitivity and high specificity, mass spectrometry is nowadays widely used as a powerful tool for measurements in life science,[1] biological engineering,[2,3] clinical diagnosis,[4–7] and in the pharmaceutical industry.[8–12] An accelerating trend in mass spectrometry is to create sample ions outside the vacuum system using atmospheric pressure ionization methods[13–18] such that the samples are easily accessible at ambient conditions and can be conveniently reloaded in a high-throughput fashion. Electrospray ionization (ESI)[14] charges neutral sample molecules infused along the spray solvent, which results in a relatively weak tolerance of matrices. Usually, a dilute sample is required for ESI; therefore a process for sample cleanup is demanded before a practical biological sample analysis. Similar to ESI, neutral sample molecules can be directly infused along with the solvent to generate ions by atmospheric pressure chemical ionization (APCI),[15,19] which generally results in a higher sensitivity owing to the chemical ionization mechanism. However, the sensitivity decreases substantially within a minute when a sample (e.g. urine) is introduced to an APCI source[20,21] because of the ion suppression effect caused by the matrices. Alternatively, ions

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of biomolecules can be generated by desorption/ionization techniques, for which the sample is usually placed on a solid substrate. In such a case, ions can be generated by a laser (e.g. PPI, matrix-assisted laser desorption/ionization (MALDI)), charged particles (e.g. desorption electrospray ionization (DESI), SIMS, atmospheric pressure desorption chemical ionization (DAPCI)), or atom bombardment (e.g. FAB, direct analysis in real time (DART)). In MALDI,[11,16,22] biological samples are deposited on a metal plate as a small spot and co-crystallized with suitable matrices. Generally, ion yield depends heavily on the sample molecules, the matrix, and how they are prepared.[23] Owing to the abundant matrix peaks, analytes of small molecular weight ($\leq$1000 Da) are extremely difficult to be characterized with MALDI.[24] Recently, progress in desorption/ionization on silicon (DIOS) has shown that some relatively small molecules ($\geq$500 Da) can be detected without a matrix.[24] However, no promising application for metabolomics has yet appeared in the literature. An interesting development was made by Cooks and coworkers[17,25] in 2004 with the invention of DESI, with which high-throughput analyses of various ambient samples was demonstrated for the first time without sample pretreatment. In DESI, ions are generated via the reactive collisions occurring between the neutral sample molecules and the charged particles (e.g. protons, fine droplets) produced by the electrospray. Unlike ESI or APCI, analytes for DESI are separated from the electrospray solvent,[26,27] which is usually methanol : water solution (1 : 1) plus some dilute organic acid (e.g. formic acid, acetic acid) or base (e.g. NH_3), if necessary. Theoretically, ion suppression can be reduced by separating the sample from the spray solvent; thus biological samples (e.g. urine,[20,21] animal tissue,[28] plant tissue,[29] sudan dyes in foods[30]) could be directly analyzed by DESI without tedious sample cleanup. Analogous to DESI, the primary ions generated from APCI can be directed to impact a sample surface for DAPCI.[31–33] Taking advantage of APCI for a high yield of primary ions, the sensitivity of DAPCI is higher than that of other desportion/ionization methods.[31–33] Another way to generate ions is to bombard the sample surface using metastable argon or helium atoms, which are usually created by a well-designed corona discharge such as in DART.[18] Experimental findings indicated that DART was less sensitive (e.g. for tablet analysis) than DESI,[31] probably because the overall ionization efficiency, which depends on the density of the metastable atoms and the efficiency of the ionization process, was low.

In principle, all these new desorption/ionization techniques have in common the fact that the sample is present as a solid, although the physical state of the original sample can also be different.[21,34] Motivated by the need for direct analysis of liquid samples with complex matrices, extractive electrospray ionization (EESI)[35] was introduced in 2006. Basically, neutral samples, even in dirty matrices, are dispersed in a spatial cross-section formed by a sample beam and an electrospray beam. Analyte molecules undergo interactions and collisions with the primary ions produced by electrospraying a pure solvent (e.g. acetic acid/methanol water solution) and become ionized for further mass spectral analysis. EESI is therefore an embodiment of a secondary ionization method operating at atmospheric pressure. Apparently, ion suppression is drastically reduced by distributing the matrices over a relatively wide space. Suffering from no ion suppression, pure solvents such as methanol/water mixture maintain constant ion yield in ESI for a long time, which ensures stable signals of analyte molecules in EESI so that matrices such as raw urine or milk can be directly infused to generate constant signal for more than 7 h.[35–37] With a homemade EESI source coupled to a linear ion trap (LTQ) mass spectrometer, it has been demonstrated that EESI is of similar sensitivity as ESI.[35] Another merit of EESI is that a neutral sample (i.e. biological subject) is safely isolated from any high voltage or from direct bombardment by charged particles, and therefore biological samples can be analyzed *in vivo*, with neither sample pretreatment nor chemical contamination. For certain types of applications, these features are essential, e.g. when the samples do not tolerate chemical contamination or interaction with charged particles. By implementing EESI in a commercially available quadrupole time-of-flight mass spectrometer (QTOF-MS) without any hardware modification, we have demonstrated that human breath can be directly analyzed *in vivo* and that nonvolatile compounds up to relatively high molecular weight ($\geq$1000 Da) are detectable in breath.[38] Therefore, metabolism dynamics can be followed easily and metabolic changes can be measured *in vivo* by EESI-MS. Besides analytical applications, EESI has been used to reduce the charge state of biopolymers either by ion–molecule reactions[39] or ion–ion reactions[40] in open air. Thus, a promising new area emerges, showing numerous potential applications of EESI in multiple disciplines.

From the point of view of sample phase, liquid samples were initially demonstrated in EESI by real-time monitoring of complex biological fluids; gaseous samples including breath aerosol were successfully analyzed without suffering from any notable ion suppression. Most biological samples are a mixture of fluids, with high salt concentration, supported by a solid structure such as tissue, skin, blood vessel, etc. Many biological samples contain a wide variety of chemicals, which are commonly accompanied by microorganisms. It is a challenge to analytical techniques for rapidly analyzing these biological samples, especially under conditions such as high/low temperature and in dangerous environment. Rapid analyses and differentiation of complex biological samples are required in many situations: for example for food quality monitoring, in airport security, in the battlefield and for clinical diagnosis. However, for many such applications there is no satisfactory analytical technology available. It is highly desirable to develop a new method for direct analysis of real-world biological samples without any pretreatment. Therefore, combining the advantage of desorption sampling techniques such as DESI and DAPCI with the feature of low ion suppression effect of EESI, a simple approach is proposed here to allow interrogation of virtually any type of sample by a gentle stream of air or gas, followed by efficient ionization of the neutral molecules released in an EESI step. The sample molecules are transported to the EESI source using the normal 'desolvation gas' line, a standard feature of every commercial ESI instrument. Ion formation in the method presented here does not suffer from serious matrix

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and memory effects, resulting in sensitive detection of both volatile and nonvolatile compounds of fairly high molecular weight (>1000 Da) in biological samples.[41] The mass spectral fingerprints yield comprehensive information about the molecular basis for the physiological states of biological samples and for the metabolic dynamics of microorganisms[42–44] associated with the samples. The difference between samples in different physiological states can be further visualized with principal component analysis (PCA).[21,45] This study is anticipated to be a starting point for more sophisticated applications in multiple disciplines, where analytes in complex matrices can be conveniently sampled in real time or *in vivo*.

EXPERIMENTAL

Reagents and materials

All chemical reagents, such as acetic acid, and all the biogenic polyamines were bought from Fluka (Buchs, Switzerland) with the highest purity available for direct use without any further purification. Deionized water was provided by ETH chemistry facilities. For the meat samples, turkey was fresh and packaged (cut from the breast); beef was fresh and packaged (cut from the haunch); pork was fresh and packaged (filet from the back); lamb was fresh and packaged (cut from the haunch); and fish was frozen (New Zealand petersfish filet). All types of meat were bought from local stores. Except for the meat loaf, meat samples were cut into 3–5 pieces (25–100 g each). Meat was kept frozen and sealed in Teflon bags for storage at −20 °C after exposure to air at room temperature (22 °C) for 0, 1 or 2 days to generate different spoilage stages. All meat samples were maintained at −20 °C (using an air bath controlled by dry ice) during measurements. Note that the old meat cannot be recognized by its smell when it is frozen.

Escherichia coli (*E. coli*) strains were a gift from Dr Chunmei Li (Institute of Molecular Systems Biology, ETH Zurich, Switzerland). All the *E. coli* cells were *E. coli TG1* strains, and they were centrifuged at 1000 rpm (*ca* 60 CRF) and flushed with water three times to get rid of the media. Spinach samples were purchased from a local food store and separated into two groups (150 g each): the control group was kept at 5 °C before use to keep it fresh, and the experimental group was sprayed with a suspension of *E. coli* (*ca* 10 mg in 5 ml water) and kept at 30 °C for 6 h before analysis. All the spinach samples were still wet on their surfaces and were directly used without further treatment. For analysis of unknown samples, extra caution must be taken to avoid contamination by the potentially more virulent strains of *E. coli*.

Skin samples were provided by healthy, nonsmoker, male volunteers. Skin was directly sampled without any pretreatment.

Method for surface sampling

Room-temperature (22 °C) dry nitrogen gas from desolvation gas outlet in the QTOF-MS instrument was directly used as the desorption gas to impact the biological samples, such as the icy surface of the meat, which were kept at −20 °C using an air bath cooler powered by dry ice. Part of the ice on the meat surface melted upon impact of the gas flow; thus an air mixture containing desorbed molecules such as metabolites produced by the microorganisms on the meat surface was generated. The air plume was automatically directed into the EESI source through the desolvation gas line for ionization. To facilitate the desorption, a sharp jet (i.d. 1 mm) was formed at the end of the nitrogen gas line so that a high gas speed (about 10 m/s) was created using a gas flow of 1–3 l/min. A V-shaped collecting tube (i.d. 10 mm) was mounted in front of the desolvation gas line so that more aerosol could be collected. The angles between the axes of the desorption gas flow and the collecting tube were optimized within the limits $\alpha = 45–70^{\circ}$, $\beta = 60–45^{\circ}$ (Fig. 1). The distance between the gas jet tip to the surface was 2–10 mm.

For other samples such as spinach and skin, the samples were kept in open air and at room temperature for neutral gas desorption sampling, and then the air plume was directed to the EESI source for ionization.

EESI-TOF mass analysis

Before measurement, the ESI-QTOF-MS instrument (ESI-QTOF Ultima, Waters Micromass, Manchester, UK) was calibrated for positive ion detection. For the instrument calibration, a sodium formate solution in 90/10 2-propanol/H_20 (10 mg/l) was used. A mass accuracy of 10 ppm and a resolution of better than 8000 were achieved. Source conditions were used as for normal ESI operation. The electrospray was operated with a voltage of 3 kV. The solvent was introduced at 2 μl/min. The source block was heated to 80 °C. The cone and first ion tunnel RF1 voltages were optimized for maximum signal intensity at 50 and 45 V, respectively. Full-scan spectra were recorded without the collision gas. Ions were detected with a multichannel plate (MCP) detector set at 2300 V.

The EESI was implemented at the interface of a commercially available ESI-QTOF-MS instrument without hardware modification. Details of the procedure have been given previously.[38,46] To ensure a 'green' analysis process and to avoid contamination by toxic agents, nitrogen alone was used to liberate metabolites from the meat. A 10% (v/v) acetic acid/water mixture was infused at 5 μl/min as the electrospray solution. Undergoing collisions with protons generated by spraying an acetic acid/water solution, analytes in the gaseous sample stream were ionized in the EESI source, and then mass-analyzed by the TOF analyzer. The ESI source was maintained at 80 °C. All spectra were background-subtracted and represent a 1.0 min average. The background subtraction was performed by acquiring 60 s of scan time with and without introducing the sample. The background subtraction algorithm from Mass Lynx software (version 4.0) was used.

For tandem MS studies, parent ions were isolated with 1 mass/charge unit width and collision-induced dissociation (CID) was performed with 10–25 units of collision energy.

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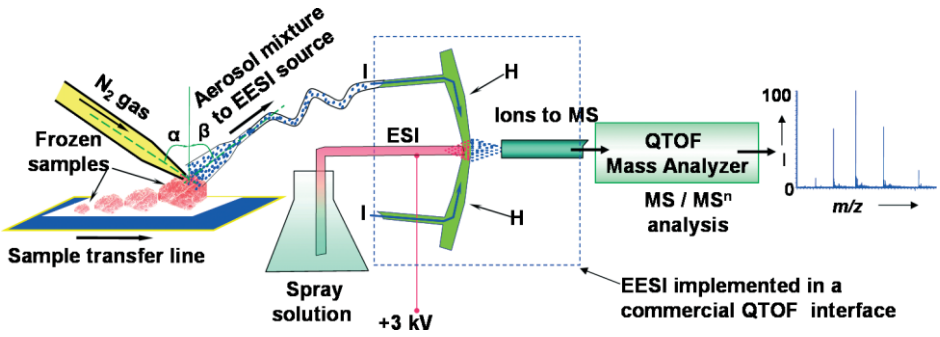

Figure 1. Schematic diagram of atmospheric pressure neutral desorption extractive electrospray ionization mass spectrometry: the compounds from the surface of biological samples such as frozen meat are desorbed without sample pretreatment by a room temperature nitrogen gas flow, which creates a neutral aerosol mixture containing molecular metabolites. The aerosol is transported to the EESI source through the desolvation gas inlet (I). The aerosol passes through the heated region (H), which is maintained at 80 °C. The distance between the desorption gas flow tip and the sample surface was 2–10 mm; the desorption (α) and collecting (β) angles were both 60°. The aerosol transfer line is a flexible Teflon tube (i.d. 5 mm and 120 cm in length), thereby demonstrating the possibility for remote analysis.

Principal component analysis (PCA)

Similar to previous studies,[21,36] raw mass spectral data obtained in. txt format were directly imported into a commercial multivariate numerical analyses software (MVSP, V3.1, Kovach Computing Services, Wales, UK) for PCA analysis. PCA results were then obtained on the basis of the normalized mass spectral data. The principal components for output were automatically determined by the software based on Kaiser's rule, and the accuracy for PCA was set to be high (1×10^{-8}). Typically, the first two principal components represent about 85% of the total variance.

For supervised PCA, mass spectral peaks of interest were selected manually, and then imported to MVSP for PCA as described above.

RESULTS AND DISCUSSION

On-line analysis of frozen meat

Food quality monitoring, which is of paramount importance to global economy, human health[47,48] and homeland security[49] (see Supplementary Information for details), challenges analytical science in terms of sensitivity, throughput, and feasibility for on-line monitoring.[50,51] Typically, quality monitoring of meat, which is industrially stored at −18 °C, requires defrosting the meat, followed by extraction and separation steps before sample analysis. Conventional analysis is therefore characterized by low throughput and off-line measurements, which furthermore often focus on detecting only 1–2 compounds (e.g. histamine) in a single run.[43,52] In contrast, fingerprinting of many molecules of low to intermediate molecular weight occurring in biological samples, such as metabolites, would give much more information about the physiological and/or pathological status, or the sample origin.

For meat samples, the EESI mass spectra were directly collected from frozen meat (−20 °C) without sample pretreatment, using a neutral gas beam for desorption (see 'Experimental' for details). Figure 2 shows different mass spectral fingerprints observed in artificially spoiled fish meat. Peaks of interest such as m/z 122, 88, 116, etc. are tentatively assigned, from their MS/MS spectra (insets in Fig. 2), to be $-NH_2$ and/or $-SH$ containing compounds. For example, the compound of the highest intensity (m/z 122) in the mass spectrum (Fig. 2(a)) is interpreted as protonated $CH_3CH_2OC(O)NHSH$ (MW 121), which loses CH_3 to yield m/z 107, CO to yield m/z 94, and CO_2 to yield m/z 88 as major fragments (inset in Fig. 2(c)). It could be cystein (MW 121); however, protonated cysteine loses water to generate a major fragment of m/z 104. But this fragment (m/z 104) was not observed. The second most abundant peak (m/z 88) is assigned to protonated C_4H_9ON (MW 87), which loses water in the MS/MS spectrum (inset in Fig. 2(a)) to yield ions of m/z 70 or alternatively loses CH_2CO to give a fragment of m/z 46. Peak at m/z 116 (Fig. 2(b)) was tentatively identified as protonated 4-amino-2-hydroxycyclopentanone (MW 115) since it loses NH_3 to yield a small peak at m/z 99 while it loses CO to give a major peak at m/z 88, which further loses water to give an abundant peak at m/z 70 (inset of Fig. 2(b)). By comparing with the MS/MS data of authentic compounds (Table 1), many peaks detected are biogenic amines, which are biomarkers known for microorganisms growing on meat,[42,43,53,54] and can be used for following the spoilage of the food. For example, trimethylamine (MW 59) shows a high signal, while dimethylamine (MW 45) gives relatively low intensities in the fresh sample (Fig. 2(a)). All the alkylamines except trimethylamine are undetectable in fish samples after exposure to room temperature. Similar dynamic changes were also found for the peaks at m/z 122 and 88 owing to the metabolic dynamics of the microorganisms.[43,44,55] We could not smell a typical 'fresh fish odor', probably because the fresh fish sample was not truly 'fresh'.[56] Chemically, the change in smell was mainly due to variable levels of alkylamines and other predominant flavors such as the compounds detected at m/z 122 and 88 (Fig. 2(a)). The peaks detected reflect the chemical dynamics of the changes in the smell. Interestingly, the peak at m/z 57, probably protonated $C_2H_4N_2$ (MW 56), is quite strong in the fish sample exposed to room temperature for two days (3.27×10^6 counts) and one day (1.06×10^5 counts) (Fig. 2(b), (c)) while it was not detected in fresh fish. The precursor ions of m/z 57 yields

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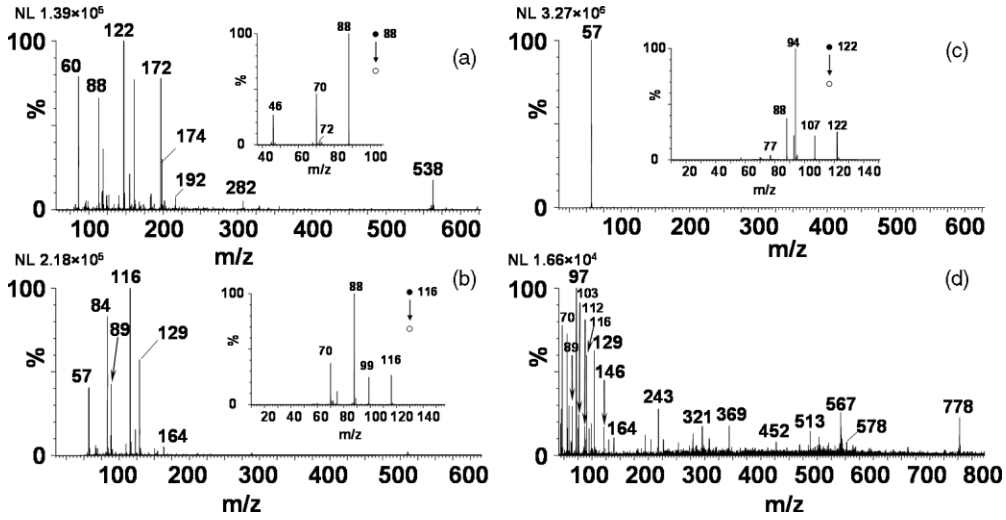

Figure 2. Desorption extractive electrospray ionization mass spectra of fish meat at different stages: (a) frozen fish not exposed to room temperature; (b) frozen fish after exposure to room temperature (22 °C) for 1 day; (c) frozen fish after exposure to room temperature for 2 days; (d) zoomed view of the spectrum in (c) showing that numerous peaks were detected in mass range from *m/z* 70 to 800. Tyramine (MW 137), tryptamine (MW 160) and spermine (MW 202) were not detectable in samples after exposure for less than 2 days, but they were detected as protonated molecules with relatively low intensities in sample after exposure for 2 days. Insets show the MS/MS spectra of the ions of interest.

Table 1. MS/MS data of identified molecular markers detected in fish meat at different stages of spoilage

Molecular markers	Molecular weight[a]	Number of days samples were exposed to room temperature
Trimethylamine[b]	59	0
Dimethylamine[b]	45	0
Dimethylacetylamine[b]	73	0
N-Methylpyrolidine[b]	117	0
$CH_3CH_2OC(O)NHSH$[c]	121	0
$CH_3NHCH_2C(CH_2)OH$[c]	87	0
N-Methylcyanoamide[c]	56	1, 2
Putrescine[b,d]	88	1, 2
Cadaverine[b,d]	102	1, 2
Histamine[b,d]	111	0, 1, 2
$C_2N_2H_8$[c]	60	0, 1
Amino-2-hydroxyclyclopentanone[c]	115	1, 2
Tyramine[b]	137	2
Spermidine[b,d]	145	2
Tryptamine[b]	160	2
Spermine[b]	202	2
Pentanethiol	104	2
$C_2H_4N_2$[c]	57	1, 2

[a] All compounds are detected as protonated molecules in EESI-MS.

[b] Compounds identified with reference compounds using MS/MS.

[c] Compounds tentatively identified from EESI-MS and MS/MS data without confirmation by reference compounds.

[d] Compounds also commonly found in beef, lamb, and turkey meat samples, especially in samples at advanced stages of spoilage.

a small peak of *m/z* 41 (by the loss of CH_3) and no other significant fragments in MS/MS in our instrument, and so it might be methylcyanamide. According to our literature survey, it has so far not been reported in spoiled fish. However, it is difficult to speculate the molecular structure without further evidence. Meat spoilage results from the growth of microorganisms that produce characteristic metabolites such as biogenic amines.[42–44,54] For instance, histamine is a typical molecular marker for various spoiled food,[43,53–55] and is the main toxin involved in health problems such as histamine fish poisoning[57] and other biochemical disorders.[58] Histamine (*m/z* 112) and putrescine (*m/z* 89) were detected in fish exposed for 1 day. All the typical biogenic amines including putrescine, cadaverine, histamine, tyramine (MW 137), spermidine (MW 145), tryptamine (MW 160), and spermine (MW 202) were detected as protonated molecules with relatively

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increased intensities in fish exposed to room temperature for 2 days (Fig. 2(d)). The biogenic amines[42–44,54] and other strong peaks such as those at m/z 116 and 57 are proposed to account for the typical putrescent smell of spoiled fish at room temperature. The multiple peaks shown in the mass spectral fingerprints of spoiled fish suggest that there is actually more than one component (i.e. histamine) in the spoiled fish. If spoiled fish is consumed, many chemicals that are more toxic than pure histamine (including toxins generated from the metabolism of the microorganisms) are ingested. Thus, this might give useful hints in understanding the somewhat surprising finding that histamine consumed in spoiled fish is more toxic than pure histamine taken orally.[57]

Specificity is generally provided by tandem mass spectrometry. In EESI-MS, specificity of detection can be further improved by using selective ion/molecule reactions, which was demonstrated in breath analysis with Ag^+ cationization.[38] In principle, additional selective reactions can be implemented in the desorption process by adding reactive reagents into the nitrogen gas; however, this was not tried in the meat analysis in order to avoid sample contamination by the reagent.

Differentiation of frozen meat samples

It is difficult to detect biogenic amines in a spoiled frozen fish sample by a sensory method[59] owing to insufficient sensitivity. However, the differentiation of frozen meat samples can be done easily by the mass spectral fingerprints (Fig. 2) and then further visualized by statistical analysis such as PCA. Figure 3 shows the PCA score plot of fish meat at three different spoilage stages. Different fish meat cuts are classified into three tight clusters distinguished by their freshness. By using the same instrument for data collection and same statistical approach, long-term reproducibility of the PCA results was demonstrated using fruit samples.[46] In the PCA loading plots (Fig. S1), many peaks differentiating the fish samples show up in the first principal component (PC), PC1. A very strong peak at m/z 57 shows up in PC2, providing good agreement with the mass spectral data (Fig. 2(c)). Some common differential peaks were identified by MS/MS data with reference compounds when available (Table 1). Supervised PCA[21,45] was also performed using the differential peaks. Fish samples were also differentiated according to their state of spoilage (shown in Fig. S2). It is conceivable that such molecular markers could be monitored individually for on-line monitoring of meat quality in the industry, by using a simple and cheap mass spectrometric sensor tuned to one or several diagnostic signals; a full mass spectrum is not required.

To further evaluate this strategy for differentiating stages of meat spoilage, many additional experimental runs were done with beef, pork, lamb, and turkey. Mass spectral fingerprints of beef samples at different spoilage stages were obtained in a similar fashion (Fig. 4). Surprisingly, the difference between the mass spectral fingerprints are so clear that it is easy to differentiate all beef samples even without further data processing, although none of the beef samples could be distinguished by a sensory method, thereby providing another example that a mass spectrometer

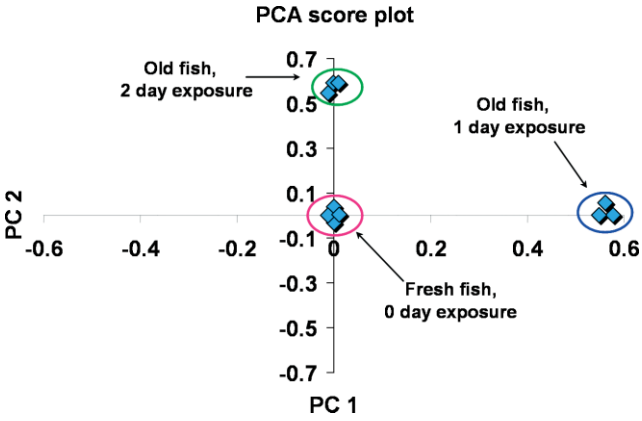

Figure 3. Score plots of principal component analysis of the full set of mass spectral data obtained from fish meat at different stages. Each cluster contains five points referring to five desorption EESI-MS spectra obtained from five fish cuts.

is the best 'electronic nose'[60] for food analysis. Lamb and turkey meat samples were successfully differentiated by the mass spectral fingerprints of lamb (Fig. 5) and turkey (Fig. 6) samples at different spoilage states. Proteins[61] were not found in the mass range investigated: probably they were not easy to be multiply charged in EESI. Note that many nonvolatile biogenic amines and high molecular weight compounds (Fig. 2(a), (d)) were incorporated into the aerosol mixture created by the gas flow during the collision-desorption of the icy surface, which were then carried into the EESI source for ionization. The detection of non-volatile compounds by EESI-MS is in good agreement with previous studies.[38,46] Regarding the biogenic amines, only putrescine (m/z 89) and cadaverine (m/z 103) were detected in spoiled beef and pork. This is in contrast to the results obtained in fish. Similarly, spoilage stages of lamb and turkey meat samples were successfully differentiated.

To further test the proposal to use peaks detected in EESI-MS for biogenic amines as biomarkers for meat spoilage, supervised PCA[21,45] was performed using typical biogenic amines found in 15 meat samples. In the PCA score plot (Fig. 7), all fresh meat samples are clustered at the center, and nonfresh samples are distributed outside. The cluster is not so tight because biogenic amines are present in different fresh meat samples in variable amounts.[43] Note that the spoiled meat samples are clustered according to their origins, such as beef, fish, and pork, with appreciable distance between samples at different spoilage states. From the distribution of the samples in the PCA score plot, it seems that the turkey samples at different spoil stages are well separated from each other. The turkey sample exposed to air for 1 day is more similar to the lamb sample exposed to air for 2 days. The turkey sample exposed to air for 2 days is more similar to the fish samples. The experimental findings support the notion that biogenic amines can be used as molecular markers for differentiation of the spoilage stages of different meat samples.[43]

Sensitivity

The detection limit was evaluated using several frozen fresh meat samples spiked with histamine or cadaverine. To further extend this method to other types of meat, chicken,

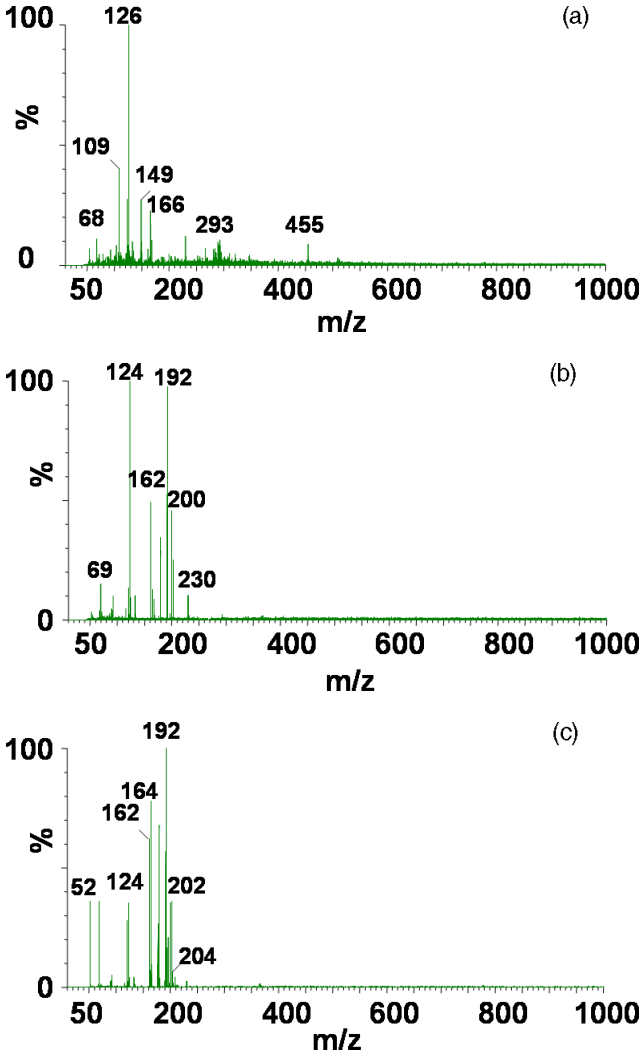

Figure 4. Desorption extractive electrospray ionization mass spectra of frozen beef samples: (a) beef without exposure to room temperature; (b) beef after exposure to room temperature for 1 day; (c) beef after exposure to room temperature for 2 days.

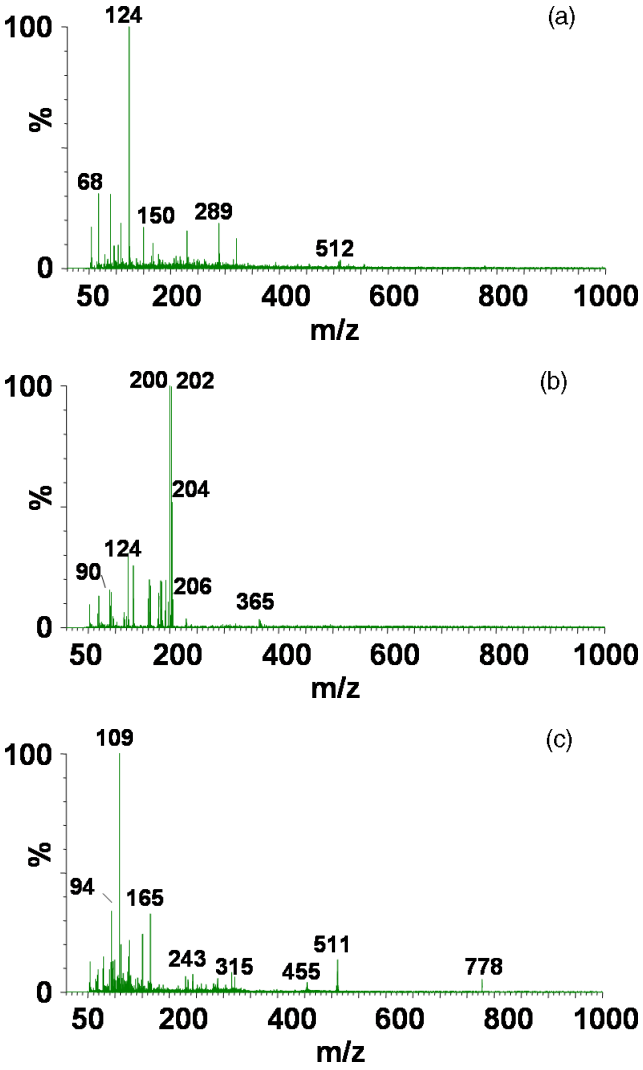

Figure 5. Desorption extractive electrospray ionization mass spectra of frozen lamb samples: (a) lamb meat without exposure to room temperature; (b) lamb meat after exposure to room temperature for 1 day; (c) lamb meat after exposure to room temperature for 2 days.

another type of popular meat, was used in one example. A detection limit of 1 pg/cm^2 (S/N = 73, RSD = 2.8% for five measurements) was found when 10 μl of a 100 ppt solution of cadaverine was deposited on the frozen chicken surface to form a *ca* 1 cm^2 spot. Similar detection limits were obtained using other types of sliced meat samples, such as beef, pork, and lamb. When it was spiked into a homogeneous meat loaf of beef or pork, the detection limit was found to be 0.1% (w/w) (S/N = 7.2, RSD = 5.8% for five measurements). For histamine, the detection limit was found to be 10 fg/cm^2 (S/N = 3, RSD = 7.2% for 10 measurements) on the surface of frozen turkey and 0.03% (w/w) (S/N = 8, RSD = 4.6% for 10 measurements) in meat loaf.

Real-time on-line monitoring

Food analysis requires high throughput and on-line feasibility. Therefore, it is highly desirable to establish a convenient method for real-time on-line monitoring of food degradation processes, especially when the metabolic dynamics are of particular interest. Desorption EESI-MS provides a practical way for real-time monitoring of solid surfaces because it tolerates complex matrices such as frozen meat without sample preparation or matrix cleanup. Total ion current (TIC) traces of each component present in the frozen fish sample are representatively shown in Fig. 8 for measurements of multiple samples (each measurement was distinguished by different file names, such as Fish_1_04, etc; each individual sample was represented by a single peak of TIC in different files). It is clear from Fig. 8 that TIC traces of components in the fish meat were promptly recorded in the desorption EESI-QTOF-MS experiments in different measurements, providing an example of real-time monitoring of the solid sample surfaces and showing the promising prospect of desorption EESI-MS for on-line monitoring of meat quality.

Analysis speed

Analysis speed in mass spectrometry is typically high; data are generated within seconds. EESI-QTOF-MS is no exception. The rate-limiting factor is aerosol transport, which

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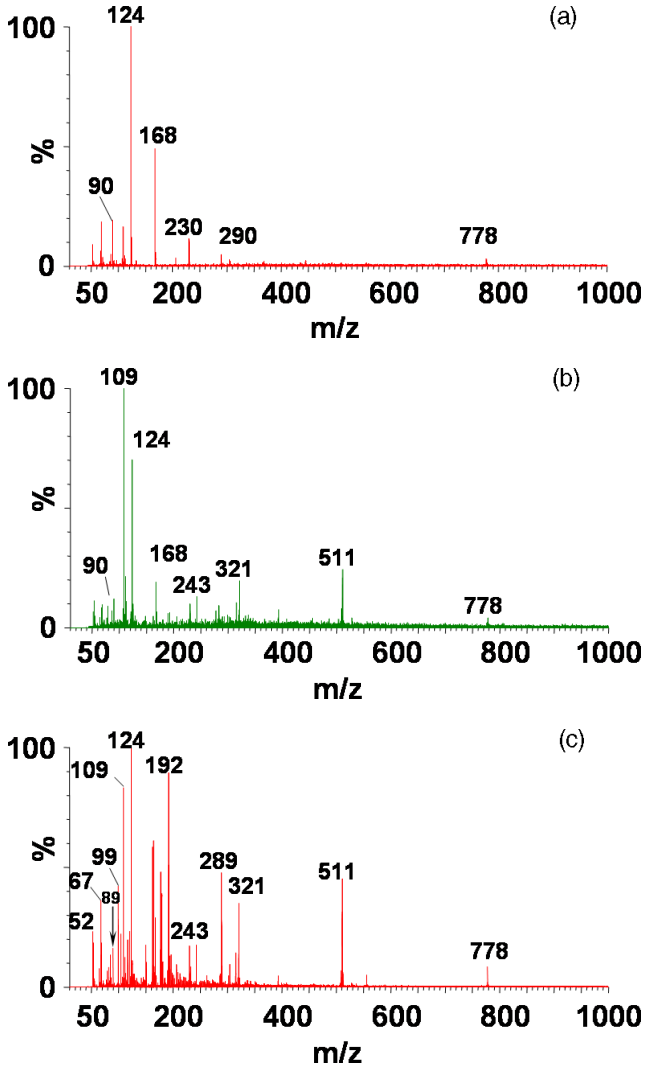

Figure 6. Desorption extractive electrospray ionization mass spectra of frozen turkey samples: (a) turkey meat without exposure to room temperature; (b) turkey meat after exposure to room temperature for 1 day; (c) turkey meat after exposure to room temperature for 2 days.

requires 1–2 s when a 120-cm tube is used. However, the long flexible aerosol transfer line allows remote sampling, which is convenient in industrial process monitoring applications. Generally, desorption EESI-MS generates data within 1–2 s, and it can be faster if the aerosol transport is accelerated. Obviously, this technique is much faster and more convenient than traditional methods which require sample thawing, extraction, and separation before sample analysis.

Rapid differentiation of spinach samples

We extended our analytical strategy to on-line screening of spinach samples. A spinach sample contaminated by *E. coli* is differentiated unmistakably from normal green spinach and old spinach by the EESI mass spectral fingerprints (Fig. 9) even without PCA, providing a practical and convenient method for on-line screening of contaminated vegetables. The two fresh green spinach samples smelled identical, although one sample had been contaminated by *E. coli*. In the fresh spinach sample (Fig. 9(a)), the mass

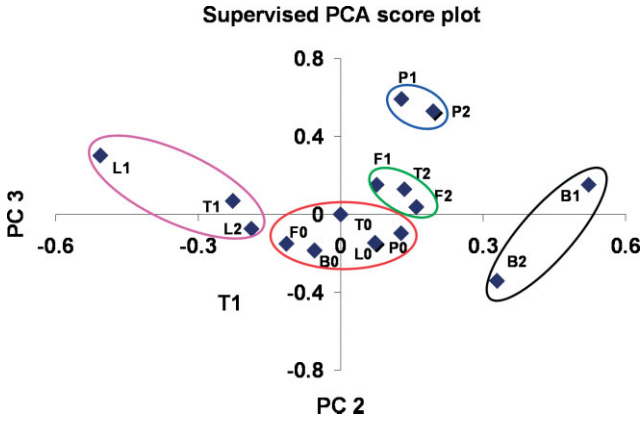

Figure 7. Score plots of supervised PCA of 15 meat samples at different stages of spoilage. Fresh samples are clustered together regardless of their origins. Old meat samples such as beef, fish, lamb and pork are clustered according to their origins with considerable distance between samples at different spoilage states. The abbreviations of B0, B1, B2, F0, F1, F2, P0, P1, P2, L0, L1, L2, T0, T1, T2 represent beef, fish, pork, lamb, and turkey after exposure to room temperature for 0, 1, 2 days, respectively. Note that each single point in this plot denotes to an average value from five individual measurements.

spectrum is dominated by a peak at m/z 189, which yields major fragments at m/z 172, 171, and 147 by loss of NH_3, H_2O, and $CH_2{=}C{=}O$ (inset in Fig. 9(a)). For fresh spinach contaminated by *E. coli*, the mass spectrum changes significantly (shown in Fig. 9(b)). In this sample, the peak at m/z 189 is considerably less intense, while a new peak at m/z 282 becomes predominant. Compared to the normal spinach sample, there are more peaks detected in the contaminated spinach sample. Metabolites produced by the growing *E. coli* bacteria are thought to be deposited on the surface of the spinach leaves, and can therefore be detected by the neutral desorption EESI-MS. Peaks detected in the relatively high mass range could be due to peptides associated with the *E. coli* strains used (inset in Fig. 9(b)); these signals are absent in normal fresh spinach. The spectral fingerprint of the old spinach sample is shown in Fig. 9(c). A few typical peaks found in the spinach samples are summarized in Table 2. These peaks might not be suitable biomarkers for every spinach sample; however, it is obvious that all three samples tested here are differentiated successfully. A further neutral desorption EESI-MS experiment is performed to record the mass spectral fingerprints of a slightly yellow spinach sample. From the EESI mass spectrum (shown in Fig. 9(c)), in contrast to the fingerprints obtained from a fresh green spinach sample, the peak at m/z 189 is decreased considerably, while the peak at m/z 248 is detected with dramatically increased intensity. However, no peak is detected in the relatively high mass range (inset in Fig. 9(c)), presenting a similarity to the fresh sample without *E. Coli* contamination. Figure 9(d) shows a mass spectral fingerprint of spinach sprayed with media only, providing a very different spectral pattern, as shown in Fig. 9(c), since there is no signal detectable in the high mass range. Therefore, the intensity ratio of peak at m/z

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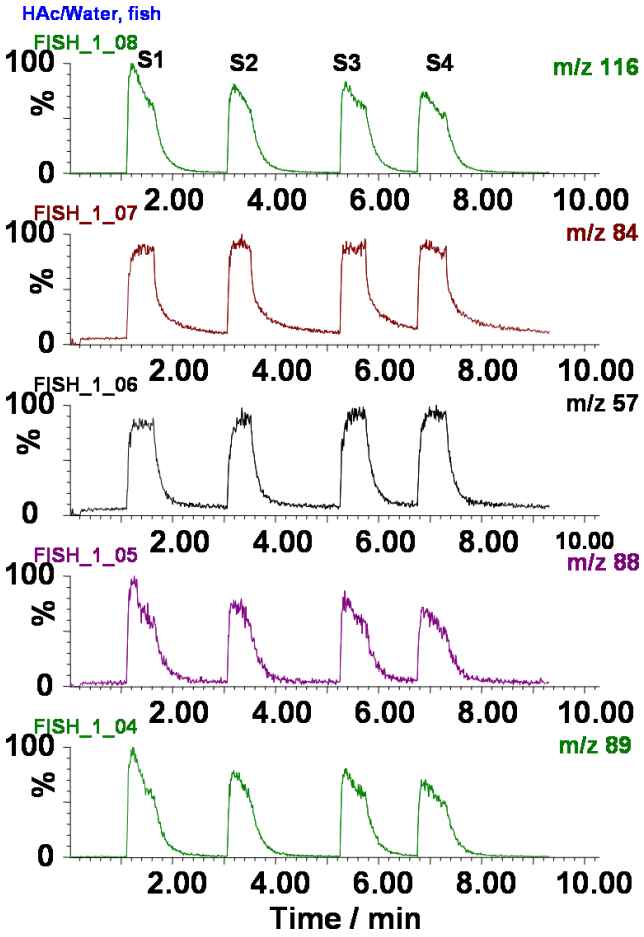

Figure 8. The total ion current traces of each component present in the frozen fish sample obtained in different measurements using water/acetic acid as electrospraying solution.

189 and 248 is proposed to differentiate the freshness of the spinach samples. Other typical peaks differentiating the spinach samples are summarized in Table 2.

In vivo skin analysis

Finally, we also show that metabolites in different areas of human skin can be fingerprinted *in vivo* using this technique. An EESI-MS spectral fingerprint obtained from the forehead skin of a male adult is shown in Fig. 10(a). Numerous peaks are detected in the mass range from m/z 50 to 1000 in the positive ion detection mode. Similarly, EESI mass spectral fingerprints are recorded from the abdominal skin, the forearm skin, and the foot skin of the same volunteer (Fig. 10(b–d)). Obviously, the spectral patterns of the different skin areas are quite different although some common peaks (e.g. m/z 282) are detected on the forehead, forearm, and abdominal skin, while the peak at m/z 538 is commonly detected on the skin of the forehead, abdomen, and the foot. The peak at m/z 181 detected in the forehead skin is identified, on the basis of a reference compound, to be protonated glucose, which losses HCHO and H_2O as major fragments in CID (inset in Fig. 10(a)). Interestingly, the amount of glucose on the skin of the forehead is much higher than that on the hand, abdomen, or the foot skin samples, indicating that the difference of the metabolites (e.g. glucose) excreted from different skin areas might be due to the differential metabolic activities of the adjacent organs, which consumes glucose with differential speed due to different metabolic status.[62] Under the experimental conditions used, the foot skin presents a much simpler fingerprint than other skin samples, probably because the feet have harder skin and therefore fewer metabolites can be released to their surface.

The fingerprints recorded from the same volunteer, but from different parts of the skin or with a different desorption reagent (e.g. acetone instead of nitrogen), provide different spectral patterns, which reveal rich molecular information. For example, acetone vapor is added into the nitrogen gas beam for skin desorption sampling, and Fig. 11(a), (b) shows the different mass spectral fingerprints obtained from the same area of a hand skin before and after addition with acetone in the gas beam. As a result, the intensity of peak at m/z 538 is dramatically enhanced so that it becomes the predominant peak and forms a proton bound cluster (m/z 598) with acetic acid. Potentially, this feature is very useful for chemical classification and metabolites profiling, especially when a specially designed reagent gradient is used for desorption sampling.

Skin is a large organ exposed directly to the ambient environment, and therefore it absorbs chemicals from the ambient air after exposure. For example, it is also possible to detect RDX, a typical explosive, on skin after exposure to air containing traces of RDX. An RDX methanol/water (1 : 1) solution (2 ml, 10 ppt) is prayed into the open air above a bare hand, which is exposed to this plume for 2 min to receive part of the RDX sprayed into the air. The hand skin is directly analyzed using the neutral gas desorption EESI-MS, and a mass spectrum is shown in Fig. 12. The peak at m/z 223 is identified as protonated RDX, which yields a major fragment at m/z 177 by loss of NO_2 during CID (inset of Fig. 12). Successful rapid detection of RDX, a typical explosive, provides an example

Table 2. Signal intensity for typical peaks found in spinach samples

	Signal intensity (CPS)[a]		
Peaks (m/z)	Spinach fresh, normal	Spinach green-yellow, old	Spinach contaminated by *E. Coli*
189	23, 300	36	4, 830
282	26	160	20, 300
256	14	88	5, 190
538	16	16	7, 870

[a] Signal intensity is a mean value of five measurements for each sample.

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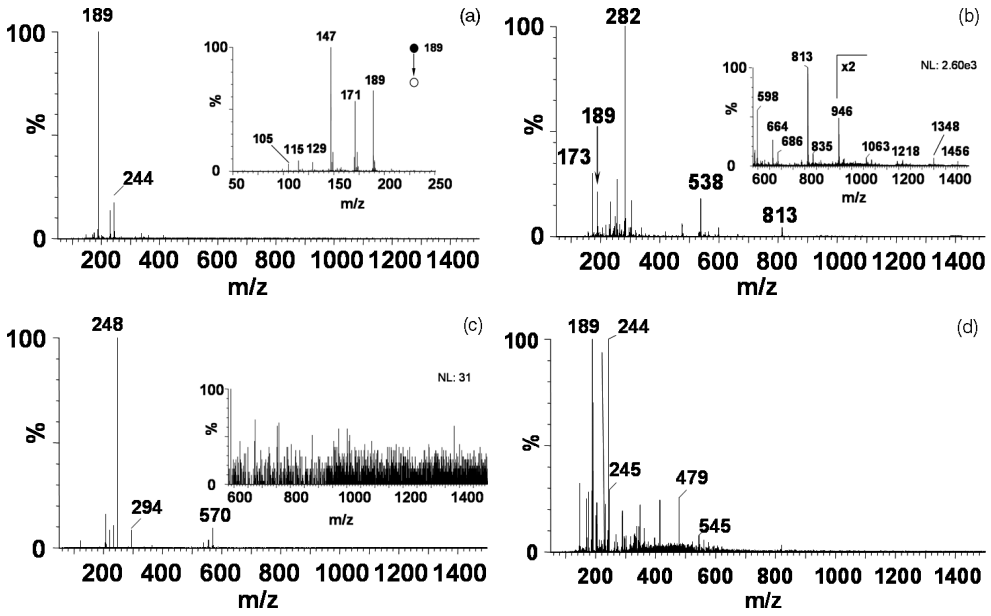

Figure 9. Extractive electrospray ionization mass spectral fingerprints of spinach samples: (a) fresh, green spinach leaves. The inset shows the CID spectrum of the predominant peak at *m/z* 189; (b) fresh, green spinach leaves contaminated by *E. coli* strain TG1. The inset shows multiple peaks in the relatively high mass range; (c) old, yellow-green spinach leaves. Inset shows no peak detectable in the relatively high mass range when the spinach sample is not contaminated by *E. coli*, (d) fresh, green spinach leaves sprayed with the *E. coli* media only.

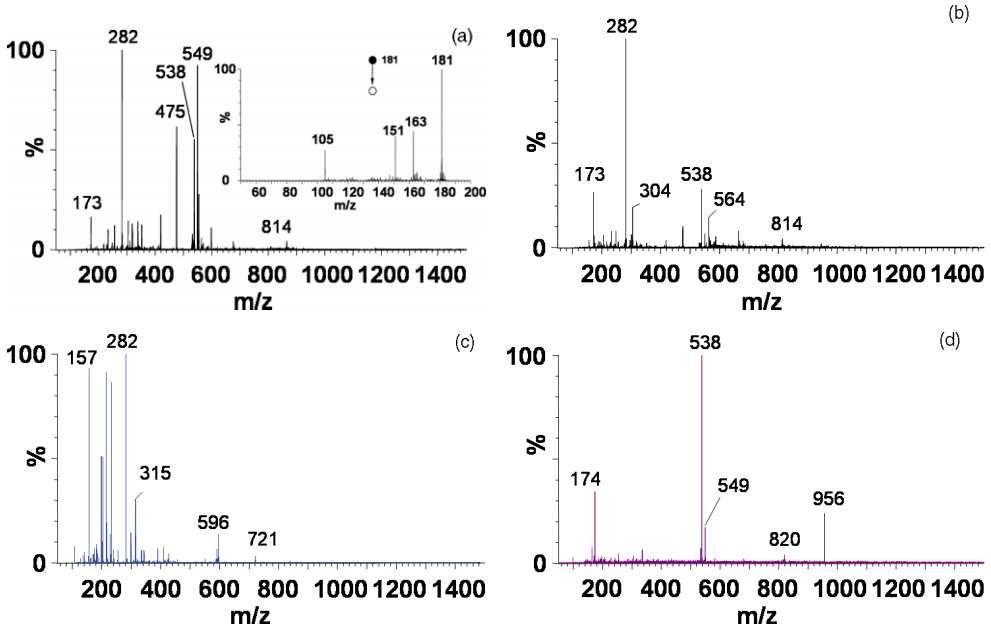

Figure 10. Extractive electrospray ionization mass spectral fingerprints recorded from the same volunteer but different skin areas during *in vivo* skin analysis: (a) fingerprints recorded from the forehead skin. The inset shows the CID spectrum of a peak at *m/z* 181, which is identified as due to protonated glucose; (b) fingerprint obtained from abdominal skin; (c) fingerprint obtained from the forearm skin; (d) fingerprint obtained from the foot skin.

for fast screening of dangerous chemical or biological hazard reagents such as anthrax bacteria, explosives, and chemical warfare agents, or for rapid clinical diagnosis, showing promising applications in homeland security programs.

CONCLUSIONS

In conclusion, a simple approach is developed to allow interrogation of virtually any type of surface by a gentle stream of air or gas, followed by efficient ionization of the neutral molecules released in an EESI step. Examples such as frozen meat, spinach, and human skin are sampled directly and in an on-line fashion by the gentle desorption of neutrals coupled with EESI-MS for rapid monitoring, without any chemical contamination or sample pretreatment. The mass spectral fingerprints display metabolites originating either from growing microorganisms or from the sample itself, and therefore molecular signatures for a wide variety of biological

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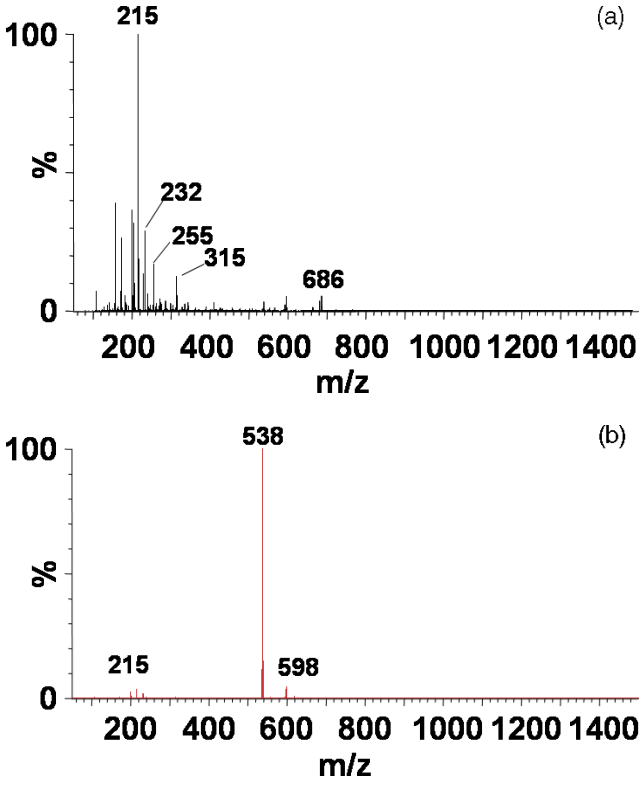

Figure 11. Extractive electrospray ionization mass spectral fingerprints obtained from the same skin area but different desorption reagents: (a) nitrogen as desorption gas; (b) nitrogen and acetone vapor as desorption gas.

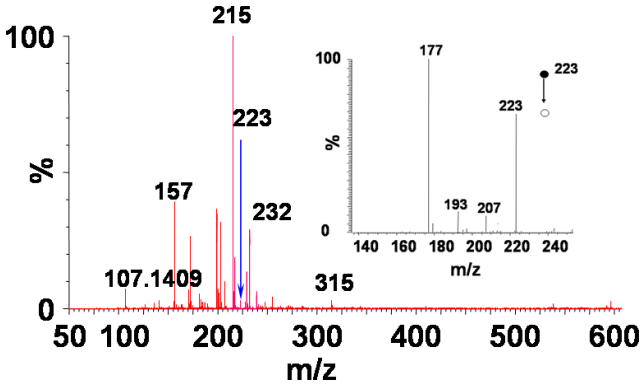

Figure 12. *In vivo* detection of explosive contaminant RDX in a skin sample. Protonated RDX (*m/z* 223) is detected in a forearm skin exposed to RDX contaminated air; the inset shows the CID spectrum of the peak at *m/z* 223.

samples are favorably obtained. This novel metabolomics-based strategy represents a 'green' procedure for fast food quality assessment. It was validated by 15 meat samples from different origins, and was successfully applied to fast screening of spinach samples contaminated by *E. coli*, and to *in vivo* analysis of human skin. The physiological and/or pathological status of animals or plants can potentially be diagnosed *in vivo* on the basis of a molecular signature[63] using the new technique reported here. We expect this strategy to be used in many disciplines, including but not limited to food quality monitoring, homeland security, *in vivo* metabolomics, and clinical diagnosis.

Supplementary material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/1076-5174/suppmat/.

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REFERENCES

1. Dhingra V, Gupta M, Andacht T, Fu ZF. New frontiers in proteomics research: a perspective. *International Journal of Pharmaceutical* 2005; **299**: 1.
2. Kumar S, Mohan A, Guleria R. Biomarkers in cancer screening, research and detection: present and future: a review. *Biomarkers* 2006; **11**: 385.
3. Nordhoff E, Kirpekar F, Roepstorff P. Mass spectrometry of nucleic acids. *Mass Spectrometry Reviews* 1996; **15**: 67.
4. Downes MR, Byrne JC, Dunn MJ, Fitzpatrick JM, Watson RWG, Pennington SR. Application of proteomic strategies to the identification of urinary biomarkers for prostate cancer: A review. *Biomarkers* 2006; **11**: 406.
5. Smith L, Lind MJ, Welham KJ, Cawkwell L. Cancer proteomics and its application to discovery of therapy response markers in human cancer. *Cancer* 2006; **107**: 232.
6. Pusch W, Flocco MT, Leung SM, Thiele H, Kostrzewa M. Mass spectrometry-based clinical proteomics. *Pharmacogenomics* 2003; **4**: 463.
7. Pandor A, Eastham J, Beverley C, Chilcott J, Paisley S. Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review. *Health Technology Assessment* 2004; **8**: 1.
8. Rubin AE, Tummala S, Both DA, Wang CC, Delaney EJ. Emerging technologies supporting chemical process R&D and their increasing impact on productivity in the pharmaceutical industry. *Chemical Reviews* 2006; **106**: 2794.
9. Hsieh Y, Fukuda E, Wingate J, Korfmacher WA. Fast mass spectrometry-based methodologies for pharmaceutical analyses. *Combinatorial Chemistry and High Throughput Screening* 2006; **9**: 3.
10. Geoghegan KF, Kelly MA. Biochemical applications of mass spectrometry in pharmaceutical drug discovery. *Mass Spectrometry Reviews* 2005; **24**: 347.
11. Corona G, Toffoli G. High throughput screening of genetic polymorphisms by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Combinatorial Chemistry and High Throughput Screening* 2004; **7**: 707.
12. Gelpi E. Contributions of liquid chromatography-mass spectrometry to ''highlights'' of biomedical research. *Journal of Chromatography. A* 2003; **1000**: 567.
13. Raffaelli A, Saba A. Atmospheric pressure photoionization mass spectrometry. *Mass Spectrometry Reviews* 2003; **22**: 318.
14. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray Ionization for Mass-Spectrometry of Large Biomolecules. *Science* 1989; **246**: 64.
15. Carroll DI, Dzidic I, Stillwell RN, Haegele KD, Horning EC. Atmospheric-pressure ionization mass-spectrometry – corona discharge ion-source for use in liquid chromatograph mass spectrometer-computer analytical system. *Analytical Chemistry* 1975; **47**: 2369.
16. Karas M, Bachmann D, Hillenkamp F. Influence of the wavelength in high-irradiance ultraviolet-laser desorption mass-spectrometry of organic-molecules. *Analytical Chemistry* 1985; **57**: 2935.
17. Takats Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* 2004; **306**: 471.

18. Cody RB, Laramee JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Analytical Chemistry* 2005; **77**: 2297.
19. Carroll DI, Nowlin JG, Stillwell RN, Horning EC. Adduct ion formation in chemical ionization mass-spectrometry of non-volatile organic-compounds. *Analytical Chemistry* 1981; **53**: 2007.
20. Pan ZZ, Gu HW, Talaty N, Chen HW, Shanaiah N, Hainline BE, Cooks RG, Raftery D. Principal component analysis of urine metabolites detected by NMR and DESI-MS in patients with inborn errors of metabolism. *Analytical and Bioanalytical Chemistry* 2007; **387**: 539.
21. Chen HW, Pan ZZ, Talaty N, Raftery D, Cooks RG. Combining desorption electrospray ionization mass spectrometry and nuclear magnetic resonance for differential metabolomics without sample preparation. *Rapid Communications in Mass Spectrometry* 2006; **20**: 1577.
22. Moyer SC, Marzilli LA, Woods AS, Laiko VV, Doroshenko VM, Cotter RJ. Atmospheric pressure matrix-assisted laser desorption/ionization. (AP MALDI) on a quadrupole ion trap mass spectrometer. *International Journal of Mass Spectrometry* 2003; **226**: 133.
23. Zenobi R, Knochenmuss R. Ion formation in MALDI mass spectrometry. *Mass Spectrometry Reviews* 1998; **17**: 337.
24. Peterson DS. Matrix-free methods for laser desorption/ionization mass spectrometry. *Mass Spectrometry Reviews* 2007; **26**: 19.
25. Cooks RG, Ouyang Z, Takats Z, Wiseman JM. Ambient mass spectrometry. *Science* 2006; **311**: 1566.
26. Takats Z, Wiseman JM, Cooks RG. Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. *Journal of Mass Spectrometry* 2005; **40**: 1261.
27. Chen HW, Talaty NN, Takats Z, Cooks RG. Desorption electrospray ionization mass spectrometry for high-throughput analysis of pharmaceutical samples in the ambient environment. *Analytical Chemistry* 2005; **77**: 6915.
28. Wiseman JM, Ifa DR, Song QY, Cooks RG. Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. *Angewandte Chemie-International Edition* 2006; **45**: 7188.
29. Talaty N, Takats Z, Cooks RG. Rapid in situ detection of alkaloids in plant tissue under ambient conditions using desorption electrospray ionization. *The Analyst* 2005; **130**: 1624.
30. Chen HW, Zhang X, Luo MB. Desorption electrospray ionization mass spectrometry for fast detection of Sudan dyes in foods without sample pretreatment. *Chinese Journal of Analytical Chemistry* 2006; **34**: 464.
31. Williams JP, Patel VJ, Holland R, Scrivens JH. The use of recently described ionisation techniques for the rapid analysis of some common drugs and samples of biological origin. *Rapid Communications in Mass Spectrometry* 2006; **20**: 1447.
32. Chen HW, Lai JF, Zhou YF, Huan YF, Li JQ, Zhang X, Wang ZC, Luo MB. Surface desorption atmospheric pressure chemical ionization mass spectrometry: instrumentation and characterization. *Chinese Journal of Analytical Chemistry* 2007; **35**: 1036.
33. Chen HW, Zheng J, Zhang X, Wang ZC, Luo MB, Qiao XL. Surface desorption atmospheric pressure chemical ionization mass spectrometry for direct ambient sample analysis without toxic chemical contamination. *Journal of Mass Spectrometry* 2007; DOI: 10.1002/jms.1235.
34. Mulligan CC, Justes DR, Noll RJ, Sanders NL, Laughlin BC, Cooks RG. Direct monitoring of toxic compounds in air using a portable mass spectrometer. *Analyst* 2006; **131**: 556.
35. Chen HW, Venter A, Cooks RG. Extractive electrospray ionization for direct analysis of undiluted urine, milk and other complex mixtures without sample preparation. *Chemical Communications* 2006; 2042.
36. Gu H, Chen H, Pan Z, Jackson AU, Talaty N, Xi B, Kissinger C, Duda C, Mann D, Raftery D, Cooks RG. Monitoring diet effects from biofluids and their implications for metabolomics studies. *Analytical Chemistry* 2007; **79**: 89.
37. Zhou Z, Jin M, Ding J, Zhou Y, Zheng J, Chen H. Rapid detection of atrazine and its metabolite in raw urine by extractive electrospray ionization mass spectrometry. *Metabolomics* 2007; DOI:10.1007/s11306-006-0050-2.
38. Chen HW, Wortmann A, Zhang WH, Zenobi R. Rapid in vivo fingerprinting of non-volatile compounds in breath by extractive electrospray ionization quadrupole time-of-flight mass spectrometry. *Angewandte Chemie Internatioanl Edition* 2007; **46**: 580.
39. Chen H, Zheng J, Jecklin M, Touboul D, Luo M, Zenobi R. Manipulation of charge states of biopolymer ions by atmospheric pressure ion/molecule reactions. *European Journal of Mass Spectrometry* 2007 (in press).
40. Zhou YM, Ding JH, Zhang X, Chen HW. Ion-ion reactions for charge reduction of biopolymer at atmospheric pressure ambient. *Chinese Chemical Letters* 2007; **18**: 115.
41. Chen HW, Yang SP, Wortmann A, Zenobi R. Neutral desorption sampling of living objects for rapid analyses by extractive electrospray ionization mass spectrometry. *Angewandte Chemie Intnational Edition* 2007 (in press).
42. Santos MHS. Biogenic amines: their importance in foods. *International Journal of Food Microbiology* 1996; **29**: 213.
43. Vinci G, Antonelli ML. Biogenic amines: quality index of freshness in red and white meat. *Food Control* 2002; **13**: 519.
44. Halasz A, Barath A, Simonsarkadi L, Holzapfel W. Biogenic-amines and their production by microorganisms in food. *Trends Food Science and Technology* 1994; **5**: 42.
45. Johnson RA, Wichern DW. *Applied Multivariates Statistical Analysis*, 4th ed. Prentice Hall: Upper Saddle River, 1998.
46. Chen H, Sun Y, Wortamann A, Gu H, Zenobi R. Differentiation of maturity and quality of fruit using non-invasive extractive electrospray ionization quadrupole time-of-flight mass spectrometry. *Analytical Chemistry* 2007; **79**: 1447.
47. Cowie J. Genetic modification and the meat market. *Nature* 2000; **404**: 921.
48. McAuley P. Meat – a growth industry. *Nature* 2005; **435**: 128.
49. Rubery E. When food kills: BSE, E-coli and disaster science. *Nature* 2003; **425**: 561.
50. Communities COTE In http://ec.europa.eu/dgs/health_consumer/library/pub/pub06_en.pdf, 2000.
51. Sun M. United-states meat inspection needs modernization. *Science* 1985; **229**: 450.
52. Paleologos EK, Kontominas MG. On-line solid-phase extraction with surfactant accelerated on-column derivatization and micellar liquid chromatographic separation as a tool for the determination of biogenic amines in various food substrates. *Analytical Chemistry* 2004; **76**: 1289.
53. Masson F, Talon R, Montel MC. Histamine and tyramine production by bacteria from meat products. *International Journal of Food Microbiology* 1996; **32**: 199.
54. Vidalcarou MC, Izquierdopulido ML, Martinmorro MC. Marinefont. Histamine and tyramine in meat-products – relationship with meat Spoilage. *Food Chemistry* 1990; **37**: 239.
55. Dalgaard P, Madsen HL, Samieian N, Emborg J. Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone belone) – effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology* 2006; **101**: 80.
56. Huss HH. *Quality and Quality Changes in Fresh Fish*. Food and Agriculture Organization of the United Nations: Rome, 1995.
57. Lehane L, Olley J. Histamine fish poisoning revisited. *International Journal of Food Microbiology* 2000; **58**: 1.
58. Whitlock FA. Histamine and the biochemistry of affective-disorder. *American Journal of Psychiatry* 1981; **138**: 1395.
59. Koutsoumanis K, Lampropoulou K, Nychas GJE. Biogenic amines and sensory changes associated with the microbial flora of mediterranean gilt-head sea bream (Sparus aurata) stored aerobically at 0, 8, and 15 degrees C. *Journal of Food Protection* 1999; **62**: 398.

60. Schaller E, Bosset JO, Escher F. Practical experience with 'electronic nose' system for monitoring the quality of dairy products. *Chimia* 1999; **53**: 98.
61. Bendixen E. The use of proteomics in meat science. *Meat Science* 2005; **71**: 138.
62. Lund ST, Bohlmann J. The molecular basis for wine grape quality – a volatile subject. *Science* 2006; **311**: 804.
63. Herwig C, Marison I, von Stockar U. On-line stoichiometry and identification of metabolic state under dynamic process conditions. *Biotechnology and Bioengineering* 2001; **75**: 345.