Quantitation with Ambient Ionization Imaging for Flavors, Fragrance and Foodstuffs Workshop Report

Wednesday 23rd May 2012 5:45 pm to 7:00 pm Room 217-219, 75 people in attendance
Eric Handberg (East China Institute of Technology, mainland China) presiding

Overview

This year at ASMS we enjoyed many events:

1 plenary lecture, The Secret Life of Food
2 oral sessions: 1. ThOA pm: Food Safety: Advances in MS for Characterization of Additives and Contaminants and ThOD pm: Food "omics": MS characterization of Food and Nutritional Supplements
134 Interest group posters in 22 sessions

The workshop began with a request for two volunteers for the workshop coordinators.

In the workshop we studied 12 publications about the state-of-the-art methods in imaging mass spectrometry (IMS) for foods and flavors with a checklist. IMS of foods and flavors was performed with either a vacuum MALDI source or an ambient ionization source, but food imaging could be either a 2D or 1D image; a profile, or a text description. Quantitation was performed with an endogenous ion and normalization; external calibration curves; a colorimetric kit; and an internal standard. Isobaric interference was overcome or was not an issue. Ion suppression may have occurred, but it was overcome with silver adducts, extraction, tandem mass spectrometry, and the combination of micro dissection and chromatography. The estimate for a research-grade ion source was affordable at 5k$. IMS of foods and flavors with ambient ionization sources is a growing field.

The workshop ended by collecting the workshop surveys and with a short pitch for the development of ambient ionization methods in food safety with graduate school-style rigor from Brian Musselman of Ionsense, who sells DART ion sources.

Introduction

Ambient ionization is both fast and semi-quantitative, and it complements standardized, hyphenated methods (GC-MS/MS and LC-MS/MS). IMS oral sessions and poster session were given during the meeting, but the content applies to a portion of interest group. For instance, tissue images of a drug or drug metabolite are limited to meat analysis. Ambient ionization oral sessions and poster sessions were given during the meeting and the content does apply to the interest group. However IMS of food, flavors and fragrance is rarely the content.

In this workshop, we studied 12 publications about IMS with foods and flavors using a checklist to compare the publications fairly. Also, the audience posed questions, which were discussed. The Discussion and Conclusions are observations from the Results section, which are the Checklists.

Method

Twelve groups were formed around discussion leaders, and ach group consisted of between 5 and 10 people. The participants were given a copy of the publication and a Publication Checklist, and terms in the Publication Checklist were defined. For instance, a profile is two or more mass spectra at two or more corresponding locations in the same tissue, and an image is a photo with pixels and a color-intensity scale. After discussion, the discussion leader reported the answers from their group to the workshop.

Publication Checklist

1. Source? _____
2. Estimated cost of the ion Source? _____
3. Desorption location? _____
4. Ionization location? _____
5. Desorption method? _____
6. Primary ions? _____
7. Secondary ions? _____
8. Sample? _____

9. Image/profiling? _____
10. Suppression? _____
11. Quantitation? _____

Results

1. Probe ESI as an Ambient FD[1]

Source? PESI
Estimated cost of the ion Source? 5k$
Desorption location? pin spot
Ionization location? Taylor cone
Desorption method? contact transfer or extraction
Primary ions? sample ions
Secondary ions? N/A
Sample? dry, green potato and 1$ bill
Image/profiling? Profile potato and 1D image of 1$ bill
Suppression? not observed
Quantitation? not observed for cocaine and solanine on 1$ bill and green, dry potato

2. Atmospheric Pressure Infrared MALDI Imaging Mass Spectrometry for Plant Metabolomics[2]

Source? AP MALDI
Estimated cost of the ion Source? IR laser 20-40 k$ and stage 10-20k$
Desorption location? laser spot
Ionization location? above the laser spot
Desorption method? bulk flow from impulse
Primary ions? sample ions
Secondary ions? N/A
Sample? banana, tomato, strawberry, cilantro, potato, onion, garlic and almonds
Image/profiling? Image and profile of lily and profiles of other samples
Suppression? Although the mass spectra are complex, triacylgrylcerols from almond, malic acid from potato, and α-ketoglutamic acid were distinguished. Other analytes may be been suppressed.
Quantitation? no

3. Visualization of anthocyanin species in rabbiteye blueberry Vaccinium ashei by matrix-assisted laser desorption/ionization imaging mass spectrometry[3]

Source? vacuum MALDI
Estimated cost of the ion Source? PESI<x<LAESI
Desorption location? laser spot
Ionization location? laser spot
Desorption method? bulk flow from impulse
Primary ions? matrix ions
Secondary ions? sample ions
Sample? banana, tomato, strawberry, cilantro, potato, onion, garlic and almonds
Image/profiling? image of m/z 433, 449 and 493
Suppression? The extraction mitigates ion suppression
Quantitation? Normalized quantitation

4. Detection of PAHs in Seafood Using MALDI Imaging[4]

Source? vacuum MALDI
Estimated cost of the ion Source? PESI<x<LAESI
Desorption location? laser spot on sample
Ionization location? laser spot above the sample
Desorption method? bulk flow from laser desorption
Primary ions? matrix ions
Secondary ions? sample ions
Sample? shrimp
Image/profiling? Image of m/z 76, 98 and 118 without assignments

Suppression? gut juice may ionize easier than a PAH metabolite
Quantitation? none

5. Enzymatic Removal of Surface Layer on Plant Tissue Followed by Mass Spectrometric Imaging[5]
Source? vacuum MALDI
Estimated cost of the ion Source? PESI<x<LAESI
Desorption location? laser spot
Ionization location? above the laser spot
Desorption method? bulk flow from impulse
Primary ions? matrix ions
Secondary ions? sample ions
Sample? Arabidopsis leaf
Image/profiling? Image of digested area
Suppression? Not observed
Quantitation? internal standards on liver

6. Metabolic Profiling and Imaging Metabolite Distribution of Pea Leaves by Mass Spectrometry[6]
Source? vacuum MALDI
Estimated cost of the ion Source? PESI< 52-100k$<LAESI
Desorption location? laser spot on leaves
Ionization location? Plume above the leaves
Desorption method? Bulk flow from impulse
Primary ions? 3 matrix ions
Secondary ions? sample ions
Sample? pea leaf
Image/profiling? Image of digested area
Suppression? Silver adducts mitigated ion suppression of linoleic acid
Quantitation? none

7. MALDI mass spectrometry imaging of secreted lipopeptides in a bacterial biofilm colonizing plant roots[7]
Source? vacuum MALDI
Estimated cost of the ion Source? PESI<x<LAESI
Desorption location? laser spot
Ionization location? laser spot
Desorption method? Bulk flow from impulse
Primary ions? matrix ions
Secondary ions? sample ions
Sample? Tomato root rhizosphere
Image/profiling? image of secretin from tomato root
Suppression? No comment
Quantitation? none

8. In Situ Profiling of Glycoside Isoforms in Intact Stevia Plant Leaves[8]
Source? DESI
Estimated cost of the ion Source? PESI< 30-100k$ <LAESI
Desorption location? ESI spray area
Ionization location? Above the leaf
Desorption method? Bulk flow from MeOH spray
Primary ions? Spray ions from MeOH
Secondary ions? Deprotonated sample ions and chlorine adduct ions
Sample? Stevia glycosides in Stevia leaves
Image/profiling? Profiles of leaf species
Suppression? We fail to observe Rebaudioside F or Dulcoside A and observe little Rebaudioside D; the strong signals for the other Steviol glycosides suggests that the concentration of these chemicals was low in the leaves, not that ion suppression occurred.
Quantitation? Normalized intensities of m/z 311, an endogenous ion

9. High-resolution spatial and temporal analysis of phytoalexin production in oats[9]
Source? ESI
Estimated cost of the ion Source? PESI<x<LAESI
Desorption location? N/A
Ionization location? the cytoplasm of a leaf cell was dissected, spiked and injected into an LC-ESI-MS/MS method
Desorption method? N/A
Primary ions? sample ions
Secondary ions? N/A
Sample? Oat leaves
Image/profiling? Image of 5 cells before and after LC-ESI-MS/MS; Profile of avenanthramides A and B
Suppression? Mitigated with both micro dissection and chromatography
Quantitation? Calibration curves of avenanthramides A and B from 0 to 30 nM with 10 mM [8',9'-$^{13}C_2$] avenanthramides A and B

10. Enhanced Detection of olefins using ambient ionization mass spectrometry: Ag+ adducts of biologically relevant alkenes[10]
Source? DESI
Estimated cost of the ion Source? PESI< 80 k$ <LAESI
Desorption location? ESI spray area
Ionization location? Above the sample
Desorption method? Bulk flow from spray
Primary ions? ESI ions
Secondary ions? sample ions
Sample? Canine bladder
Image/profiling? Image of ions for dog bladder cancer
targeted, non-targeted or suspect list ? targeted
Suppression? mitigated with silver ion
Quantitation? LOD

11. Authenticity assessment of beef origin by principal component analysis of matrix-assisted laser desorption/ionization mass spectrometric data[11]
Source? MALDI
Estimated cost of the ion Source? 350k$
Desorption location? laser spot on sample
Ionization location? Plume above the laser spot
Desorption method? Bulk flow from impulse
Primary ions? matrix ions
Secondary ions? sample ions
Sample? beef
Image/profiling? Image of triacylglycerol
targeted, non-targeted or suspect list ? Non-targeted
Suppression? N/A
Quantitation? Triacylglycerol with colorimetric kit and normalization of MS/MS spectra

12. LAESI brochure[12]
Source? LAESI
Estimated cost of the ion Source? 60-100k$
Desorption location? laser spot
Ionization location? laser spot
Desorption method? Bulk flow from impulse
Primary ions? ESI ions
Secondary ions? sample ions
Sample? liver
Image/profiling? both

Suppression? Not discussed
Quantitation? internal standards on liver

Discussion

IMS was performed on plants, fruits, vegetables, herbs, nuts, and meat. Hiraoka et al., and Wiseman et al. and Jun et al. and Debois et al. reported images and profiles for plants respectively[1, 5, 7-8]. Li et al. reported both images and profiles for lily leaves and profiles for banana, tomato, strawberry, cilantro, potato, onion, garlic and almonds.[2] Yoshimuro et al. reported images for phytochemicals in blueberries,[3] and Song et al. reported classes of chemicals in text for pea leaves.[6] Salla et al., Zaima et al., Jackson et al, and Proteo reported images for shrimp, beef, dog bladder and liver.[4, 10-12]

Although the results of imaging experiments in foods and flavors and drug metabolite IMS are sometimes similar, usually the results of imaging experiments in foods and flavors are less formal than results in the IMS literature. Many IMS reports are images of a drug or drug metabolite in a tissue. The image is necessary to understanding the biological effect of the drug or drug metabolite. Table 1 is a list of the result types from the publications in the workshop. Izumi et al. performed cell-specific imaging like some drug metabolite reports,[9] and nine other publications included images.[1-5, 7-12] However, the five of the nine other food and flavor publications are less formal in four ways. First, Hirako et al.'s imaging result was a 1D image of a 1$ bill, not a 2D image.[1] Second, Li et al.[2] and the Protea group[12] augmented the images with profiles. Third, Salla et al.'s image was a non-targeted ion image.[4] Finally, Song et al. gave a verbal description of the results and not an image or a profile.[6]

The ion source estimates are summarized in Table 1 and in Figure 1. The estimates were between 5k$ for PESI and 350k$ for vacuum MALDI. Although one vacuum MALDI group failed to provide an estimate[3], a mean vacuum MALDI estimate was calculated to be 116k$ from the other five estimates (see Table 1); the mean DESI estimate was 73k$. Figure 1 is a plot of the mean estimate versus ion source; the mean vacuum MALDI estimate is the highest estimate, which matches its popularity. The estimates from the discussion leaders may not reflect the market price. For instance, the author obtained a verbal quote for the LAESI ion source from Protea of around 250 k$ with negotiation, but the discussion leader's estimate was 80k$.

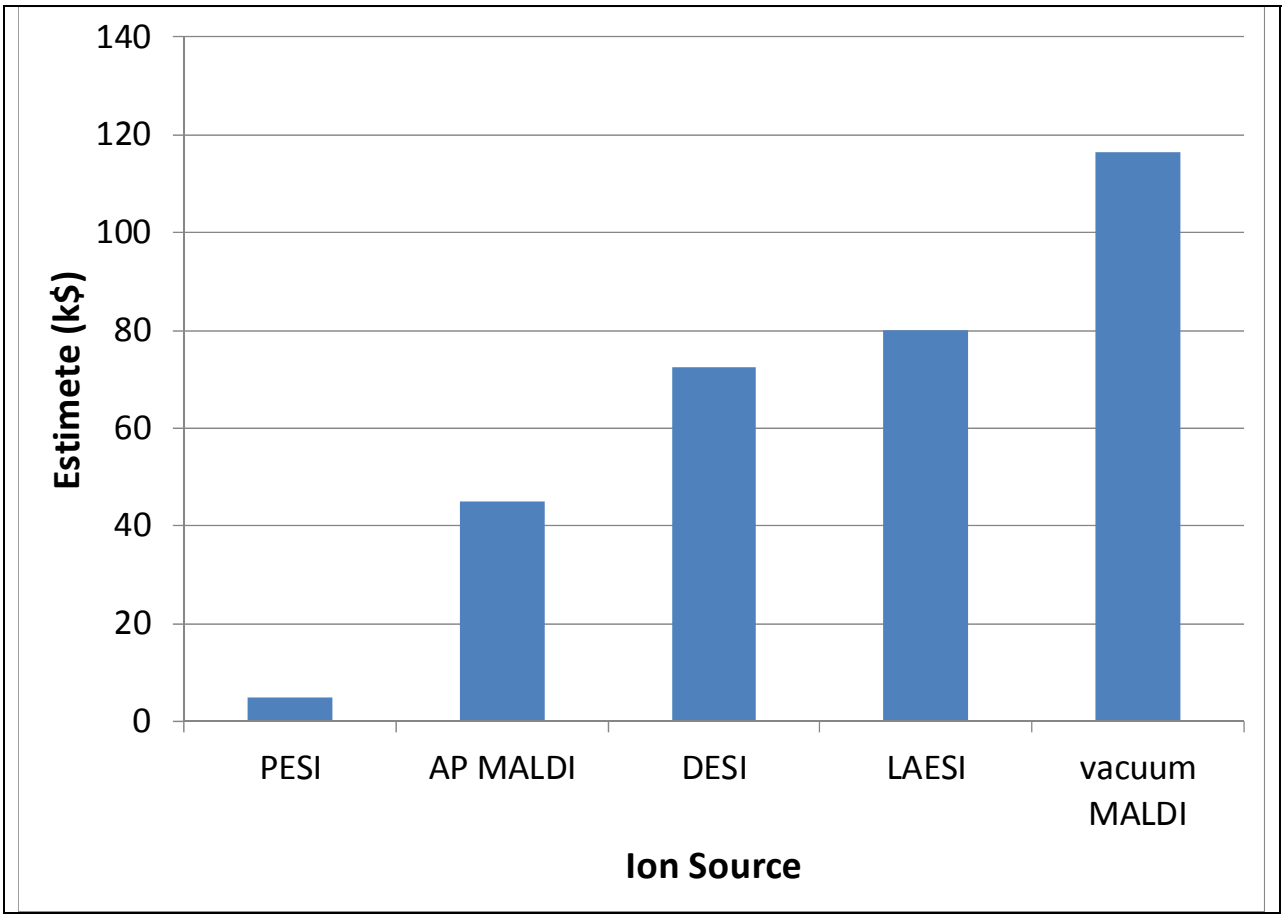

Figure 1. Bar plot of the estimate versus ion source.

Table 1. Table of references.

Ion Source	Mean (k$)	Source mean (k$)	Result type	Ref
PESI	5	5	Profile of a potato; 1D image of a 1$ bill	1
AP MALDI	45	45	Image and profile of a lily	2
vacuum MALDI		116	Image of blueberry	3
" "	50		Image of shrimp gut	4
" "	76		Image of digested pea leaf	5
" "	30		Description of primary alcohols in adaxial epidermis; alkane and secondary alcohols in abaxial epidermis; and sucrose, inositol and lineinic acid in mesophyll	6
" "	76		Image of surfactin C12-C15 Biofilm	7
" "	350		Image of triacylglycerol	11
DESI	65	72	Profile of leaf species	8
" "	80		Image of dog bladder	10
LAESI	80	80	Image and profile of liver	12

Quantitation was performed with an endogenous ion and normalization; external calibration curves; a colorimetric kit; and an internal standard. Quantitation with an endogenous ion and normalization was performed with Stevia leaves with DESI.[8] The limit of detection of fatty acid standards

and prostaglandin standards were determined with DESI.[10] Quantitation of triacylglycerol was determined with a colorimetric kit.[11] Quantitation of avenanthramindes A and B was performed with calibration curves of avenanthramides A and B from 0 to 30 nM with 10 mM [8',9'-$^{13}C_2$] avenanthramides A and B.[9]

Isobaric inference during IMS of foods and flavors was overcome with a combination of tandem mass spectrometry, silver ion adducts and the theoretical isotopic distribution of the isobars or was not an issue. First, isobaric interference was observed during the analysis of linoleic acid-silver adduct and oleic acid-silver adduct. The overlap was confirmed with the theoretical isotopic profiles and tandem mass spectrometry. Second, isobaric interference was observed during the analysis of Stevia despite both chloride adducts. (M-H)- Steviolbioside/Rubusoside; (M+Cl)- Steviolbioside/Rubusoside; (M-H)- Stevioside/Rebaudioside B; (M+Cl)- Stevioside/Rebaudioside B; (M-H)- Rebaudioside A/E; (M-Cl)- Rebaudioside A/E; and (M+Cl)- Rebaudioside A/E are isobar pairs and are isobaric interference in the mass spectrum. However, the goal of the analysis was the selection of the best species of Stevia among 4462-93, HG and s5831, and DESI analysis with normalization allowed assessment of the relative abundances of the steviol glycosides in the three distinct plant species.

Ion suppression may have occurred during IMS of food and flavors, but ion suppression was mitigated with silver adducts, extraction, tandem mass spectrometry, and the combination of micro dissection and chromatography. Hiraoka et al. reported that cocaine and solanine are the base peaks in the mass spectra of the 1$ bill and the green, dry potato, but other ions may have been suppressed.[1] Although Li et al. observed triacylgrycerols from almond, malic acid from potato and α-ketoglutamic acid from lily, the mass spectra are complex, so other ions may have been suppressed[2]. Salla et al. observed benzo(a)pyrene spiked on shrimp gut cross-section, m/z 76, m/z 98 and m/z 188 in a shrimp gut cross-section, but the group failed to observe PAH metabolites in the shrimp gut cross-sections, so the PAH metabolites may have been suppressed.[4] Debois et al. observed surfactins in the rhizosphere of tomato roots, but failed to observe fengycins.[7] Because both surfactins and fengycins have a polar head and non-polar tail, their ionization efficiency should be comparable. Still the fengycins may have suppressed. Wiseman et al. fail to observe Rebaudioside F or Dulcoside A and observed little Rebaudioside D; the strong signals for the other Steviol glycosides suggest that the concentration of these chemicals was low in the leaves, not that ion suppression occurred.[8] Still, Rebaudioside F, Dulcoside A and Rebaudioside D may have been suppressed. In contrast to these reports, other authors mitigated ion suppression with silver adducts, extraction, tandem mass spectrometry and the combination of micro dissection and chromatography. Jackson et al. reported both DESI with dog bladder[13] and DESI with silver adducts[10] with dog bladder and observed 50-fold improvement in alkene signal and a new triacylglyceride signal.[10] Yoshimura et al. delipidated blueberries with a hexane-ethanol mixture before imaging to eliminate interference from lipid components.[3] Zaima et al. failed to provide a single-scan mass spectrum of an imaged beef sample, so the workshop could not determine the complexity of the mass spectrum except from the lipid extract spectra, which showed complexity.[11] Still, Zaima et al. used tandem mass spectrometry of phosphatidylcholine, diacyl 34:1 and 36:1, and the workshop thinks that tandem mass spectrometry minimized the effect of the complex spectrum. Izumi et al. used the combination of micro dissection and chromatography to eliminate interference from the cytoplasm sample.[9]

Silver adduct ions were used to identify both triacylglycerides and linoleic acid. Jackson et al. with DESI provided an acylglyceride image with the silver ion and used the isotopic distribution of silver, theoretical calculations and tandem mass spectrometry to show that both linoleic and oleic acid contributed to the m/z 389 ion abundance.[10] Jun et al. used collodial silver as a matrix with vacuum MALDI to provide a linoleic acid image.[5]

Imaging of foods and flavors is done, but chemists do not always use an ambient ionization source. Vacuum MALDI is the most popular method followed by DESI. Six vacuum MALDI articles,[3-7, 11] two DESI publications[8, 10] an AP IR MALDI article[2], a LAESI brochure[12] and elected slides from the PESI oral session[1] were discussed.

Ambient ionization IMS in foods and flavors is a growing field. Commercialized ion sources are well represented. Half of the publications used a vacuum MALDI ionization source. Because the vacuum MALDI ion source is both commercialized and popular, the coordinator found more publications for it than

for DESI, AP MALDI, LAESI, or PESI in the imaging literature for foods and flavors. The PESI ion source, the ambient desorption/ionization mass spectrometry source (ADI-MS)[14] and the surface desorption atmospheric pressure chemical ionization (SDAPCI) source[15] are research-grade ionization sources in the imaging literature and were considered for the workshop.

Conclusion

IMS of foods and flavors was performed. It was less formal than IMS of drugs and drug metabolites in tissue. Although an estimate failed to match the market price, the estimated ion source price range was 5k$ for PESI and 350k$ for vacuum MALDI. Quantitation was performed with an endogenous ion and normalization; external calibration curves; a colorimetric kit; and an internal standard. Isobaric interference was overcome or was not an issue. Ion suppression may have occurred, but it was overcome with silver adducts, extraction, tandem mass spectrometry, and the combination of micro dissection and chromatography. Silver adducts identified both triacylglycerides and linoleic acid. Imaging of foods and flavors was done, but chemists do not always use an ambient ionization source. IMS with ambient ionization sources of foods and flavors is a growing field.

Workshop survey

Overall the discussion worked well and the majority of people (~90 %) felt that the format of the workshop was successful. It gave everyone the opportunity to get involved and initiated some animated discussion.

Acknowledgements

We thank ASMS for the workshop opportunity and their help. The 12 discussion leads were important for a successful discussion and active participation I thank them for their help and enthusiasm.

1. Dr. K. Hiraoka group representative [1]
2. Dr. Bindesh [2]
3. Dr. Nobuhiro Zaima group representative [3, 11]
4. Dr. Kermit K Murray group representative [4]
5. Dr. Lee Young Jin group representative [5-6]
6. Dr. Edwin De Pauw group representative [7]
7. Prosalia group representative [8]
8. Dr. Yoshihiro Izumi group representative [9]
9. Cooks group representative [10]
10. Protea group representative [12]

Publications

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4. Salla, V.; Hayes, J. M.; Murray, K. K. In *Detection of PAHs in Seafood Using MALDI Imaging*, American Conference for Mass Spectrometry, Denver, Denver, 2011.
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