Neutral Desorption Sampling of Living Objects for Rapid Analyses by Extractive Electrospray Ionization Mass Spectrometry

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Importance of food quality regulation

Food quality and food safety are extremely important topics in politics, economy, science and technology as well as for daily life[1-23]. Technically, food regulation challenges analytical science in terms of sensitivity, specificity and high throughput. Tools for food analysis are highly desirable to be minimally invasive, capable of online operation, and should cause no contamination by toxic compounds.

World meat production has more than quadrupled in the past half-century to some 220 million tons annually (US Department of Agriculture, World Markets and Trends 1998). Meat has long formed an important part of the European diet, providing a high quality source for European consumers` protein requirements. The EU consumes roughly 35 million tons each year of various meat types, amounting to around 92 kilograms per capita per year on average. The EU produces 16% of the meat consumed worldwide (Agriculture in the European Union — Statistical and economic information 2003, available at http://ec.europa.eu/agriculture/publi/fact/meat/2004-eu.pdf). However, spoiled meat is often found in the markets. For example, in Germany, up to 50 tons of spoiled meat were distributed by a company in Munich in the summer of 2006. The date of expiry was found to be more than 4 years ago. Shortly thereafter, similar findings were made in other countries. The amount of spoiled meat already distributed added up to more than 100 tons (www.wikipedia.de).

The European Union's food policy is built around high food safety standards, which serve to protect, and promote, the health of the consumer. The production and consumption of food is central to any society, and has economic, social and, in many cases, environmental consequences. Food quality is directly linked to foodborne diseases, which have a huge impact on human society. The recent food scandals in the U.S. concerning E. coli contaminated vegetables, and in Germany concerning rotten meat (only two of over 200 examples of food borne problems in one year, see http://web.worldbank.org), demonstrate that current measures to monitor and to guarantee food quality for consumers are still not sufficient. The large

amount of meat consumed annually, approximately 30 million tons in the U.S., 45 million tons in Europe and 220 million tons worldwide (source: Economic Research Service / USDA, FAO World agriculture: towards 2015/2030), however, shows the difficulty of this task. The US Center for Disease Control and Prevention (CDC) estimates that 76 million people suffer foodborne illnesses each year in the United States, accounting for 325,000 hospitalizations and more than 5,000 deaths (http://www.niaid.nih.gov/factsheets/foodbornedis.htm). Foodborne diseases are also very costly. Health experts estimate that the yearly cost of all foodborne diseases in the US is 5 to 6 billion dollars in direct medical expenses and loss of productivity. Thus, it is reasonable to guess that the situation about food quality and foodborne diseases in developing countries should be ever worse than that in developed countries such as USA and Germany.

Besides meat products, vegetables are also important to human society. For example, the E. coli outbreak caused huge financial losses in the US. For example, in September 2006, there was an outbreak of food-borne illness caused by Escherichia coli (E. coli) bacteria found in uncooked spinach in 26 U.S. states (http://en.wikipedia.org/wiki/List_of_United_States_foodborne_illness_outbreaks),(http://www.fda.gov/oc/opacom/hottopics/spinach.html). As of October 06, 2006, 199 people had been infected, including three people who died and 31 who suffered a type of kidney failure called hemolytic uremic syndrome after eating spinach contaminated with the E. coli O157:H7, a potentially deadly bacterium that causes bloody diarrhea and dehydration. In 2005, the spinach crop in California was valued at $258.3 million, and each acre lost amounts to a roughly $3,500 loss for the farmer. According to ***New York Times***, 5,000 deaths, 325,000 hospitalizations and 76 million illnesses are caused by food poisoning within the US every year (http://query.nytimes.com/gst/fullpage.html?sec=health&res=9900E3D8123DF93BA25750C0A9679C8B63).

Obviously, it is not a good solution to urge people not to eat all spinach[24, 25], and other vegetables. Therefore, it is urgent to develop a practical convenient method to monitor the food quality.

Tandem MS and specificity

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. For example, the compound of the highest intensity (m/z 122) in the mass spectrum (Figure 3 a) is tentatively interpreted as protonated $C_3H_7O_2NS$ (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 (Figure S2). However, it could not be protonated cysteine, because the protonated cystein loses water first to generate a fragment of m/z 103. This fragment (m/z 103) was not observed in the MS/MS spectrum using the precursor ions of m/z 122. The second most abundant peak (m/z 88) is assigned to protonated C_4H_9ON (MW 87), which loses water in MS/MS

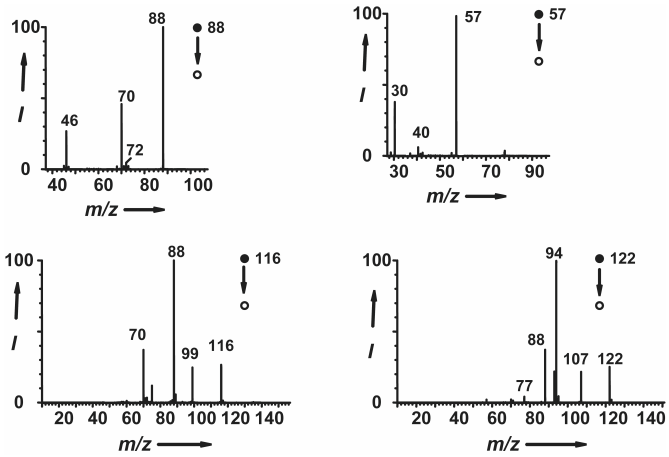

Figure S2 Tandem mass spectra of compounds of interest

(Figure S2) 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 (Figure 3 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 big peak at m/z 88, which further loses water to give a major peak at m/z 70 (Figure S2). Interestingly, the peak at m/z 57 is quite strong in the 2-day (8.16×10^6), 1-day (1.66×10^5) exposed fish samples while it was not detectable in fresh fish (Figure 3 a). This peak could be protonated $CN\text{-}NHCH_3$ (MW 56), however, it only yielded a

small peak of m/z 40 (by the loss of NH_3) and m/z 30 (by the loss of HCN) and no other significant fragments in MS/MS in our instrument (Figure S2). According to our literature survey, it has so far not been reported in spoiled fish. Theoretically, the peaks detected can be identified by using accurate mass measurement and comparison with tandem mass data of reference compounds. However, it is beyond the scope of this communication to identify all the compounds.

Differentiation of frozen meat samples

For food analysis, EESI-MS provides the desired characteristics, i.e., no requirement for any sample preparation, fast acquisition times and high sensitivity. The ability to detect quality degradation in a variety of food samples is demonstrated, even in the frozen state. Implementing this technique on a commercial electrospray mass spectrometer does not require any hardware modifications, thus providing a good example for food industry for products online monitoring.

Real time online monitoring of frozen meat

A single mass scan can be completed in a few milliseconds with most commercial mass spectrometers. It is demonstrated that a single sample analysis can be completed in 1-2 s in a real time, online fashion (Fig. S3). This provides a high throughput mass spectrometric detection of multiple trace components present in biological samples with complex matrices. Taking advantage of sampling biological samples, even living objects, at ambient pressure, this method provides real-world analytical capabilities for high throughput real-time analysis of biological samples *in vivo*.

Food analysis requires high throughput and online feasibility. Therefore, it is highly desirable to establish a convenient method for real time online monitoring of food degradation process, especially when the

metabolic dynamics are of particular interest. By tolerating complex matrices, 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 clean-up. Total ion current (TIC) traces of each component present in the frozen fish sample are representatively shown in Figure S2 for measurements of multiple samples (each measurement is 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 Figure S3 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 a promising prospective of desorption EESI-MS for online monitoring of meat quality.

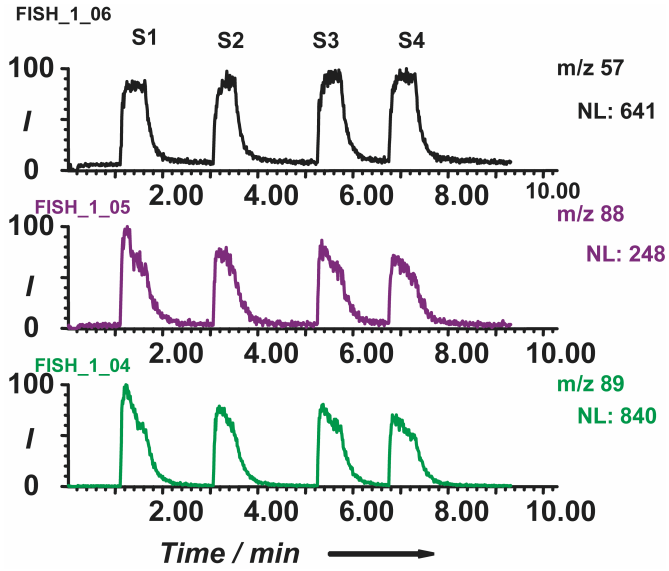

Figure S3. The total ion current traces of each component present in the frozen fish sample obtained in different measurements using water/acetic acid as electrospraying solution.

Sensitivity and analysis speed

The detection limit was evaluated using several frozen fresh meat samples spiked with histamine or cadaverine. A detection limit of 1 pg/cm^2 (S/N=73) was found when 10 µL of a 100 ppt solution of cadaverine was deposited on the frozen meat surface to form a ca. 1cm^2 spot. 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). For histamine, the detection limit was found to be 10 fg/cm^2 (S/N=3) on the surface of frozen turkey and to be 0.03% (w/w) (S/N=8) in meat loaf.

Analysis speed in mass spectrometry is typically fast, data are generated within milliseconds. EESI-QTOF-MS is no exception. The aerosol transport may need about 1-2 seconds to pass the 120 cm long tube in our case. However, the long flexible aerosol transfer line allows remote sampling, which is convenient in practical industrial process monitoring applications. Generally, desorption EESI-MS generates data within 1-2 seconds, 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.

Table S1. Signal intensities from different skin areas

Signal (m/z)	Head	Abdomen	Foot
181[a]	5.4×10^{2}	74	51
186[b]	78	8	1.6×10^{2}
282	2.2×10^{5}	2.1×10^{5}	1.54×10^{4}
475	9.43×10^{3}	1.08×10^{4}	1.47×10^{3}
538	4.06×10^{3}	2.11×10^{4}	4.04×10^{4}
549	6.02×10^{3}	1.01×10^{4}	9.46×10^{2}

[a] identified as protonated glucose by MS/S using reference compound; [b] tentatively identified by MS/MS as protonated serine phosphate. All the data are average values of 10 measurements.

Table S2 MS/MS data of identified molecular markers detected in fish meat sample at different stages of spoilage.

Molecular markers	Molecular weight [a]	Number of days for samples exposure to room temperature
trimethylamine [b]	59	0
dimethylamine [b]	45	0
dimethylacetylamine [b]	73	0
N-methylpyrolidine [b]	117	0
$C_3H_7O_2NS$ [c]	121	0
C_4H_9ON [c]	87	0
$C_2H_4N_2$ [c]	56	1, 2
putrescine [b]	88	1, 2
cadaverine [b]	102	1, 2
histamine [b]	111	0, 1, 2
$C_2H_8N_2$ [c]	60	0, 1
$C_5H_9NO_2$ [c]	115	1, 2
tyramine [b]	137	2
spermidine [b]	145	2
tryptamine [b]	160	2
spermine [b]	202	2
Pentanethiol [b]	104	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.

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