Food Analytical Methods

Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry

--Manuscript Draft--

Manuscript Number:	FANM-D-17-01194R1
Full Title:	Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry
Article Type:	Original Research
Keywords:	mass spectrometry; Surface Acoustic Wave Nebulization; Vinegar, Wine, Beer - Analysis
Corresponding Author:	Thomas Schneider, Ph.D. University of Washington Seattle, WA UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Washington
Corresponding Author's Secondary Institution:	
First Author:	Thomas Schneider, Ph.D.
First Author Secondary Information:	
Order of Authors:	Thomas Schneider, Ph.D.
	Benjamin L Oyler, B.S.
	Sung Hwan Yoon, Ph.D.
	Tao Liang, B.S.
	Gloria S Yen, Ph.D.
	David P Kilgour, Ph.D.
	Erik Nilsson, B.S.
	David R Goodlett, Ph.D.
Order of Authors Secondary Information:	
Funding Information:	National Institutes of Health (1R01 GM111066-01) — Prof. David R Goodlett
	National Science Foundation (1330459) — Mr. Erik Nilsson
	School of Pharmacy, University of Maryland Mass Spectrometry Center (SOP1841-IQB2014) — Prof. David R Goodlett
Abstract:	Rapid food product analysis is of great interest for quality control and assurance during the production process. Conventional quality control protocols require time and labor intensive sample preparation for analysis by state-of-the-art analytical methods. To reduce overall cost and facilitate rapid qualitative assessments, food products need to be tested with minimal sample preparation. We present a novel and simple method for assessing food product compositions by mass spectrometry using a novel surface acoustic wave nebulization method. This method provides significant advantages over conventional methods requiring no pumps, capillaries, or additional chemicals to enhance ionization for mass spectrometric analysis. In addition, the surface acoustic wave nebulization - mass spectrometry method is ideal for rapid analysis and to investigate certain compounds by using the mass spectra as a type of species-specific fingerprint analysis. We present for the first time surface acoustic wave nebulization generated mass spectra of a variety of fermented food products from a small selection

	of vinegars, wines, and beers.

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

28 February 2018

Prof. David Rodríguez-Lázaro
Editor-in-Chief, Food Analytical Methods
Microbiology Division,
Department of Biotechnology and Food Science,
Faculty of Science,
University of Burgos,
Burgos, Spain
Email: drlazaro@ubu.es

Re: Schneider T., et al., "Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry"

Dear Prof. Rodríguez-Lázaro,

We thank you for the opportunity to revise our manuscript according to the reviewer's comments and suggestions. We have addressed each reviewer's point-by-point below and believe that our revisions satisfy the reviewer's comments. Changes to the manuscript and supporting information are highlighted in red with the track changes tool. In addition, we are submitting a clean version of the revised manuscript and supporting information.

Thank you for consideration of our manuscript.

With kind regards,

Below are the comments by the reviewers addressed point-by-point.

Reviewer 1:

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

The article describes a relatively novel (SAWN) technology that is still being developed, and being investigated for its use in new fields of application. As such SAWN deserves wider understanding and scrutiny by our scientific community. The authors point out the potential large market interest (food products) for such a simple sampling system, that rapidly develops gas-phase ions to be measure by MS. if successful this would fit in to existing analytical workflows employed world-wide. Thus as a first time article for Food Analytical Methods I find it a valuable contribution in its current form.

Comments to be addressed that would make the article better for the reader.

Response:

We thank the reviewer for his/her helpful comments and critiques and hope that our responses successfully address the reviewers' concerns.

Comment 1-1:

Abstract:

"We present for the first time surface acoustic wave nebulization generated mass spectra of a variety of fermented food products."

The authors could be more specific than merely stating "...a variety of food products."? It would help those who are looking to find specific information about a food type or product, if this was mentioned somewhere in the abstract or introduction (also not mentioned here). I suggest to state something like "... a small selection of different vinegars, wines, and beers."

Response 1-1:

We amended the last sentence of the abstract to include a more focused statement of the types of fermented food products used in our study. The revised sentence reads: "We present for the first time surface acoustic wave nebulization generated mass spectra of a variety of fermented food products from a small selection of vinegars, wines, and beers."

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

Comment 1-2:

P 6

As above, we are still clueless as to what is measured here and, besides the news about SAWN technology, what is reported here?

Same here: please mention at least what is measured, such as a small selection of

vinegars, wines, and beers.

Response 1-2:

In addition to the abstract changes, we modified the first sentence in the last paragraph of the introduction to highlight the type of samples that were used in our study. The revised sentence reads: "Here we present for the first time the rapid analysis of food products by SAWN-MS that include a selection of fermented food products such as vinegars, wines, and beers".

Comment 1-3:

P 8

Why was assignment and verification of selected compounds conducted on both a Finnigan LTQ and on a Waters Synapt G2-S? As no reason is given at all, it might be best to just state that choice of instrument was based on instrument availability, or otherwise some (compelling?) technical ability of a particular instrument? One is left guessing now…

Response 1-3:

We thank the reviewer for pointing this out and we have included an instrument availability statement in the first sentence of the third paragraph under section 2.2 of the materials and methods. The revised sentence reads: "Assignment and verification of selected compounds was conducted with ESI by collision-induced dissociation (CID) on a Finnigan LTQ (Thermo Scientific, San Jose, CA, USA) retrofitted with a bespoke ion funnel (Canterbury et al. 2014) and on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation), chosen based on instrument availability at the time of analysis."

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

Comment 1-4:

Figures

It was not always clear to me when a SAWN spectra was discussed, or ESI. Could this clearly be stated in ALL figure legends and throughout the text? Sometimes it was chosen to mention the vendor of the instrument. For example: "Figure S5. Fragmentation of oligosaccharides in samples from Bud Light acquired by ESI (LTQ)". Is this 'LTQ' important? Pls omit or fill in for all figure legends.

Response 1-4:

We revised the legends of the main text figures 2 and 3 to include the word SAWN in front of "mass spectra" to clarify the source of sample transfer where the clear distinction was missing. In addition, we revised the figure captions of the supplemental information to include further information of the type of mass spectrometer used (shown in the tracked changes portion of the revised texts and the cleaned version of the finalized revision of the manuscript and supplemental text):

- In figures S1, S4 – S6: the data was acquired on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel
- In figure S2: the data was acquired on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation)
- In figures S3 and S8: the data was acquired by SAWN MS.

Comment 1-5:

**Figure S1. **

The figure shows a panel marked A) and B), with the following text: "Fragmentation of sodium adducts of disaccharides found at m/z 365 (a) and glucose/fructose at m/z 203 (b) in samples from rice vinegar acquired by ESI (LTQ). Shown are the precursor ions (MS2 at CE 0, black spectra) and fragment ions (MS2 at CE 30, red spectra)."

There seems to be some information missing here, as A) shows two mass spectra and B) does as well. However, A) lists a CE of 20eV, whereas B) lists 30eV. What's wrong here? Note that nowhere is CE defined. This also difference in nomenclature to Fig S2 where (e.g.) CID 25eV is used.

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

Response 1-5:

We understand the source of confusion for the reader in this figure caption and have revised the text of the figure caption accordingly. The terms normalized collision energy (NCE) and collision-induced dissociation (CID) were defined at the end of the materials and methods section of the manuscript. The nomenclature for tunable parameters in CID is instrument vendor-specific. The NCE and CID collision energy values are not directly comparable between the two manufacturers and are hence given as reference to compare to fragmentation patterns for the interested reader. The revised caption to figure S1 reads: "Figure S1. Fragmentation of sodium adducts of disaccharides found at m/z 365 (a) and glucose/fructose at m/z 203 (b) in samples from rice vinegar acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel. Shown are the precursor ions (MS2 at NCE of 0 %, black spectra) and fragment ions (MS2 at NCE of 20 % in (a) and 30 % in (b), red spectra). Note that the differences in the collision energy used in this experiment are due to differences in energy required to fragment mono- and disaccharides."

Comment 1-6:

Figure S2.

Again this needs some clean up. There are A, B, C, and D (with three spectra) panels

shown, but the legend only gives information on two, namely A and C. Please provide missing information.

Response 1-6:

We thank the reviewer to point out the incomplete caption and have revised the figure caption to clarify the different spectra. The revised caption reads: "Figure S2. MS spectra from direct infusion in (+) ion mode of rice vinegar acquired by ESI on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation). Shown are the MS1 spectrum (a) and the fragmentation spectra (MS2) of sodium and potassium adducts of disaccharides found at m/z 365 (b) and m/z 381 (c), and glucose/fructose at m/z 203 (d). The fragmentation spectra were acquired with different collision-induced dissociation (CID) energies to show the fragment ions from the different precursor ions. The masses measured were: $C_{12}H_{22}O_{11}Na$, measured: 365.1051, exact: 365.1060, error: 2.5 ppm; $C_{12}H_{22}O_{11}K$, measured: 381.0741, exact: 381.0799, error: 15.2 ppm."

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

Comment 1-7:

**Figure S3. **

Why an extra x-axis numerical marking in the middle of the figure? Is this somehow

different from the one at the bottom?

Response 1-7:

We revised the figure to exclude the additional x-axis labels.

Reviewer 2:

The manuscript by T. Schneider et al. describes application of SAWN, a novel ambient ionization technique for mass spectrometry, to food product analysis. This is an interesting contribution that contains relevant data and will be interesting for the readers of the journal. The manuscript is well-written without any major improvements being necessary. It clearly shows the potential applicability of the SAWN ionization in food analysis. In the same time, it is fair to say that the work also demonstrates some of the general limitations of ambient ionization (compare to established techniques such as GCMS or LSMS).

I recommend publication of this manuscript in Food Analytical Methods as it is or after a minor revision. Here are my comments for consideration by the authors for the revised manuscript as well as for the future work:

Response:

We thank the reviewer for the kind remarks and comments on our work and a grateful for the helpful suggestions to improve our manuscript.

Comment 2-1:

-The figures that show the positive and negative mode spectra flipped upside down have questionable information value. Comparing flipped spectra makes sense if one seeks to either demonstrate differences between mostly identical spectra or to show that two spectra are completely the same. It is

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

not surprising that positive and negative mode spectra will have all the peaks different so I am not sure what is to be shown here. It would make more sense to use this graphical interpretation of the data to compare spectra of two different samples with the same source polarity.

Response 2-1:

We understand the argument for upright versus flipped (or upside down) spectra in direct comparison of spectra acquired in similar polarity. We used upside down and stacked graphs to show a comparison of spectra acquired by the same polarity throughout the supplemental materials section of our manuscript. Our intent was to show the direction of the polarity during acquisition as represented by the flipped spectra (i.e., upside down) in the main part of the manuscripts and we believe that this is a valuable means of presentation. Additionally, these flipped spectra show ions detected in both modes, which correspond to the same molecule, adding confidence to identifications. As such, we acknowledge the reviewers concerns and politely disagree on the type of representation of spectra.

Comment 2-2:

-Spectrum S2 has incorrect description in the caption (Panel A is apparently a full range MS spectrum, tandem MS spectra start with panel B)

Response 2-2:

We revised this figure caption. The revised caption reads: "Figure S2. MS spectra from direct infusion in positive ion mode of rice vinegar acquired by ESI on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation). Shown are the MS1 spectrum (a) and the fragmentation spectra (MS2) of sodium and potassium adducts of disaccharides found at *m/z* 365 (b) and *m/z* 381 (c), and glucose/fructose at *m/z* 203 (d). The fragmentation spectra were acquired with different collision-induced dissociation (CID) energies to show the fragment ions from the different precursor ions. The masses measured were: $[C_{12}H_{22}O_{11}Na]^+$, measured: 365.1051, exact: 365.1060, error: 2.5 ppm; $[C_{12}H_{22}O_{11}K]^+$, measured: 381.0741, exact: 381.0799, error: 15.2 ppm."

Comment 2-3:

-It is interesting that ions at m/z 365 and 381 in QTOF spectra in Figure S2 have a big difference in mass error. The two ions with very close m/z values arrived to the detector within a fraction of a microsecond.

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

In the absence of space charge issues one has to wonder why the difference in mass error. Were other options, non-potasiated species, also considered as candidates during identification?

Response 2-3:

We considered other obvious options for species that might be present during identification and did not come up with other possibilities. Additionally, the tandem mass spectra for each of these ions is characteristic for each assignment. These are not very large differences in error for a TOF analyzer, but one possibility other than space-charge effect is the presence of chemical noise or isobars at low abundance skewing the data a bit. Furthermore, despite calibration of the Q-TOF instrument, we have not used a lock mass ion during ESI and SAWN experiments to post-calibrate the results. The mass shift of 9 mmu for Na+ and 58mmu for K+ may be attributed to the experiment.

Comment 2-4:

-It is actually not uncommon to use [M-Cl]- ions to detect and quantify sugars in ESI-MS analysis. The adducts are stable in ESI and using [M-Cl]- ions dramatically increases sensitivity and LOD compare M- ions. The limitation in LCMS is not the fragility during ESI ionization, but compatibility with basic pH mobile phase. [M-Cl]-. The problem in LCMS of sugars is with reducing sugars that undergo mutarotation. Separation of alpha and beta anomers then results in very bad peak broadening, often beyond detectability However, the anomers can be collapsed into one peak by using high pH mobile phase (e.g. 0.1 % NH_4OH). But higher pH suppresses the formation/stability of chloride adducts in ESI. Although I believe the authors that SAWN provides softer ionization in a sense that the internal energy of ions that originate from SAWN is lower then in ESI (as also claimed for many alternative techniques such as ESSI and some other ambient techniques), I am not sure that it causes better survival rates of the mentioned ion adducts. If there is really a significant difference between SAWN and ESI in abundance of ion adducts (the manuscript however does not provide sufficient evidence for this), it could be also caused by some other factor, for instance different local concentrations of the cationization/anionization species or different pH.

Response 2-4:

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

We thank the reviewer for his/her thoughtful remarks. The study of different pH during ionization or sample transfer during SAWN-MS is of interest to us. However, we have not explored this area deeply. We are aware of the differences in pH in the samples of vinegar and wine we studied but a much deeper analytical study into such variations and the impact on sample transfer and ionization was beyond the scope of our study.

Comment 2-5:

-From the terminological perspective, ESI is not usually called ambient ionization (as it is in pg 14 ln46), although it operates at ambient pressure. See relevant papers from Cooks group for the arguments and definition of ambient ionization.

Response 2-5:

We agree with the assessment of the reviewer regarding the terminology of the methods and have revised the first sentence in 5th paragraph of the introduction as follows:

"All of the novel ionizations methods listed here that operate at ambient pressures are widely used in research settings."

Comment 2-6:

-The advantage of QTOFs for ambient ionization is that they can quickly obtain accurate mass spectra, which allow determination of molecular formula and thus provide more confidence in identification. This is a feature that is not sufficiently explored in this work. One potential difficulty is the absence of predefined mass lock specie (such as leucine-eucefaline) that could be utilized for accurate mass internal calibration.

Response 2-6:

We understand that the addition of lock mass compounds as described by the reviewer would increase the mass measurement accuracy for our identifications. However, we have not included lock mass species in our initial studies shown here out of an abundance of caution to retain clean spectra from the different samples we tested. Additionally, the identifications we made using tandem MS at accurate

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY

David R. Goodlett, Ph.D.
Professor of Pharmaceutical Sciences
Pharmacy Hall North Room 631
20 N. Pine Street
Baltimore, MD 21201

mass, along with MS1 TOF mass spectra provide sufficient information without lock mass to identify these relatively small ions. The QTOF instrument used in these experiments is not sufficiently accurate, even with lock mass, to define empirical formulae based on precursor mass alone for very many ions in any of our spectra. We agree with the reviewer that the QTOF's speed is a major advantage to its use in acquiring ambient ionization MS data.

Comment 2-7:

-The comparison between IPA and Bud Light, that shows almost no difference between identified species, in my opinion demonstrates the limitation of ambient ionization. A properly performed untargeted LCMS study with high resolution mass spec would likely identified hundreds of compounds in both beers as well as differences between them. Thus, the SAWN data show how preferential ionization and ion suppression, which are common in all ambient techniques, make it relatively difficult to find practical applications of ambient ionizations. (Unless the authors actually wanted to conclude that good IPA is the same as Bud Light…)

Response 2-7:

We refrained from making an assessment to conclude that the IPA used in our study is the same as Bud Light. The main focus here was that we could observe the fragmentation of oligosaccharides to a greater extent in our study when compared to the referenced study conducted by ESI. The conclusion for us was simply that spontaneous fragmentation was observed to a lesser degree in SAWN-MS compared to ESI-MS. This was in support of earlier studies with SAWN-MS that were able to show "softer" ionization compared to alternative methods, which can be of benefit in the analysis of labile compounds. While we agree that online separation often provides an advantage with respect to the number of confident compound identifications, we would note that we did not attempt to identify all monoisotopic ions in the mass spectra; there are hundreds. So, while the spectra are qualitatively similar between Bud Light and Goose IPA (they are both beer, after all), it is possible that there are features, or relative abundances of features, in the mass spectra which could reliably differentiate between the two types of beer. Figure S8 shows a few *m/z* regions wherein some of these differences can be observed. Development of computational tools for differentiation of mass spectra would be necessary to determine the feasibility of this platform for the aforementioned use.

Food Analytical Methods

Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry

Thomas Schneider[a]*, Benjamin L. Oyler[b], Sung Hwan Yoon[c], Tao Liang[a], Gloria S. Yen [d], David P. A. Kilgour[e], Erik Nilsson[d], David R. Goodlett [a,d]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA

[b] Department of Toxicology, School of Medicine, University of Maryland, Baltimore, MD, 21201, USA

[c] Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, MD, 21201, USA

[d] Deurion LLC, Seattle, WA 98103, USA.

[e] Chemistry and Forensics, School of Science & Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK

Contact Information:

*Corresponding Author: T. Schneider (tschneid@uw.edu)

Abstract

Rapid food product analysis is of great interest for quality control and assurance during the production process. Conventional quality control protocols require time and labor intensive sample preparation for analysis by state-of-the-art analytical methods. To reduce overall cost and facilitate rapid qualitative assessments, food products need to be tested with minimal sample preparation. We present a novel and simple method for assessing food product compositions by mass spectrometry using a novel surface acoustic wave nebulization method. This method provides significant advantages over conventional methods requiring no pumps, capillaries, or additional chemicals to enhance ionization for mass spectrometric analysis. In addition, the surface acoustic wave nebulization – mass spectrometry method is ideal for rapid analysis and to investigate certain compounds by using the mass spectra as a type of species-specific fingerprint analysis. We present for the first time surface acoustic wave nebulization generated mass spectra of a variety of fermented food products from a small selection of vinegars, wines, and beers.

Keywords

Mass Spectrometry; Surface Acoustic Wave Nebulization; Vinegar, Wine, Beer - Analysis

1. Introduction

Food production and manufacturing has the largest share in gross manufacturing output in the U.S., reaching 957 billion US$ in 2015 (Nicholson 2017). Consumers expect quality and consistency from food products and manufacturers use quality and consistency as competitive tools to gain and maintain market share. This is especially true in the beverage and condiment industries, where quality control is a significant production cost. In addition, product authentication is important to both producers and customers (Tesfaye et al. 2002).

Traditional methods for analysis and quality control in the spirit and beverage industry include liquid and gas chromatography (LC and GC), photometry, and enzymatic analysis (Phillips et al. 2006). To reduce quality control costs, new methods have been proposed that require less sample preparation and time to obtain qualitative and quantitative results. These methods include the use of principle component analysis combined with high-resolution nuclear magnetic resonance (NMR) or Fourier-transform infrared (FT-IR) spectroscopy (Duarte et al. 2004; Lachenmeier 2007).

Another technique that can reduce analysis cost and time while increasing accuracy, sensitivity, and the number of analytes per measurement is mass spectrometry (MS). Conventional atmospheric pressure ionization MS relies mostly on two methods to introduce a sample into the mass spectrometer for analysis, namely electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) (Nordhoff et al. 1996). While ESI and MALDI are extremely useful techniques for gas-phase ion generation, the use of these specific sample transfer methods can limit the type of sample that can be analyzed and impose challenges such as capillary clogging and matrix interference of the target analyte's signal (Cohen and Chait 1996),

sometimes requiring dedication of considerable time and effort to troubleshooting and method development. MS has been applied together with high resolution chromatography in the study of beer flavors in the 1980's (Peppard 1985), while beer phenols have recently been reported for the first time in a detailed study by high resolution MS (Quifer-Rada et al. 2015).

The advancements in novel ambient ionization methods over the past ten years, including desorption electrospray ionization (DESI) (Takats et al. 2004), direct analysis in real time (DART) (Cody et al. 2005), laser ablation electrospray ionization (LAESI) (Nemes and Vertes 2007), rapid evaporative ionization mass spectrometry (REIMS) (Schäfer et al. 2009), paper spray ionization (Wang et al. 2010), and others have greatly expanded MS to new applications at ambient conditions, including the analysis of varying surfaces and living tissues under real life conditions (Dill et al. 2011; Zhang et al. 2017). Several variations of these methods have been developed since then, but few of these techniques have found applications in the food and beverage industry. Notably, DESI has been applied to fruit peels and food stuff extracts to trace agrochemicals (Garcia-Reyes et al. 2009). DART has been used for metabolomic profiling of different beer samples in a study aimed at the development of cost-effective methods to help authenticate food products based on the origin of their ingredients (Cajka et al. 2011).

All of the novel ionizations methods listed here that operate at ambient pressures are widely used in research settings. However, these methods generally require some type of support medium (*e.g.*, carrier gas, ESI) or thermal heating / ionization through a hot electrode, a corona needle, or a laser optical setup in order to achieve sample transfer to the MS (Van Berkel et al. 2008). Therefore, further simplifying and improving the sample transfer into the mass spectrometer will greatly enhance the capability of MS as a low cost and powerful analytical method for the food and beverage industry. In order to address challenges in the sample transfer

methods, Goodlett and colleagues recently introduced a novel ambient sample transfer method called surface acoustic wave nebulization (SAWN) (Heron et al. 2010). SAWN is expected to help addressing the challenges complex samples face in MS analyses.

Evolved from the telecommunication and semiconductor industry (Campbell 1989), surface acoustic waves (SAWs) have been applied to a variety of applications including surface patterning, fluidic mixing, sample transport and focusing, and jetting and nebulization (Länge et al. 2008; Yeo and Friend 2014). SAWN takes advantage of the SAW effect that is induced in piezoelectric materials by metallic electrodes (interdigital transducers, IDTs; **Fig. 1**) at high frequencies to create a fine plume of droplets (Heron et al. 2010; Ho et al. 2011; Huang et al. 2012; Yen et al. 2016). These droplets are readily introduced into the vacuum interface region of a mass spectrometer, further desolvated, and subsequently analyzed. Recently, we showed its application as a versatile tool for the fast analysis of hydrophobic lipid A (Yoon et al. 2012, 2016; Liang et al. 2017), a major component of the outer membrane of Gram-negative bacteria, which is recognized by the host immune system as an endotoxin (Coats et al. 2009). We have further developed the SAWN technology using a standing wave configuration to achieve higher nebulization efficiency (Huang et al. 2016; Liang et al. 2017).

Here we present for the first time the rapid analysis of food products by SAWN-MS that include a selection of fermented food products such as vinegars, wines, and beers. While standard methods for quality control and targeted analysis exist based on GC-MS and LC-MS (Flamini and Traldi 2010), such methods require significantly more time than SAWN-MS for rapid spot-checking analysis of sample quality during the beverage and condiment manufacturing process. SAWN-MS holds the unique advantage of being an ambient nebulization method, which can be conducted with complex samples. Conventional sample transfer methods

such as ESI and nanospray ionization (NSI) require careful consideration of the solvent/sample composition to avoid potential interactions that can lead to clogging of capillary tips, which increases the time required for analysis. SAWN-MS relies on a planar chip surface that is used to nebulize the sample and is therefore not as restricted in its use as ESI, and unlike ESI no voltage is applied directly to the sample making SAWN inherently less likely to break labile bonds during ionization (Huang et al. 2012). This is especially useful when analyzing complexes held together by electrostatic forces (e.g. ion clusters) and/or compounds with very labile functional groups (e.g., nucleotides or phospholipids) (Yoon et al. 2012).

2. Materials and Methods

2.1 Food Samples

Different fermented food products were used as received and diluted up to 100-fold in ultra-purified water (18.2 MΩ; MilliQ, Millipore, Milford, MA, USA) immediately before SAWN-MS analysis. The food products included vinegars, wines, and beer. Vinegars were purchased in local grocery stores: Heinz Distilled White Vinegar (H.J. Heinz Company, L.P, King of Prussia, PA, USA), Rice Vinegar (Kikkoman Sales USA, Inc., San Francisco, CA, USA), and Aged Balsamic Vinegar (Pepper Palace, Sevierville, TN, USA). Wines and beer were sourced from local restaurants: Tapena Red Wine (Freixenet USA, Sonoma, CA, USA), Le Rime Pinot Grigio (Banfi srl, Siena, IT), Goose IPA (Goose Island Beer Company, Chicago, IL, USA), and Bud Light (Anheuser-Busch, St. Louis, MO, USA).

2.2 Surface Acoustic Wave Nebulization – Mass Spectrometry

The Standing Wave (SW) SAWN chips used in our current study were manufactured at the Washington Nanofabrication Facility (University of Washington, Seattle, WA, USA) following an established procedure (Huang et al. 2016; Liang et al. 2017). A detailed summary of the chip fabrication is provided in the **Supplemental Material.** The SW-SAWN chips were placed in a custom chip holder designed and built using 3D Computer Aided Design Software (123D Design, Autodesk, San Rafael, CA, USA) and a Simple Metal 3D printer (Printrbot, Lincoln, CA, USA). A custom designed PCB board was used to connect the chip through RF cables to the SAWN Controller v1.0 (Deurion LLC, Seattle, WA, USA), which provided control over power and duration of the SAWN (**Fig. 1**). All experiments in the present study were conducted at a SAW frequency of 9.56 MHz and a power output of 11 W.

The food samples were analyzed on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation, Milford, MA, USA; **Fig. 1a**) in sensitivity mode, with positive and negative ion mode acquisition. The source block temperature was set to 150°C. Sample aliquots of 1 μL were pipetted directly into the delay region of the SAWN chip (i.e., in between the IDT's, see **Fig. 1b**) in a discontinuous fashion and the data from five aliquots were averaged to enhance signal-to-noise ratio. Typical SAWN experiments last 2-3 s.

Assignment and verification of selected compounds was conducted with ESI by collision-induced dissociation (CID) on a Finnigan LTQ (Thermo Scientific, San Jose, CA, USA) retrofitted with a bespoke ion funnel (Canterbury et al. 2014) and on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation), chosen based on instrument availability at the time of analysis. The same samples used for SAWN-MS were used for compound assignment. The spectral data were acquired over 1 min at different normalized collision energies (NCE = 0 – 30 %).

3. Results and Discussion

The advantages of the SAWN-MS analysis are its flexibility and speed. This technique requires minimal sample pre-processing (*e.g.*, dilution in water or organic solvent prior spotting) and is not limited by the challenges of conventional sample transfer methods for ESI/NSI and MALDI-MS (*e.g.*, clogging of capillaries, the requirement for syringe pumps, laser-optical setups, the use of chemical additives for sample ionization, and matrix interferences at low *m/z*). In the present work, we focused our investigation on the potential of the SAWN-MS method for rapid analysis of complex food products from the beverage and condiment industry. Some of the fermented food products we have investigated here have been studied in detail before by conventional LC-MS and tandem MS methods. The focus of these studies was often on the identification of specific individual compounds found in vinegars, wines, or beer based on their ion fragments in tandem MS experiments (Araújo et al. 2005; Chinnici et al. 2009). We confirmed key compounds found in our spectra by ESI-CID experiments (see **Supplemental Material Fig. S1, S2, S4-S6**), while the assignment of other compounds relied on prior assignments published in the literature.

Vinegars are produced through bacterial fermentation of ethanol and contain mainly acetic acid and water. Vinegars are important ingredients for cooking and as preservatives of food through pickling processes. While acetic acid produces ions below 100 *m/z* that are a general indicator of the presence of vinegar in a sample, it was not of interest for our investigation. Instead, we focused our study on a larger *m/z* range to investigate the difference between low cost distilled white vinegar (made from corn) and more aromatic rice vinegar (made

from rice) and balsamic vinegars (made from concentrated grape juice and must or wine vinegar with the addition of caramel and other additives, respectively). The full mass spectra for these three different vinegar styles are shown in **Fig. 2**.

In the positive ion mode mass spectrum of distilled white vinegar we found repeating ions, spaced equally apart by Δ*m/z* 216, indicating the presence of oligomers with sodium adducts $[M+Na]^+$ (*i.e.*, *m/z* 455, 671, and 887) and potassium adducts $[M+K]^+$ (*i.e.*, *m/z* 471, 687, and 903; **Fig. 2a**). Similarly spaced ions are present in the mass spectra of rice and balsamic vinegar (**Fig. 2a-c**). All vinegars showed ions at *m/z* 203 and 219 which are sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of glucose or fructose (Konda et al. 2012; Lee et al. 2012). Other ions that were visible in all vinegars and wines were identified as sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of disaccharides through tandem MS (*i.e.*, ions at *m/z* 365 and 381, compare **Fig. 2** and **3, see Supplemental Material Fig. S1 & S2**).

The negative ion mode mass spectra of vinegars showed a variety of sample-specific ions, especially at low *m/z*, which can be associated with organic acids. A prominent ion at *m/z* 195 $[M-H]^-$ was found in all vinegars (**Fig. 2**) as well as in red wine (**Fig. 3a**), and has been previously associated with gluconic acid, which occurs naturally in fruits, honey, and wine (Felipe et al. 2014). The differences in intensity of gluconic acid can be used as a strong indicator of the type of sample that is being analyzed as it varies strongly between vinegars and wines (see **Supplemental Material Fig. S3**). Several organic acids known to be present in grape-based food products may be associated with the ions shown in the negative ion mode mass spectra of vinegars and wines. For example, an ion found at *m/z* 115 could be associated with fumaric acid $[M-H]^-$ and is present in balsamic vinegar (**Fig. 2c**). Fumaric acid, a common food additive, is used as an acidity regulator and is often used to replace tartaric acid, another food

additive used as acidity regulator, antioxidant, and a primary component of wine grapes and fermented wines (Bravdo et al. 1985). Tartrate can be associated with *m/z* 149 (Amorisco et al. 2012) and is dominant in balsamic vinegar as well as both wines tested, and has low abundance in white vinegar (**Fig. 2** and **3**). A hydroxycinnamic acid, caffeic acid, is known to be present in red wines and to produce an $[M-H]^-$ ion at *m/z* 179 (Perez-Magarino et al. 1999). We found these ions in the mass spectra of balsamic vinegar, as well as in the mass spectra of the other two vinegars tested, albeit at lower relative abundances.

The mass spectra of balsamic vinegar showed a complex number of potential compounds dominant at low *m/z*, particularly in negative ion mode acquisition (**Fig. 2c**). The mass spectra of the wines were less complex in comparison to balsamic vinegar. The dominant species found in the positive ion mode mass spectra of wines are sodium and potassium adducts of glucose or fructose at *m/z* 203 and 219. The main difference in the mono- and disaccharide mass spectral peaks between vinegars and wines are their relative intensities and intensity ratios (**Fig. 2** and **3**). This difference in relative intensity is ideal for mass spectral fingerprint analyses to help distinguish different samples.

At low *m/z* in the positive ion mode mass spectra, an ion at *m/z* 116 was present dominantly in both wines, as well as the balsamic vinegar, but absent in rice vinegar and distilled vinegar (**Fig. 3**). The compound was assigned to the amino acid proline (based on CID, see **Supplemental Material Fig. S4**), which is abundant in grape berries and commonly found in wines even after fermentation (Ough 1968; Costin et al. 2004).

In contrast to the mass spectra acquired from vinegars and wines are those from the two beers tested in our study. The beer mass spectra showed several ions in both positive and

negative ion acquisition mode that are equally spaced by Δ*m/z* 162, indicating the presence of di- and oligosaccharides. The ions in the positive acquisition mode mass spectrum of Bud Light and Goose IPA indicate the presence of O-linked saccharides (**Fig. 4**). These di- and oligosaccharides are the sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of maltose, maltotriose, and maltotetraose at *m/z* of 365 and 381, *m/z* of 527 and 543, and *m/z* of 689 and 705, respectively (**Fig. 4**) (Araújo et al. 2005; Belitz et al. 2009). Larger oligosaccharides are present in our SAWN-mass spectra that were confirmed by tandem MS (see **Supplemental Material Fig. S5 and S6**). Similarly spaced peaks (Δ*m/z* 162) were found in mass spectra for both beers acquired in negative ion mode, indicating the presence of chloride adducts $[M+Cl]^-$ for the same saccharides at *m/z* 377, 539, and 701, respectively. These ions showed the natural isotope distribution pattern of chlorine (^{35}Cl and ^{37}Cl; **see Supplemental Material Fig. S7**). Sodium and potassium adducts of glucose (*m/z* 203 and 219) were present in relative low abundance in both beers. Compared to recent fingerprinting studies of beer with ESI (Araújo et al. 2005), our SAWN-MS method was able to show sodium and potassium adducts in the positive ion acquisition mode of additional oligosaccharides in both beers tested, including maltopentaose (*m/z* 851 and 867), maltohexaose (*m/z* 1013 and 1029), and maltoheptaose (*m/z* 1175 and 1191). Similarly, chloride adducts of the oligosaccharides were found in the negative ion mode acquired mass spectra (**Fig. 4**). The main differences between the two beers investigated here was in the intensity ratios of the sodium and potassium adducts, which are ideal for distinguishing different types of beer.

In negative ion mode we also found several compounds that can be tentatively associated with phenolic acids (Quifer-Rada et al. 2015). Among the compounds found in both beer samples are humulones, a resin component of mature hops, and a key ingredient in the brewing

process which gives beer a bitter taste and has known bioactivity (Tagashira et al. 1995). Different humulones were tentatively identified, including Cohumulone I and II and Iso-α-cohumulone (*m/z* 347), as well as Ad-humulone and n-humulone (*m/z* 341) (Hofte and Hoeven 1998; García-Villalba et al. 2006; Quifer-Rada et al. 2015). Other compounds found in both mass spectra of beer at low signal/noise can be associated to phenolic acids such as caffeic acids (*m/z* 179 and 341) and caffeoylquinic acids (*m/z* 353), as well as apigenins (*m/z* 431 and 593) which are commonly found in barley, a key ingredient in the beer brewing process (see **Supplemental Material Fig. S8**) (Frangne et al. 2002; Quinde-Axtell and Baik 2006; Quifer-Rada et al. 2015).

4. Conclusions

We have presented the first mass spectra acquired by SAWN-MS for different vinegars, wines, and beers. The mass spectra were acquired within minutes directly after a simple dilution in water, without the need for sample preparation steps such as centrifugation, extraction, or purification. The SAWN-MS method presented is ideal for spot-checking of samples during production, and can significantly reduce process analysis costs, as it requires no pumps, capillaries, lasers, or chemical enhancers. Compared to other ambient ionization methods such as ESI, SAWN-MS is energetically softer (Huang et al. 2012), leading to less fragmentation during ionization, and allowing more direct composition analysis. SAWN is also compatible with CID to allow structure analysis where needed for identification of target compounds (Yoon et al. 2012). The advantage of softer ionization is exemplified in the SAWN-MS spectra of beer in our study that provided more information than prior ESI reports on beer analysis. Specifically,

distinct oligomeric ions series and ratios of sample specific adducts (*e.g.*, sodium, potassium, chloride) can be used from SAWN spectra as direct indicators in targeted fingerprint analysis of food samples. Thus, we believe we have shown here that SAWN-MS can be a powerful tool to reduce quality control costs while helping to increase product quality, consistency, and authenticity.

5. Acknowledgements

The authors acknowledge the support of the NINN at the University of Washington for microfabrication of the SAWN chips.

6. Appendix A. Supplementary data

Supplemental data associated with this article can be found in the online version (ESI-CID compound assignments).

7. Compliance with Ethical Standards

Funding: This study was funded by the National Institute of Health (Grant 1R01 GM111066-01, D.R.G) and National Science Foundation (Grant 1330459; Deurion LLC). Parts of the study are based upon work supported by the National Science Foundation (Grant # IIP-1059286 to the American Society for Engineering Education). Additional thank is due to the University of Maryland School of Pharmacy Mass Spectrometry Center (SOP1841-IQB2014).

Conflict of Interest: D.R.G. has financial interests in Deurion LLC.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Not applicable.

8. References

Amorisco A, Locaputo V, Pastore C, Mascolo G (2012) Identification of low molecular weight organic acids by ion chromatography/hybrid quadrupole time-of-flight mass spectrometry during Uniblu-A ozonation. Rapid Commun Mass Spectrom 27:187–199. doi: 10.1002/rcm.6429

Araújo AS, da Rocha LL, Tomazela DM, et al (2005) Electrospray ionization mass spectrometry fingerprinting of beer. Analyst 130:884–889. doi: 10.1039/b415252b

Belitz HD, Grosch W, Schieberle P (2009) Food chemistry, 4th edn. Springer-Verlag, Berlin

Bravdo B, Hepner Y, Loinger C, et al (1985) Effect of Crop Level and Crop Load on Growth, Yield, Must and Wine Composition, and Quality of Cabernet Sauvignon. Am J Enol Vitic 36:125–131.

Cajka T, Riddellova K, Tomaniova M, Hajslova J (2011) Ambient mass spectrometry employing a DART ion source for metabolomic fingerprinting/profiling: A powerful tool for beer origin recognition. Metabolomics 7:500–508. doi: 10.1007/s11306-010-0266-z

Campbell CK (1989) Applications of surface acoustic and shallow bulk acoustic wave devices. Proc IEEE 77:1453–1484. doi: 10.1109/5.40664

Canterbury JD, Merrihew GE, MacCoss MJ, et al (2014) Comparison of data acquisition strategies on quadrupole ion trap instrumentation for shotgun proteomics. J Am Soc Mass Spectrom 25:2048–2059. doi: 10.1007/s13361-014-0981-1

Chinnici F, Guerrero ED, Sonni F, et al (2009) Gas chromatography-mass spectrometry (GC-

MS) characterization of volatile compounds in quality vinegars with protected European geographical indication. J Agric Food Chem 57:4784–4792. doi: 10.1021/jf804005w

Coats SR, Jones JW, Do CT, et al (2009) Human Toll-like receptor 4 responses to P. gingivalis are regulated by lipid A 1- and 4'-phosphatase activities. Cell Microbiol 11:1587–99. doi: 10.1111/j.1462-5822.2009.01349.x

Cody RB, Laramee J a, Durst HD, et al (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem 77:2297–2302. doi: 10.1021/ac050162j

Cohen SL, Chait BT (1996) Influence of matrix solution conditions on the MALDI-MS analysis of peptides and proteins. Anal Chem 68:31–7. doi: 10.1021/ac9507956

Costin JW, Barnett NW, Lewis SW (2004) Determination of proline in wine using flow injection analysis with tris(2,2-bipyridyl)ruthenium(II) chemiluminescence detection. Talanta 64:894–898. doi: 10.1016/j.talanta.2004.03.065

Dill AL, Eberlin LS, Ifa DR, Cooks RG (2011) Perspectives in imaging using mass spectrometry. Chem Commun (Camb) 47:2741–2746. doi: 10.1039/c0cc03518a

Duarte IF, Barros A, Almeida C, et al (2004) Multivariate Analysis of NMR and FTIR Data as a Potential Tool for the Quality Control of Beer. J Agric Food Chem 52:1031–1038. doi: 10.1021/jf030659z

Felipe D, Brambilla L, Porto C, et al (2014) Phytochemical Analysis of Pfaffia glomerata Inflorescences by LC-ESI-MS/MS. Molecules 19:15720–15734. doi: 10.3390/molecules191015720

Flamini R, Traldi P (2010) Mass Spectrometry in Grape and Wine Chemistry. John Wiley &

Sons, Inc.

Frangne N, Eggmann T, Koblischke C, et al (2002) Flavone glucoside uptake into barley mesophyll and Arabidopsis cell culture vacuoles. Energization occurs by H(+)-antiport and ATP-binding cassette-type mechanisms. Plant Physiol 128:726–733. doi: 10.1104/pp.010590

Garcia-Reyes JF, Jackson AU, Molina-Diaz A, Cooks RG (2009) Desorption Electrospray Ionization Mass Spectrometry for Trace Analysis of Agrochemicals in Food. Anal Chem 81:820–829. doi: 10.1021/ac802166v

García-Villalba R, Cortacero-Ramírez S, Segura-Carretero A, et al (2006) Analysis of Hop Acids and Their Oxidized Derivatives and Iso-α-acids in Beer by Capillary Electrophoresis−Electrospray Ionization Mass Spectrometry. J Agric Food Chem 54:5400–5409. doi: 10.1021/jf060207x

Heron SR, Wilson R, Shaffer SA, et al (2010) Surface acoustic wave nebulization of peptides as a microfluidic interface for mass spectrometry. Anal Chem 82:3985–3989. doi: 10.1021/ac100372c

Ho J, Tan MK, Go DB, et al (2011) Paper-based microfluidic surface acoustic wave sample delivery and ionization source for rapid and sensitive ambient mass spectrometry. Anal Chem 83:3260–3266. doi: 10.1021/ac200380q

Hofte AJP, Hoeven RAM Van Der (1998) Characterization of Hop Acids by Liquid Chromatography with Negative Electrospray Ionization Mass Spectrometry. J Am Soc Brew Chem 56:118–122. doi: 10.1094/ASBCJ-56-0118

Huang Y, Heron SR, Clark A, et al (2016) Standing Waves Improve Surface Acoustic Wave

Nebulization Performance. J Mass Spec 51:424–429. doi: 10.1002/jms.3766

Huang Y, Yoon SH, Heron SR, et al (2012) Surface acoustic wave nebulization produces ions with lower internal energy than electrospray ionization. J Am Soc Mass Spectrom 23:1062–1070. doi: 10.1007/s13361-012-0352-8

Konda C, Bendiak B, Xia Y (2012) Differentiation of the stereochemistry and anomeric configuration for 1-3 linked disaccharides via tandem mass spectrometry and 18O-labeling. J Am Soc Mass Spectrom 23:347–358. doi: 10.1007/s13361-011-0287-5

Lachenmeier DW (2007) Rapid quality control of spirit drinks and beer using multivariate data analysis of Fourier transform infrared spectra. Food Chem 101:825–832. doi: 10.1016/j.foodchem.2005.12.032

Länge K, Rapp BE, Rapp M (2008) Surface acoustic wave biosensors: A review. Anal Bioanal Chem 391:1509–1519. doi: 10.1007/s00216-008-1911-5

Lee S, Valentine SJ, Reilly JP, Clemmer DE (2012) Analyzing a mixture of disaccharides by IMS-VUVPD-MS. Int J Mass Spectrom 309:161–167. doi: 10.1016/j.ijms.2011.09.013

Liang T, Schneider T, Yoon SH, et al (2017) Optimized Surface Acoustic Wave Nebulization Facilitates Bacterial Phenotyping.

Nemes P, Vertes A (2007) Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. Anal Chem 79:8098–8106. doi: 10.1021/ac071181r

Nicholson JR (2017) 2015: What is Made in America.

Nordhoff E, Kirpekar F, Roepstorff P (1996) Mass spectrometry of nucleic acids. Mass Spectrom Rev 15:67–138. doi: 10.1002/(sici)1098-2787(1996)15:2<67::aid-mas1>3.0.co;2-8

Ough C (1968) Proline content of grapes and wines. Vitis 7:321–331.

Peppard TL (1985) The Application of Mass Spectrometry in Beer Flavour Studies. J Inst Brew 91:16–24. doi: 10.1002/j.2050-0416.1985.tb04299.x

Perez-Magarino S, Revilla I, Gonzalez-SanJose ML, Beltran S (1999) Various applications of liquid chromatography-mass spectrometry to the analysis of phenolic compounds. J Chromatogr A 847:75–81. doi: 10.1016/S0021-9673(99)00255-1

Phillips KM, Patterson KY, Rasor AS, et al (2006) Quality-control materials in the USDA National Food and Nutrient Analysis Program (NFNAP). Anal Bioanal Chem 384:1341–1355. doi: 10.1007/s00216-005-0294-0

Quifer-Rada P, Vallverdú-Queralt A, Martínez-Huélamo M, et al (2015) A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC-ESI-LTQ-Orbitrap-MS). Food Chem 169:336–43. doi: 10.1016/j.foodchem.2014.07.154

Quinde-Axtell Z, Baik BK (2006) Phenolic compounds of barley grain and their implication in food product discoloration. J Agric Food Chem 54:9978–9984. doi: 10.1021/jf060974w

Schäfer K-C, Dénes J, Albrecht K, et al (2009) In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. Angew Chemie - Int Ed 48:8240–8242. doi: 10.1002/anie.200902546

Tagashira M, Watanabe M, Uemitsu N (1995) Antioxidative Activity of Hop Bitter Acids and Their Analogs. Biosci Biotechnol Biochem 59:740–742. doi: 10.1271/bbb.59.740

Takats Z, Wiseman JM, Gologan B, Cooks RG (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science (80-) 306:471–473. doi: 10.1126/science.1104404

Tesfaye W, Morales ML, García-Parrilla MC, Troncoso AM (2002) Wine vinegar: Technology,

authenticity and quality evaluation. Trends Food Sci Technol 13:12–21. doi: 10.1016/S0924-2244(02)00023-7

Van Berkel GJ, Pasilis SP, Ovchinnikova O (2008) Established and emerging atmospheric pressure surface sampling/ionization techniques for mass spectrometry. J Mass Spectrom 43:1161–1180. doi: 10.1002/jms.1440

Wang H, Liu J, Cooks RG, Ouyang Z (2010) Paper spray for direct analysis of complex mixtures using mass spectrometry. Angew Chem Int Ed Engl 49:877–880. doi: 10.1002/anie.200906314

Yen GS, Edgar JS, Yoon SH, et al (2016) PDMS microchannels coupled to surface acoustic wave nebulization mass spectrometry. Rapid Commun Mass Spectrom 30:1096–100. doi: 10.1002/rcm.7531

Yeo LY, Friend JR (2014) Surface AcousticWave Microfluidics. Annu Rev Fluid Mech 46:379–406. doi: 10.1146/annurev-fluid-010313-141418

Yoon SH, Huang Y, Edgar JS, et al (2012) Surface acoustic wave nebulization facilitating lipid mass spectrometric analysis. Anal Chem 84:6530–6537. doi: 10.1021/ac300807p

Yoon SH, Liang T, Schneider T, et al (2016) Rapid Lipid A Structure Determination via Surface Acoustic Wave Nebulization and Hierarchical Tandem Mass Spectrometry Algorithm. Rapid Commun Mass Spectrom 30:2555–2560.

Zhang J, Rector J, Lin JQ, et al (2017) Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. Sci Transl Med 9:eaan3968. doi: 10.1126/scitranslmed.aan3968

9. Figure Captions

Figure 1. a) SAWN Chip setup coupled to the inlet of a Waters Synapt G2-S. b) Sketch of SAWN principle. Counter-propagating SAW's generated by interdigital transducers (IDTs) on a piezoelectric material induce strong acoustic streaming and recirculation in the sample droplet which leads to its vertical nebulization.

Figure 2. Comparison of three different vinegars based on their mass spectra. Shown are the SAWN mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Heinz White Vinegar (a), Rice Vinegar (b), and Balsamic Vinegar (c).

Figure 3. Comparison of red and white wine based on their mass spectra. Shown are the SAWN mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Tapena Red Wine (a) and Le Rime White Wine (b).

Figure 4. Comparison of beer SAWN mass spectra. Shown are the mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Goose IPA (a) and Bud Light (b).

Supplemental Information:

Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry

Thomas Schneider[a]*, Benjamin L. Oyler[b], Sung Hwan Yoon[c], Tao Liang[a], Gloria S. Yen [d], David. P. A. Kilgour[e], Erik Nilsson[d], David R. Goodlett [a,d]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA

[b] Department of Toxicology, School of Medicine, University of Maryland, Baltimore, MD, 21201, USA

[c] Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, MD, 21201, USA

[d] Deurion LLC, Seattle, WA 98103, USA.

[e] Chemistry and Forensics, School of Science & Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK

Contact Information:

*Corresponding Author: T. Schneider (tschneid@uw.edu)

1. Materials and Methods

SAWN Chip Fabrication

The Interdigital transducer (ITD) design was created by computer aided design software (AutoCAD) and subsequently converted to a chrome mask (Heidelberg μPG 101 Laser Pattern Generator; Heidelberg Instruments Mikrotechnik GmbH, Heidelberg, Germany) at the University of Washington Nanotech User Facility. Lithium Niobate wafers ($LiNbO_3$ 128 Y-cut, X-propagating, 3-inch; Crystal Technology, Inc., Palo Alto, CA, USA) were coated ~1 μm positive photoresist (AZ 1512; AZ Electronic Materials, Somerville, NJ, USA), followed by exposure (Oriel mask aligner; Newport Corporation, Irvine, CA, USA) to create a sacrificial layer containing the SAW transducer. The SAW chip design used in this study consisted of two IDT pairs of electrodes to create counter-propagating SAW's. Each IDT pair consisted of 20 electrode pairs 100 μm wide, spaced 100 μm apart, and with a 5 mm aperture. The delay region between the two IDT pairs was 8.1 mm. The operating frequency of the transducer used in this study was 9.56MHz. The IDT microelectrodes were created through heated vapor deposition of a 20 nm chrome adhesion layer followed by a 60 nm layer of gold. Subsequently, the photoresist was removed through acetone rinse leaving behind the patterned electrodes on the $LiNbO_3$ wafer.

2. Supplemental Figures

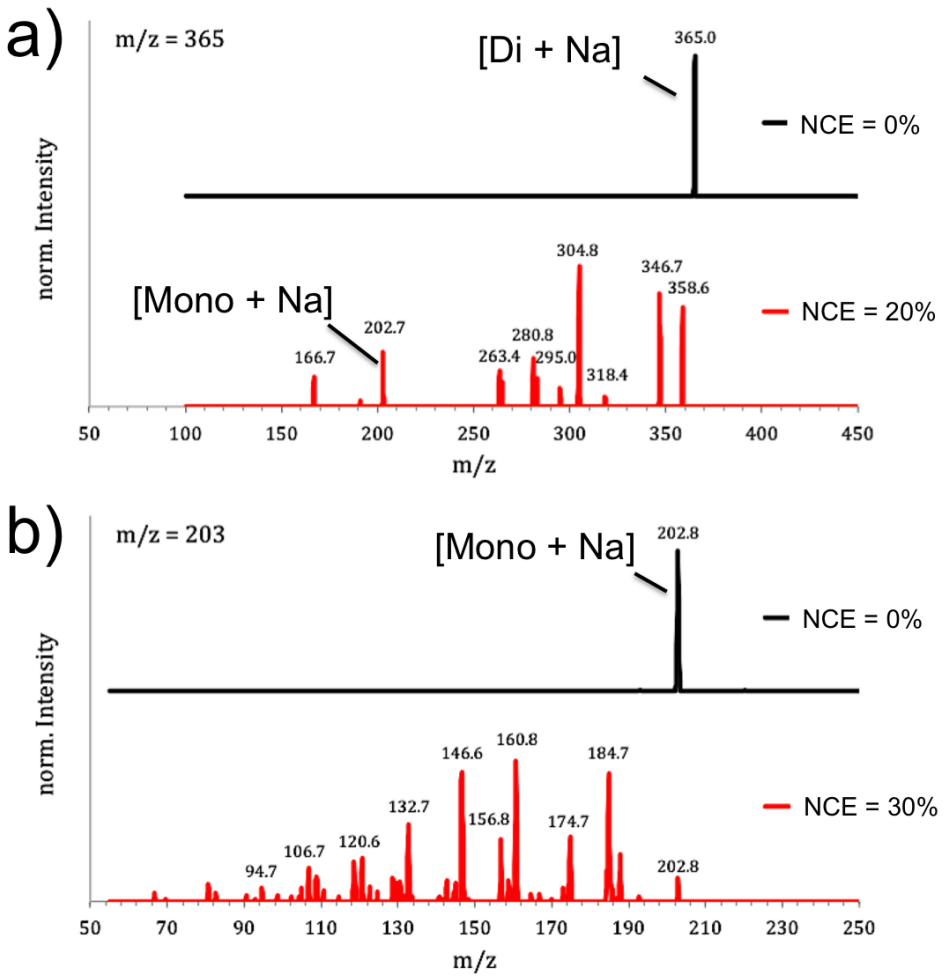


Figure S1. Fragmentation of sodium adducts of disaccharides found at *m/z* 365 (a) and glucose/fructose at *m/z* 203 (b) in samples from rice vinegar acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel. Shown are the precursor ions (MS^2 at NCE of 0 %, black spectra) and fragment ions (MS^2 at NCE of 20 % in (a) and 30 % in (b), red spectra). Note that the differences in the collision energy used in this experiment are due to differences in energy required to fragment mono- and disaccharides.

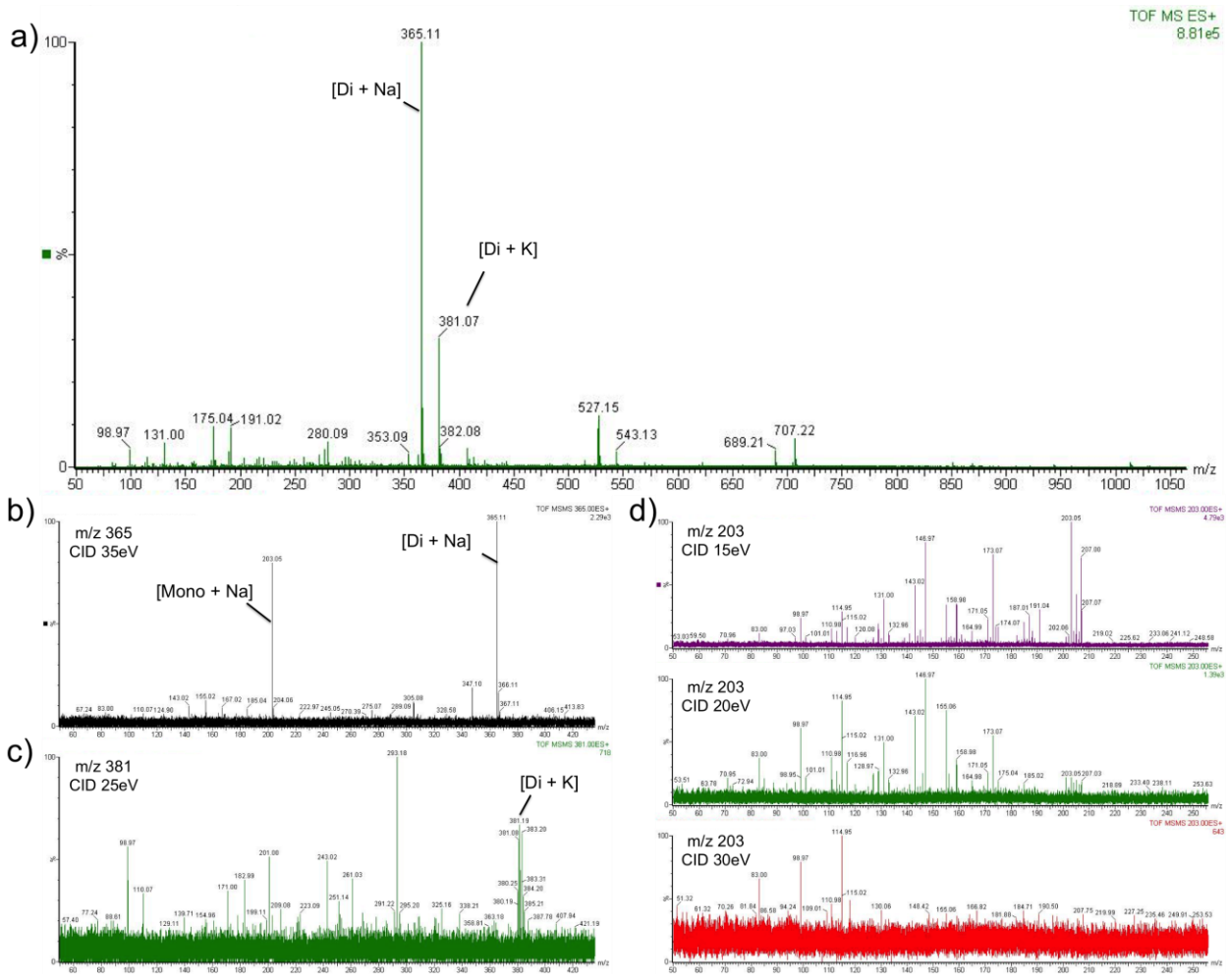


Figure S2. MS spectra from direct infusion in positive ion mode of rice vinegar acquired by ESI on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation). Shown are the MS1 spectrum (a) and the fragmentation spectra (MS2) of sodium and potassium adducts of disaccharides found at *m/z* 365 (b) and *m/z* 381 (c), and glucose/fructose at *m/z* 203 (d). The fragmentation spectra were acquired with different collision-induced dissociation (CID) energies to show the fragment ions from the different precursor ions. The masses measured were: $[C_{12}H_{22}O_{11}Na]^+$, measured: 365.1051, exact: 365.1060, error: 2.5 ppm; $[C_{12}H_{22}O_{11}K]^+$, measured: 381.0741, exact: 381.0799, error: 15.2 ppm.

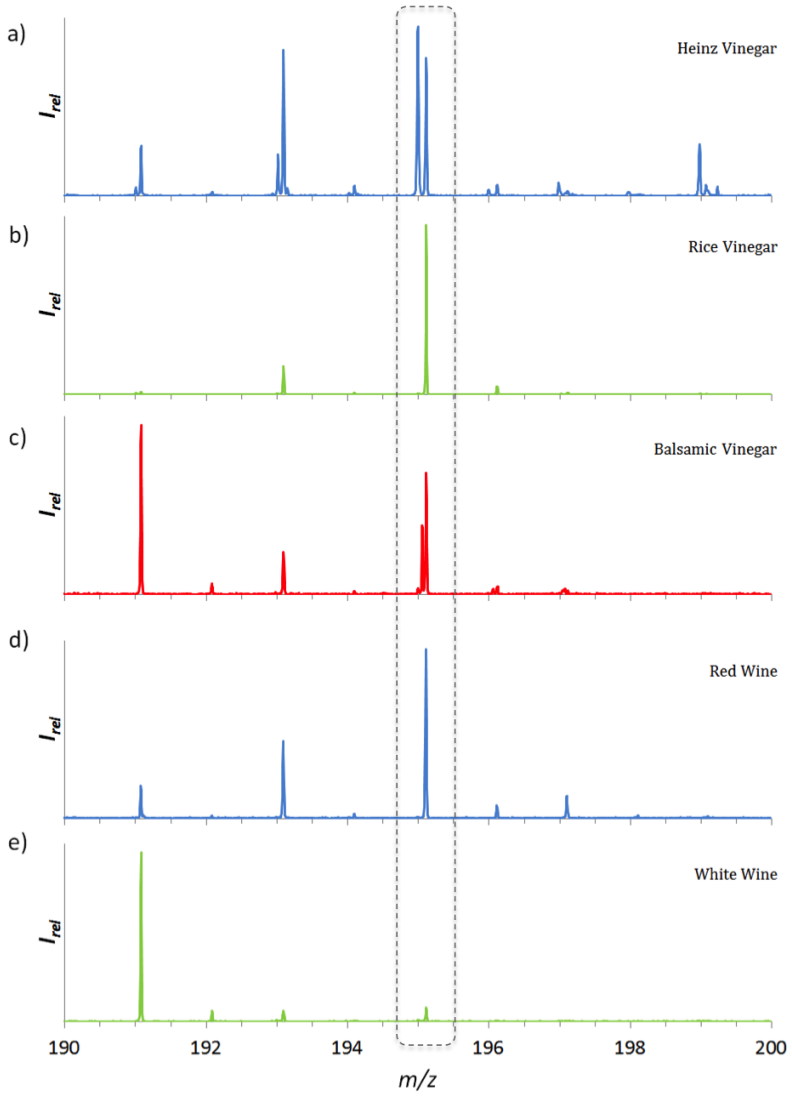


Figure S3. Comparison of changes in gluconate (*m/z* 195) in the negative ion mass spectra of different vinegars and wines acquired by SAWN MS.

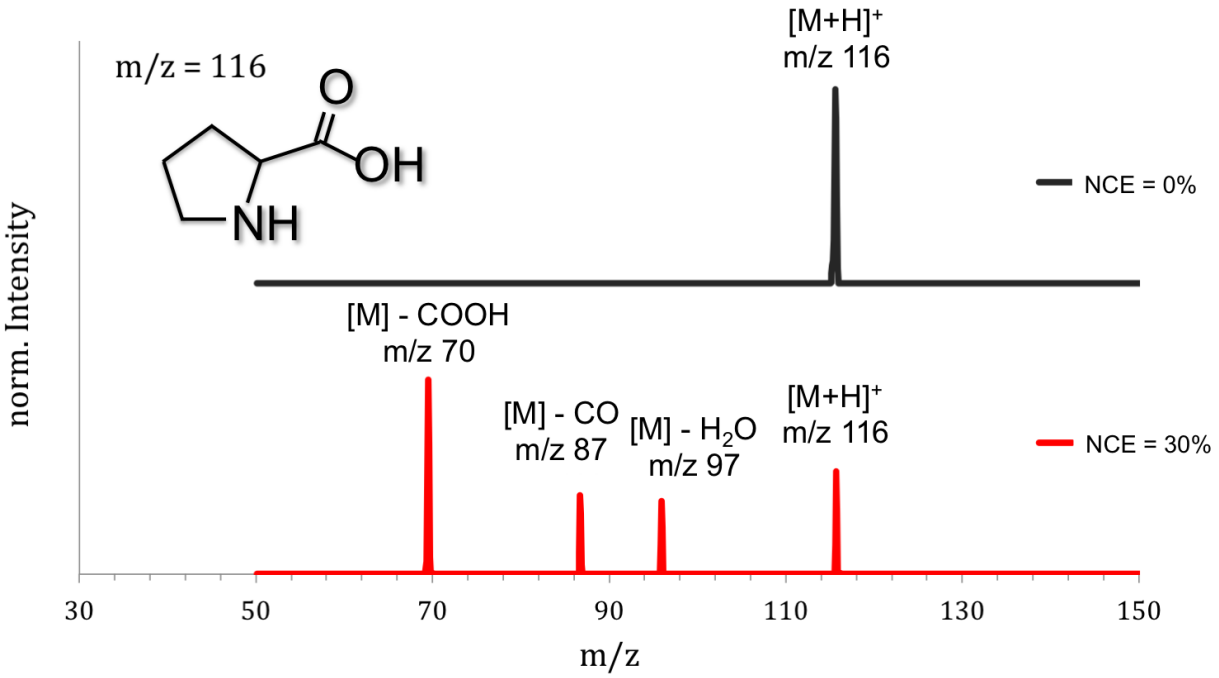


Figure S4. Fragmentation of proline found in samples from balsamic vinegar acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel. Shown are the precursor ions (MS^2 at NCE = 0 %, black spectrum) and fragment ions (MS^2 at NCE = 30 %, red spectrum).

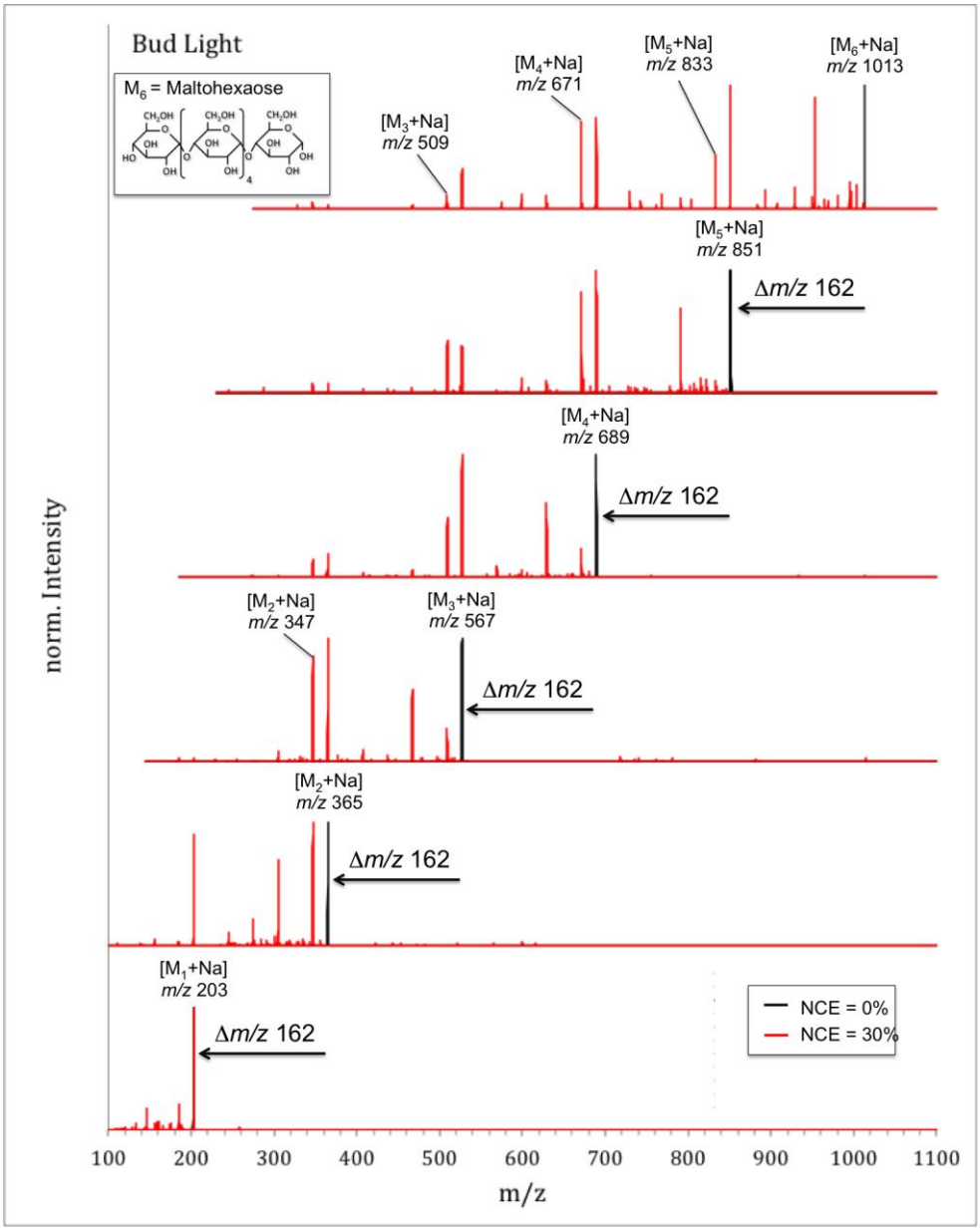


Figure S5. Fragmentation of oligosaccharides in samples from Bud Light acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel. Shown are the precursor ions (MS^2 at NCE = 0 %, black spectra) and fragment ions (MS^2 at NCE = 30 %, red spectra).

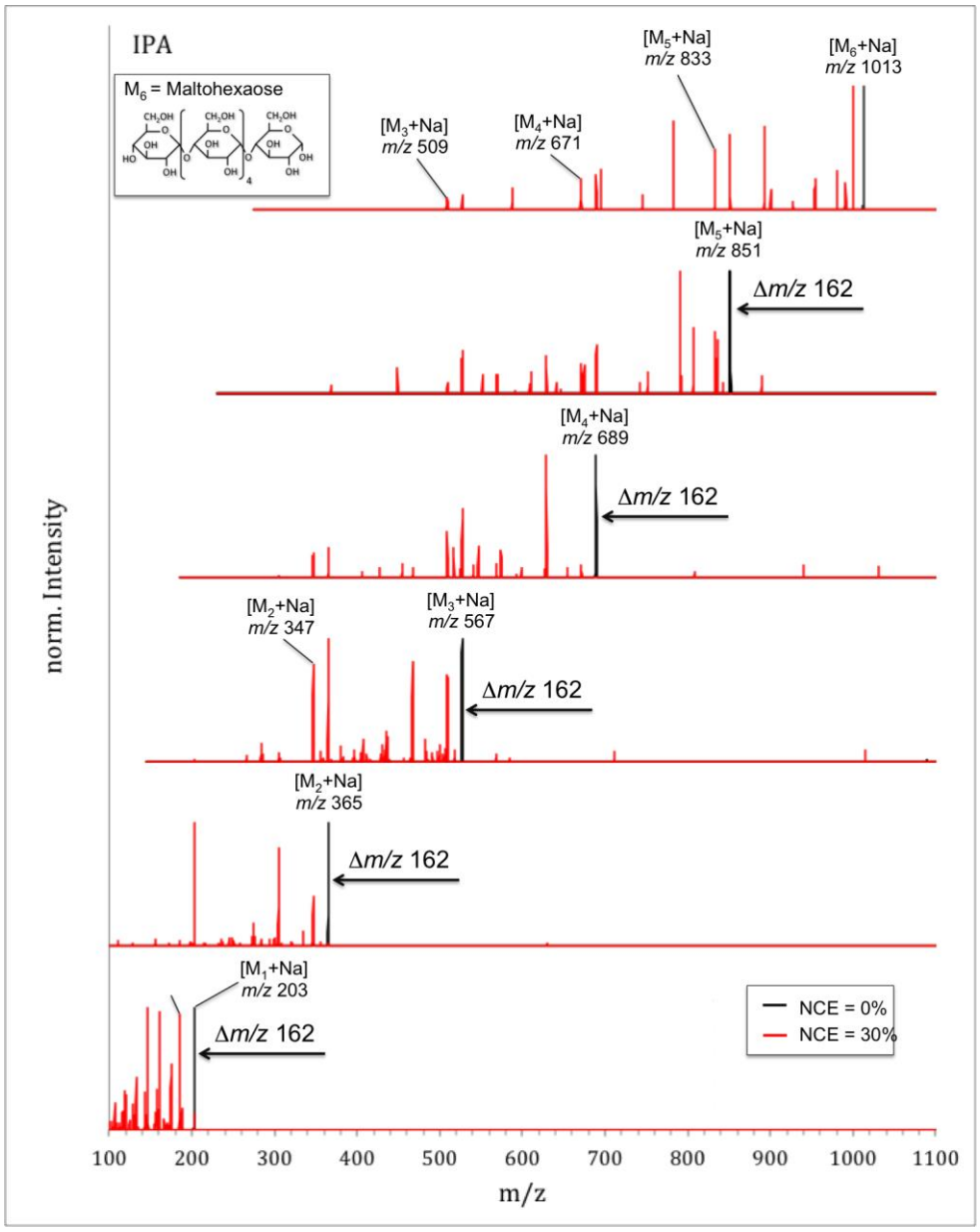


Figure S6. Fragmentation of oligosaccharides in samples from IPA acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel. Shown are the precursor ions (MS^2 at CE 0, black spectra) and fragment ions (MS^2 at CE 30, red spectra).

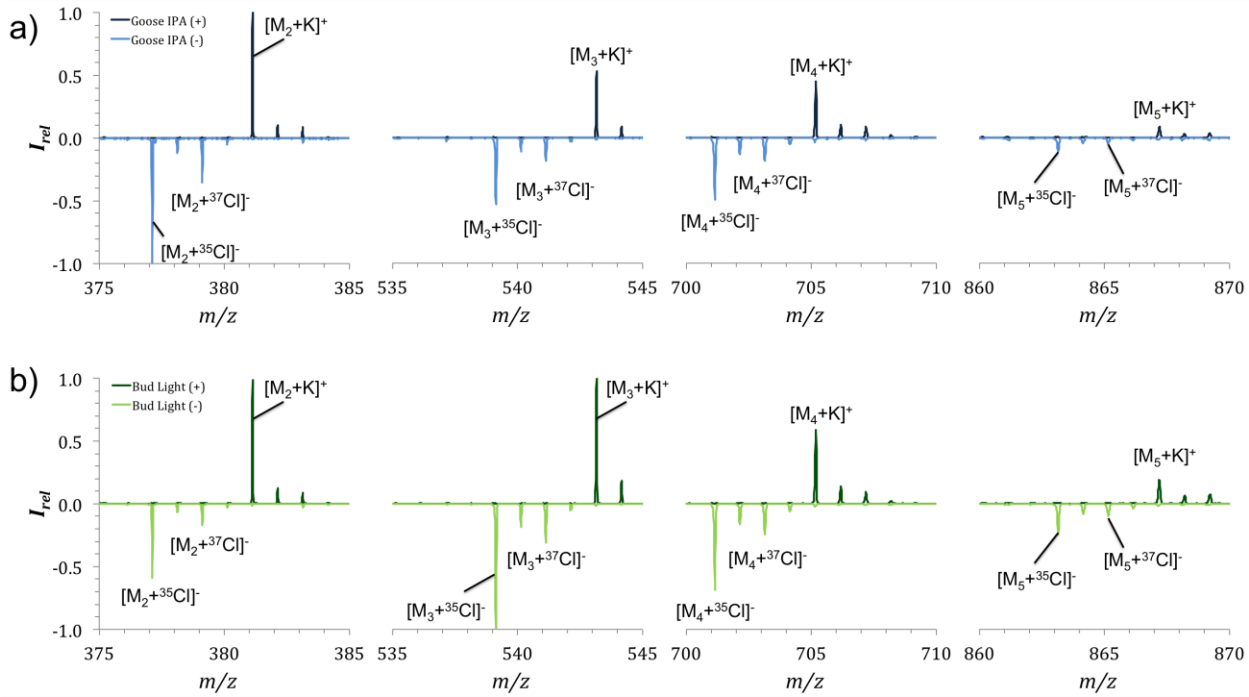


Figure S7. Natural isotope distribution pattern of chlorine in the oligosaccharide adducts of beer samples (Goose IPA (a) and Bud Light (b)) analyzed by SAWN-MS in negative ion mode (bottom graphs, light colors). M_2 = maltose, M_3 = maltotriose, M_4 = maltotetraose, and M_5 = maltopentaose.

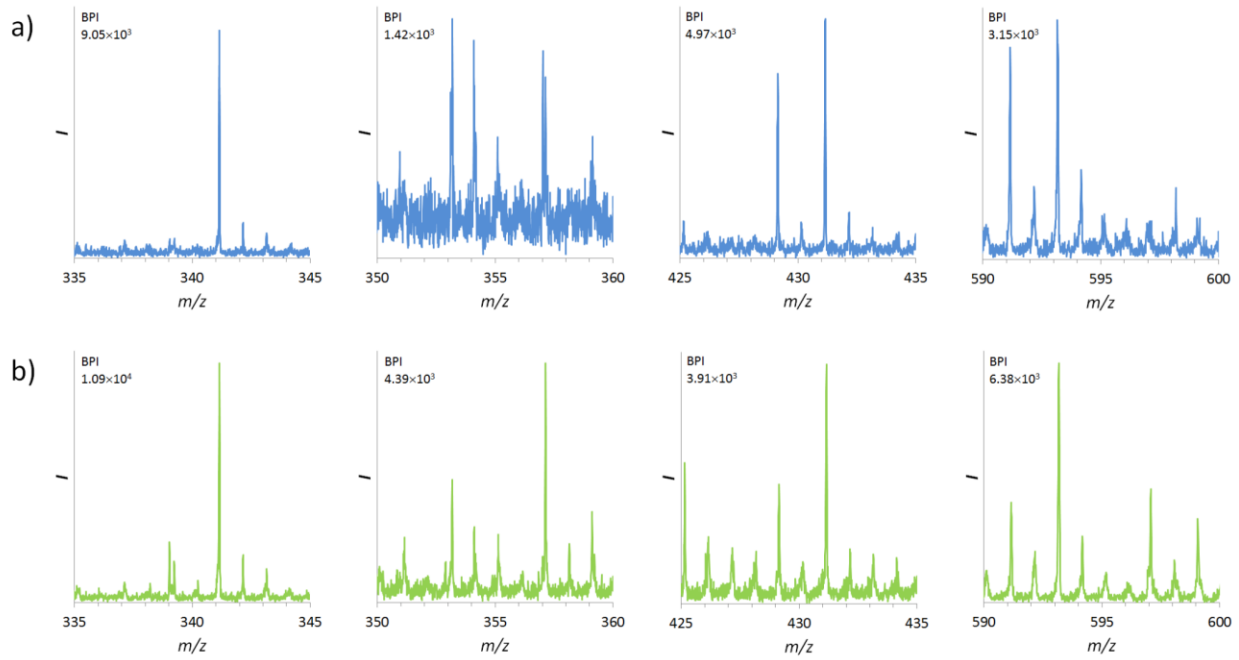


Figure S8. Expanded negative ion mass spectra for low S/N species in samples of Goose IPA (a) and Bud Light (b) acquired by SAWN MS.

Food Analytical Methods

Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry

Thomas Schneider[a]*, Benjamin L. Oyler[b], Sung Hwan Yoon[c], Tao Liang[a], Gloria S. Yen [d], David P. A. Kilgour[e], Erik Nilsson[d], David R. Goodlett [a,d]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA

[b] Department of Toxicology, School of Medicine, University of Maryland, Baltimore, MD, 21201, USA

[c] Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, MD, 21201, USA

[d] Deurion LLC, Seattle, WA 98103, USA.

[e] Chemistry and Forensics, School of Science & Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK

Contact Information:

*Corresponding Author: T. Schneider (tschneid@uw.edu)

Abstract

Rapid food product analysis is of great interest for quality control and assurance during the production process. Conventional quality control protocols require time and labor intensive sample preparation for analysis by state-of-the-art analytical methods. To reduce overall cost and facilitate rapid qualitative assessments, food products need to be tested with minimal sample preparation. We present a novel and simple method for assessing food product compositions by mass spectrometry using a novel surface acoustic wave nebulization method. This method provides significant advantages over conventional methods requiring no pumps, capillaries, or additional chemicals to enhance ionization for mass spectrometric analysis. In addition, the surface acoustic wave nebulization – mass spectrometry method is ideal for rapid analysis and to investigate certain compounds by using the mass spectra as a type of species-specific fingerprint analysis. We present for the first time surface acoustic wave nebulization generated mass spectra of a variety of fermented food products from a small selection of vinegars, wines, and beers.

Keywords

Mass Spectrometry; Surface Acoustic Wave Nebulization; Vinegar, Wine, Beer - Analysis

1. Introduction

Food production and manufacturing has the largest share in gross manufacturing output in the U.S., reaching 957 billion US$ in 2015 (Nicholson 2017). Consumers expect quality and consistency from food products and manufacturers use quality and consistency as competitive tools to gain and maintain market share. This is especially true in the beverage and condiment industries, where quality control is a significant production cost. In addition, product authentication is important to both producers and customers (Tesfaye et al. 2002).

Traditional methods for analysis and quality control in the spirit and beverage industry include liquid and gas chromatography (LC and GC), photometry, and enzymatic analysis (Phillips et al. 2006). To reduce quality control costs, new methods have been proposed that require less sample preparation and time to obtain qualitative and quantitative results. These methods include the use of principle component analysis combined with high-resolution nuclear magnetic resonance (NMR) or Fourier-transform infrared (FT-IR) spectroscopy (Duarte et al. 2004; Lachenmeier 2007).

Another technique that can reduce analysis cost and time while increasing accuracy, sensitivity, and the number of analytes per measurement is mass spectrometry (MS). Conventional atmospheric pressure ionization MS relies mostly on two methods to introduce a sample into the mass spectrometer for analysis, namely electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) (Nordhoff et al. 1996). While ESI and MALDI are extremely useful techniques for gas-phase ion generation, the use of these specific sample transfer methods can limit the type of sample that can be analyzed and impose challenges such as capillary clogging and matrix interference of the target analyte's signal (Cohen and Chait 1996),

sometimes requiring dedication of considerable time and effort to troubleshooting and method development. MS has been applied together with high resolution chromatography in the study of beer flavors in the 1980's (Peppard 1985), while beer phenols have recently been reported for the first time in a detailed study by high resolution MS (Quifer-Rada et al. 2015).

The advancements in novel ambient ionization methods over the past ten years, including desorption electrospray ionization (DESI) (Takats et al. 2004), direct analysis in real time (DART) (Cody et al. 2005), laser ablation electrospray ionization (LAESI) (Nemes and Vertes 2007), rapid evaporative ionization mass spectrometry (REIMS) (Schäfer et al. 2009), paper spray ionization (Wang et al. 2010), and others have greatly expanded MS to new applications at ambient conditions, including the analysis of varying surfaces and living tissues under real life conditions (Dill et al. 2011; Zhang et al. 2017). Several variations of these methods have been developed since then, but few of these techniques have found applications in the food and beverage industry. Notably, DESI has been applied to fruit peels and food stuff extracts to trace agrochemicals (Garcia-Reyes et al. 2009). DART has been used for metabolomic profiling of different beer samples in a study aimed at the development of cost-effective methods to help authenticate food products based on the origin of their ingredients (Cajka et al. 2011).

All of the novel ~~ambient~~ ionizations methods listed here that operate at ambient pressures are widely used in research settings. However, these methods generally require some type of support medium (*e.g.*, carrier gas, ESI) or thermal heating / ionization through a hot electrode, a corona needle, or a laser optical setup in order to achieve sample transfer to the MS (Van Berkel et al. 2008). Therefore, further simplifying and improving the sample transfer into the mass spectrometer will greatly enhance the capability of MS as a low cost and powerful analytical method for the food and beverage industry. In order to address challenges in the sample transfer

methods, Goodlett and colleagues recently introduced a novel ambient sample transfer method called surface acoustic wave nebulization (SAWN) (Heron et al. 2010). SAWN is expected to help addressing the challenges complex samples face in MS analyses.

Evolved from the telecommunication and semiconductor industry (Campbell 1989), surface acoustic waves (SAWs) have been applied to a variety of applications including surface patterning, fluidic mixing, sample transport and focusing, and jetting and nebulization (Länge et al. 2008; Yeo and Friend 2014). SAWN takes advantage of the SAW effect that is induced in piezoelectric materials by metallic electrodes (interdigital transducers, IDTs; **Fig. 1**) at high frequencies to create a fine plume of droplets (Heron et al. 2010; Ho et al. 2011; Huang et al. 2012; Yen et al. 2016). These droplets are readily introduced into the vacuum interface region of a mass spectrometer, further desolvated, and subsequently analyzed. Recently, we showed its application as a versatile tool for the fast analysis of hydrophobic lipid A (Yoon et al. 2012, 2016; Liang et al. 2017), a major component of the outer membrane of Gram-negative bacteria, which is recognized by the host immune system as an endotoxin (Coats et al. 2009). We have further developed the SAWN technology using a standing wave configuration to achieve higher nebulization efficiency (Huang et al. 2016; Liang et al. 2017).

Here we present for the first time the rapid analysis of food products by SAWN-MS that include a selection of fermented food products such as vinegars, wines, and beers. While standard methods for quality control and targeted analysis exist based on GC-MS and LC-MS (Flamini and Traldi 2010), such methods require significantly more time than SAWN-MS for rapid spot-checking analysis of sample quality during the beverage and condiment manufacturing process. SAWN-MS holds the unique advantage of being an ambient nebulization method, which can be conducted with complex samples. Conventional sample transfer methods

such as ESI and nanospray ionization (NSI) require careful consideration of the solvent/sample composition to avoid potential interactions that can lead to clogging of capillary tips, which increases the time required for analysis. SAWN-MS relies on a planar chip surface that is used to nebulize the sample and is therefore not as restricted in its use as ESI, and unlike ESI no voltage is applied directly to the sample making SAWN inherently less likely to break labile bonds during ionization (Huang et al. 2012). This is especially useful when analyzing complexes held together by electrostatic forces (e.g. ion clusters) and/or compounds with very labile functional groups (e.g., nucleotides or phospholipids) (Yoon et al. 2012).

2. Materials and Methods

2.1 Food Samples

Different fermented food products were used as received and diluted up to 100-fold in ultra-purified water (18.2 MΩ; MilliQ, Millipore, Milford, MA, USA) immediately before SAWN-MS analysis. The food products included vinegars, wines, and beer. Vinegars were purchased in local grocery stores: Heinz Distilled White Vinegar (H.J. Heinz Company, L.P, King of Prussia, PA, USA), Rice Vinegar (Kikkoman Sales USA, Inc., San Francisco, CA, USA), and Aged Balsamic Vinegar (Pepper Palace, Sevierville, TN, USA). Wines and beer were sourced from local restaurants: Tapena Red Wine (Freixenet USA, Sonoma, CA, USA), Le Rime Pinot Grigio (Banfi srl, Siena, IT), Goose IPA (Goose Island Beer Company, Chicago, IL, USA), and Bud Light (Anheuser-Busch, St. Louis, MO, USA).

2.2 Surface Acoustic Wave Nebulization – Mass Spectrometry

The Standing Wave (SW) SAWN chips used in our current study were manufactured at the Washington Nanofabrication Facility (University of Washington, Seattle, WA, USA) following an established procedure (Huang et al. 2016; Liang et al. 2017). A detailed summary of the chip fabrication is provided in the **Supplemental Material.** The SW-SAWN chips were placed in a custom chip holder designed and built using 3D Computer Aided Design Software (123D Design, Autodesk, San Rafael, CA, USA) and a Simple Metal 3D printer (Printrbot, Lincoln, CA, USA). A custom designed PCB board was used to connect the chip through RF cables to the SAWN Controller v1.0 (Deurion LLC, Seattle, WA, USA), which provided control over power and duration of the SAWN (**Fig. 1**). All experiments in the present study were conducted at a SAW frequency of 9.56 MHz and a power output of 11 W.

The food samples were analyzed on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation, Milford, MA, USA; **Fig. 1a**) in sensitivity mode, with positive and negative ion mode acquisition. The source block temperature was set to 150°C. Sample aliquots of 1 µL were pipetted directly into the delay region of the SAWN chip (i.e., in between the IDT's, see **Fig. 1b**) in a discontinuous fashion and the data from five aliquots were averaged to enhance signal-to-noise ratio. Typical SAWN experiments last 2-3 s.

Assignment and verification of selected compounds was conducted with ESI by collision-induced dissociation (CID) on a Finnigan LTQ (Thermo Scientific, San Jose, CA, USA) retrofitted with a bespoke ion funnel (Canterbury et al. 2014) and on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation), ~~due to~~chosen based on instrument availability at the time of analysis. The same samples used for SAWN-MS were used for compound assignment. The spectral data were acquired over 1 min at different normalized collision energies (NCE = 0 – 30 %).

3. Results and Discussion

The advantages of the SAWN-MS analysis are its flexibility and speed. This technique requires minimal sample pre-processing (*e.g.*, dilution in water or organic solvent prior spotting) and is not limited by the challenges of conventional sample transfer methods for ESI/NSI and MALDI-MS (*e.g.*, clogging of capillaries, the requirement for syringe pumps, laser-optical setups, the use of chemical additives for sample ionization, and matrix interferences at low *m/z*). In the present work, we focused our investigation on the potential of the SAWN-MS method for rapid analysis of complex food products from the beverage and condiment industry. Some of the fermented food products we have investigated here have been studied in detail before by conventional LC-MS and tandem MS methods. The focus of these studies was often on the identification of specific individual compounds found in vinegars, wines, or beer based on their ion fragments in tandem MS experiments (Araújo et al. 2005; Chinnici et al. 2009). We confirmed key compounds found in our spectra by ESI-CID experiments (see **Supplemental Material** ~~Fig~~ Fig. ~~xxx~~S1, S2, S4-S6), while the assignment of other compounds relied on prior assignments published in the literature.

Vinegars are produced through bacterial fermentation of ethanol and contain mainly acetic acid and water. Vinegars are important ingredients for cooking and as preservatives of food through pickling processes. While acetic acid produces ions below 100 *m/z* that are a general indicator of the presence of vinegar in a sample, it was not of interest for our investigation. Instead, we focused our study on a larger *m/z* range to investigate the difference between low cost distilled white vinegar (made from corn) and more aromatic rice vinegar (made

from rice) and balsamic vinegars (made from concentrated grape juice and must or wine vinegar with the addition of caramel and other additives, respectively). The full mass spectra for these three different vinegar styles are shown in **Fig. 2**.

In the positive ion mode mass spectrum of distilled white vinegar we found repeating ions, spaced equally apart by Δ*m/z* 216, indicating the presence of oligomers with sodium adducts $[M+Na]^+$ (*i.e.*, *m/z* 455, 671, and 887) and potassium adducts $[M+K]^+$ (*i.e.*, *m/z* 471, 687, and 903; **Fig. 2a**). Similarly spaced ions are present in the mass spectra of rice and balsamic vinegar (**Fig. 2a-c**). All vinegars showed ions at *m/z* 203 and 219 which are sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of glucose or fructose (Konda et al. 2012; Lee et al. 2012). Other ions that were visible in all vinegars and wines were identified as sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of disaccharides through tandem MS (*i.e.*, ions at *m/z* 365 and 381, compare **Fig. 2** and **3, see Supplemental Material Fig. S1 & S2**).

The negative ion mode mass spectra of vinegars showed a variety of sample-specific ions, especially at low *m/z*, which can be associated with organic acids. A prominent ion at *m/z* 195 $[M-H]^-$ was found in all vinegars (**Fig. 2**) as well as in red wine (**Fig. 3a**), and has been previously associated with gluconic acid, which occurs naturally in fruits, honey, and wine (Felipe et al. 2014). The differences in intensity of gluconic acid can be used as a strong indicator of the type of sample that is being analyzed as it varies strongly between vinegars and wines (see **Supplemental Material Fig. S3**). Several organic acids known to be present in grape-based food products may be associated with the ions shown in the negative ion mode mass spectra of vinegars and wines. For example, an ion found at *m/z* 115 could be associated with fumaric acid $[M-H]^-$ and is present in balsamic vinegar (**Fig. 2c**). Fumaric acid, a common food additive, is used as an acidity regulator and is often used to replace tartaric acid, another food

Formatted: Font: Bold

Formatted: Font: Bold

additive used as acidity regulator, antioxidant, and a primary component of wine grapes and fermented wines (Bravdo et al. 1985). Tartrate can be associated with *m/z* 149 (Amorisco et al. 2012) and is dominant in balsamic vinegar as well as both wines tested, and has low abundance in white vinegar (**Fig. 2**~~**, Fig.**~~ and **3**). A hydroxycinnamic acid, caffeic acid, is known to be present in red wines and to produce an $[M-H]^-$ ion at *m/z* 179 (Perez-Magarino et al. 1999). We found these ions in the mass spectra of balsamic vinegar, as well as in the mass spectra of the other two vinegars tested, albeit at lower relative abundances.

Formatted: Font: Bold

Formatted: Font: Bold

The mass spectra of balsamic vinegar showed a complex number of potential compounds dominant at low *m/z*, particularly in negative ion mode acquisition (**Fig. 2c**). The mass spectra of the wines were less complex in comparison to balsamic vinegar. The dominant species found in the positive ion mode mass spectra of wines are sodium and potassium adducts of glucose or fructose at *m/z* 203 and 219. The main difference in the mono- and disaccharide mass spectral peaks between vinegars and wines are their relative intensities and intensity ratios (**Fig. 2** and **3**). This difference in relative intensity is ideal for mass spectral fingerprint analyses to help distinguish different samples.

At low *m/z* in the positive ion mode mass spectra, an ion at *m/z* 116 was present dominantly in both wines, as well as the balsamic vinegar, but absent in rice vinegar and distilled vinegar (**Fig. 3**). The compound was assigned to the amino acid proline (based on CID, see **Supplemental Material Fig. S4**), which is abundant in grape berries and commonly found in wines even after fermentation (Ough 1968; Costin et al. 2004).

In contrast to the mass spectra acquired from vinegars and wines are those from the two beers tested in our study. The beer mass spectra showed several ions in both positive and

negative ion acquisition mode that are equally spaced by Δ*m/z* 162, indicating the presence of di- and oligosaccharides. The ions in the positive acquisition mode mass spectrum of Bud Light and Goose IPA indicate the presence of O-linked saccharides (**Fig. 4**). These di- and oligosaccharides are the sodium adducts $[M+Na]^+$ and potassium adducts $[M+K]^+$ of maltose, maltotriose, and maltotetraose at *m/z* of 365 and 381, *m/z* of 527 and 543, and *m/z* of 689 and 705, respectively (**Fig. 4**) (Araújo et al. 2005; Belitz et al. 2009). Larger oligosaccharides are present in our SAWN-mass spectra that were confirmed by tandem MS (see **Supplemental Material Fig. S5 and S6**). Similarly spaced peaks (Δ*m/z* 162) were found in mass spectra for both beers acquired in negative ion mode, indicating the presence of chloride adducts $[M+Cl]^-$ for the same saccharides at *m/z* 377, 539, and 701, respectively. These ions showed the natural isotope distribution pattern of chlorine (^{35}Cl and ^{37}Cl; **see Supplemental Material Fig. S7**). Sodium and potassium adducts of glucose (*m/z* 203 and 219) were present in relative low abundance in both beers. Compared to recent fingerprinting studies of beer with ESI (Araújo et al. 2005), our SAWN-MS method was able to show sodium and potassium adducts in the positive ion acquisition mode of additional oligosaccharides in both beers tested, including maltopentaose (*m/z* 851 and 867), maltohexaose (*m/z* 1013 and 1029), and maltoheptaose (*m/z* 1175 and 1191). Similarly, chloride adducts of the oligosaccharides were found in the negative ion mode acquired mass spectra (**Fig. 4**). The main differences between the two beers investigated here was in the intensity ratios of the sodium and potassium adducts, which are ideal for distinguishing different types of beer.

In negative ion mode we also found several compounds that can be tentatively associated with phenolic acids (Quifer-Rada et al. 2015). Among the compounds found in both beer samples are humulones, a resin component of mature hops, and a key ingredient in the brewing

process which gives beer a bitter taste and has known bioactivity (Tagashira et al. 1995). Different humulones were tentatively identified, including Cohumulone I and II and Iso-α-cohumulone (*m/z* 347), as well as Ad-humulone and n-humulone (*m/z* 341) (Hofte and Hoeven 1998; García-Villalba et al. 2006; Quifer-Rada et al. 2015). Other compounds found in both mass spectra of beer at low signal/noise can be associated to phenolic acids such as caffeic acids (*m/z* 179 and 341) and caffeoylquinic acids (*m/z* 353), as well as apigenins (*m/z* 431 and 593) which are commonly found in barley, a key ingredient in the beer brewing process (see **Supplemental Material Fig. S8**) (Frangne et al. 2002; Quinde-Axtell and Baik 2006; Quifer-Rada et al. 2015).

4. Conclusions

We have presented the first mass spectra acquired by SAWN-MS for different vinegars, wines, and beers. The mass spectra were acquired within minutes directly after a simple dilution in water, without the need for sample preparation steps such as centrifugation, extraction, or purification. The SAWN-MS method presented is ideal for spot-checking of samples during production, and can significantly reduce process analysis costs, as it requires no pumps, capillaries, lasers, or chemical enhancers. Compared to other ambient ionization methods such as ESI, SAWN-MS is energetically softer (Huang et al. 2012), leading to less fragmentation during ionization, and allowing more direct composition analysis. SAWN is also compatible with CID to allow structure analysis where needed for identification of target compounds (Yoon et al. 2012). The advantage of softer ionization is exemplified in the SAWN-MS spectra of beer in our study that provided more information than prior ESI reports on beer analysis. Specifically,

distinct oligomeric ions series and ratios of sample specific adducts (*e.g.*, sodium, potassium, chloride) can be used from SAWN spectra as direct indicators in targeted fingerprint analysis of food samples. Thus, we believe we have shown here that SAWN-MS can be a powerful tool to reduce quality control costs while helping to increase product quality, consistency, and authenticity.

5. Acknowledgements

The authors acknowledge the support of the NINN at the University of Washington for microfabrication of the SAWN chips.

6. Appendix A. Supplementary data

Supplemental data associated with this article can be found in the online version (ESI-CID compound assignments).

7. Compliance with Ethical Standards

Funding: This study was funded by the National Institute of Health (Grant 1R01 GM111066-01, D.R.G) and National Science Foundation (Grant 1330459; Deurion LLC). Parts of the study are based upon work supported by the National Science Foundation (Grant # IIP-1059286 to the American Society for Engineering Education). Additional thank is due to the University of Maryland School of Pharmacy Mass Spectrometry Center (SOP1841-IQB2014).

Conflict of Interest: D.R.G. has financial interests in Deurion LLC.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Not applicable.

8. References

Amorisco A, Locaputo V, Pastore C, Mascolo G (2012) Identification of low molecular weight organic acids by ion chromatography/hybrid quadrupole time-of-flight mass spectrometry during Uniblu-A ozonation. Rapid Commun Mass Spectrom 27:187–199. doi: 10.1002/rcm.6429

Araújo AS, da Rocha LL, Tomazela DM, et al (2005) Electrospray ionization mass spectrometry fingerprinting of beer. Analyst 130:884–889. doi: 10.1039/b415252b

Belitz HD, Grosch W, Schieberle P (2009) Food chemistry, 4th edn. Springer-Verlag, Berlin

Bravdo B, Hepner Y, Loinger C, et al (1985) Effect of Crop Level and Crop Load on Growth, Yield, Must and Wine Composition, and Quality of Cabernet Sauvignon. Am J Enol Vitic 36:125–131.

Cajka T, Riddellova K, Tomaniova M, Hajslova J (2011) Ambient mass spectrometry employing a DART ion source for metabolomic fingerprinting/profiling: A powerful tool for beer origin recognition. Metabolomics 7:500–508. doi: 10.1007/s11306-010-0266-z

Campbell CK (1989) Applications of surface acoustic and shallow bulk acoustic wave devices. Proc IEEE 77:1453–1484. doi: 10.1109/5.40664

Canterbury JD, Merrihew GE, MacCoss MJ, et al (2014) Comparison of data acquisition strategies on quadrupole ion trap instrumentation for shotgun proteomics. J Am Soc Mass Spectrom 25:2048–2059. doi: 10.1007/s13361-014-0981-1

Chinnici F, Guerrero ED, Sonni F, et al (2009) Gas chromatography-mass spectrometry (GC-

MS) characterization of volatile compounds in quality vinegars with protected European geographical indication. J Agric Food Chem 57:4784–4792. doi: 10.1021/jf804005w

Coats SR, Jones JW, Do CT, et al (2009) Human Toll-like receptor 4 responses to P. gingivalis are regulated by lipid A 1- and 4'-phosphatase activities. Cell Microbiol 11:1587–99. doi: 10.1111/j.1462-5822.2009.01349.x

Cody RB, Laramee J a, Durst HD, et al (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem 77:2297–2302. doi: 10.1021/ac050162j

Cohen SL, Chait BT (1996) Influence of matrix solution conditions on the MALDI-MS analysis of peptides and proteins. Anal Chem 68:31–7. doi: 10.1021/ac9507956

Costin JW, Barnett NW, Lewis SW (2004) Determination of proline in wine using flow injection analysis with tris(2,2-bipyridyl)ruthenium(II) chemiluminescence detection. Talanta 64:894–898. doi: 10.1016/j.talanta.2004.03.065

Dill AL, Eberlin LS, Ifa DR, Cooks RG (2011) Perspectives in imaging using mass spectrometry. Chem Commun (Camb) 47:2741–2746. doi: 10.1039/c0cc03518a

Duarte IF, Barros A, Almeida C, et al (2004) Multivariate Analysis of NMR and FTIR Data as a Potential Tool for the Quality Control of Beer. J Agric Food Chem 52:1031–1038. doi: 10.1021/jf030659z

Felipe D, Brambilla L, Porto C, et al (2014) Phytochemical Analysis of Pfaffia glomerata Inflorescences by LC-ESI-MS/MS. Molecules 19:15720–15734. doi: 10.3390/molecules191015720

Flamini R, Traldi P (2010) Mass Spectrometry in Grape and Wine Chemistry. John Wiley &

Sons, Inc.

Frangne N, Eggmann T, Koblischke C, et al (2002) Flavone glucoside uptake into barley mesophyll and Arabidopsis cell culture vacuoles. Energization occurs by H(+)-antiport and ATP-binding cassette-type mechanisms. Plant Physiol 128:726–733. doi: 10.1104/pp.010590

Garcia-Reyes JF, Jackson AU, Molina-Diaz A, Cooks RG (2009) Desorption Electrospray Ionization Mass Spectrometry for Trace Analysis of Agrochemicals in Food. Anal Chem 81:820–829. doi: 10.1021/ac802166v

García-Villalba R, Cortacero-Ramírez S, Segura-Carretero A, et al (2006) Analysis of Hop Acids and Their Oxidized Derivatives and Iso-α-acids in Beer by Capillary Electrophoresis−Electrospray Ionization Mass Spectrometry. J Agric Food Chem 54:5400–5409. doi: 10.1021/jf060207x

Heron SR, Wilson R, Shaffer SA, et al (2010) Surface acoustic wave nebulization of peptides as a microfluidic interface for mass spectrometry. Anal Chem 82:3985–3989. doi: 10.1021/ac100372c

Ho J, Tan MK, Go DB, et al (2011) Paper-based microfluidic surface acoustic wave sample delivery and ionization source for rapid and sensitive ambient mass spectrometry. Anal Chem 83:3260–3266. doi: 10.1021/ac200380q

Hofte AJP, Hoeven RAM Van Der (1998) Characterization of Hop Acids by Liquid Chromatography with Negative Electrospray Ionization Mass Spectrometry. J Am Soc Brew Chem 56:118–122. doi: 10.1094/ASBCJ-56-0118

Huang Y, Heron SR, Clark A, et al (2016) Standing Waves Improve Surface Acoustic Wave

Nebulization Performance. J Mass Spec 51:424–429. doi: 10.1002/jms.3766

Huang Y, Yoon SH, Heron SR, et al (2012) Surface acoustic wave nebulization produces ions with lower internal energy than electrospray ionization. J Am Soc Mass Spectrom 23:1062–1070. doi: 10.1007/s13361-012-0352-8

Konda C, Bendiak B, Xia Y (2012) Differentiation of the stereochemistry and anomeric configuration for 1-3 linked disaccharides via tandem mass spectrometry and 18O-labeling. J Am Soc Mass Spectrom 23:347–358. doi: 10.1007/s13361-011-0287-5

Lachenmeier DW (2007) Rapid quality control of spirit drinks and beer using multivariate data analysis of Fourier transform infrared spectra. Food Chem 101:825–832. doi: 10.1016/j.foodchem.2005.12.032

Länge K, Rapp BE, Rapp M (2008) Surface acoustic wave biosensors: A review. Anal Bioanal Chem 391:1509–1519. doi: 10.1007/s00216-008-1911-5

Lee S, Valentine SJ, Reilly JP, Clemmer DE (2012) Analyzing a mixture of disaccharides by IMS-VUVPD-MS. Int J Mass Spectrom 309:161–167. doi: 10.1016/j.ijms.2011.09.013

Liang T, Schneider T, Yoon SH, et al (2017) Optimized Surface Acoustic Wave Nebulization Facilitates Bacterial Phenotyping.

Nemes P, Vertes A (2007) Laser ablation electrospray ionization for atmospheric pressure, in vivo, and imaging mass spectrometry. Anal Chem 79:8098–8106. doi: 10.1021/ac071181r

Nicholson JR (2017) 2015: What is Made in America.

Nordhoff E, Kirpekar F, Roepstorff P (1996) Mass spectrometry of nucleic acids. Mass Spectrom Rev 15:67–138. doi: 10.1002/(sici)1098-2787(1996)15:2<67::aid-mas1>3.0.co;2-8

Ough C (1968) Proline content of grapes and wines. Vitis 7:321–331.

Peppard TL (1985) The Application of Mass Spectrometry in Beer Flavour Studies. J Inst Brew 91:16–24. doi: 10.1002/j.2050-0416.1985.tb04299.x

Perez-Magarino S, Revilla I, Gonzalez-SanJose ML, Beltran S (1999) Various applications of liquid chromatography-mass spectrometry to the analysis of phenolic compounds. J Chromatogr A 847:75–81. doi: 10.1016/S0021-9673(99)00255-1

Phillips KM, Patterson KY, Rasor AS, et al (2006) Quality-control materials in the USDA National Food and Nutrient Analysis Program (NFNAP). Anal Bioanal Chem 384:1341–1355. doi: 10.1007/s00216-005-0294-0

Quifer-Rada P, Vallverdú-Queralt A, Martínez-Huélamo M, et al (2015) A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC-ESI-LTQ-Orbitrap-MS). Food Chem 169:336–43. doi: 10.1016/j.foodchem.2014.07.154

Quinde-Axtell Z, Baik BK (2006) Phenolic compounds of barley grain and their implication in food product discoloration. J Agric Food Chem 54:9978–9984. doi: 10.1021/jf060974w

Schäfer K-C, Dénes J, Albrecht K, et al (2009) In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. Angew Chemie - Int Ed 48:8240–8242. doi: 10.1002/anie.200902546

Tagashira M, Watanabe M, Uemitsu N (1995) Antioxidative Activity of Hop Bitter Acids and Their Analogs. Biosci Biotechnol Biochem 59:740–742. doi: 10.1271/bbb.59.740

Takats Z, Wiseman JM, Gologan B, Cooks RG (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science (80-) 306:471–473. doi: 10.1126/science.1104404

Tesfaye W, Morales ML, García-Parrilla MC, Troncoso AM (2002) Wine vinegar: Technology,

authenticity and quality evaluation. Trends Food Sci Technol 13:12–21. doi: 10.1016/S0924-2244(02)00023-7

Van Berkel GJ, Pasilis SP, Ovchinnikova O (2008) Established and emerging atmospheric pressure surface sampling/ionization techniques for mass spectrometry. J Mass Spectrom 43:1161–1180. doi: 10.1002/jms.1440

Wang H, Liu J, Cooks RG, Ouyang Z (2010) Paper spray for direct analysis of complex mixtures using mass spectrometry. Angew Chem Int Ed Engl 49:877–880. doi: 10.1002/anie.200906314

Yen GS, Edgar JS, Yoon SH, et al (2016) PDMS microchannels coupled to surface acoustic wave nebulization mass spectrometry. Rapid Commun Mass Spectrom 30:1096–100. doi: 10.1002/rcm.7531

Yeo LY, Friend JR (2014) Surface AcousticWave Microfluidics. Annu Rev Fluid Mech 46:379–406. doi: 10.1146/annurev-fluid-010313-141418

Yoon SH, Huang Y, Edgar JS, et al (2012) Surface acoustic wave nebulization facilitating lipid mass spectrometric analysis. Anal Chem 84:6530–6537. doi: 10.1021/ac300807p

Yoon SH, Liang T, Schneider T, et al (2016) Rapid Lipid A Structure Determination via Surface Acoustic Wave Nebulization and Hierarchical Tandem Mass Spectrometry Algorithm. Rapid Commun Mass Spectrom 30:2555–2560.

Zhang J, Rector J, Lin JQ, et al (2017) Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. Sci Transl Med 9:eaan3968. doi: 10.1126/scitranslmed.aan3968

9. Figure Captions

Figure 1. a) SAWN Chip setup coupled to the inlet of a Waters Synapt G2-S. b) Sketch of SAWN principle. Counter-propagating SAW's generated by interdigital transducers (IDTs) on a piezoelectric material induce strong acoustic streaming and recirculation in the sample droplet which leads to its vertical nebulization.

Figure 2. Comparison of three different vinegars based on their mass spectra. Shown are the SAWN mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Heinz White Vinegar (a), Rice Vinegar (b), and Balsamic Vinegar (c).

Figure 3. Comparison of red and white wine based on their mass spectra. Shown are the SAWN mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Tapena Red Wine (a) and Le Rime White Wine (b).

Figure 4. Comparison of beer SAWN mass spectra. Shown are the mass spectra acquired in positive (top graphs, dark colors) and negative (bottom graphs, light colors) acquisition mode of Goose IPA (a) and Bud Light (b).

Supplemental Information:

Rapid Food Product Analysis by Surface Acoustic Wave Nebulization Coupled Mass Spectrometry

Thomas Schneider[a]*, Benjamin L. Oyler[b], Sung Hwan Yoon[c], Tao Liang[a], Gloria S. Yen [d], David. P. A. Kilgour[e], Erik Nilsson[d], David R. Goodlett [a,d]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA

[b] Department of Toxicology, School of Medicine, University of Maryland, Baltimore, MD, 21201, USA

[c] Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, MD, 21201, USA

[d] Deurion LLC, Seattle, WA 98103, USA.

[e] Chemistry and Forensics, School of Science & Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK

Contact Information:

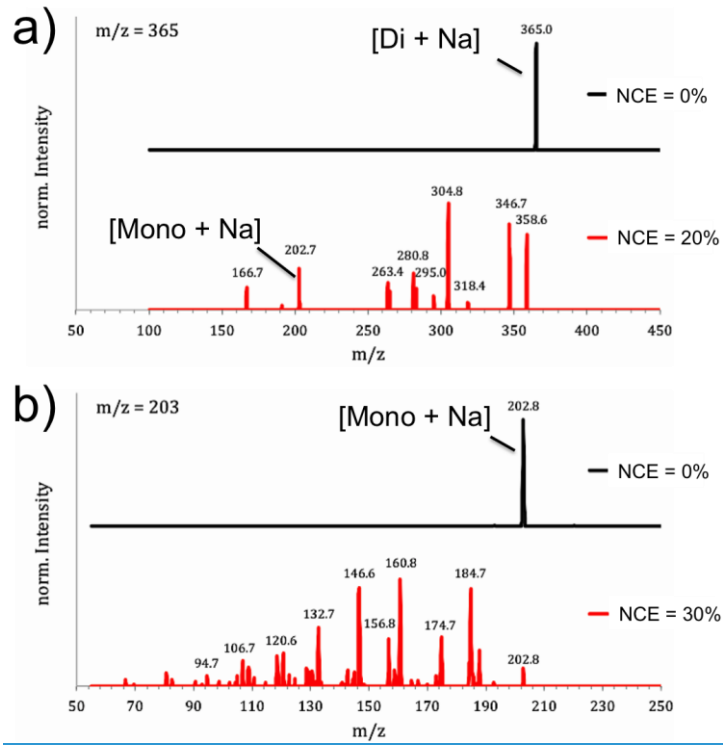
*Corresponding Author: T. Schneider (tschneid@uw.edu)

1. Materials and Methods

SAWN Chip Fabrication

The Interdigital transducer (ITD) design was created by computer aided design software (AutoCAD) and subsequently converted to a chrome mask (Heidelberg μPG 101 Laser Pattern Generator; Heidelberg Instruments Mikrotechnik GmbH, Heidelberg, Germany) at the University of Washington Nanotech User Facility. Lithium Niobate wafers ($LiNbO_3$ 128 Y-cut, X-propagating, 3-inch; Crystal Technology, Inc., Palo Alto, CA, USA) were coated ~1 μm positive photoresist (AZ 1512; AZ Electronic Materials, Somerville, NJ, USA), followed by exposure (Oriel mask aligner; Newport Corporation, Irvine, CA, USA) to create a sacrificial layer containing the SAW transducer. The SAW chip design used in this study consisted of two IDT pairs of electrodes to create counter-propagating SAW's. Each IDT pair consisted of 20 electrode pairs 100 μm wide, spaced 100 μm apart, and with a 5 mm aperture. The delay region between the two IDT pairs was 8.1 mm. The operating frequency of the transducer used in this study was 9.56MHz. The IDT microelectrodes were created through heated vapor deposition of a 20 nm chrome adhesion layer followed by a 60 nm layer of gold. Subsequently, the photoresist was removed through acetone rinse leaving behind the patterned electrodes on the $LiNbO_3$ wafer.

2. Supplemental Figures


a)
m/z = 365
[Di + Na]
365.0
NCE = 0%
norm. Intensity
[Mono + Na]
202.7
166.7
304.8
346.7
358.6
280.8
263.4
295.0
318.4
NCE = 20%
50
100
150
200
250
300
350
400
450
m/z
b)
m/z = 203
[Mono + Na]
202.8
NCE = 0%
norm. Intensity
146.6
160.8
184.7
132.7
156.8
174.7
106.7
120.6
94.7
202.8
NCE = 30%
50
70
90
110
130
150
170
190
210
230
250
m/z

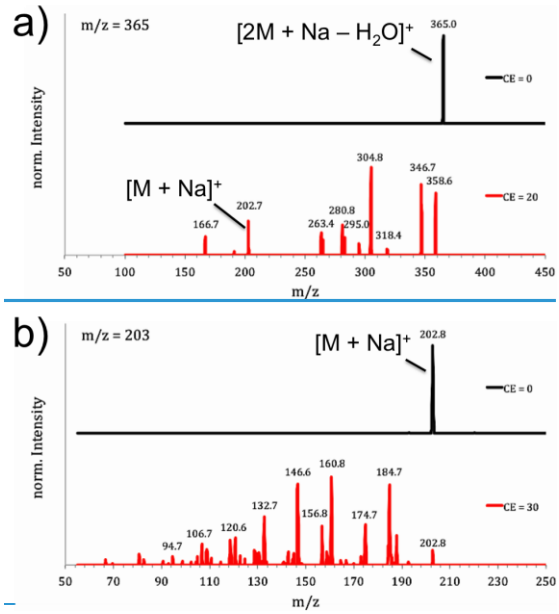
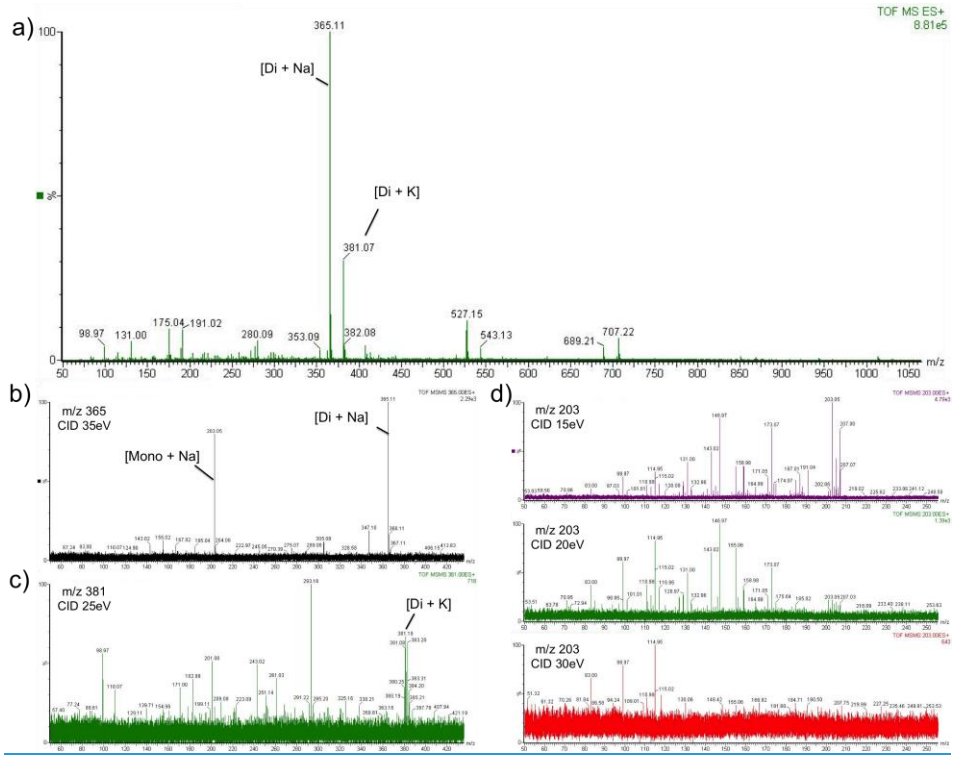


Figure S1. Fragmentation of sodium adducts of disaccharides found at *m/z* 365 (a) and glucose/fructose at *m/z* 203 (b) in samples from rice vinegar acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel~~(LTQ)~~. Shown are the precursor ions (MS^2 at ~~collision energy,~~ NCE~~,~~ of 0 %, black spectra) and fragment ions (MS^2 at NCE of 20 % in (~~b~~a) and 30 % in (b), red spectra). Note that the differences in the collision energy used in this experiment are due to differences in energy required to fragment mono- and disaccharides.


a)
TOF MS ES+
8.81e5
365.11
[Di + Na]
[Di + K]
381.07
527.15
543.13
707.22
689.21
382.08
353.09
280.09
175.04
191.02
131.00
98.97
m/z
b)
m/z 365
CID 35eV
[Di + Na]
[Mono + Na]
c)
m/z 381
CID 25eV
[Di + K]
d)
m/z 203
CID 15eV
m/z 203
CID 20eV
m/z 203
CID 30eV

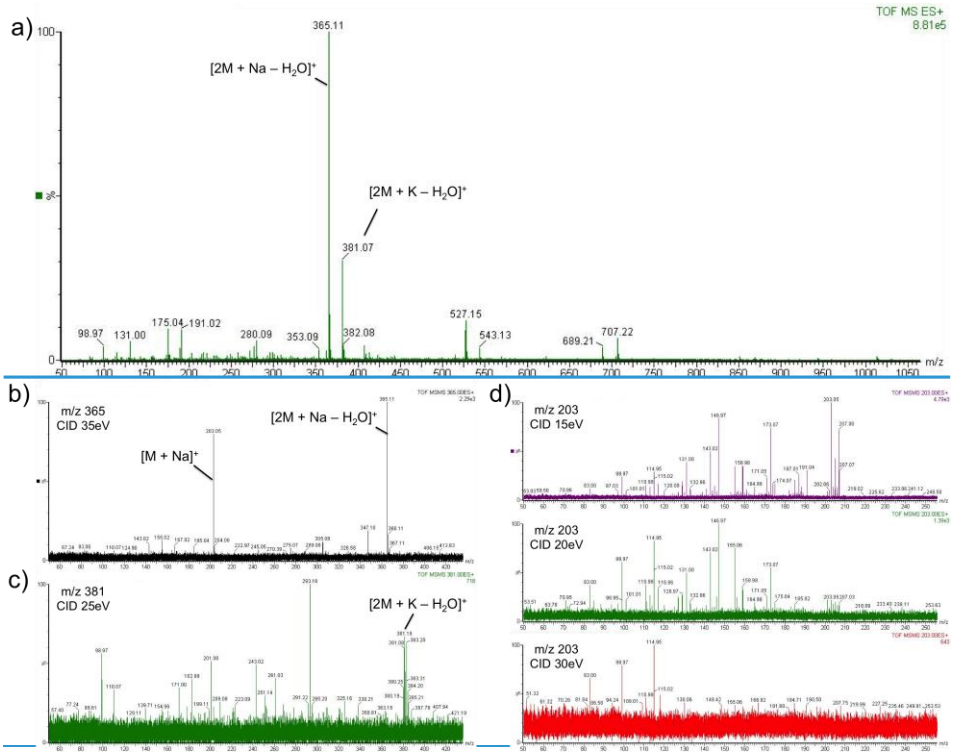
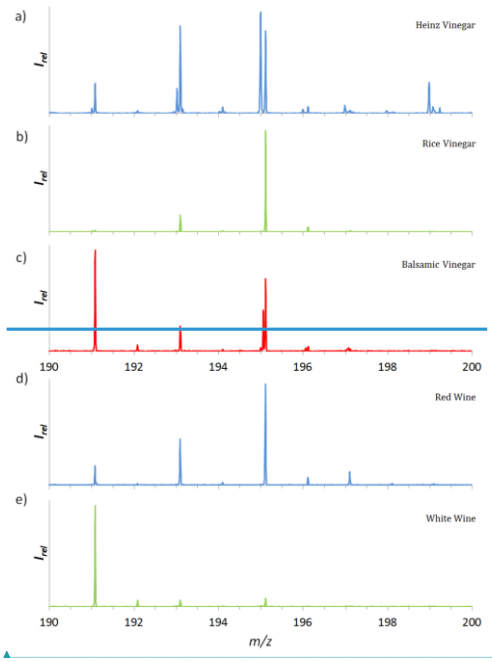


Figure S2. MS spectra from direct infusion in ~~(+)~~ positive ion mode of rice vinegar acquired by ESI on a Waters Synapt G2-S HDMS Q-IMS-oaTOF mass spectrometer (Waters Corporation). ~~-~~ Shown are the MS1 spectrum (a) and the fragmentation spectra (MS2) of sodium and potassium adducts of disaccharides found at *m/z* 365 (~~a~~b) and *m/z* 381 (c), and glucose/fructose at *m/z* 203 (~~e~~d). The fragmentation spectra were acquired with different collision-induced dissociation (CID) energies to show the fragment ions from the different precursor ions. ~~in samples from rice vinegar acquired by ESI (Synapt).~~ The masses measured were: $[C_{12}H_{22}O_{11}Na]^+$, measured: 365.1051, exact: 365.1060, error: -2.5 ppm; $[C_{12}H_{22}O_{11}K]^+$, measured: 381.0741, exact: 381.0799, error: -15.2 ppm.

Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Superscript
Formatted: Subscript
Formatted: Subscript
Formatted: Subscript
Formatted: Superscript



Formatted: Font: (Default) Times New Roman, 12 pt

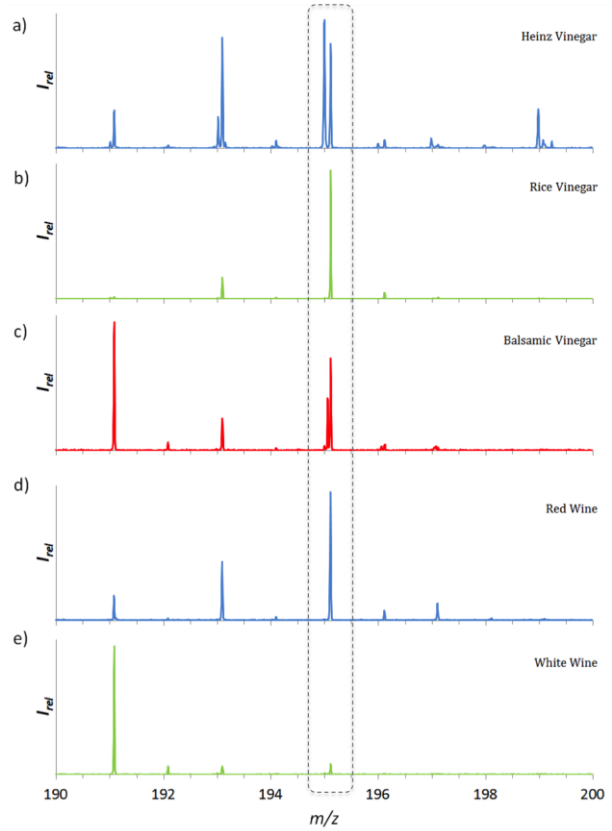


Figure S3. Comparison of changes in ~~gluconic acid~~gluconate (*m/z* 195) in the negative ion mass spectra of different vinegars and wines acquired by SAWN MS.

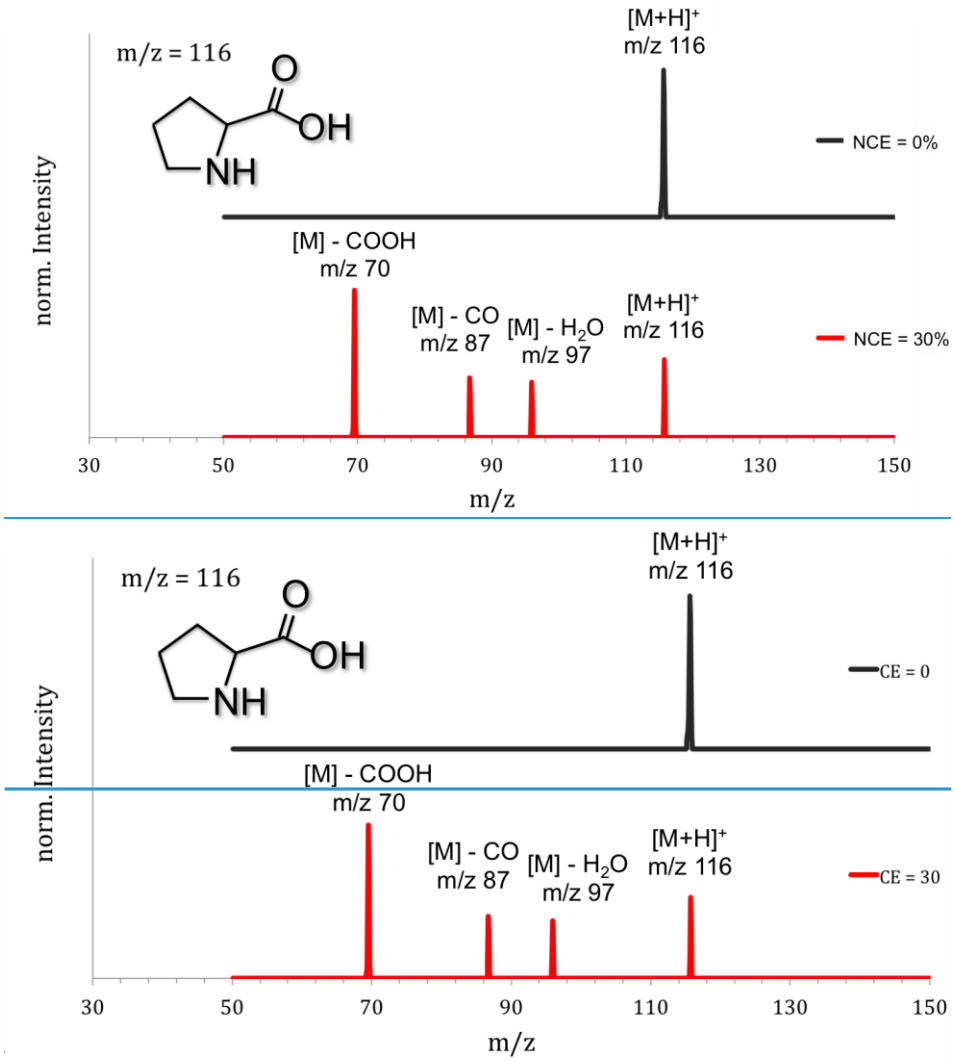
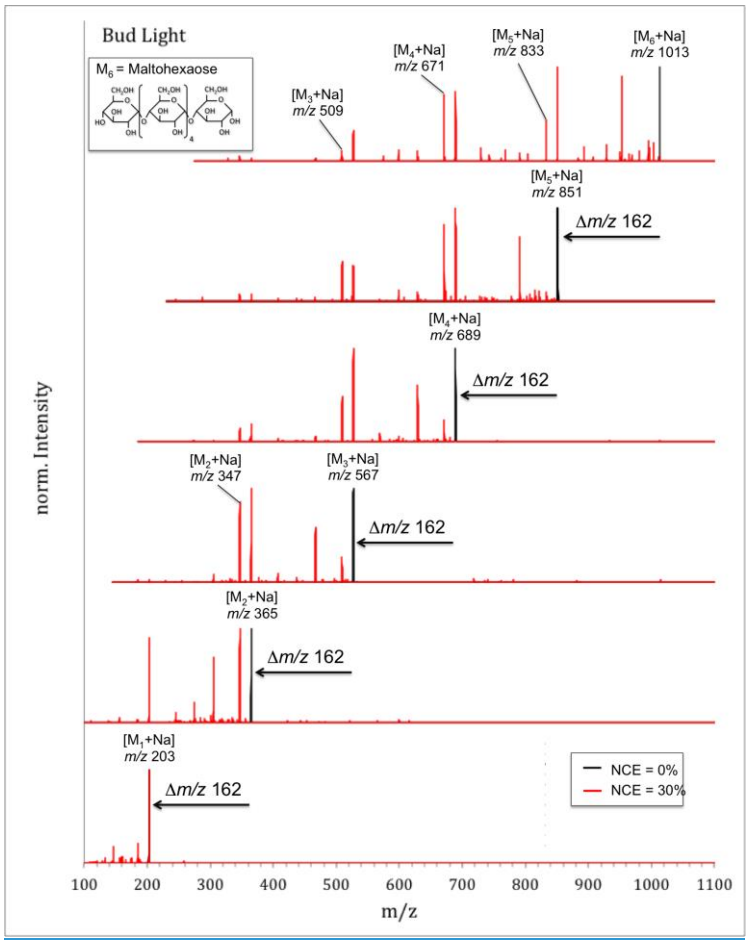


Figure S4. Fragmentation of proline found in samples from balsamic vinegar acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel~~(LTQ)~~. Shown are the precursor ions (MS^2 at NCE = 0 %, black ~~spectra~~spectrum) and fragment ions (MS^2 at NCE = 30 %, red ~~spectra~~spectrum).


Bud Light
M6 = Maltohexaose
[M3+Na] m/z 509
[M4+Na] m/z 671
[M5+Na] m/z 833
[M6+Na] m/z 1013
[M5+Na] m/z 851
Δm/z 162
[M4+Na] m/z 689
Δm/z 162
[M2+Na] m/z 347
[M3+Na] m/z 567
Δm/z 162
[M2+Na] m/z 365
Δm/z 162
[M1+Na] m/z 203
Δm/z 162
NCE = 0%
NCE = 30%
norm. Intensity
100 200 300 400 500 600 700 800 900 1000 1100
m/z

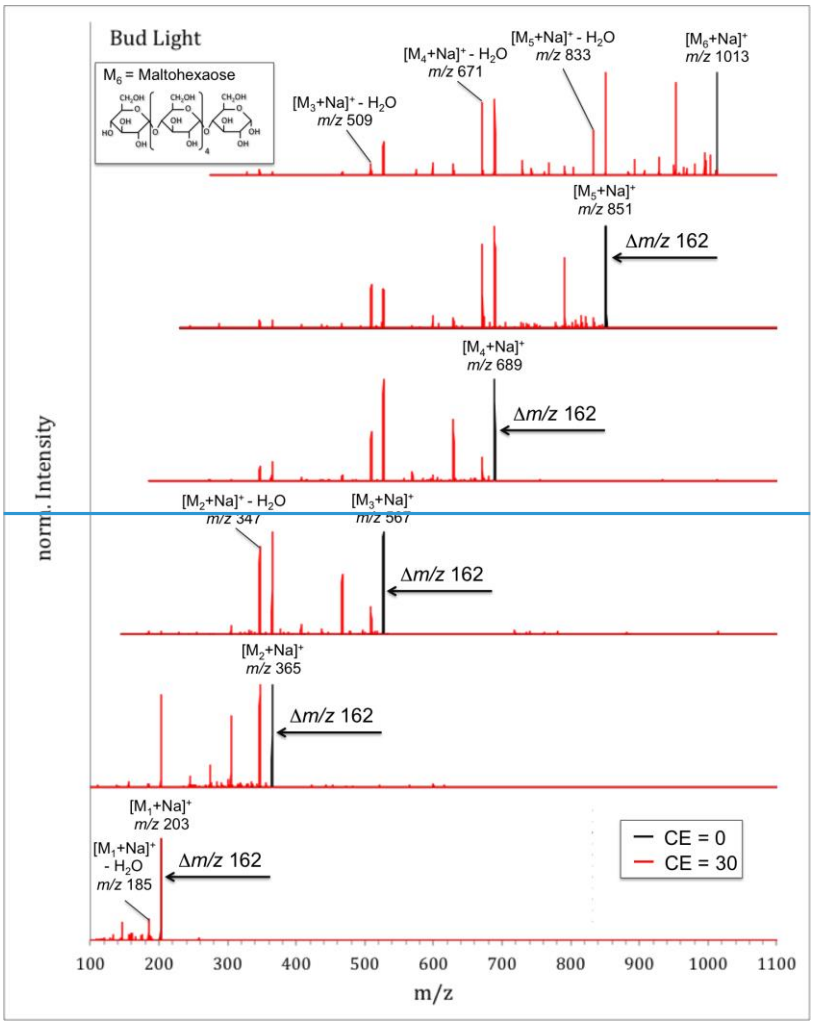
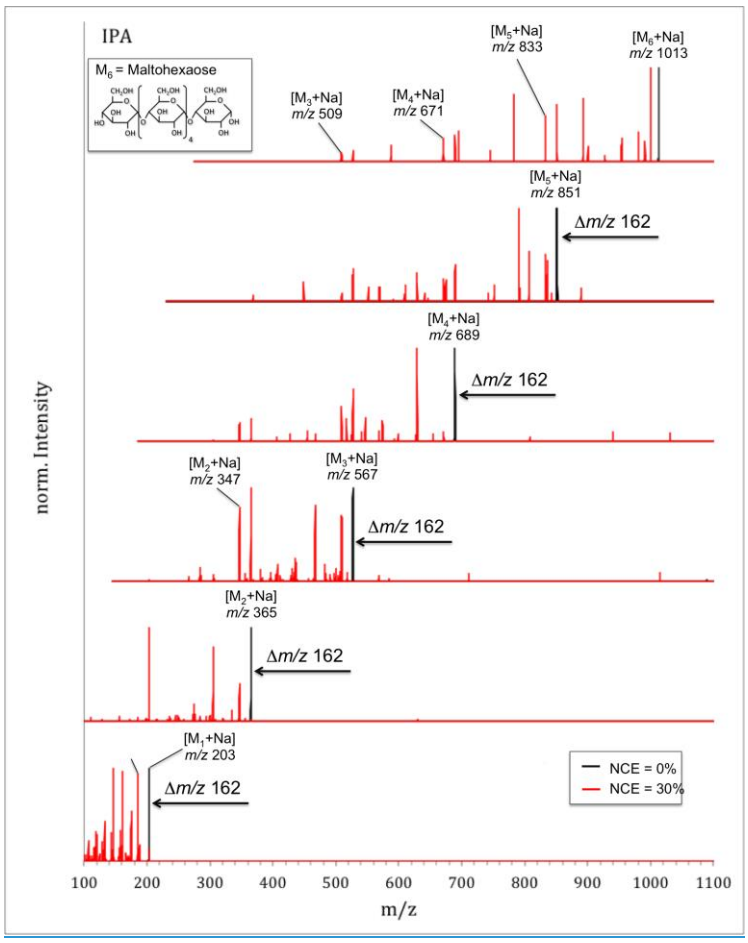


Figure S5. Fragmentation of oligosaccharides in samples from Bud Light acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel~~(LTQ)~~. Shown are the precursor ions (MS^2 at NCE = 0 %, black spectra) and fragment ions (MS^2 at NCE = 30 %, red spectra).


IPA
M6 = Maltohexaose
[M3+Na] m/z 509
[M4+Na] m/z 671
[M5+Na] m/z 833
[M6+Na] m/z 1013
[M5+Na] m/z 851
Δm/z 162
[M4+Na] m/z 689
Δm/z 162
[M2+Na] m/z 347
[M3+Na] m/z 567
Δm/z 162
[M2+Na] m/z 365
Δm/z 162
[M1+Na] m/z 203
Δm/z 162
NCE = 0%
NCE = 30%
norm. Intensity
100 200 300 400 500 600 700 800 900 1000 1100
m/z

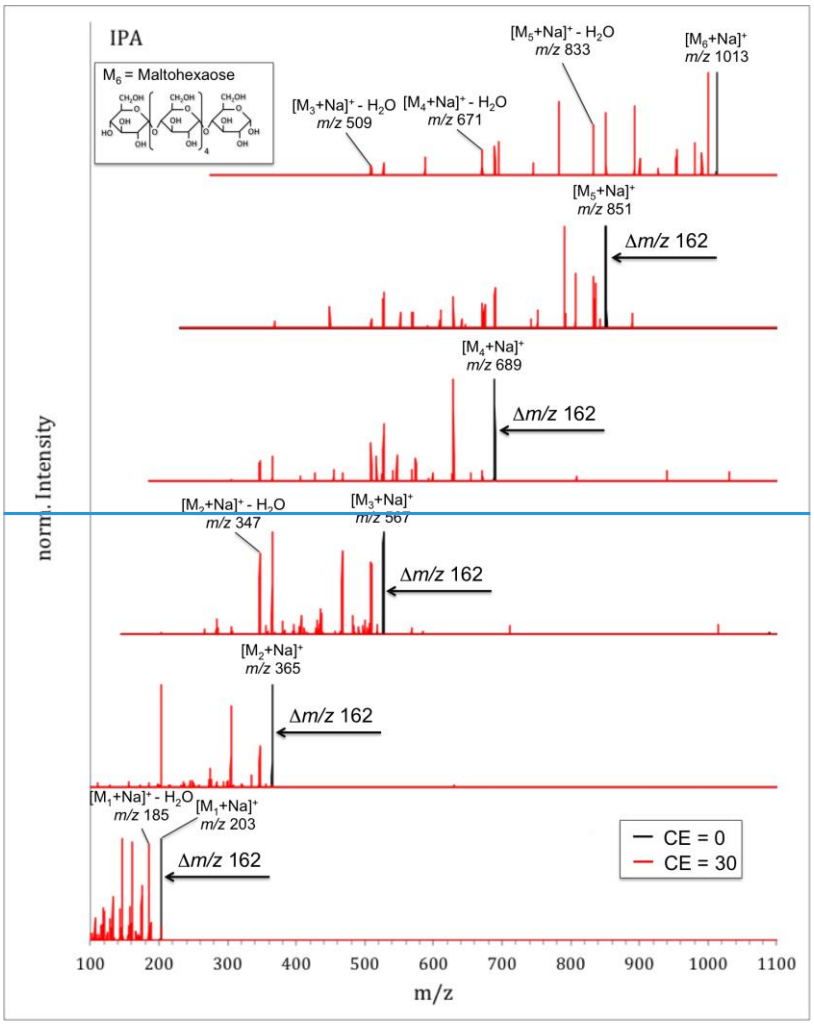


Figure S6. Fragmentation of oligosaccharides in samples from IPA acquired by ESI on a Finnigan LTQ (Thermo Scientific) retrofitted with a bespoke ion funnel~~(LTQ)~~. Shown are the precursor ions (MS^2 at CE 0, black spectra) and fragment ions (MS^2 at CE 30, red spectra).

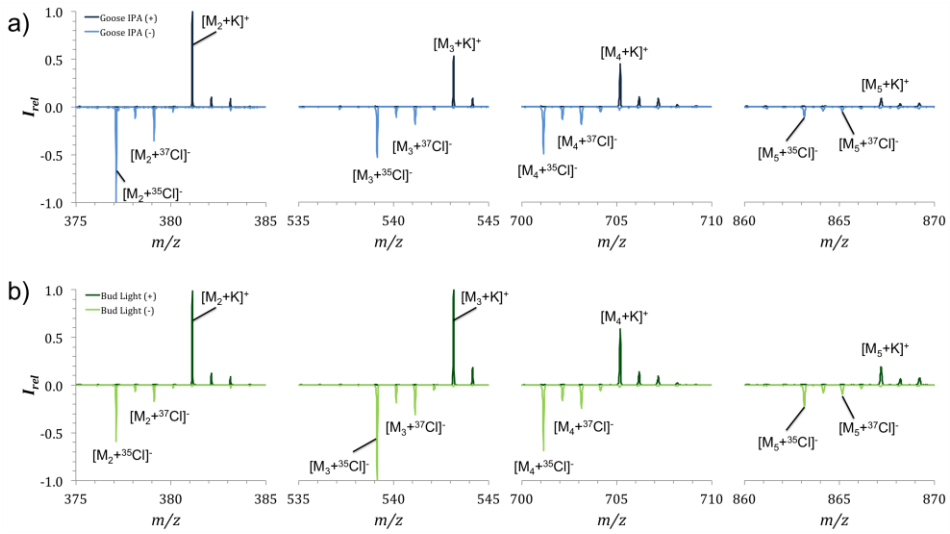


Figure S7. Natural isotope distribution pattern of chlorine in the oligosaccharide adducts of beer samples (Goose IPA (a) and Bud Light (b)) analyzed by SAWN-MS in negative ion mode (bottom graphs, light colors). M_2 = maltose, M_3 = maltotriose, M_4 = maltotetraose, and M_5 = maltopentaose.

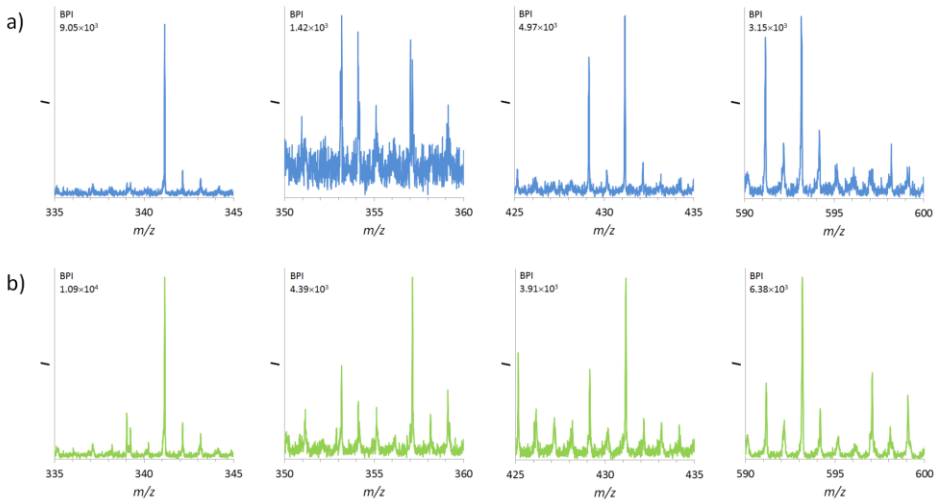


Figure S8. Expanded negative ion mass spectra for low S/N species in samples of Goose IPA (a) and Bud Light (b) acquired by SAWN MS.

Figure 1

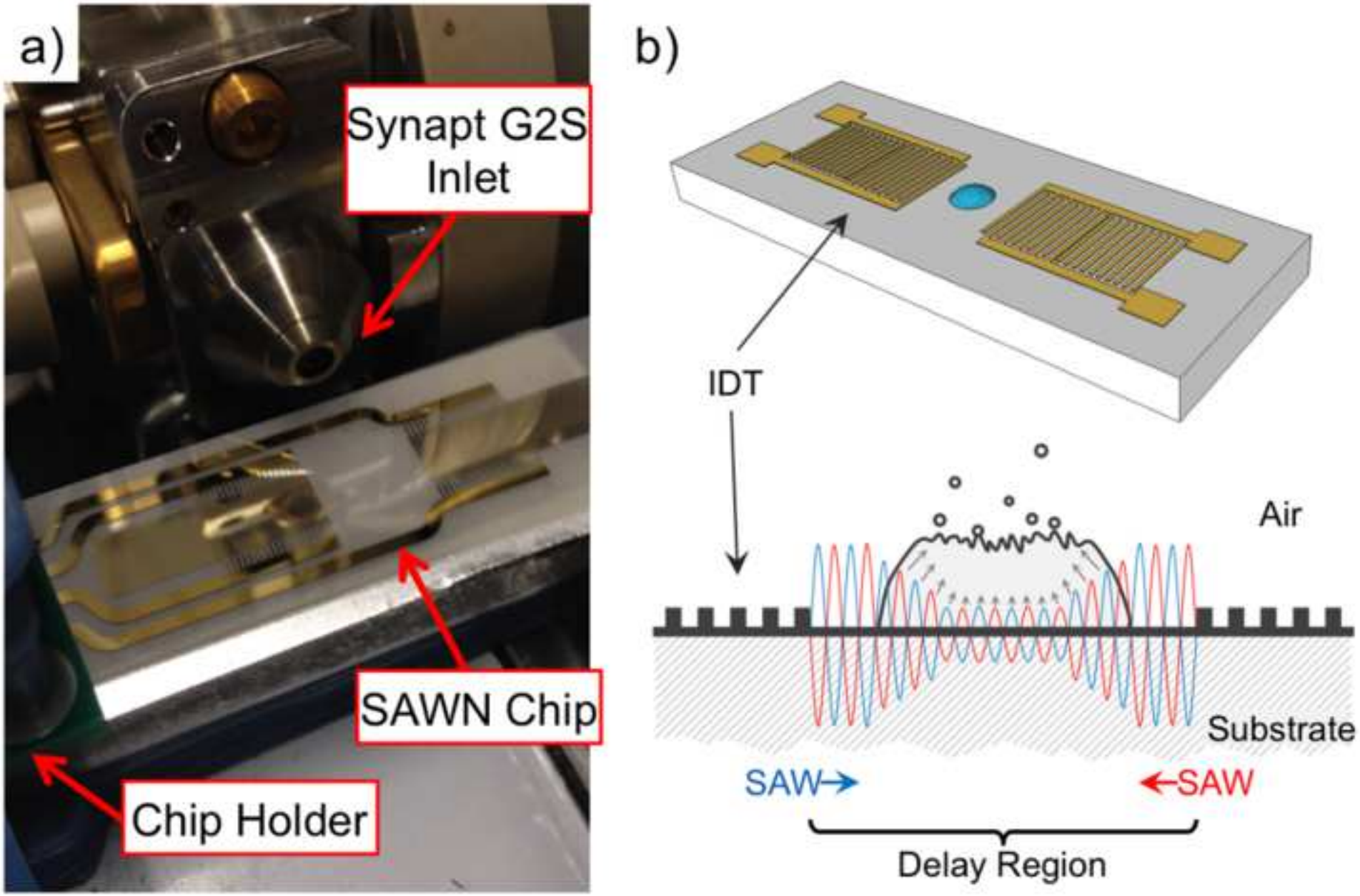


Figure 2

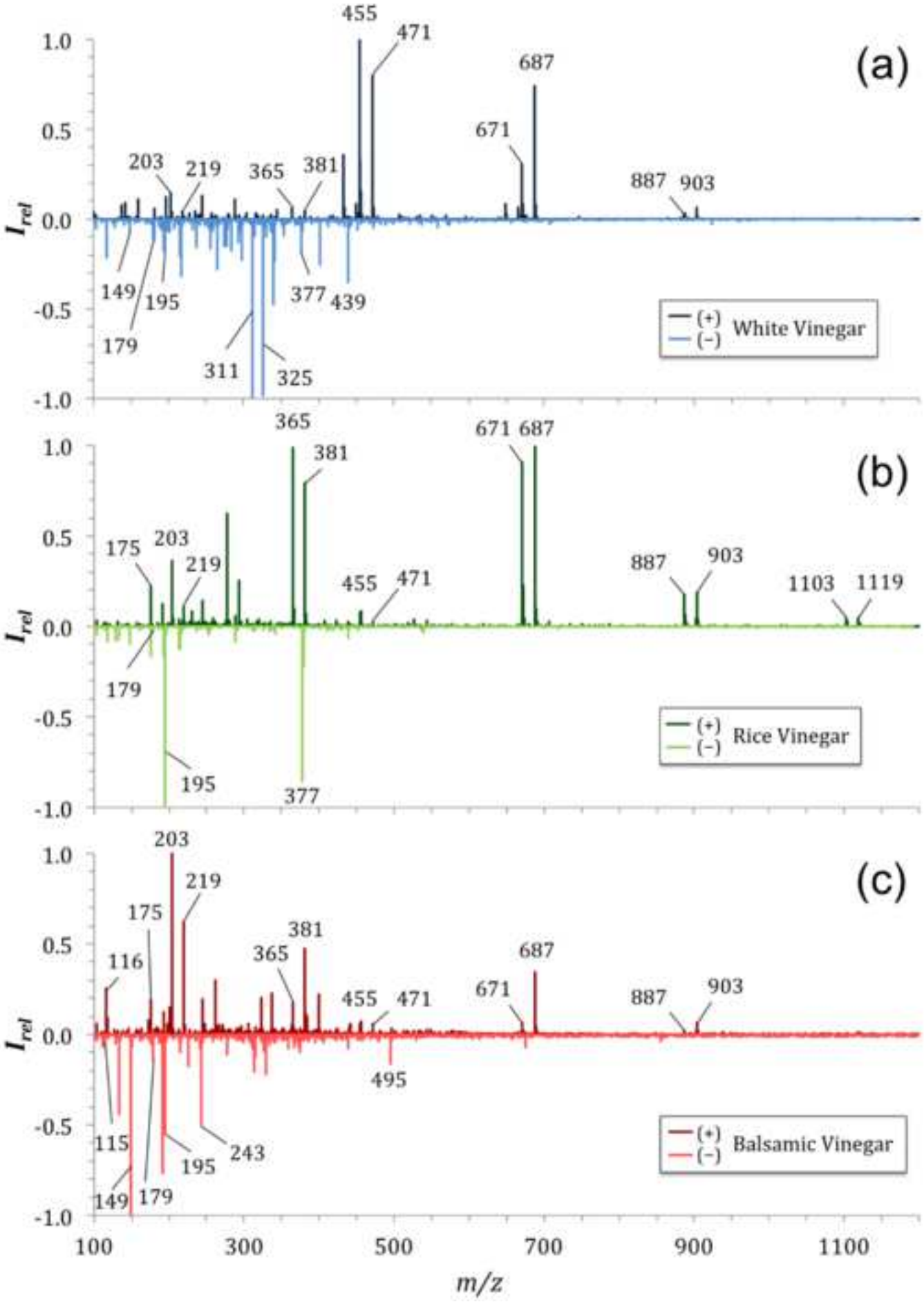


Figure 3

Click here to download Figure Figure 3_300dpi_092817.tif

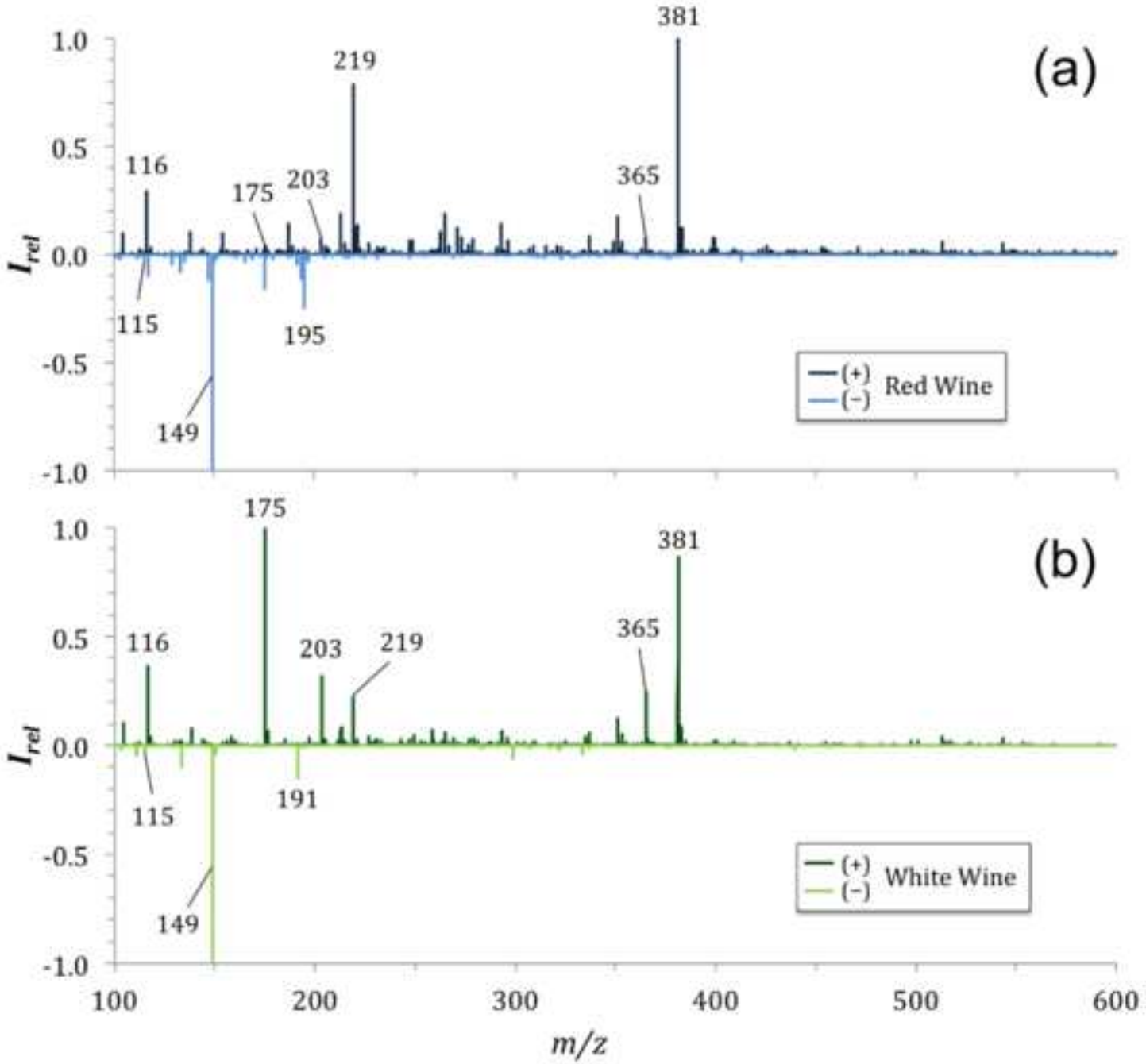


Figure 4

Click here to download Figure Figure 4_300dpi_092817.tif