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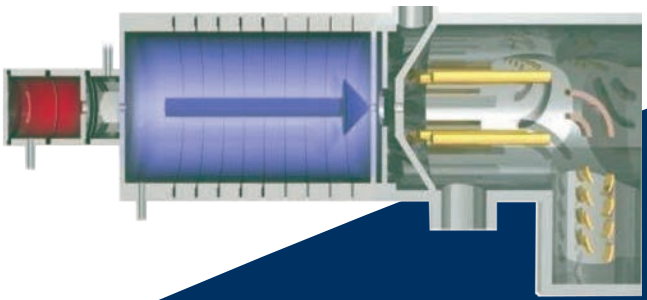

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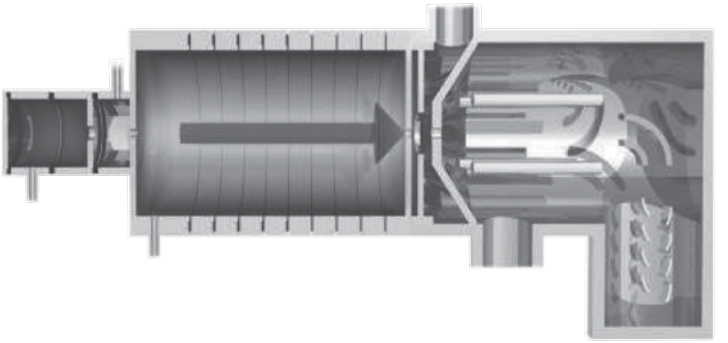

Contributions

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Foreword

PTR-MS (Proton Transfer Reaction - Mass Spectrometry) is a technology developed at the Institute of Ion Physics and Applied Physics at the University of Innsbruck 20 years ago. PTR-MS has been found to be an extremely powerful and promising technology for the in-situ detection of volatile organic compounds (VOCs) at trace levels (pptv) in gaseous media. Today the most recently developed PTR3-TOF instrument reaches limits of detection in the sub-ppq range monitoring semi volatile and even non-volatile compounds. PTR-MS has been successfully employed in many fields of research & technology including environmental research, life sciences, and food science.

In 1998 the spin-off company Ionicon Analytik GmbH (www.ionicon.com) was founded to provide PTR-MS instruments to a growing user community and to develop the technology further. Today many research institutions and companies use this technology throughout the world.

The intent in initiating and organizing the 1st International PTR-MS Conference in January 2003 in Igls, Austria was to bring together active scientists and technology experts involved in mass spectrometric measurements of VOCs. The 7th PTR-MS conference continues this series to provide a discussion forum for PTR-MS users and scientists from both academia and industry. More than 100 conference participants are expected at the conference site in Obergurgl. This year's conference is organized in plenary sessions and focused parallel sessions. The program will start with a plenary session with keynote speakers presenting interdisciplinary overviews in environmental science, food science and medicine. On the following days the conference topics PTR-MS in Environmental Science, Food Science, Medicine & Biotechnology, will be discussed in parallel sessions.

I would like to thank the session chairs Thomas Karl (Environmental Science, biogenic VOC), Franco Biasioli (Food Science) and Jonathan Beauchamp (Medicine and Biotechnology) for putting together an exciting programme, which exemplifies the growing number of PTR-MS applications in various scientific disciplines.

Special thanks goes to Jürgen Dunkl and Sandra Naschberger, who worked very hard to organise this conference. Finally I would like to thank the UNIVERSITY of INNSBRUCK and IONICON ANALYTIK for support. IONICON ANALYTIK also sponsors the poster award.

Armin Hansel

Innsbruck, December 2015

Applications in Environmental Science

Plant volatiles in a changing world

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Abstract

Plants and other organisms produce an abundant and diverse array of metabolites including many volatile organic compounds that are released into the atmosphere. These compounds participate in numerous chemical reactions that influence the atmospheric abundance of important air pollutants and short-lived climate forcers including organic aerosol, ozone and methane. The production and release of these organics are strongly influenced by environmental conditions including air pollution, temperature, solar radiation, and water availability and they are highly sensitive to stress and extreme events. As a result, releases of biogenic organics to the atmosphere have an impact on, and are sensitive to, air pollution and climate leading to potential feedback couplings. Their role in linking air pollution and climate is conceptually clear but an accurate quantitative representation is needed for predictive models. Progress towards this goal will be presented including numerical model development and assessments of the predictive capability of the Model of Emission of Gases and Aerosols from Nature (MEGAN). Recent studies of processes controlling the magnitude and variations in biogenic organic emissions will be described and observations of their impact on atmospheric composition, air pollution and climate will be discussed. Recent advances and priorities for future research will be discussed including laboratory process studies, long-term measurements, multi-scale regional studies, global satellite observations, and the development of a next generation model for simulating land-atmosphere chemical exchange.

Monitoring food crop stress via VOC analysis

L. Cappellin[1,2], A. Algarra Alarcon[1], V. Lazazzara[1], P.L. Bianchedi[1], M. Perazzolli[1], V. Giacomuzzi[3], S. Angeli[3], K. McKinney[2], and F. Biasioli[1]

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Abstract

Grapevine and apple are two of the most important fruit crops globally, both for fresh produce (apples, table grapes) and processed items (juice, wines). In nature, crops are challenged by abiotic and biotic stresses, which often cause damage and limit crop yields if compensatory plant responses do not occur. Plants have evolved complex mechanisms to adapt to these stresses. Generic responses to stresses include the production of excess reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radicals, superoxide, and hydrogen peroxide. Excessive ROS accumulation can lead to extensive oxidation of important components such as nucleic acids, proteins and lipids which can further exacerbate ROS accumulation leading to programmed cell death [1].

Other responses are dependent on the stress mechanism. For example, it is suggested that upon herbivore attack plants can release volatile organic compounds referred to as herbivore-induced plant volatiles (HIPVs). These volatiles may either directly repel the herbivore; or they can be perceived by neighbouring plants and by distal parts of the attacked plant inducing the activation of direct defence mechanisms; or they can attract insect natural enemies such as predators or parasitoids thus indirectly defending the plant. Plants also emit VOCs in response to pathogen infections, especially green leaf volatiles (GLVs), terpenes and terpenoids, methyl salycilate, methyl jasmonate, and aldehydes. The role of such VOCs in the defense mechanisms against pathogens is still relatively unknown. It is suggested that some pathogen-induced volatiles could exert direct antimicrobial effects. For example β-caryophyllene, 2-carene, 2-hexenal, 2-nonenal, and C9-aldehydes have shown direct suppression of *Botrytis cinerea* infections [2]. Some studies have also suggested within-plant signaling and plant-plant communication. For instance, upon *Colletotrichum lindemuthianumin* attacks, resistant bean plants emitted VOCs able to enhance resistance in neighboring susceptible genotypes [3]. In the last two decades, research advances in this area have been possible thanks to the availability of new techniques for head-space sampling, e.g. solid phase micro-extraction coupled to GC-MS, and new instruments for real time analysis, such as PTR-MS.

In the talk, a series of studies will be presented.

In the first study a new methodology to assess the effects of stresses on grapevine VOC emission using in-vitro plants has been developed. Plants grown *in-vitro* were employed to ensure axenic conditions and avoid the interference of potential contaminant microorganisms. *In-vitro* grapevines were inoculated with *Plasmopara viticola*, the agent causing downy mildew, one of the most damaging diseases in grapevine. *Plasmopara viticola* infection, symptom development, and sporulation was permitted by the constant high humidity caused by the axenic conditions. In the experiments three different grapevine genotypes, one susceptible to *P. viticola* and two resistant ones,

were employed. The susceptible grapevine genotype was Pinot Noir, belonging to *Vitis vinifera*, while the two resistant genotypes were the American hybrids SO4 and Kober 5BB. These genotypes were screened for VOC emission by PTR-ToF-MS at different time points after inoculation and the emission pattern of VOCs was compared to the level of disease severity [4]. Our method discriminated the three tested grapevine genotypes based on VOC emissions, and showed significant differences between the resistant genotypes and the susceptible *V. vinifera* genotype upon *Plasmopara viticola* inoculation. The infection induced emissions of monoterpenes by SO4 plants and of sesquiterpenes by Kober 5BB and SO4 plants. In contrast, the susceptible genotype Pinot Noir did not display any variations in terpene emissions and suffered the greatest level of disease severity after inoculation under the same conditions. These results imply that monoterpenes and sesquiterpenes play a role in plant resistance to this pathogen and highlight the necessity of further studies on the role of volatile terpenes in the defense response of resistant grapevine genotypes against *Plasmopara viticola*. Clarifying this role requires the assessment of direct toxic effects of these terpenes on *P. viticola* and/or the testing of the activation of plant resistance via within-plant signaling and plant-plant communication.

In another series of experiments using both GC-MS and PTR-ToF-MS we investigated the response of grapevines and apple plants grown under control conditions to several herbivore insect attacks. Different HIVB were found depending on the fruit crop and on the herbivore insect. These included GLV, terpenoids and benzenoid compounds. The emission of the latter class of VOCs from crops and plants in general has recently been shown to possibly have an impact at the global level [5]. Recent in-field investigations showing the direct emissions of benzenoid and other stress compounds will also be presented.

Acknowledgements

L.C. acknowledges funding from the European Commission's H2020 Framework Programme under Grant Agreement Number 659315.

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Cycling of Volatile Organic Compounds by Marine Plankton using PTR-TOF/MS

Cleo L. Davie-Martin[1], Kimberly Halsey[1], Jing Sun[1], and Stephen J. Giovannoni[1]

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Abstract

Volatile organic compounds (VOCs), such as acetone and methanol, are produced and/or consumed by marine plankton. Our work uses proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF/MS) in combination with dynamic seawater stripping chambers to investigate the cycling of VOCs by marine phytoplankton and bacterioplankton. Our initial research involving cultures of the chemoheterotrophic bacteria *Pelagibacter*, the most abundant bacterioplankton in the surface ocean, showed that dimethylsulfide and methanethiol were immediately released by cells exposed to dimethylsulfoniopropionate (DMSP), even when cells were previously cultured in the absence of DMSP. Additionally, cultures of the model green algae *Dunaliella tertiolecta* suspended in filtered natural seawater were found to produce large quantities of acetone and methanol. Our ongoing work involves four field campaigns to the North Atlantic Ocean, where we will focus on the cycling of VOCs in natural seawater suspensions during different stages of the annual phytoplankton bloom cycle. This work will help to clarify the roles of VOCs in ocean ecosystems and quantify marine VOC sources and sinks.

Introduction

Oceans are considered to be both a source and sink for atmospheric VOCs, such as acetaldehyde and methanol. In the atmosphere, VOCs are known to form radicals and other atmospherically important species.[1,2] One such compound, dimethylsulfide, has been implicated in the formation of aerosols and cloud condensation nuclei,[3] which influence cloud formation/radiative budgets and thus, have consequences for global climate change.

Atmospheric VOCs have both abiotic and biological origins. For example, photochemical transformations such as those involving chromatophoric dissolved organic matter are known to produce acetone and acetaldehyde.[4] Furthermore, marine plankton such as *Pelagibacter* are important vectors for the production of atmospheric dimethylsulfide.[5] However, the biological mechanisms responsible for mediating the flux of VOCs between the ocean surface and the atmosphere are not well constrained. Moreover, the extent to which VOCs are produced and consumed by marine microbial populations and the overall contribution of VOCs to the marine carbon cycle is unknown.

This project aims to investigate the cycling of VOCs by marine plankton in the ocean surface. In particular, we have ongoing field studies exploring VOC production (sources) and consumption (sinks) in the North Atlantic Ocean during different stages of the annual phytoplankton bloom cycle. Here we present an overview of our preliminary results, which utilize both laboratory-based experiments with axenic culture suspensions and field-based measurements with natural seawater.

Experimental Methods

Dynamic stripping chambers

Our dynamic stripping chambers consist of 200 mL polycarbonate tubes each fitted with a 2-2.5 µm sintered glass frit at the base. Cultures, natural seawater suspensions, or media only controls (100 mL) are added to each chamber and bubbled with synthetic air containing 390 ppm CO_2 (Figure 1). The chambers are placed in incubators through which exposure to different temperature (3-21 °C) and light (20-2000 µmol photons m^{-2} s^{-1}) conditions is controlled. Our set-up allows for six chambers to run simultaneously, whereby the gases evolved from a single chamber are directed into the multiport switching inlet at 1.5 min intervals.

Figure 1: Dynamic stripping chambers containing 100 mL of culture, natural seawater, or media only controls bubbled with synthetic air containing CO_2 at 390 ppm.

Real-time measurements of VOCs by PTR-TOF/MS

1. Laboratory-based experiments with *Pelagibacter* were carried out to investigate the mechanisms of DMSP uptake and metabolism. HTCC1062 strain was grown in autoclaved, filtered artificial seawater amended with nutrients and excess vitamins. Cells were harvested and re-suspended in artificial seawater ($3\sim5\times10^6$ cells mL^{-1} final concentration). 1 µM DMSP was added to the suspensions and incubated at 16 °C in the dynamic stripping chambers.

2. Pilot-scale experiments with *Dunaliella tertiolecta*, which are known to produce VOCs, were trialed to test the magnitude of VOC production. *D. tertiolecta* grown in L1 media on board the research vessel were added to filtered natural seawater and incubated at the surface seawater temperature.

3. Preliminary field-based measurements of VOC cycling in natural seawater suspensions were investigated in the North Atlantic Ocean during a campaign in November 2015. During the cruise, discrete natural seawater samples were collected from either the surface water flow-through system during transit periods or from different depths during the CTD rosette casts while on station. We conducted experiments under different blue and white light levels at temperatures corresponding to the sea surface temperature at the time of collection. At some stations, we

collected water from deeper within or below the mixed layer (50-200 m), which were usually incubated in the dark. We experimented with VOC additions to chambers containing natural seawater. ^{13}C-labeled or unlabeled acetone or methanol were added to the chambers to help tease apart their consumption rates from production rates.

A commercially available PTR-TOF 1000 (IONICON Analytik, Innsbruck, Austria) was used to quantify VOCs. Primary H_3O^+ ions were produced from pure water vapor in the hollow cathode ion source at a flow rate of 5 sccm. Sample gases evolved from the dynamic stripping chambers were introduced to the drift tube, where proton transfer reactions occurred between H_3O^+ and VOCs with proton affinities greater than that of water (691 kJ mol^{-1}):

$$VOC + H_3O^+ \rightarrow VOC{\cdot}H^+ + H_2O$$

Within the drift tube, the pressure, temperature, and voltage conditions were kept constant at 2.3 mbar, 80 °C, and 600 V, respectively, which equated to a field strength (*E/N*) of 133 Td (where Td = 10^{-17} cm^2 V molecule^{-1}). Mass spectra were recorded up to 250 amu at 1 to 10 s integration intervals. Preliminary quantification of gas-phase VOCs was achieved using the relative transmission (kinetic) approach and additionally accounted for the influence of the hydrated water cluster at m/z 37 (due to the high sample humidity introduced by bubbling air through seawater). Collision rates were obtained from the literature where possible; otherwise a default collision rate constant of 2.00x10^{-9} cm^{-2} was assumed. Gas calibration standards were used throughout the November field campaign and will be used to obtain more reliable concentrations.

Results and Discussion

VOC cycling from culture experiments

Pelagibacter HTCC1062 was found to simultaneously produce methanethiol and dimethylsulfide when exposed to 1 μM DMSP. While the release of methanethiol was unexpected, we noted that as the supply of intracellular DMSP exceeded the cellular demands for the reduced sulfur required for biosynthesis, increasing amounts of dimethylsulfide were also released (Figure 2). These findings suggest that DMSP supply and demand relationships in *Pelagibacter* metabolism are important for determining rates of oceanic dimethylsulfide production.

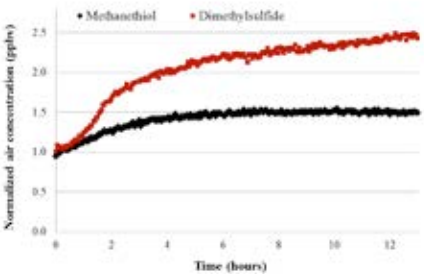

Figure 2: Production of methanethiol and dimethylsulfide from HTCC1062 cell suspensions following addition of 1 μM DMSP. Measurements are presented in relative concentration units and were normalized to the gas-phase concentrations of methanethiol and dimethylsulfide at t=0

Experiments with *D. tertiolecta* suspended in filtered natural seawater were found to rapidly produce acetone and methanol upon exposure to light (Figure 3).

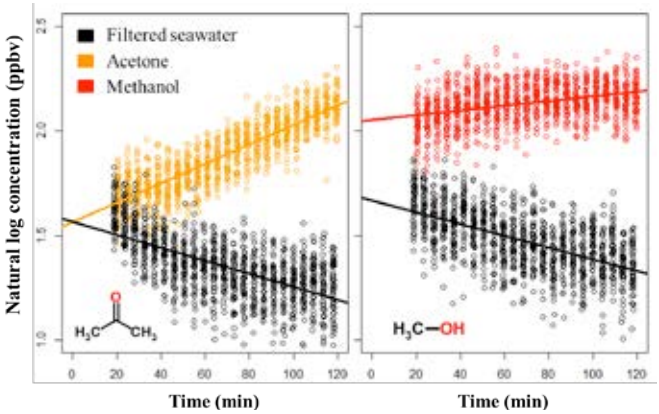

Figure 3: Real-time gas-phase production of acetone and methanol by D. tertiolecta.

VOC cycling in natural seawater

Our preliminary data suggests that VOC production and consumption rates were very low during our November cruise in the North Atlantic. This was likely attributable to the low microbial biomass present at that time in the surface ocean. Nevertheless, these data provide us with an excellent baseline to build upon in future cruises and laboratory-based experiments. Based on our experiments with cultures, we expect VOC signals will be more easily resolved with the heightened plankton biomass associated the seasonal bloom cycle, which we hope to capture in upcoming field campaigns.

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Bi-directional exchange of BVOC in the Mediterranean periurban forest

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The Presidential Estate of Castelporziano is a 6000 ha forest near the coast of Tyrrhenian sea, 20 Km from Rome downtown. The Estate hosts protected areas representative of the main Mediterranean forest ecosystems. Since decades, the Estate hosts intensive field campaigns aimed at investigating BVOC emission from Mediterranean vegetation. An international field campaign in 2012 was carried out in a mixed Oak and Pine forest. Eddy covariance measurements using a PTR-TOF-MS showed that this ecosystem is a large monoterpene emitter. Data were used to parameterize a chemical transport model (CLM) linked to MEGAN (Fares et al. 2013). Results showed that a monoterpene-emitting forest has different implications for air quality in terms of minor photochemical ozone production at regional level compared with an isoprene emitting ecosystem. More recently, two field campaigns were carried out in January and August 2014 to explore BVOC fluxes in a Holm oak forest in two seasons with different climate conditions and physiological activity of plants. Each half-hour, we switched between measurements at high frequency above the canopy and sampling through a 5-levels gradient from soil to above the canopy. We used the eddy covariance technique to calculate fluxes above the canopy, while gradient measurements were used to estimate in-canopy source and sink distribution by applying an Inverse Lagrangian Transport Model (Karl et al., 2004). Low temperatures lead to almost negligible BVOC fluxes during Winter. Summer fluxes were largely represented by monoterpenes. Supporting laboratory experiments using plant enclosures and PTRMS measurements helped to understand the capacity of leaves to sequestrate reaction products of primarily emitted BVOC with atmospheric oxidants, and the potential of BVOC to form ozone in a VOC-/NOx-limited environment. Both field and laboratory experiments were crucial to better understand the importance of forest canopies in the interaction between VOC and secondary pollutants in a peculiar Mediterranean site where the sea-land breeze circulation allows a strong mixing between contaminated air from the city and cleaner air from the sea under high UV radiations and air temperatures.

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Semivolatile organic compounds measured by thermal desorption denuder sampling

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Abstract

A thermal desorption denuder sampling device has been combined with a PTR-TOF8000 instrument and tested for the first during the Southern Oxidant Aerosol Study (SOAS) in Centreville, Alabama, United States in the summer of 2013.

More than 200 different ions exhibited significantly enhanced signals and for 80% of these ions the typical ambient air volume mixing ratio was below 20 pmol/mol. The method allowed easy detection of compounds at the sub-100 fmol/mol level, which can be further improved by at least one order of magnitude through optimizing the pre-concentration step.

First results from a commercialized 3-stage denuder system featuring a 15 minutes time resolution, optimized pre-concentration, and full integration in the PTR-manager software will be shown.

Introduction

Semivolatile organic compounds (SVOCs) originate from primary pollution (e.g. long chain hydrocarbons or polycyclic aromatic compounds), however, the vast majority of SVOCs is produced in situ by photo oxidation of volatile hydrocarbons in the atmosphere. With the addition of oxygen and nitrogen functional groups, these chemically processed compounds tend to condense on particles in the air and typically constitute 70% of accumulation mode particles (PM1) [1]. Therefore, the understanding of the life cycle of SVOCs is crucial in assessing the radiative effects of aerosol pollution, which constitutes the largest uncertainty in assessing the total anthropogenic radiative forcing [2]. Scientific progress in this field is essential to improve the accuracy of climate projections on the decennia time scales.

Up to date only few and very recent methods exist to characterize SVOCs in ambient air [3-7]. The reason for this are analytical challenges rooted in the 'stickiness' of SVOCs, the large number of different compounds, and the low concentration of individual species. In principle PTR-MS is a very suitable technique to quantify SVOCs and has a great potential to explore this new field in combination with the presented thermal-desorption denuder setup.

Experimental Methods

Semivolatile and volatile organic compounds were collected on a serial 3-stage denuder system. The setup of the denuder sampler is shown in Figure 1. The first two denuders were coated with dimethylpolysiloxane (custom made from Agilent DB-1 GC columns) and the third denuder was an activated charcoal monolith (Mast Carbon, UK). Sampling occurred for 30 minutes a flow rate of 1 liter per minute (standard conditions) and desorption over 8 minutes at a flow rate of 0.07

liters per minute, resulting in a pre-concentration factor of ~54. The instrumental background and contamination was measured by sampling ambient air, purified by a platinum catalyst operated at 500°C. Data analysis has been done with the IDL based PTRwid tool [3].

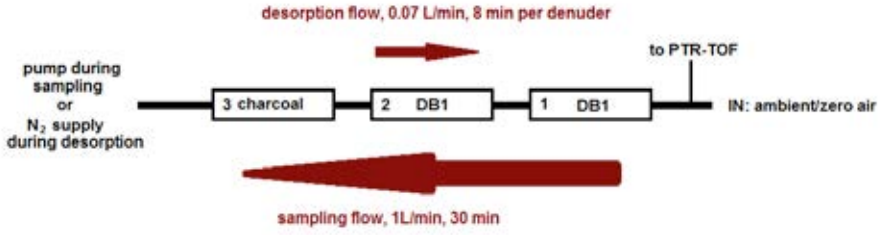

Figure 1: Conceptual setup of the thermal-desorption denuder sampler.

Results

Over 200 different ions exhibited significantly enhanced signals with respect to instrumental noise and contamination. Of these, ~150 were collected on the first DB1 denuder and are considered semivolatile. The total detected burden of SVOCs is ~5 µg/m3 and thus of the same order as measured organic aerosol.

Figure 2 shows two example ions that represent the volatile compounds methylvinylketone (MVK) and methacrolein (MACR) which are both detected at m/z 71.049 (C_4H_6O) and the semivolatile heptadecanoic acid detected at m/z 271 ($C_{17}H_{34}O_2$). Both, MVK and MACR are oxidation products of isoprene (C5H8), thus its diurnal course is controlled by photochemical production during the day and dry deposition during night. Heptadecanoic acid is an oxidation product of long-chained alkanes, which are for example emitted by diesel engines. Therefore, its diurnal cycle is more driven by transport of local and regional pollution. However, there seems to be a local background of a few hundred fmol/mol.

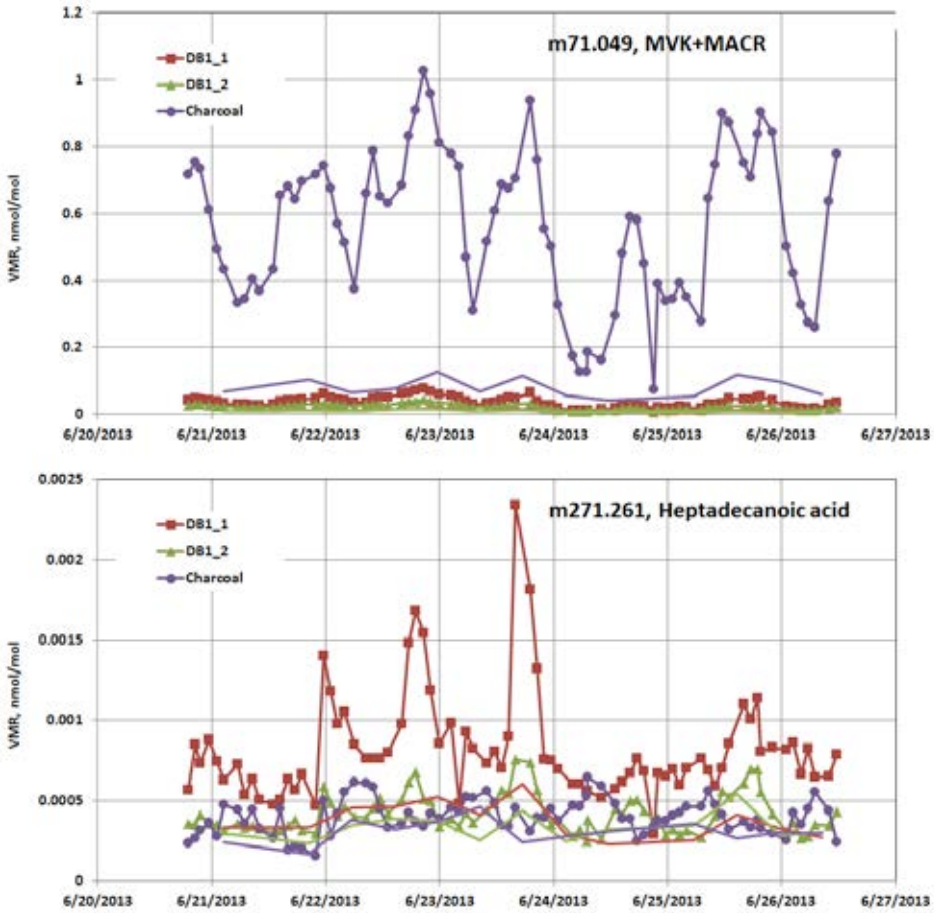

Figure 2: Course of MVK+MACR and Heptadecanoic acid during 6 days in June, 2013, at Centreville, AL, USA. The brown, green, and blue data display the signal from denuder 1, 2, and 3, respectively. Data with markers display ambient air signals, and the solid lines display background measurements. MVK and MACR are volatile compounds and pass the two DB1 denuders before being captured on the third activated charcoal denuder. Heptadecanoic acid is semivolatile and the majority is captured on the first DB1 denuder. About 15% is carried over to the second DB1 denuder.

Discussion

In this talk we will present the major findings of the first deployment of this technique during the SOAS campaign. We will discuss problems and limitations that occurred during this deployment. First measurements of an improved setup that has been developed in collaboration with IONICON Analytik GmbH will be shown and provide for the first time a comprehensive characterization of SVOCs in Innsbruck, Austria.

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Analysis of evaporative emissions from gasoline vehicles by using NO^+ chemical ionization mass spectrometry

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Abstract

For fast and sensitive measurements of multiple alkanes, the detection properties of alkanes by NO^+ chemical ionization mass spectrometry are investigated and the method was applied for the analysis of evaporative emissions from gasoline vehicles during parking and refueling. First, we conducted three continuous day diurnal breathing loss (DBL) tests on nine vehicles which have been used in Japanese market to confirm the variety of breakthrough features among different car models. Among the nine vehicles, four vehicles exhibited huge increased emissions coursed by the breakthrough emissions during the experimental period and all of them were by Japanese manufacturers. The compositions of the breakthrough and permeation emissions were analyzed in real time using NO^+ chemical ionization mass spectrometry to estimate the ozone formation potential for the evaporative emissions. The composition analysis gave an estimated maximum incremental reactivity (MIR) 20 % higher for the breakthrough than for the gasoline that was tested, but the MIR for the permeation emissions was almost the same as the MIR for the fuel. Next, refueling emissions from cars available on the Japanese market, which were not equipped with specific controlling devices, were also investigated. The results of composition analysis indicated that the maximum incremental reactivity (MIR) of refueling emissions in Japan was approximately 20 % higher than that for the tested gasoline. The emissions consist of 80 % alkanes and 20% alkenes, and aromatics and di-enes were negligible.

Introduction

Proton transfer reaction mass spectrometry (PTR-MS) is a technique that allows for fast and sensitive measurement of volatile organic compounds (VOCs) at trace levels in air [1]. Proton transfer is an example of chemical ionization: it enables soft ionization of chemical species that have a proton affinity (PA) higher than that of the reagent species (i.e., water):

$$H_3O^+ + VOC \rightarrow VOC{\bullet}H^+ + H_2O. \qquad (1)$$

PTR-MS makes possible quantitative measurement of alkenes (except ethylene), aromatics, and even oxygenated VOCs. However, the proton transfer in reaction (1) does not occur for alkanes because they have lower PAs than water. A method to measure C_{12}-C_{18} alkanes using PTR-MS was demonstrated [2]. They were, however, detected by a series of fragment ions with formula C_nH_{2n+1} and were detected not individually, but as an ensemble.

Reactions of alkanes with NO^+ have been investigated by selected ion flow tube mass spectrometry (SIFT-MS) [3]. It was reported that hydride ion transfer is a major channel in the reaction of alkanes (RH) with NO^+ [3].

$$NO^+ + RH \rightarrow R^+ + HNO. \qquad (2)$$

Recently, the PTR-MS instrument has been combined with switchable reagent ion capability, which allows for easy and fast switching between H_3O^+, NO^+, and O_2^+ ions [4]. For fast and sensitive measurements of multiple alkanes, the detection properties of alkanes by NO^+ chemical ionization mass spectrometry are investigated.

According to an estimation by Yamada (2013) [5], evaporative emissions from gasoline vehicles are substantial among the total VOC emissions in Japan; however, recent VOC reduction strategies in Japan have focused on tailpipe emissions rather than evaporative emissions. Alkanes, alkenes, and aromatics are major components of gasoline [2]. The estimation was based on the emissions of total hydrocarbons from gasoline vehicles. However, the emergence of ambient air pollutants, such as photochemical ozone and secondary organic aerosols, depends on individual VOC concentrations. In this study, the method was applied for the analysis of evaporative emissions from gasoline vehicles during parking and refueling.

Experimental Methods

A commercially available PTR-MS instrument was used for this work (IONICON Analytik). To generate NO^+ as a reagent ion, air (5.0 sccm) was directed into the hollow cathode discharge ion source. The sample air (approximately 80 sccm) was introduced from a port located beneath the ion source into the drift tube. VOCs in the sample air were ionized by reactions with NO^+ in the drift tube. A homogeneous electric field was established inside the drift tube. The pressure and the temperature of the drift tube were maintained at 2.1 mbar and 105 °C, respectively, and the field strength of the drift tube, *E/N*, where *E* is the electric field strength (V cm^{-1}) and *N* is the buffer gas number density (molecule cm^{-3}), was set to 67 Td (1 Td = 10^{-17} cm^2 V molecule^{-1}). A fraction of the reagent ion, NO^+, and the product ions, was extracted through a small orifice into a quadrupole mass spectrometer. The ions were detected by a secondary electron multiplier for ion pulse counting. The count rate of NO^+, calculated from the count rate at *m/z* 31 ($^{15}N^{16}O^+$ and $^{14}N^{17}O^+$) multiplied by 250, was typically (1.7–1.9) × 10^7 cps in the range between *E/N* values of 67 and 120 Td. The ratio of the count rate of O_2^+ to that of NO^+ was typically 0.02–0.03. However, NO_2^+ suppression was poor, and the ratio of the NO_2^+ count rate to that of NO^+ was 0.09.

Results and Discussion

We determined the normalized detection sensitivities for C_3–C_{13} normal alkanes and C_5–C_{10} branched-chain alkanes, and they are summarized in Figure 1 [6]. The normalized detection sensitivity was defined as the normalized counts per second (ncps) relative to a reagent ion count rate of 10^6 cps when 1 part per billion by volume (ppbv) of the VOC of interest was present in the sample. The C_6–C_{10} branched-chain alkane isomers that were selected were 2-methylpentane, 2-methylhexane, 2-methylheptane, 2-methyloctane, and 2-methylnonane. Aromatic compounds and many alkenes were ionized as shown in reaction 3 because they have lower ionization energies than does NO^+ [3].

$$NO^+ + M \rightarrow M^+ + NO \qquad (3)$$

The typical detection sensitivities achieved for isoprene, toluene, *p*-xylene, and 1,3,5-trimethylbenzene (7.3 ncps ppbv^{-1}) were used as the typical detection sensitivities for the alkenes and aromatic compounds. A detection sensitivity of 5.2 ncps ppbv^{-1} was used for benzene.

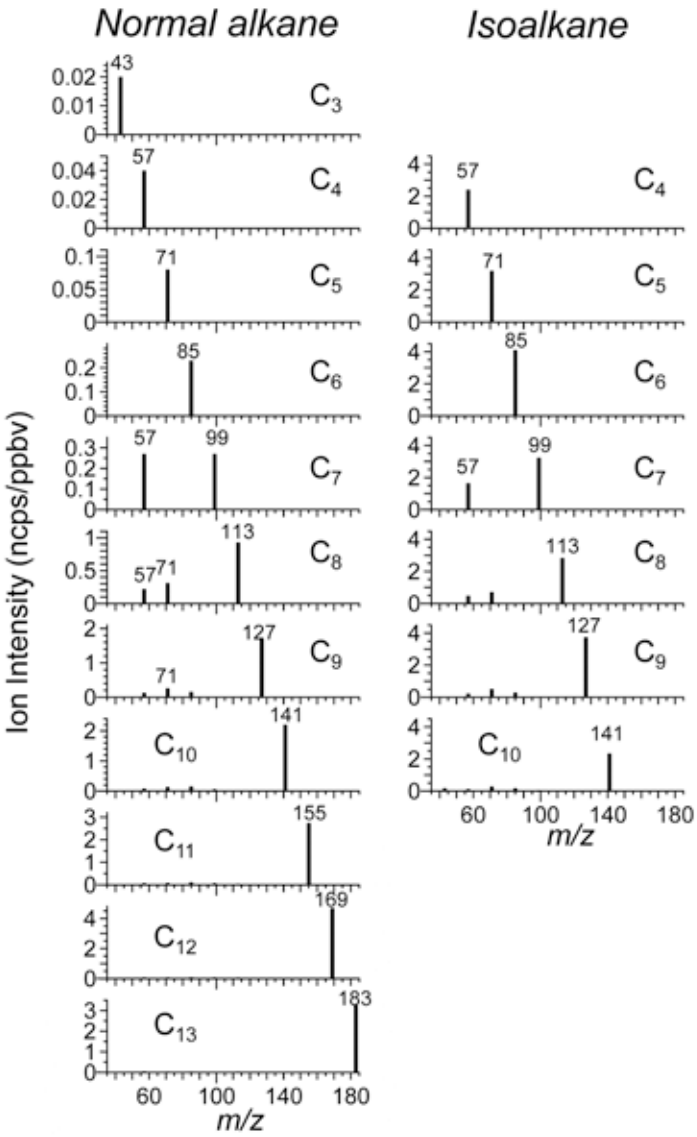

Figure 1: Normalized detection sensitivity (ncps/ppbv) for a series of n-alkanes and isoalkanes.

We conducted three continuous day diurnal breathing loss (DBL) tests on nine vehicles which have been used in Japanese market to confirm the variety of breakthrough features among different car models [6]. Two of them were made by US and European manufacturers. Both of

them meet with not only Japanese regulation but also those in each maker's native countries. The evaporative emissions from gasoline vehicles were measured by placing a test vehicle in a variable temperature sealed housing evaporative determination (SHED) unit (VSH-9353; Hitachi Technology Engineering Inc., Tokyo, Japan) which met the requirements of the Japanese approval tests for evaporative emissions. Among nine vehicles, four vehicles exhibited huge increased emissions coursed by the breakthrough emissions during the experimental period and all of them were by Japanese manufacturers. The compositions of the breakthrough and permeation emissions were analyzed in real time using NO^+ chemical ionization mass spectrometry to estimate the ozone formation potential for the evaporative emissions. The real-time measurements showed that the adsorption of hydrocarbons in a sealed housing evaporative determination unit can result in emissions being underestimated if the concentrations are monitored only before and after a DBL test. The composition analysis gave an estimated maximum incremental reactivity (MIR) 20 % higher for the breakthrough than for the gasoline that was tested, but the MIR for the permeation emissions was almost the same as the MIR for the fuel.

Refueling emissions from cars available on the Japanese market, which were not equipped with specific controlling devices, were also investigated [7]. The results of composition analysis indicated that the maximum incremental reactivity (MIR) of refueling emissions in Japan was 3.49±0.83, which is approximately 20 % higher than that for the gasoline that was tested. The emissions consist of 80 % alkanes and 20% alkenes, and aromatics and di-enes were negligible. C_4 alkene had the highest impact on the MIR of refueling emissions.

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Eddy-covariance flux measurements in a forest using PTR-ToF-MS, PTR-Q-MS, FIS and other instrumentations

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Abstract

A field campaign in a small forest in Ispra, Italy, was performed by JRC-Ispra, Italy, together with University of Innsbruck, Austria, in 2013, using PTR-ToF-MS (Proton Transfer Reaction – Time-off-Flight - Mass Spectrometry), PTR-Q-MS (Proton Transfer Reaction – Quadrupole - Mass Spectrometry), FIS (Fast Isoprene Sensor) and several other instrumentations. One of the main goals of this investigation was to compare the three techniques and to compare the results that could be extracted from each of them, focusing on the VOC-flux values that could be measured, the number of VOC's identified/measured, and finally if any oxidation products from primary emitted VOC could be identified/measured. Another goal was to look at the uptake of ozone by forest ecosystems (ozone deposition), which is attributed to both stomatal and non-stomatal pathways. The collected data were used to calculate daily and seasonal changes in ozone fluxes and also partitioning of total ozone fluxes between stomatal and non-stomatal sinks. Finally, additional results are still being extracted from the campaign.

Introduction

The forest flux station 'IT-Isp' is situated in a small and unmanaged forest inside the JRC-Ispra, situated in North Italy (location: 45.8127 N, 8.6338 E). The forest consists mostly of deciduous trees (Quercus robur L. (80%), Alnus glutinosa L. (10%), Populus alba L. (5%) and Carpinus betulus L. (3%)) with an average leaf area index of 4.2 m2/m2. It consists of a self-standing 36 m high tower with two platforms at 18 m and 36 m located at 45.8127 N, 8.6340 E. Infrastructural details: The instrumentation is placed in a 6m x 3m container with air conditioning, approx. 15 kW electrical power, local TCP/IP network to tower top with gateway to JRC network, data acquisition PCs and Campbell data loggers. The monitoring station is operated since 2012.

Experimental Methods

The main analytical techniques mainly applied here were two types of PTR-MS and one FIS. The PTR-MS technique for on-line detection of VOCs, which was developed at the University of Innsbruck, Austria, has been thoroughly reviewed elsewhere, see e.g. ref. [1,2]. Examples of the use of PTR-ToF-MS and PTR-Q-MS can e.g. be found in ref. [3,4,5]. The technique FIS and examples of the use can e.g. be found in ref. [6]. In addition, several other measurements were also performed during the campaign. *On tower top of tower measurements /sampling on top of tower:* NOx concentrations (Thermo); CO_2 & H_2O fluxes: Licor 7200 (and also the CO_2/H_2O vertical profile); O_3 fluxes; Sonic anemometer: Gill HS-100 (highly accurate vertical flow

analysis, HS-100 monitors wind speeds of 0-45 m/s and has an update rate of 100 Hz); temperature, RH, air pressure and precipitation; net radiation (measures the difference between downward/incoming and upward/outgoing radiation from Earth); Photosynthetically Active Radiation (PAR), total, diffuse (measures the spectral range (wave band) of solar radiation from 400 to 700 nanometers that photosynthetic organisms are able to use in the process of photosynthesis); fraction of Absorbed PAR, FaPAR (the Photosynthetically Active Radiation spectral region that is absorbed by a photosynthetic organism). *On the ground close to tower measurements:* Soil heat flux; soil temperature and water content profiles; ground water level; NO/NO_2 soil fluxes: automated dynamic chamber system; hemispheric photography for LAI (used to calculate solar radiation transmitted through (or intercepted by) plant canopies, as well as to estimate aspects of canopy structure such as Leaf Area Index (LAI)); litter collection (biomass) in the forest; soil respiration (CO_2 emissions from the soil). A nearby EMEP/GAW station (within 200 m) measures many other pollutants/parameters (for a complete list of the additional measurements, see ref. [7]).

Results and Discussion

Findings with PTR-ToF-MS / PTR-Q-MS / FIS:

Figure 1 shows that isoprene fluxes could easily be measured by PTR-ToF-MS. In addition, several other VOC's were identified/measured in high enough concentrations, so that the fluxes could be measured (protonated masses): 31.017 CH3O Formaldehyde; 33.034 CH5O Methanol; 42.033 C2H4N Acetonitrile; 43.017 C2H3O Acetic Acid fragment + interferences; 43.051 C3H7 Typical fragment; 45.034 C2H5O Acetaldehyde; 47.052 C2H7O Ethanol; 57.049 C2H5N2 C3H4O Instrument background + e.g. acrolein; 59.050 C3H7O Acetone / propanal; 61.030 C2H5O2 Acetic acid / methyl formate; 63.014 C2H6S DMS; 69.069 C5H9 Isoprene; 71.054 C4H7O MVK / MACR / croton-aldehyde; 73.034 C3H5O2 Propionic acid e.g.; 73.071 C4H9O Methyl-ethyl-ketone / butanal; 79.051 C6H7 Benzene; 87.056 C5H10O Unknown;; 89.055 C4H9O2 Unknown; 93.068 C7H9 Toluene; 107.078 C8H11 C8-alkylbenzenes; 121.097 C9H13 C9-alkylbenzenes; 137.145 C10H17 Monoterpene. Finally it should be mentioned, that several oxidation products from primary emitted VOC could clearly be identified, e.g. MVK/MeCr from isoprene. Figure 1 also shows that isoprene fluxes could also easily be measured with PTR-Q-MS. In addition, several other VOC's were identified/measured: Potential interesting masses identified by PTR-Q-MS with a significant count/value during the summer campaign (protonated masses): mass 33 (methanol), mass 42 (acetonitrile), mass 43 (acetic acid and fragments/interferences), mass 45 (acetaldehyde), mass 59 (acetone), mass 61 (acetic-acid/methyl-formate), mass 73 (propionic-acid/methyl-ethyl-ketone/butanal), mass 87 (unknown), mass 89 (unknown), mass 93 (toluene), mass 107 (C8-alkyle-benzene), mass 137 (monoterpenes). Finally, Figure 1 also shows that isoprene fluxes could also easily be measured with FIS. FIS is only measuring isoprene.

Some overall findings from this section:

During the summer campaign in 2013, isoprene concentrations were measured up to 16 ppb. In addition, isoprene fluxes up to 85 nmole m^{-2} s^{-1} were measured. During a winter campaign in 2013 in the same forest, no isoprene fluxes were observed (measured with PTR-Q-MS). It should be mentioned, that isoprene-flux-measurements were possible with all 3 instruments, but up to 30 % differences were observed (see Figure 1). Table 1 show that about 20-30 VOC's were clearly identified by PTR-ToF-MS and fluxes can be achieved/measured for most of them. Using PTR-Q-MS much less VOC's could be clearly identified and it was only possible to achieve fluxes (from biogenic compounds) for isoprene. It could also be seen in Table 1 that with PTR-ToF-MS several oxidation products from primary emitted VOC could clearly be identified, e.g. MVK/MeCr from isoprene.

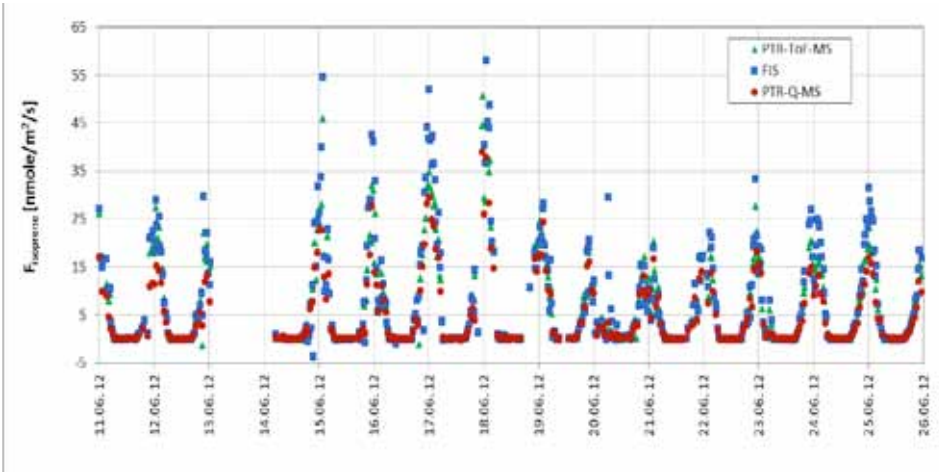

Figure 1: Isoprene fluxes measured with all 3 instruments during summer 2013. Explanation to x-axis: E.g. "18.06.12" stands for 18.06.2013 at 12:00 GMT/UTC.

Uptake of ozone (ozone deposition):

Figure 2 shows the daily averages of ozone concentrations/fluxes during summer 2013. High levels of ozone concentration were observed (up to 118 ppb as a peak value with a 30 min. average) during the measurement period of the campaign, and with ozone fluxes reaching up to -40 nmol m-2 s-1 (peak value with a 30 min. average). Total ozone fluxes varied diurnally with maximum values at midday and minimum values close to zero at night (Fig. 3). Stomatal ozone fluxes resulted in minor part of the total ozone flux over the studied forest ecosystem (23%). The measurements have shown that the ozone deposition is for the major part due to the non-stomatal sinks, however the ratio of the stomatal to total ozone flux was subject to seasonal and diurnal changes.

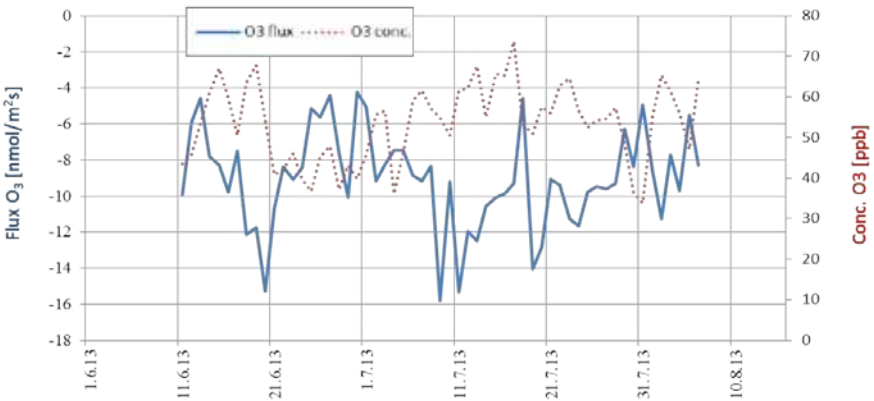

Figure 2: Daily averages of ozone fluxes and concentrations during summer 2013.

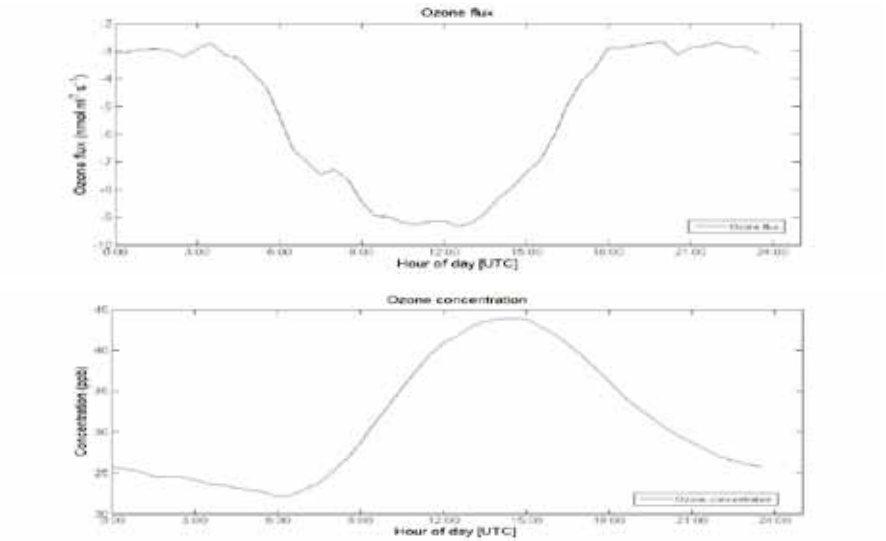

Figure 3: Diurnal cycle of ozone flux and ozone concentration (average summer 2013).

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Comparison of top-down and bottom-up reactivity of isoprene oxidation

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Abstract

The oxidative fate of non-methane volatile organic compounds (VOCs, reactive carbon) is directly coupled to the formation of secondary pollutants, such as ozone and secondary organic aerosol, that affect human health and climate. Measurements of the lifetime of the OH radical, the dominant VOC oxidant, provide a top-down constraint on the amount of reactive carbon and its processing rate [1]. Previous work has highlighted that bottom-up approaches using the sum of measured and modeled compounds often predict much lower VOC reactivity than the top-down constraint also in isoprene dominated environments[1-7].

During the 2013 Southern Oxidant and Aerosol Study (SOAS) field campaign, OH reactivity, isoprene, and key first-generation and later-generation isoprene oxidation products were measured over the South-East United States. The observed OH reactivity can largely be accounted for, with an average "missing" reactivity of ~25%. Measurement and model discrepancies are used to explore the plausibility of unknown physical and chemical carbon sinks [9]. We also discuss the role of isoprene hydroxyl hydroperoxides (ISOPOOH) and related molecules in interpreting OH reactivity and the reactive carbon budget [10].

Introduction

On a global scale, biogenically emitted isoprene is the most abundant nonmethane volatile organic compound (VOC) [8], and therefore a key source of reactive carbon in the earth's atmosphere. Because there are significant uncertainties in the oxidation mechanism of isoprene, and little work examining the deposition rates of the key oxidation products, predicting the total amount, the speciation, and the fate of isoprene oxidation products is difficult. Therefore, the fate of reactive carbon is also poorly constrained.

Previous studies in isoprene dominated environment have often shown large differences between measured VOC reactivity and that calculated from measured or modeled species [1-7]. During the 2013 Southern Oxidant and Aerosol Study (SOAS) field campaign, OH reactivity, isoprene, and a comprehensive suite of VOC oxidation products were measured, providing a unique basis for comparing measured (top-down) and calculated (bottom-up) VOC reactivity. The goal of the study is to address the question whether all sources of reactive carbon accounted for by measured and modeled volatile organic compounds (VOCs) and oxidized VOCs (OVOCS) during SOAS?

Recently, questions have arisen regarding the role of ISOPOOH both with respect to the OH-reactivity measurement method as well as with respect to speciated measurements of VOC oxidation products. Current understanding of this topic will be discussed [10].

Results

Figure 1 shows a comparison between measured and modeled OH reactivity with the model constrained to measured OVOCs. Model and measured values are well correlated (r^2=0.85), with a slope of 0.80 ± 0.02. The average missing reactivity for all measurement points is 16 ± 18%. This is less than the combined model and measurement uncertainty and, on average, we find no significant discrepancy between modeled and measured OH reactivity. However, a subset of points falls out of this range, and these points correspond to conditions with the highest monoterpene concentrations. Figure 2 shows contributions from different compound classes to the reactivity which demonstrates that the reactivity at the site is dominated by primary VOC emissions. However, when the model is not constrained to observed VOC oxidation products the modeled concentrations of most first generation VOC oxidation products are significantly (3-7 times) overpredicted compared to measurements. The measured concentrations of OVOCs suggest surprisingly little intracanopy oxidation of primary VOCs at this site, considering the measured OH concentrations. The cause remains unclear and is under further investigation.

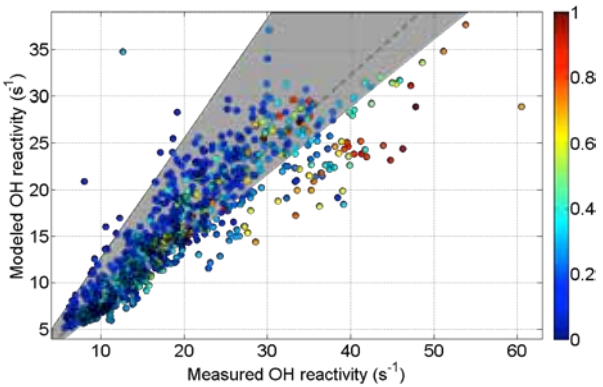

Figure 1: Modeled and measured OH reactivity. The color scale corresponds to the concentration of β-pinene in ppbv. An uncertainty-weighted linear least squares fit of the modeled reactivity gives y = 0.80 x +0.036 with an r^2 = 0.85.

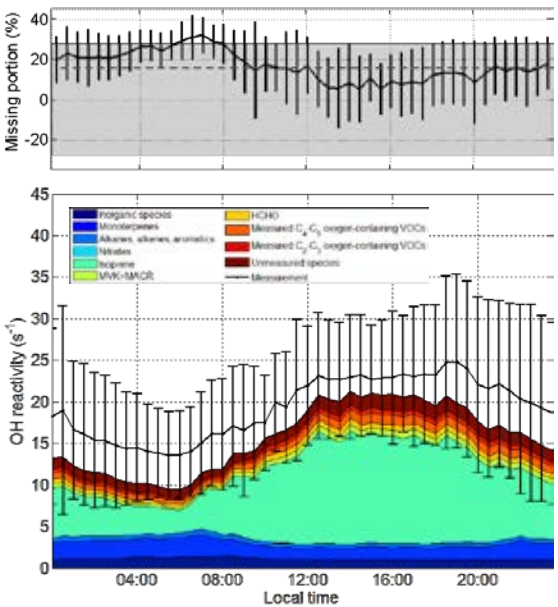

Figure 2: The top graph shows average missing OH reactivity and the bottom shows the contribution from individual measured and modeled species.

Discussion

The total OH reactivity is typically well captured, suggesting the total production rate of peroxy radicals and oxidation products and resulting ozone production is well captured. The trend in total OH reactivity with isoprene suggest small but significant missing reactive carbon that could be more important at higher total emissions. The discrepancies are potentially related to missing terpene emissions, which contribute significantly to secondary organic aerosol (SOA) at this site. Model misrepresentation of isoprene OVOCs suggests further molecule-centric studies are needed to understand SOA, as IEPOX also contributes to SOA.

An ongoing topic of research within this context is the role of compounds that rapidly recycle OH. For example, the oxidation of ISOPOOH with OH recycles OH on a very short timescale. If this timescale is shorter than the timescale of the OH reactivity measurement, the measurement will not measure the reactivity from these compounds and hence should not be included in the OH reactivity calculated from individual species. One of the open questions for OH reactivity measurements is what fraction of the OH reactivity of compounds that rapidly recycle OH is captured by the measurement. At the same time, it has now become clear that the same compounds, e.g., ISOPOOH, are observed as lower reactivity compounds in a number of methods that measure speciated compounds, potentially resulting in errors in the reactivity calculated from observations. We will present the current state of this active research area.

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Observationally Constraining Unknowns in Tropospheric Oxidation Capacity and Reactivity

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Abstract

We will discuss how uncertainty in reactive VOC observations can propagate into our ability to understand tropospheric oxidation capacity especially in high BVOC and low NO environments. In such environments, several observational studies have reported conflicting outcomes in assessing oxidation capacity and total amount of reactive gases in the boundary layer. The discussion on potential systematic discrepancies among the analytical techniques and their impacts on the modeling capacity will be discussed.

Introduction

Since the atmospheric chemistry community realized that hydroxyl radical (OH) controls chemical lifetime of most tropospheric trace gases[1], the OH oxidation reaction rates and the oxidation products of the trace gases have been intensely studied[2-5]. Analytical techniques to characterize trace gases, their oxidation products and oxidants such as OH have been also developed for laboratory and field applications[6, 7]. Anecdotal intercomparison studies indicate that isoprene the most dominant BVOC can be observed in the range of 20 – 80 % by applying different analytical techniques[8]. In addition, Lelieveld and colleagues reported higher than expected OH (~ 5 times)[9] triggered follow up studies many different aspects such as isoprene oxidation mechanisms[10]. In that context, evaluations of potential positive artifacts on OH quantifications especially in high isoprene environments were also condcuted[11]. We will review recent research outcomes on instrument evaluations on BVOCs, OVOCs and OH in field conditions and discuss implications towards tropospheric photochemistry.

Results and discussion

During the presentation, we will present two recent field observational results. The SOAS (Southern Oxidant and Aerosol Studies) campaign was conducted in Brent, Alabama USA located in the middle of an oak wood land with low to moderate NO conditions. A multiple instrumentation to quantify OH, OH reactivity, and VOCs was deployed to the ground site. Therefore, we will discuss the uncertainty from utilizing different analytical techniques in understanding tropospheric oxidation capacity. In addition, we will discuss how carefully characterized observational datasets of trace gases and oxidants can enhance our photochemical modeling capacity.

A presentation on a comprehensive VOC and oxidant dataset from the GoAmazon-2014 field campaign will be followed. With the dataset, we will examine the previous reports on oxidation capacity, which consistently claimed unaccountably high OH concentrations.

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Nighttime chemistry and morning isoprene drive urban ozone downwind of the isoprene volcano

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Abstract

Isoprene is the predominant volatile organic compound emitted to the atmosphere [1] and shapes tropospheric composition and biogeochemistry through its effects on ozone [2], other oxidants [3; 4], aerosols [5], and the nitrogen cycle [6]. Isoprene is emitted naturally by trees and shrubs during daytime, when its photochemical oxidation is rapid and in the presence of nitrogen oxides (NO_x) produces ozone and degrades air quality in polluted regions. Here we show for a city downwind of a major deciduous forest that isoprene actually peaks at night, due to transported late-afternoon emissions; ambient levels then endure owing to low nighttime OH radical concentrations. Nocturnal chemistry controls the fate of that isoprene and the likelihood of a high-ozone pollution episode the following day. When nitrate (NO_3) radicals are suppressed, high isoprene persists through the night, providing photochemical fuel upon daybreak and leading to a dramatic morning ozone peak. On nights with significant NO_3, isoprene is removed before dawn; days with low morning isoprene then have lower ozone with a more typical diurnal profile and an afternoon peak. This biogenic-anthropogenic coupling expands the daylight hours with persistently high ozone and likely has opposite ozone-NO_x response as otherwise expected, with implications for exposure and air quality management in such areas.

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Ambient air VOCs at an urban site of India: Results from first PTR-TOF-MS measurements in India

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Abstract

The proton transfer reaction-time of flight-mass spectrometry (PTR-TOF-MS) technique has been used for the measurements of ambient volatile organic compounds (VOCs) at Physical Research Laboratory (PRL), Ahmedabad (23.03oN, 72.58oE, 49 m asl), India. This is the first and only PTR-TOF-MS system available in India for the high mass resolution measurements of VOCs. In the winter season, emissions from vehicular exhaust make the major contribution to ambient levels of most of VOCs. However for several important VOCs, emissions from biogenic and secondary sources were also found to be significant. The time series trends of daily VOCs during the study period were controlled by the change in synoptic scale weather condition and the pattern of long-range transport. The mean mixing ratios of methanol, acetone and acetaldehyde were 20.6, 5.8 and 5.3 ppbv, respectively. While the levels of two aromatic VOCs namely benzene and toluene were 2.3 and 5.9 ppbv, respectively. The bimodal diurnal variation of anthropogenic VOCs was primarily due to the major contribution from local traffic. In spite of dominant emissions from anthropogenic sources at the study site, the levels of isoprene increased with the increasing ambient temperature. The similarity between the diurnal cycles of isoprene/benzene ratio and temperature emphasizes the emissions from biogenic sources as well.

Introduction

The mixing ratios of VOCs show large spatial and temporal variation from pptv levels in the remote sites to order of several ppbv in the industrial and urban locations [1]. In the South Asia region, the atmospheric chemistry is complex due to different climatic zones and also due to the coexistence of anthropogenic and natural emission sources. Indian subcontinent is experiencing severe air pollution due to the fast urbanization and ever increasing number of automotive vehicles. Consequently, the study of ambient air VOCs especially in the major cities of India is very important. Thus far, the measurements of VOCs in India are limited to several sites. In recent years, the comprehensive study of urban VOCs has been initiated in the different parts of South Asia. In addition to direct emissions from vehicular exhaust, some oxygenated-VOCs (OVOCs) are produced from the reactions of peroxy (RO2) and alkoxy (RO) radicals [2]. This study is based on the high time resolution measurements of dominant VOCs using a PTR-TOF-MS at an urban site from December 2013 to January 2014. The primary objective of this study is to characterize the role of VOCs emitted from the different sources and also the photochemical aging.

Experimental Methods

The observation site, in the campus of PRL, is located in the western region of the Ahmedabad city. This is the capital city with a population of over 6.5 million (according to the census year 2011) of Gujarat State in the western India. The climate of this region can be described as semi-

arid. The fast urban expansion and industrial activities are the major source of increasing pollutants (both gaseous and particulate) in ambient air of the city. The city has a total of around 3.2 million registered vehicles which is increasing at the rate of about 9-10% per year.

A high mass resolution PTR-TOF-MS 8000 instrument (Ionicon Analytik GmbH Innsbruck) was installed at the campus of PRL in Ahmedabad in November 2013. The drift pressure, voltage and temperature were held at 2.3 mbar, 600 V and 50 oC, respectively. These set of operating parameters correspond to an E/N ratio of about 129 Td (Where E = strength of electric field; N = number density). In the drift tube, air samples were introduced through a heated PEEK tube (1.5 m long) at a flow rate of about 60 mL min-1. The PTR-TOF-MS mass spectra up to 280 m/z were measured in the hydronium ion (H3O+) mode. The normalized sensitivity and set value (ppbv) showed excellent linear relations (r2 >0.98) for different VOCs. However, varied from compound to compound, the overall uncertainty was less than 13.5% which includes uncertainties in the flow of the gas calibration unit (GCU), relative humidity (RH) of zero air and calibration mixture (±5%).

Results and Discussion

The observation site is influenced by the long-range transport of continental pollutants due to NW-NE winds in the winter season. The large variation in the daily mixing ratios of all VOCs was mainly caused by the variation in local and synoptic weather conditions. Among the VOCs measured during this study, the contribution of OVOCs was largest of about 75% with methanol as the most abundant compound. On the diurnal scale, the mixing ratios of VOCs exhibit bimodal variation with peaks during morning and evening hours. The highest of toluene/benzene (T/B) ratio was measured during evening rush hours suggesting the fresh emissions from automotive vehicles. Some delays in the occurrence of peak concentrations of acetone and acetaldehyde from morning peak traffic time suggest the role of secondary or photochemical production. The mixing ratios of VOCs decreased rapidly with the increasing wind speed. During the daytime on clear-sky days, the levels of isoprene increased with the increasing temperature.

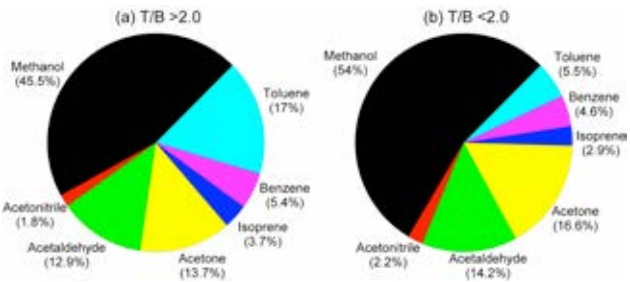

Figure 1: The mean relative contribution (%) of each compound to the total VOCs in two different air masses of (a) T/B > 2.0 ppbv ppbv^{-1} and (b) T/B < 2.0 ppbv ppbv^{-1}.

The average relative contribution of each VOC to the measured VOCs in the fresh and aged air masses is shown in Figure 1. The abundance of OVOCs in photochemically aged air masses was higher than those measured in the fresh emissions. The contributions of OVOCs in fresh (T/B > 2.0) and aged (T/B < 2.0) air masses were ~72% and 85%, respectively. In fresh air emissions, about 13% deficit of OVOCs was counterbalanced by the higher percentage contribution of primary VOCs. Among the primary VOCs measured during this study, the contribution of toluene

was highest in both fresh (17%) and aged (5.5%) air masses. The results presented in this study will provide key inputs to the researchers working in the field of tropical chemistry and national policy makers to control the air pollution. The studies of VOCs need greater attention to understand the regional importance of biogenic emissions and photochemical process in South Asia region.

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Characterization of Trace Compounds Present in Blast Furnace Gases using PTR-SRI-QiTOF-MS. A First Step towards Online Measurement On-Site

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Abstract

Up to date, blast furnace gases produced in the steel industry have found no real application as feedstock or have been recycled in an efficient way. They have been only used to preheat the ovens or to produce electricity in steam engines. The growing toughness of environmental regulations in the European Union and the increase of costs for carbon credits due to CO_2 emissions has pushed companies to rethink their processes and improve efficiency through more sustainable programs. This imposes new challenges, since the utilization of the typical gases produced in the steel industry, such as coke oven gas, blast furnace gas and converter gas can be seriously compromised due to the high number of trace compounds, which could act as a poison for any subsequent catalytic process. Although many trace compounds are found in these gases only in the lower ppm or ppb concentration range, they could deactivate a specific catalyst within minutes. Since until now, the detection of such low-concentration substances has been of little interest and a complete characterization would be time consuming and expensive, there is a lack of knowledge about their concentration fluctuations and their persistence after a conditioning process.

In order to tackle this problematic, the main aim of this study is to develop a methodology to characterize the gases of the steel industry in a container installed directly on-site, so that different catalysts for reactions, such as methanol synthesis, can be tested under real conditions. For the analysis of trace compounds, a new generation of a mass spectrometer, the so called Proton-Transfer-Reaction Selective Reagent Ionization Quadrupole ion guide Time-of-Flight Mass Spectrometer (PTR-SRI-QiTOF-MS) will be used. This prototype is equipped with additional features like a Fast-GC for the identification of possible isomers and two NDIR gas sensors for the determination of the main components CO and CO_2. Additionally, a NDIR-Gas analyzer and two gas chromatographers (GC-FID/TCD) will be used for the analysis of main the components and the performance of the methanol catalysts. The gathered information will be used for the design of tailor-made purification processes and as output, a database of the identified traces will be generated together with their spectra and their optimum ionization parameters.

Introduction

The interest for the utilization of waste gases from steel mill plants as feedstock for the production of chemicals has been growing in the last years due to economical and political reasons. Since environmental regulations in the European Union are becoming stricter and the costs for CO_2 emissions are making products and processes less competitive, a search for more sustainable ways of operation is growing. During the different steps of the steel production three different gases are generated, namely coke oven gas (50-70% H_2, 5% CO, 25-30% CH_4 and 10%

N_2), blast furnace gas (2-4% H_2, 20-30% CO, 20-25% CO_2 and 45-60% N_2) and converter gas (2% H_2, 65% CO, 15% CO_2 and 18% N_2). Apart from the main components, these gases exhibit a variety of volatile organic compounds (VOCs) in reduced or oxidized form, such as C2-C3 alkanes, sulfur-containing compounds, small and polycyclic aromatic compounds (BTEX, PAHs) and also inorganic compounds. Although the concentration of these VOCs lies normally in the ppm or ppb range they could act as potential poisons for methanol catalysts. Therefore, their removal is mandatory if the blast furnace gases are used as feedstock in subsequent downstream catalytic processes.

The usual characterization of these metallurgical gases limits mainly to the analysis of the major compounds and only offline analyses are carried out within certain time intervals for the determination of trace compounds. These offline sampling procedures exhibit many drawbacks such as, extremely long sampling times and time consuming sample preparation, which allows only an average determination of the trace compounds and thus, possible concentration peaks cannot be recorded. The measurement of these metallurgical gases with conventional techniques like gas chromatography coupled with mass spectrometry (GC-MS) after sampling with for instance, Tedlar® gas bags exhibits low sensitivity and significant recovery losses due to wall adsorption effects. More sensitive techniques like thermodesorption coupled to GC-MS offers higher sensitivities towards semi- and low-volatile compounds but it exhibits several drawbacks since depending on the strength of the adsorbent chosen, highly volatile compounds may breakthrough if the sampling time is too long and the recovery of low-volatile compounds is compromised due to strong adsorption effects. Moreover, some adsorbents exhibit high background levels and could also be reactive towards certain compounds, making identification and quantification a hard task.

For online analysis of the trace compounds in metallurgical gases, these conventional techniques seem not suitable and therefore, the application of a PTR-SRI-QiTOF-MS appears as a promising technique, which will enable the determination of low-concentration trace compounds, the assessment of concentration peaks and a better understanding of the temporal changes in the gas phase. Current work focuses on the preliminary analysis of offline samples (coke gas, blast furnace gas and converter gas), the method development and the optimization of the PTR-SRI-QiTOF-MS before the test container is taken to the steel mill plant.

Experimental Methods

For the online analysis of trace compounds present in the gases of a steel mill plant, a test container has been developed and equipped with a new generation mass spectrometer (Fig. 1); the so called Proton-Transfer-Reaction Selective-Reagent-Ionization Quadrupole ion guide Time-of-Flight Mass Spectrometer (PTR-SRI-QiTOF-MS, from IONICON). This prototype is a state-of-the-art instrument, which is equipped with additional features like a Fast-GC for the identification of possible isomers and NDIR gas sensors for CO and CO_2. Additionally, two reactor setups (Premex), two gas chromatographers (GC-FID/TCD, Agilent) and a gas analyzer (NDIR, Emerson) will be used for the analysis of main components and the performance of the methanol catalysts.

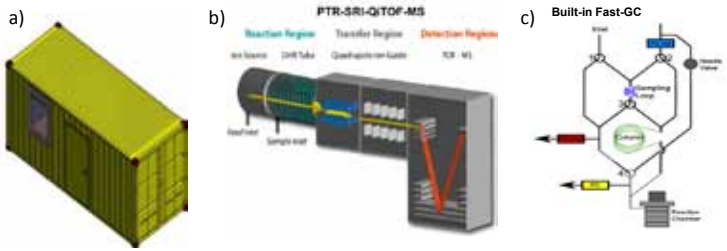

Figure 1: (a) Picture of the test container used for online measurements on-site. (b) Description of the PTR-SRI-QiTOF-MS after [1]. (c) Sketch of the built-in Fast-GC add-on taken from [2].

Preliminary offline characterizations of the different metallurgical gases using washing with liquids and solid adsorbents (not shown here) have indicated that for reliable measurements, long sampling times are needed and the use of for instance, Tedlar gas bags drives to recovery losses and introduces some cross contamination (Fig. 2b). Conventional techniques like GC-MS are not sensitive enough for the detection of most trace compounds and even the use of more sensitive methods such as thermodesorption tubes (TD-GCMS) appears as challenging due to the expected breakthrough of highly volatile compounds. For the TD-GCMS analysis preconditioned commercial Tenax TA tubes were used and measured using a TD20 and GCMS QP2010 from Shimadzu. During sampling two tubes were put in series with a flow of 100 ml/min and were loaded for 1 minute. For comparison purposes using the PTR-SRI-QiTOF-MS samples were taken using 1 L Tedlar gas bags. During measurements three primary ions (H_3O^+, NO^+ and O_2^+) were changed in order to check for those substances, which cannot be measured with H_3O^+ due to their lower proton affinities. All tests were carried out at room temperature.

Results and Discussion

Since the PTR-SRI-QiTOF-MS is a prototype, initial work has been focused on the optimization of measurement parameters and the testing of the limits of this apparatus. Figure 2a shows for instance the effect of the pressure and voltage change inside the drift tube. Regarding the primary ion H_3O^+, the drift pressure appears to have a more significant effect than the voltage. Up to 500 ml/min the inlet flow shows no influence on the compounds measured. However, for "sticky" compounds higher flows favor the signal, since equilibrium is established much faster. In Fig. 2b it is apparent that the concentrations of the compounds measured appear to be higher when using coated Tedlar® gas bags. This is very significant since typical offline sampling is carried out with such bags. The storage time is expected to have also an influence because low-volatile compounds would adsorb on the bag walls, thus reducing their recovery, whereas the concentration of some high-volatile compounds would increase if the main components (H_2, CO, CO_2, CH_4) diffuse out the gas bag. The permeability of coated bags is much lower than for clear gas bags and therefore such artefacts are minimal with this kind of bags.

The preliminary offline analyses shown in Figure 3 make clear that the utilization of TD-GCMS exhibits some drawbacks like the breakthrough of highly volatile compounds even at short sampling times (1 minute). Tenax TA was chosen for the preliminary analyses due to the known low background levels and good recoveries. However, for high-volatile organic compounds stronger adsorbents (e.g. activated carbons) are needed, which for low-volatile compounds exhibit poor recoveries. Moreover, the chromatograms in Fig. 3a indicate that longer sampling times are required since the sensitivity for compounds eluting after 20 minutes is very low. Fig 3b shows

that offline sampling with Tedlar gas bags (clear or coated) exhibit also some drawbacks due to cross contamination, since such gas bags are not 100 % inert and consequently, some volatile organic compounds (VOCs) may desorb during the storage time. Moreover, if the gas bags are not coated, labile compounds could be depleted due to photolytic reactions. Both coated and uncoated gas bags exhibit for low- and semi-volatile compounds significant wall adsorption effects, which results in poor recoveries.

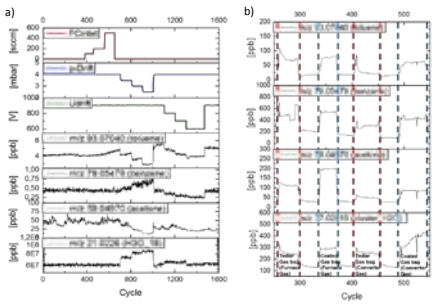

Figure 2: (a) Effect of some measurement parameters on the concentration of selected masses, (b) Comparison of two Tedlar gas bags with and without coating using blast furnace gas and converter gas.

In Fig. 3b it becomes apparent that the concentration of some compounds in clear gas bag higher is as in the coated gas bag (e.g. m/z 88 u). On the other hand, the coated gas bag shows higher concentrations for masses like m/z 61 u. Therefore, an online characterization of the metallurgical gases using PTR-SRI-QiTOF-MS appears mandatory. Additionally, the application of PTR-SRI-QiTOF-MS offers the advantage of very mild ionization by using primary ions like H_3O^+, NO^+ and O_2^+, which means that the fragmentation of the VOCs is minimal, thus making their identification and quantification possible.

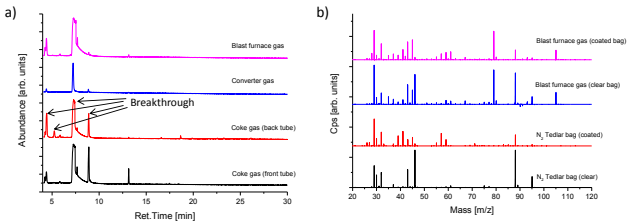

Figure 3: Offline measurements of metallurgical gases using (a) TD-GCMS and (b) PTR-SRI-QiTOF-MS.

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Primary results of VOC measurement using PTR-MS in Beijing, China

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Abstract

There are numerous species of volatile organic compounds (VOCs) in the atmosphere playing an important role in many atmospheric chemical procedures and their concentrations change all the time because of several factors such as various sources, complex photochemical reactions, and potential transports. PTR-ToF-MS offers an online and sensitive method to measure VOCs in very low concentration, which is helpful to well know and study VOCs and the related problems such as formation of ozone and fine particles as well. Recently, a PTR-Tof-MS 8000 was applied to measure VOCs in urban air of Beijing, China and here we report some of the primary results of the measured VOCs concentration and their diurnal variation characteristics in different weather and emission conditions.

Tracing plant volatile organic compound emissions and CO_2 fluxes by stable isotopes

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Introduction

Plant metabolic processes exert a large influence on global climate and air quality through the emission of the greenhouse gas CO_2 and volatile organic compounds (VOCs). Despite the enormous importance, processes controlling plant carbon allocation into primary and secondary metabolism, such as respiratory CO_2 emission and VOC synthesis, remains unclear.

The vegetation exerts a large isotopic imprint on the atmosphere through both, photosynthetic carbon isotope discrimination and fractionation during respiratory CO_2 release ($\delta^{13}C_{res}$). While the former is well understood, many processes driving carbon isotope fractionation during respiration are unknown. There are striking differences in variations of $\delta^{13}C_{res}$ between plant functional groups, which have been proposed to be related to carbon partitioning in the metabolic branching points of the respiratory pathways and secondary metabolism, which are linked via a number of interfaces including the central metabolite pyruvate. Notably, it is a known substrate in a large array of secondary pathways leading to the biosynthesis of many volatile organic compounds (VOCs), such as volatile isoprenoids, oxygenated VOCs, aromatics, fatty acid oxidation products, which can be emitted by plants.

Here we investigate if carbon isotope fractionation in light and dark respired CO_2 is associated with VOC emissions in the atmosphere. Specifically, we hypothesize that a high carbon flux through the pyruvate into various VOC synthesis pathways is associated with a pronounced ^{13}C-enrichment of respired CO_2 above the putative substrate, as it involves the decarboxylation of the ^{13}C-enriched C-1 from pyruvate.

Experimental Methods

Based on simultaneous real-time measurements of stable carbon isotope composition of branch respired CO_2 (CRDS) and VOC fluxes (PTR-MS) we traced carbon flow into these pathways by pyruvate positional labeling.

Results and Discussion

We demonstrated that in a Mediterranean shrub the ^{13}C-enriched C-1 from pyruvate is released in substantial amounts as CO_2 in the light. Simultaneously, naturally ^{13}C depleted C-2 and C-3 carbon atoms of the acetyl-moiety are emitted as a variety of VOCs. Moreover, during light-dark transitions leaf emission bursts of the oxygenated metabolite acetaldehyde were observed as part of the PDH bypass pathway in the cytosol[2]. This may be a new piece of evidence for the origin of ^{13}C-enriched $\delta^{13}CO_2$ which is released during Light-Enhanced Dark Respiration (LEDR).

Our study provides the first evidence that the isotopic signature of respired CO_2 is closely linked to carbon partitioning between anabolic and catabolic pathways and plants strategies of carbon investment into secondary compound synthesis.

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Applications in Food Science

Bioactivity of aroma compounds

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Abstract

Aroma-active compounds may not only serve as important indicators of food quality. Volatile aroma compounds, in particular, are gaining growing interest because of their potential health benefits with respect to behaviour, mood, satiety, and also the progression of several diseases such as irritable bowel syndrome, cancer, inflammation or cardiovascular diseases. Although most of the studies reported effects in rodents, there is considerable evidence that aroma compounds exert health beneficial effects in humans as well. Efficacy in humans has, for instance, been shown for a daily dose of 900 mg peppermint oil, which significantly reduced clinical symptoms of abdominal distension, bloating, abdominal pain, and diarrhoea in irritable bowel patients [1]. Of the most widely studied group of volatile aroma compounds, the terpenes, limonene and alpha-terpineol have been found to reduce the progression of experimentally initiated skin cancer in rodents, and to exert anti-inflammatory and satiating effects in experiments on human cells in culture and in human intervention studies.

While human intervention trials and animal studies are suitable for providing data on health benefits in vivo, cell culture studies may prove mechanisms of cellular uptake and action. However, pharmacokinetics, including intestinal degradation, absorption, transport and metabolic transformation in response to a given dose cannot be investigated in one cell system, but are mandatory for evaluating a compound's efficacy. Here, quantitative analysis of the parent volatile aroma compound and its metabolites not only in the preparation administered but also in target tissues plays a pivotal role in identifying in vivo-representative cell culture conditions. Alpha-terpineol, for instance, exerts it's anti-inflammatory activity prior to intestinal and metabolic transformation in buccal cells, but loses this activity after absorption into circulation.

Combining PTR-MS techniques with appropriate biochemical outcome measures will help to unravel the bioactivity and possible health beneficial effects of volatile aroma compounds.

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Linking Cocoa Beans to Chocolate: Volatile, multi-elemental and isotopic fingerprint comparisons

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Abstract

The properties of chocolate differ considerably from those of the raw cocoa beans; the identification of chemical links between the starting cocoa beans and the finished product would enhance fraud control.

Methodologies based on Mass spectrometry (MS) techniques were applied to chocolate and cocoa beans to identify chemical fingerprints of geographical origin and processing in the finished products. Resulting data were combined with chemometric analysis to discriminate the samples according to the origin and variety of the beans and the brand, and to allow a correlation between raw material and finished product. Preliminary results showed that the chocolate fingerprint is strongly affected by the conditions of production. However, the sensitivity of these techniques allowed discrimination of the chocolate by variety and origin of beans used.

The combination of MS techniques with chemometric analysis has shown to be a powerful technique emphasizing the possibility to use this methodology in finding the link between cocoa beans and chocolate.

Introduction

Food authentication ensures the composition and constituents of foods, the provenance of a product and protects from counterfeiting [1]. These measures become difficult for processed foods such as chocolate. The complexity of the processing steps and the ingredient composition make the chocolate bar differ completely from the raw cocoa beans and , therefore, more difficult to track. Identifying chemical links between the starting cocoa beans and the finished product would enhance fraud control.

Traditional techniques cannot fully satisfy the new needs of food authentication, as they just focus on specific markers or particular undesired compounds, which cannot characterize a product according to the origin or the production steps. Therefore, an analytical fingerprint approach may be more suitable. This methodology is a non-selective way of analysis and takes into account a complete spectrum or an image of the test material. Fingerprints can be generated through many analytical techniques such as gas chromatography, spectroscopic and spectral measurements [2].

For chocolate authentication, we are developing methodologies based on Mass spectrometry (MS) techniques to identify chemical fingerprints of geographical origin and processing in the finished products.

Experimental Methods

Ninety samples of dark chocolates available on the Dutch market were analysed using Proton Transfer Reaction–MS (PTR-MS) to determine the volatile organic components. The chocolates were collected considering the geographical origin and the variety of the cocoa beans, and the brand presented on the labels.

In a second step of the study, 10 different chocolates of a specific brand and the corresponding 10 beans used for them manufacturing the chocolate were analysed.

The investigation was carried out using different MS techniques:

- Proton Transfer Reaction-MS (PTR-MS)
- Inductively Coupled Plasma-MS (ICP-MS)
- Isotope ratio-MS (IRMS)

Resulting data were combined with chemometric analysis to generate fingerprints of chocolates and beans and to allow a correlation between the raw material and the finished product. Principal Component Analysis (PCA) was applied to reduce the data dimensionality in order to enable the data visualization and to find possible natural clusters, according to the chosen parameters (geographical origin, cocoa variety, brands). Partial Least Squares Discriminant Analysis (PLS-DA) was applied to classify the chocolate samples according to the beans' geographical origin (Africa, Asia, South-America), the beans' variety (Criollo, Forastero, Trinitario) and the chocolates' brand.

Results and Discussion

In the first step of the study, the PTR-MS fingerprints of 90 samples revealed a differentiation of the chocolates according to the brand and, the variety and geographical origin of beans showing a trend in the PCA samples distribution. PLS-DA further discriminated the samples according to the three classes giving good range of classification with an average efficiency of 98% for the brand, 94% for the variety and 93% for the geographical origin. However, PCA results showed cases overlapping and a zone of a mixed cluster; PLD-DA evidenced samples misclassification. The analysis of these ambiguous results stressed the interaction of the complex formulation and production process with the beans characteristics.

This approach proved that the volatile profiles of chocolates are strongly affected by the conditions of production. However, the sensitivity of these techniques allowed the discrimination of the chocolate depending on the variety and origin of beans used. This underlines that characteristic marks of the cocoa beans remain in the finished chocolate, making possible to characterize and differentiate the chocolates, and their ingredients, according to their geographical origins, brands, and types of cocoa.

To reduce the processing influence, 10 different chocolates of a specific brand were analysed in the second step of the study. In order to find a link between the chocolate bars and the raw material, the corresponding 10 beans used for them manufacturing the chocolate investigated too. The PCA plot in Figure 1 shows a correlation between chocolate and cocoa beans. Although beans and chocolates were separated in the first dimension of the PCA, in the second/third dimension the similarities between chocolates and corresponding beans became obvious. A clear correlation is evident in the Hierarchical Cluster Analysis (HCA) results (Fig.2). In this case, three different chocolates from the same origin,Trinidad, are linked with the related batch of beans, independently of the % of cocoa.

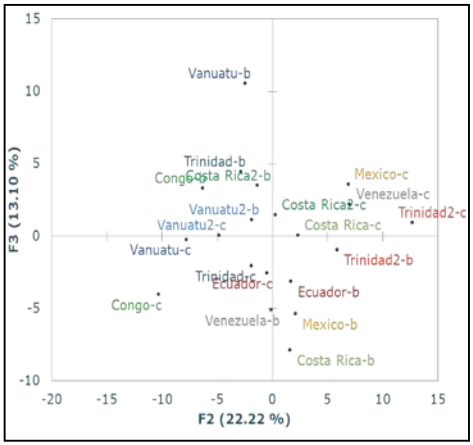

Figure 1:PCA 2D score plots (transforming: normalize) of the full PTR-MS spectra of chocolates (c) and related beans (b). Pearson Correlation coefficient between chocolate and beans scores was 0.88.

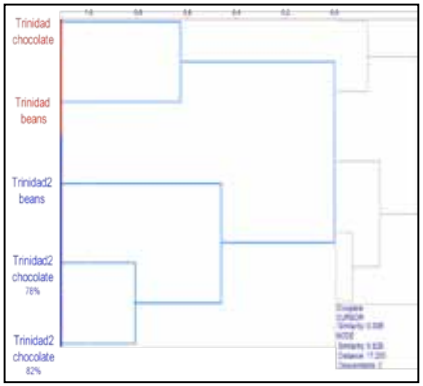

Figure2: HCA (transforming: normalize) links 3 different chocolates from Trinidad with the related batch of beans.

In conclusion we can state that the sensitivity of PTR-MS and the combination with the statistical analyses allowed a distinction of the chocolates by their brand, and the variety and geographical origin of the beans.

The first results on similarities in compositional properties between chocolates and beans of a single brand are promising and will be further explored in the near future comparing the volatile results with the multi-elemental and isotopic fingerprints.

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Measurements of aroma, fair and foul: Real-time breath measurements of aroma release and on-line detection of spoilage compounds from meat

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Introduction

Aromas and odors are the perception of VOCs by the human nose. PTR-MS is designed specifically to study VOCs and what's more, to do so in real time. Herein PTR-MS is exhibited to study pleasant aromas and foul odors. Firstly, PTR-MS is used as a tool for studying the aroma release profiles measured from the nasal cavities of a panel of taste volunteers while ingesting flavored solutions [1]. Secondly, measurements are reported as PTR-MS is used to monitor the odor profile progression from spoiling beef steak and chicken breast.

Aroma Release Profile

Highly sensitive gas detection techniques allow the temporal components of processes to be studied. From breath analysis to process monitoring, the information contained in these temporal profiles can be a challenge to uncover. Aroma release profiles are one such area of research.

A chief topic in aroma research is sampling methodology: How best to take a sample. A data scientific procedure is developed and applied to data obtained in pursuit of this topic.

Spoilage Odor Release

A further research area where temporal information is of valuable insight is the measurement of spoilage compounds contributing to the odor of spoiled meat products. Food wastage is currently at unsustainably high levels, to reduce these levels a better understanding of food spoilage is needed. To study the compounds responsible for food malodor, VOCs emitted from beef and chicken are studied using PTR-MS.

Experimental Methods

Aroma Release Profile

Results were obtained using four breath sampling protocols with two tasting solutions; milk and water, spiked with four aroma compounds (acetoin; 135 ppm, anisaldehyde; 137 ppm, ethyl butyrate; 93 ppm, ethyl octanoate; 135 ppm). Measurements of nasally exhaled breath are made with an Ionicon PTR-MS at Unilever R&D. The method of breath sampling uses a nose sampler, consisting of a heated block, short Teflon nostril tubes and a heated inlet line to the PTR-MS. Panelists rest their nose against the sampler, such that the nostril tubes sit gently in the panelists nose. The first three protocols investigate the effect of breathing control on measured aroma

release reproducibility: a free protocol is used as a baseline; a further protocol fixes the swallowing time; a third protocol fixes the swallowing time and the breathing rate. The fourth protocol investigates the effect of multiple swallowing on total aroma release. A study of eight subjects, repeating each protocol five times for both solutions produced a large data set, which was analyzed using multivariate techniques and a data pre-processing strategy.

Spoilage Odor Release

Measurements of meat spoilage odor are made using the Radboud University PTR-MS. The device matches closely the design of commercially available instruments. Meat is typically packaged in modified atmospheres; high concentrations of carbon dioxide in a balance gas of oxygen or nitrogen. Oxygen is used for red meat, to maintain its 'bloody' appearance, while nitrogen is used for non-red meat.

To accommodate the use of high carbon dioxide concentrations the PTR-MS is first calibrated for use in this scenario, the effect of varying carbon dioxide concentration is measured for a calibration mixture of 8 compounds with varying characteristics across a range of functional groups: Methanol, acetaldehyde, acetone, isoprene, benzene, toluene, o-xylene, α-pinene. The effect on fragmentation and detection sensitivity is observed as a function of both reduced electric field and carbon dioxide concentration.

To measure samples of beef steak and chicken breast, pre-packaged product is bought from a local supermarket and divided into 4 or 5 samples of approximately 35-40 g each. These samples are sealed in glass cuvettes, that could be selectively measured with the PTR-MS by using an automated valve system. Samples were measured in turn from the day of purchase to several days after the best before date. Sampling times were between 20-25 minutes per measurement. A background cuvette was measured in the intervening time between each cuvette for typically 15-20 minutes, this ensured a background measurement before each sample was measured.

Results & Discussion

Aroma Release Profile

A data scientific procedure is developed, that allows data analysis time to be significantly shortened. Firstly, a baseline correction is applied to the data to correct for any baseline drift (Figure 1A). After this, each breath peak is identified by its starting and ending points by locating minima of the acetone profile (Figure 1B). Starting and ending points corresponding to peaks with areas less than 25 % of the average peak area are discarded. Step 3 transforms the data to breath-by-breath values by integrating the ethyl octanoate function using a trapezoidal integration function using the limits found in step 2 (Figure 1C). The final step removes the background intensity of ethyl octanoate, leaving a breath-by-breath profile of the data (Figure 1D).

Ten aroma release profile parameters were extracted from the data; two from intensity measurements and eight from breath-by-breath analysis. These parameters were analyzed multivariately using multivariate partial least squares data analysis (ML-PLS-DA) to detect differences in the products under study. Ethyl octanoate revealed the greatest difference in product, having the highest logP (lipophilicity). Differences in the protocols were searched for using principle component analysis (PCA).

Comparing the pooled variance for each protocol and for each kinetic parameter it is possible to conclude that the protocol with the greatest amount of control (controlled breathing rate and swallow time) is optimal and recommended by this study.

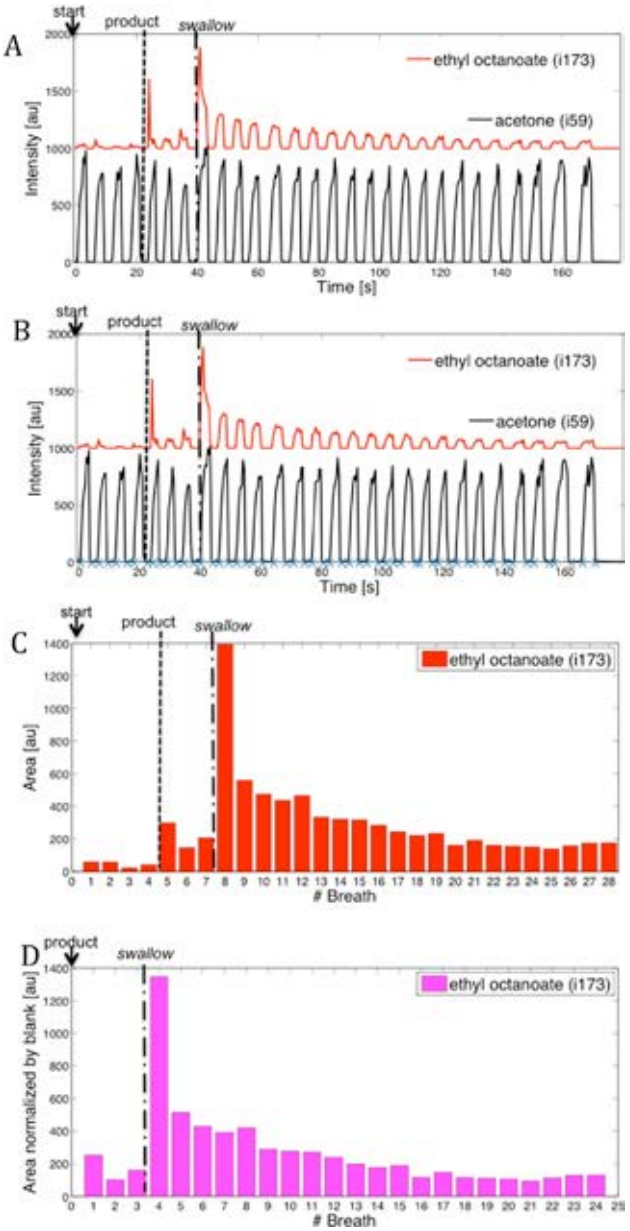

Figure 1: Transformation of a real time breath profile to a breath-by-breath profile. In panels A & B ethyl octanoate is offset by 1000 arbitrary units.

Spoilage Odor Release

To investigate the effect of carbon dioxide sample concentration, variations in carbon dioxide were made between 0 – 50 % in steps of 10 % while measuring a constant concentration of calibration gases. The trace gas flow was altered as necessary to maintain a constant drift tube pressure for changing carbon dioxide concentration to allow easy comparison of reduced electric fields across a range of carbon dioxide concentrations. The introduction of high carbon dioxide concentrations into the drift tube lessened the drift tube energy; the ion mobility is reduced by the introduction of carbon dioxide. The major effect of this lessened energy is to increase the abundance of ionized water clusters. At high carbon dioxide concentrations the sensitivity to compounds lowly reactive with the protonated water cluster is reduced, i.e. benzene, toluene, o-xylene. Compounds that react readily with the water cluster, either because of high proton affinity (α-pinene) or because of a high dipole moment (acetone) show increased sensitivity at higher carbon dioxide concentrations, possibly due to increased reaction times caused by the reduced ion velocity.

Regarding the measurements of meat, chicken breast meat was studied in a range of four atmospheres; mixing air, carbon dioxide and nitrogen, and at two refrigerated temperatures; 4 and 8 °C. In oxygen deprived atmospheres the most important markers for spoilage were ethanol and acetic acid. Where oxygen is present methanethiol becomes the most important spoilage compound.

In measurements of beef steak, a single modified atmosphere at 30:70 carbon dioxide and oxygen mixing ratio is used; beef steak being packaged under much less variable atmospheres. The most prominent compound observed in beef steak spoilage was 2,3 butanedione. In these experiments the effect of sampling on the measurements is examined by testing a range of sampling techniques: continuous flushing headspace analysis, flushing headspace analysis only during sample collection and quasi-static headspace analysis.

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Rapid assessment of aromatic mineral oil constituents contamination in paper-based food packaging

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Abstract

Mineral oil hydrocarbons (MOH) are volatile constituents of mineral oil that include mineral oil aromatic hydrocarbons (MOAH) – of which some are suspected carcinogens – that can make their way into paper-based food packaging and subsequently contaminate food. The source of MOAH in paperboard packaging is primarily from printing inks that remain in the pulp of recycled paper used to produce the packaging. The current procedure for determining the presence of these compounds in food and packaging involves the use of high-performance liquid chromatography combined with gas chromatography (LC-GC) [1], but such analyses are time-consuming, require manual sample extraction and are accompanied by a relatively low sensitivity and inability to detect specific MOAH compounds. Moreover, classical headspace GC-MS has limited capability to specifically target the MOAH fraction within the complex MOH mixture. The use of proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOFMS) for on-line, non-destructive analysis of the presence of MOAH compounds in paperboard packaging was therefore investigated as a suitable alternative. Packaging samples were spiked with a solution containing several polycyclic aromatic hydrocarbons (PAHs), which were used as surrogates for MOAH compounds. Real-time headspace analyses were performed by PTR-TOFMS at different PAH concentrations to establish the detectability and linearity range. This presentation will outline the methodology and the promising results obtained.

Introduction

Contamination of foods with mineral oil hydrocarbons (MOH) – comprising mineral oil aromatic and saturated hydrocarbons (MOAH and MOSH, respectively) –received much attention in the press of late, due to the potential health problems they pose. In particular, MOSH compounds have been shown to cause damage to the liver, heart valves and lymph nodes in rats [2,3]. Dry foods packaged in paperboard cartons, such as rice or pasta, are particularly susceptible to contamination due to the lack of barrier materials between the paperboard packaging and the product, the large surface area of the food, and the typically long shelf-life leading to lengthy storage and thereby an enhanced likelihood of migration.

Currently, the standard method to determine the degree of contamination in either the packaging material or the food product itself uses high performance liquid chromatography (HPLC) combined with gas chromatography-flame ionisation detection (GC-FID), but this LC-GC methodology delivers only the composite sum of the MOSH and MOAH fractions and not a

selective detection of the individual compounds. This is mainly due to the high complexity of the MOH mixture, with co-elution posing a major problem, as evident in the chromatogram shown in figure 1.

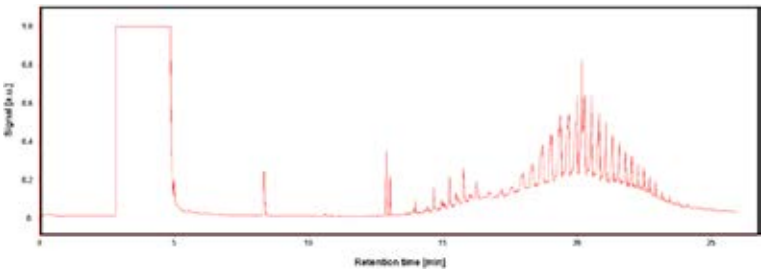

Figure 1: A LC-GC-FID chromatogram of the MOSH fraction in contaminated recycled paperboard, clearly showing co-elution of multiple compounds at retention times between ~14 and 25 min.

The shortcomings of the HPLC-GC-FID technique and classical headspace GC-MS present a challenge to both the food/packaging industries and the regulatory authorities in ensuring that such packaged foods do not present a risk to public health. Thus there is a great need to develop an analytical system that can detect and quantify the individual MOAH compounds specifically and in a rapid manner for use as a non-destructive screening tool.

To address this challenge, we investigated the potential of using PTR-TOFMS as an analytical tool for screening samples for the presence of MOAH compounds via headspace analysis. Tests were performed on PAH compounds as surrogates for MOAH substances due to the current lack of knowledge on the exact nature of the latter, although PAHs belong to this group of substances.

Experimental Methods

The following PAHs were used as MOAH surrogate compounds (chemical purities are given in parentheses): naphthalene (98 %), 1-ethylnaphthalene (99 %), benzophenone (99 %), 4-methylbenzophenone (99 %), 2,7-diisopropylnaphthalene (95 %), 1-methylnaphthalene (97 %), and phenanthrene (98 %).

The analyses of MOAH-surrogate compounds were performed using a high resolution, high sensitivity PTR-TOFMS (PTR-TOF 8000; IONICON Analytik GmbH, Innsbruck, Austria), as well as an HPLC-GC-FID (Axel Semrau GmbH & Co. KG, Sprockhövel, Germany; LC 1260 Infinity, Agilent Technologies, Santa Clara, USA, Master GC, DANI INSTRUMENTS, Milan, Italy). Fragmentation studies by PTR-TOFMS were initially performed on the seven PAH surrogate substances listed above to ascertain the predominant fragments detected. This was achieved by dynamic headspace measurements of 50 mL vials containing the individual compounds that were flushed with 1 L/min zero-air generated by an advanced gas calibration unit (GCU-a; IONICON Analytik) and with a PTR-TOFMS sampling flow of 100 mL/min. Fragmentation analyses were performed at a reaction chamber temperature of 60 °C, pressure of 2.2 mbar, and varying drift voltages to achieve a range of *E/N* conditions (~88-132 Td).

Simulated MOAH-contaminated cardboard samples were prepared by spiking the surrogate PAH compounds onto a mineral oil-free matrix (filter paper from Schleicher & Schuell, Chalfont St. Giles, UK). All seven substances were individually mixed in *n*-hexane and the pure cardboard samples were spiked with 100 µL of mixed solutions at ten different concentrations, namely at

0.006, 0.012, 0.024, 0.048, 0.06, 0.096, 0.12, 0.19, 0.38 and 0.76 mg/kg. Samples were placed in 60 mL septum-capped glass vials, and triplicate PTR-TOFMS static headspace analysis was made for each concentration. Gas-phase volume mixing ratios (in ppb_v) were calculated from a theoretical approach using compound-specific reaction rates reported in the literature [4] where available, or a standardised reaction rate of 2.0×10^{-9} cm^3 s^{-1}.

Results

All of the seven compounds were detected at the parent ion; only one compound, namely 1-methyl naphthalene, produced a single fragment (by loss of a water molecule). The distinctive peaks determined for each compound thereby made the unique detection and quantitation of the individual compounds in the samples possible. The gas-phase concentrations detected in the headspace of the samples related linearly to the liquid-phase spiked concentrations (figure 2; example of the detection of naphthalene). The PTR-TOFMS detection limits for the PAH compounds were found to be sufficiently low to enable detection of all compounds mostly down to the lowest dilution stages (compound dependent). For naphthalene, for example, the limit of detection was determined to be ~75 ppb_v, corresponding to a concentration in paperboard of ~9.5 µg kg^{-1}. This outperformed the limit of detection of ~71 µg kg^{-1} for naphthalene assessed by HPLC-GC-FID in this study.

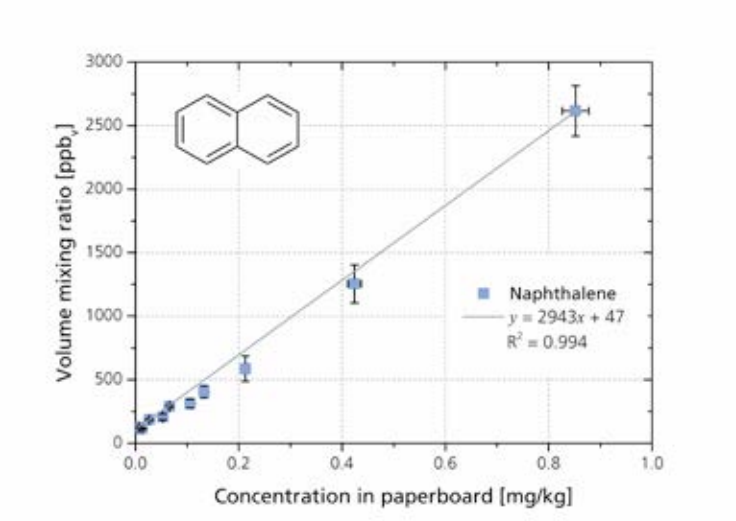

Figure 2: PTR-TOFMS analysis of the headspace of paperboard spiked with a dilution series of PAHs; naphthalene is shown as an example of the detection linearity.

Conclusions

PTR-TOFMS was found to be a suitable tool to quickly and sensitively detect and quantify specific MOAH-related substances in the headspace of paper-based samples, whereby all of the MOAH surrogate compounds were individually detectable, albeit with different detection limits. By comparison, although HPLC-GC-FID was confirmed to be a good method to determine composite MOAH fractions, individual substances could not be detected. PTR-TOFMS will also

likely outperform classical headspace GC-MS for the detection of such surrogate compounds, given the soft and selective protonation that reduces the interferences from MOSH compounds. Further work is needed to establish analytical procedures to determine non-MOAH-related substances (e.g. phthalates and benzophenone-derivatives, which are likely to be present in paper and board), and also to optimise the PTR-TOFMS operating conditions and standardise the sample handling and measurement procedures. This feasibility study nevertheless demonstrates the potential for PTR-TOFMS to be used as a rapid screening tool to determine the level of contamination of MOAHs in both packaging and food products, which could assist the manufacturer in performing quality assurance procedures and similarly provide regulatory authorities with a method to rapidly screen samples and identify likely contaminated ones.

Acknowledgements

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Aroma release from coffee beverages is affected by matrix composition and human saliva during *in vitro* analysis in model mouth device coupled with PTR-ToF-MS

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Abstract

The aim of this work was to evaluate the effect of coffee beverage composition on aroma release profile and particle size distribution in conditions mimicking human mouth. Five coffee/creamer matrices differing by their level of fat and salt were investigated. Aroma release in the presence of human saliva or water was followed in a salivary reactor device coupled with PTR-ToF-MS. In parallel, changes in the structure of the matrices were evaluated by laser granulometry. Aroma release mainly depends (i) on fat content and the hydrophobicity of the aroma volatile, i.e. higher fat content, less release of hydrophobic aroma compound, (ii) on salt content, i.e. higher salt content leads to an increase of aroma compound release of hydrophobic compounds due to salting out effect. Particle size distribution analysis shows that differences in aroma release is mostly due to fat droplet flocculation phenomena either in water or in saliva.

Introduction

In humans, the mouth is the first organ to perceive food and the different signalling phenomena associated to food breakdown [1]. Events that contribute to in-mouth emulsion breakdown are shear forces due to the compression of tongue and palate, tongue movements, heat transfer and action of saliva. For the last, some salivary proteins (mucins), enzymes (amylase) and ions have been suggested as key components in the in-mouth emulsion destabilization. These components can provoke fat droplet flocculation or coalescence by depletion phenomena and/or by electrostatic attraction and can hydrolyse the emulsion stabilizers located either at the surface of the oil droplets or in the continuous medium. However, these effects are related to the type and concentration of emulsifying proteins at the oil–water interfaces [2]. To date, most of the studies conducted on in-mouth emulsion breakdown were conducted on model food and little is known about hot coffee/creamer emulsions. In this context, the aim of the present study was to understand how human oral physiology may govern the in-mouth breakdown of coffee/creamer emulsions with its consequence on food bolus structure and aroma release and to focus in particular on saliva role. On this purpose, an *in vitro* study was performed in a saliva reactor device in the presence of water or human saliva coupled with PTR-ToF-MS for aroma release analysis. In parallel, structure of the coffee beverage in the salivary reactor was evaluated through particle size analysis. Relationships between structure changes and aroma release were established through multivariate analysis.

Experimental Methods

Aroma release analysis

9 ml of water or human saliva (37°C) were transferred into a saliva bioreactor and then 63 ml of coffee/creamer (55°C) were added. PTR-ToF-MS (8000 from Ionicon Analytik GmbH (Innsbruck/Austria)) acquisition began 2 min before the introduction of the mix. Flow and temperature were set throughout all experiments at 55 ml/min, 110°C, in the drift tube. Acquisition be (2.3 mbar; 480 V) resulting in an E/N ratio of 122 Td. With a ToF extraction frequency of 37 kHz and an acquisition rate of 1 Hz, a mass range m/z of 0 – 249.67 was covered each 0.108 s for each extraction pulse. The sampled headspace was diluted with nitrogen gas to avoid saturation effect. 7 aroma compounds were followed: 2-methylfuran (LogP=1.91), 3-methylbutanal (LogP=1.23), furfural (LogP=0.83), pyridine (LogP=0.80), acetoin (LogP=-0.36), 2,3-pentanedione (LogP=-0.85), diacetyl (LogP=-1.34) respectively named in the present text 2MF, 3MB, FUR, PYR, 2,3PT, AC and DCTL. We used PTR-MS viewer for the extractions and Microsoft Excel for signal smoothing and parameter extraction from the release curves (Figure 1).

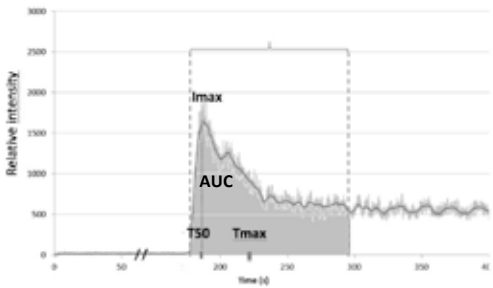

Figure 1: Typical PTR-ToF-MS aroma release curve profile from the reactor. AUC: quantity of aroma released at 120 seconds, Imax: maximum intensity, Tmax (s): time to reach maximum intensity (Tmax in seconds), T50 (s): time to reach 50% of the AUC.

Matrix studied

Five matrices were studied. They are described in the table 1 below.

Matrices	M1	M2	M3	M4	M5
Coffee + sugar + aroma + thickener	x	x	x	x	x
Salt	x	x	x	x	**0**
Creamer (Fat)	x	**x/2**	**2x**	**x/2***	x

* half fat in the creamer

Particle size distribution analysis (PSD)

PSD of the coffee/creamer was determined by laser light scattering granulometry with a Malvern Mastersizer Hydro 2000 SM (Malvern Instrument Ltd., Worcestershire, UK) at 5% obscuration. PSD were reported as the volume mean diameter d_{43} (µm).

Statistical analysis

All experiments were conducted in triplicates. PSD data were submitted to analysis of variance to determine significant effects of the studied factors (medium and matrix). PLS-DA (Partial Least

Square Discriminant Analysis) was also applied to examine the relationship between aroma release data and medium & matrix samples.

Results

Aroma release

In the presence of water, we observe a direct relation between the level of fat and the hydrophobicity of the aroma compounds (Fig 2A). The most hydrophilic compounds (DCTL, 2,3PT & AC) are more released for M3 (high fat) than for M2 & M4 (low fat) which released more hydrophobic compounds (PYR, FUR, 2MF, 3MB). Moreover, T50 and Tmax are higher for M3 and M1 than for M2 and M4. In the presence of saliva (Fig 2A'), we observe also a clear and similar effect of fat content on aroma release for M1, M2 and M3. However, for M4, the projection is different. This matrix releases less of all the aroma compounds compared to M1 & M2 and with longer times (T50 and Tmax) compared to M3. Changing the level of salt affects also aroma release. In water, a high level of salt (M1) leads to a higher release of the most hydrophobic compounds (2MF & 3MB) (Fig 2B). In the presence of saliva, similar results were obtained but, in this case, for most of the aroma compounds. Higher is the level of salt in the matrix, higher is the aroma release (Fig 2B').

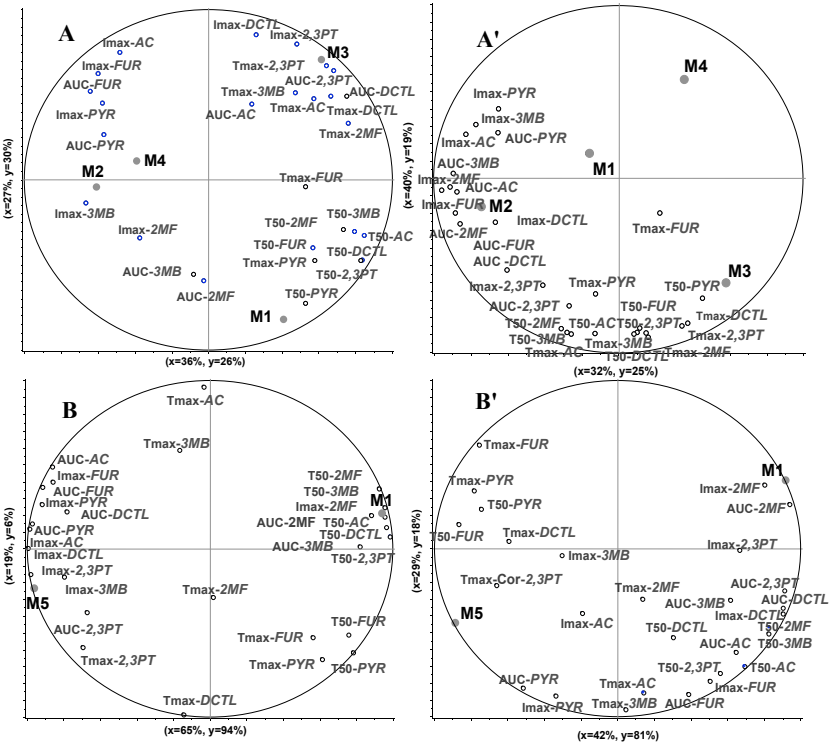

Figure 2: Result of PLS-DA calibration modelling in water (A & B) and human saliva (A' & B') for X-variables (aroma release), and their inner relationships with coffee/creamer matrices for the first two latent variables. A & A': matrices differing by fat level (M1, M2, M3, M4). B & B': matrices differing by salt level (M1, M5)

PSD analysis

The average PSD observed for each of the matrices is presented in table 2. For M1, we observed a significant decrease of particle size in the presence of saliva while it was the opposite for M4 and M5. M3 was the matrix the less modified by saliva. Further analysis showed that these particles were the result of flocculation instead of coalescence phenomena (data not shown).

Table 2: Average d_{43} and d_{32} values in the 5 coffee matrices. Values in italic bold indicate a significant difference between saliva and water * =0.01<p<0.05, **=0.001<p<0.01, ***=p<0.001

	M1		**M2**		**M3**		**M4**		**M5**	
	Water	Saliva	Water	Saliva	Water	Saliva	Water	Saliva	Water	Saliva
d_{43}	13.5	***6.7****	10.1	6.4	3.8	2.2	6.5	***15.3****	7.3	***8.8****

Discussion

We observed a significant effect of coffee/creamer composition on aroma release and PSD. This effect was different depending on the medium (water or saliva) used to conduct *in vitro* experiments. In water, fat level explains the most aroma release differences that are linked rather to the hydrophobicity of the molecule than to PSD, even these last are different among the matrices. This was often observed in the literature [3]. However, with saliva M4 PSD was doubled compared to other matrices. In this case, the exchange surface between the droplet and the medium decrease leading to a decrease in aroma release which could explain the particular behaviour of M4 with saliva. Enhancing the salt content leads to an increase of hydrophobic aroma compound release either in water or saliva. It is likely that release was mostly due to salting out effect commonly observed for this kind of aroma compounds [3]. Regarding PSD, M1 has twice the particle size than M5 in water. Similar results have been described for cheese, *i.e.* matrices with a higher salt content have a higher fat particle size [4]. Interestingly, with saliva, we observed an important decrease of particle size for M1 (saltiest matrix). This result may explain a higher salting out effect for M1 in the presence of saliva compared to water. Decrease of particle size leads to a higher exchange surface between fat particles and water, thus a higher transfer of aroma from oil phase to water phase and then a higher amount of aroma release in the vapour phase due to the salting out effect.

In conclusion, our salivary reactor coupled with PTR-ToF-MS appears to be an interesting device for the characterization of coffee beverages in an *in vitro* manner closest to what occurs during *in vivo* conditions. Moreover, this work highlights the impact of saliva in aroma release and matrix structure change. Food matrix/saliva interaction should be taken into account when investigating aroma release to relate aroma release to food sensory properties.

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Real-time monitoring of volatile organic compounds as possible spoilage markers from modified atmosphere packaged chicken meat

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Abstract

Traditional procedures to determine meat spoilage are microbial analysis in combination with sensory assessments. These methods are both time-consuming and costly, and results are typically only available after a few days. Considerable effort has been made over the years to find more rapid and efficient options for shelf-life prediction. One such method is the use of food freshness indicators (FFIs), which have been developed to monitor the spoilage processes of meat by reacting with volatile organic compounds (VOCs) released from the meat to cause a colour change that indicates that the meat is spoiled. Nevertheless, the use of FFIs is scarce, owing to the complexity of meat and the current lack in understanding of the spoilage processes. This study presents a measurement configuration that allows VOCs from modified atmosphere packaged (MAP) chicken breast to be monitored in real-time using proton-transfer-reaction mass spectrometry (PTR-MS) at a controlled storage temperature. The release profiles obtained offer a visualisation of the meat spoilage process that aids in finding possible volatile spoilage markers and provides a better understanding about the release processes involved.

Introduction

Meat is a highly perishable food that can be spoiled within a few days. Due to its chemical composition many microorganisms can grow on the surface of meat but under aerobic conditions it is usually spoiled by *Pseudomonas* [1,2]. To prolong the shelf-life of the meat it is often placed in modified atmosphere packaging (MAP), whereby air is completely substituted by defined amounts of oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2). The ratio of these gases depends on the type of meat: for poultry an atmosphere of 30 % CO_2 and 70 % O_2 is typically used in Germany. CO_2 has a bacteriostatic effect on a wide range of microorganisms, mostly gram negatives like *Pseudomonas*. O_2 is usually used to maintain the red colour of the meat [3–5] and inhibits the growth of pathogenic organisms. Under modified atmosphere conditions the microflora shifts towards *Br. thermosphacta* and lactic acid bacteria [6–8].

The shelf-life of meat is traditionally determined via microbiological tests, but these methods are time-consuming and expensive. In the last years great effort has been made to find fast and cheap alternatives. One procedure is to use food freshness indicators (FFIs) as part of intelligent packaging. FFIs are sensitive to volatile organic compounds (VOCs) that are formed and released during the spoilage process of meat and indicate to the consumer that the meat is off via a colour change. Although many FFIs have been patented, only rarely do they make it to the market. One reason for this limited success could relate to the use of conventional gas chromatography-mass spectrometry (GC-MS) analysis in developing the FFIs, which provides only limited information on the release dynamics of the target VOCs. In this study we present a measurement configuration

that can be used for monitoring VOCs released during the spoilage of MAP chicken meat in real-time by proton-transfer-reaction mass spectrometry (PTR-MS). This system has high potential for contributing to a better understanding of the meat spoilage process by generating highly time-resolved release curves and thereby allowing the release kinetics to be more fully understood.

Experimental Methods

The measurement configuration allows for real-time monitoring of VOCs from four samples in series using PFA beakers (1 L). Two in-line mass flow controllers provided a constant flow rate for the modified atmosphere gas and the downstream dilution gas (zero-air). Two-way solenoid valves were incorporated into the set-up to allow selective sampling, which was automatically controlled by the PTR-MS control software during the measurements.

Prior to the measurements single fresh chicken breast fillets with a declared shelf-life of 7 days were placed in three of the four beakers. The fourth beaker was used for the measurement of the background signal (blank, control). The headspace of the samples in the beakers was initially flushed with modified atmosphere gas to completely replace the air inside. The headspace of each sample was flushed with modified atmosphere gas only during active sampling, which proceeded at a slow and constant rate of 10 mL/min. Each sample was measured for 5 cycles in scan mode, from *m/z* 20-160 with a dwell time of 500 ms per *m/z*, resulting in a sampling time of less than 6 min. In the headspace the modified atmosphere gas that also served as carrier gas was enriched with the volatiles to be detected. The carrier gas containing the VOCs was diluted with zero-air at a ratio of 1:10. The experiments were performed for 12 days at 4 °C and for 7 days at 10 °C. The *m/z* signals were tentatively identified by headspace solid-phase micro-extraction (HS-SPME) GC-MS and by comparison with literature reports.

Results

A total of 12 different *m/z* signals were detected, including various groups of compounds like alcohols, hydrocarbons, aldehydes, ketones and sulphides. A summary of the most prominent *m/z* signals is shown in Table 1. Higher concentrations of some VOCs were observed at 10 °C. Most VOCs showed an exponential increase and looked similar to the typical growth curves of microorganisms (lag phase, exponential phase). At 10 °C a shorter lag phase was observed than for 4 °C. Some VOCs followed a linear behaviour. Whereas some VOCs were present in all samples at both temperatures, some were found to vary only in individual samples. The signal at *m/z* 59, tentatively identified as acetone, was present at high amounts from the beginning of the experiments. Other VOCs appeared only after a few days, depending on the temperature.

Table 1: Tentative identification of the most prominent VOCs

m/z signal	Tentative identification of VOCs by GC-MS and literature	Reference
47	ethanol[#]	[9]
59	acetone[#]	[9]
61	acetic acid[#], 1-propanol[#], 2-propanol[#]	
63	dimethyl sulphide[#]	[9]
69	1-octen-3-ol[#]	
87	2,3-butanedione (diacetyl)[#], 3-methyl butanal[#]	
89	3-hydroxy-2-butanone (acetoin)[#], 3-methylbutanol[#]	[10–13]

[#]detected by HS-SPME GC-MS in this study

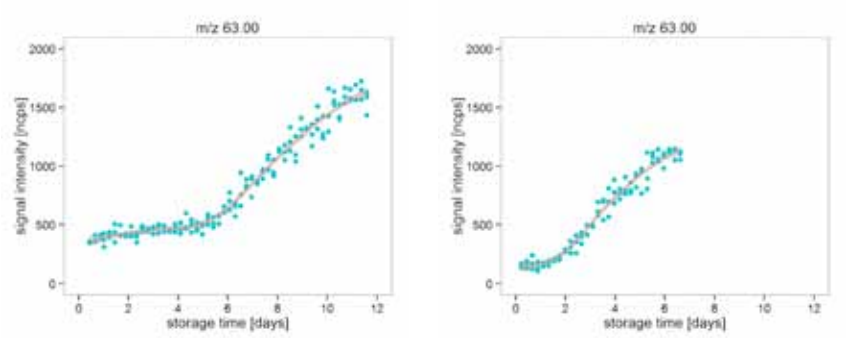

Figure 1: The signal of *m/z* 63 (tentatively identified as dimethyl sulphide) in the headspace of meat at 4 °C *(left)* and 10 °C *(right)*.

Discussion

A particular challenge in monitoring VOCs released into a modified atmosphere by PTR-MS is the high CO_2 content, which changes the ion mobilities in the drift tube [14] and the high O_2 content, which promotes charge exchange to compete with proton transfer reactions [15]. This challenge was overcome by diluting the sample gas prior to sampling by PTR-MS, offering the additional advantage of creating only a slow flushing rate through the sample beakers themselves, thus minimising potential altering effects due to a compositional change in the headspace gas. Despite this dilution step, the detection of VOCs that are present down to low ppb_v levels is still achievable.

The experimental temperatures of 4 °C and 10 °C were chosen to reflect real-life conditions. Chicken meat should be stored at 4 °C, whereas 10 °C simulates conditions when the cold-chain is interrupted, e.g., due to consumer misuse. This presented a further challenge to the PTR-MS analysis in that the number of VOCs that might be expected to be present was greatly reduced. The data revealed that higher concentrations of some VOCs were detected at 10 °C compared with at 4 °C, which might be explained by two reasons: first, at 10 °C the vapour pressure is higher, resulting in higher concentrations of the VOCs released into the headspace. Second, the metabolic activity of the microorganisms is higher at elevated temperatures. Differences in the release of VOCs might also be attributable to other factors, such as glucose content, pH, initial microflora of the meat, and the growth and development of the microflora during storage. The meat was mainly spoiled by *Br. thermosphacta*, suggesting that the majority of the VOCs detected derived from this microorganism. *Br. thermosphacta* is known to use only glucose as an energy source under modified atmosphere conditions [7]. In the present study, the main products of glucose metabolism (e.g., 3-hydroxy-2-butanone, 3-methylbutanol, 3-methylbutanal, acetic acid) were detected.

This novel set-up allows for a visualisation of the release of VOCs that extends our knowledge on the processes involved in meat spoilage and will ultimately improve the methods for determining the shelf-life of meat by helping to find possible volatile spoilage markers that can be used in the development of FFIs.

Acknowledgements

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FastGC PTR-ToF-MS analysis of yeast VOCs

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Abstract

For the first time in this study proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) equipped with a prototype fast-GC system and a multipurpose head-space automated sampler was used to investigate variability between four meiotic segregants of M28 natural *Saccharomyces cerevisiae* strains and two mating type of BY strains of *S. cerevisiae* strains during their growing in a rapid and non-invasive way. The technique was successful in characterizing selected yeast strains.

Introduction

Yeast metabolism plays a key role in the production of flavor compounds in alcoholic beverages thus affecting their final quality and sensory profile. Volatile compound concentration is influenced by the growth characteristics of yeast strains. For this reason a rapid and non-invasive screening of the yeast volatilome is of outmost relevance. Proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) showed promising results in monitoring *Saccharomyces cerevisiae* volatile production in dough and bread (Makhoul et al. 2014). However, this technique does not allow separating structural and spatial isomers that complicates the identification and quantification of the compounds. Furthermore due to high ethanol production during fermentation processes PTR-ToF-MS parameters should be changed drastically or an inert gas should be introduced in order to prevent hydronium ion depletion and the generation of ethanol dimers and trimers, clusters between ethanol and water, and their fragments. In the present work a new approach is proposed for the analysis of yeast volatile organic compound (VOCs) by the coupling of PTR-ToF-MS both to a prototype fastGC system and a multipurpose head-space automated sampler. In this case the chromatographic separation provided by fastGC permits to discriminate compounds with the same chemical formula as well as to eliminate the undesired effect of high ethanol concentration.

Experimental Methods

Four meiotic segregants of the M28 natural *Saccharomyces cerevisiae* strain (M28-1A, M28-1B, M28-1C and M28-1D) and two BY *S. cerevisiae* strains (BY4741 – MAT *a* and BY4742 – MAT *α*) were selected for studying their VOCs profiles by a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) coupled both to a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) and a prototype fastGC system (Ionicon Analytik GmbH, Innsbruck, Austria) with a short polar GC column (MXT-WAX Cap. Column 6m, 0.25mm ID, 0.25um, Restek, Bellefonte, PA). The samples (twelve biological replicates of each yeast strain, the substrate used for their growth (solid YPD, 1% Yeast Extract,

2% Peptone, 2% Dextrose) and lab air) were left for 12 days in dark at 30°C with regular aeration. The headspace of each sample was measured at seventh and twelfth day of the experiment for 15 seconds which guaranteed total replacement of headspace by pure air. The injection time to fastGC sample loop was set to 4 seconds which was enough for its complete filling. The fastGC temperature ramp was from 40°C till 220°C and lasted 130 seconds, which was optimal for the separation of the investigated VOCs.

Data processing of PTR-ToF-MS spectra consisted of dead time correction, external calibration and peak extraction (Cappellin et al. 2010). Retention time shift has been accounted for by aligning the chromatogram to the peak of oxygen (O_2+, m/z 31.989).

Results and discussion

The preliminary analysis of fastGC chromatograms showed the possibility to distinguish between every M28 meiotic segregants and BY strains in the size of the chromatographic peaks (i.e. m/z 41.038, 43.017, 43.054, 57.069, 61.028, 71.085, 89.059, and others). In general BY strains produced more VOCs than M28 ones. In particular BY4742 showed higher total VOC emissions than BY4741 one. M28-1A and 1C colonies were more active VOC emitters than other two M28 ones.

The comparison of the results of two time-points demonstrated the significant decrease of ethanol and other VOC emissions by all yeast colonies, especially in M28-1B and M28-1D colonies, which can be explained by different growth characteristics and resistance to metabolic starving [3]. However the production of methanol (m/z 33.033) and acetone (m/z 59.049) augmented for all yeast samples during second time-point measurement most probably due to aging of yeast colonies.

In this work, for the first time, PTR-ToF-MS coupled both to a multipurpose headspace automated sampler and a prototype fastGC system was applied for a rapid and non-invasive analysis of the yeast colonies. The technique was successful in characterizing different yeast strains and identifying differences in the release of important classes of compounds.

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Aroma volatile metabolites at olfactory mucosa level evidenced by *in vitro* PTR-Tof-MS studies

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Abstract

Olfactory mucosa can metabolize odorants through various enzymatic mechanisms participating in their clearance and therefore in the termination of the olfactory signal. Preliminary *ex-vivo* studies using headspace-GC revealed the formation of volatile metabolites when odorant molecules were injected above a fresh explant of rat olfactory mucosa. However, this method did not allow accessing the data during the first five minutes of contact between the odorant and the mucosa, thus limiting the olfactory biological significance. Using a direct-injection mass spectrometry technique (PTR-MS) we have been able for the first time to investigate the first moments of the enzymatic process of the metabolic capacity of *ex-vivo* rat olfactory mucosa in real time. Using various odorous substrates, we demonstrated that they can be metabolized by an *ex-vivo* olfactory mucosa within seconds, producing volatile metabolites. Significance for human olfaction has to be investigated and will be discussed.

Introduction

Humans as well as animals detect and discriminate thousands of odorous volatile organic compounds (VOCs). To be perceived, these VOCs need first to reach and bind their olfactory receptors proteins located on the cilia of olfactory sensory neurons in the olfactory mucosa (OM). VOCs, as other potentially toxic xenobiotics, are mainly hydrophobic molecules that easily penetrate into OM cells where Xenobiotic Metabolizing Enzymes (XMEs), present in high amount, participate in their clearance. This major detoxification process is organized in three principal enzymatic steps catalysing the biotransformation of the xenobiotic in hydrophilic inactivated metabolites prompt to be eliminated (Figure 1).

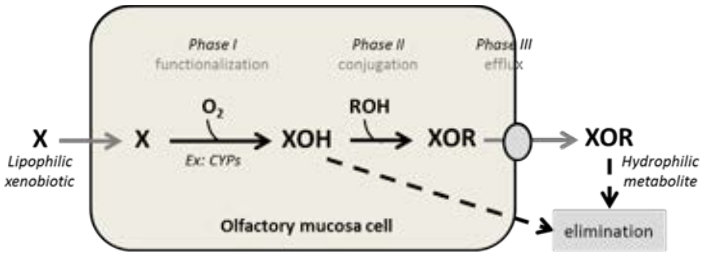

Figure 1: Hypothesized metabolism of a lipophilic volatile xenobiotic in the olfactory mucosa cell by the xenobiotic metabolizing enzymes pathway.

It has been shown that XMEs, are involved in olfactory signal termination by metabolizing odorant molecules, avoiding receptors saturation and thus maintaining olfactory sensitivity [1-3]. After the first functionalization step, hydrophilic volatile metabolites may sometimes be directly eliminated out of the cell by transporters (Figure 1). If these volatile metabolites are odorous, it can be hypothesized that they could modify olfactory detection and be implied in overall aroma sensory perception during the course of food consumption (Figure 2). This hypothesis was reinforced by observing at biological and behavioural levels the action of certain metabolic enzymes secreted in the nasal mucus of mice [4].

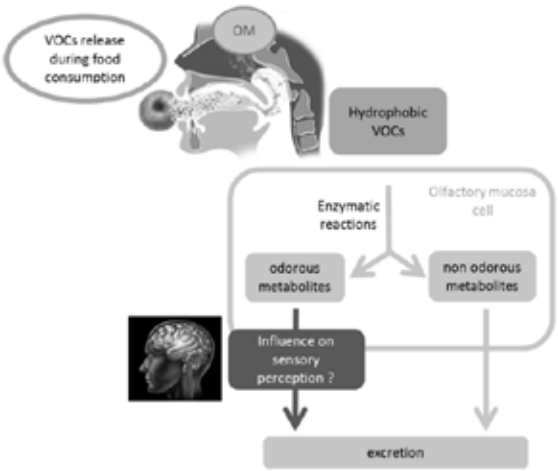

Figure 2: Production of odorous metabolites at olfactory mucosa level and their hypothetical role in sensory perception during food consumption in humans.

We recently developed an ex-vivo headspace-gas chromatography (HS-GC) methodology allowing easy and reproducible measurement of odorant olfactory metabolism. Using this method we demonstrated that a volatile metabolite (ethanol) was formed when the odorant ethyl acetate was injected in the headspace above a fresh explant of rabbit olfactory mucosa, together with the significant decrease of the initial odorant, probably by the action of carboxylesterases [5]. Experiments conducted with fresh explant of rat OM revealed the same results. However, this HS-GC method did not allow accessing the data during the very first minutes of contact between the odorant and the mucosa, rendering the results not really relevant for the physiological time scale. Therefore we turned to a direct-injection mass spectrometry technique such as PTR-MS that is a dedicated technique for real-time sensitive analysis of VOCs. Metabolism kinetics of ethyl acetate was explored in real time by PTR-MS analysis of the headspace above fresh explant of rat OM exposed to the odorant. A discontinuous method using headspace vials allowing discrete sampling was first implemented. The results demonstrated for the first time that an odorant molecule can be metabolized by an ex-vivo olfactory mucosa within seconds, producing an identified volatile metabolite. To access true real-time data, an on-line method was developed that allowed odorant delivery in two independent circuits continuously monitored by a PTR-ToF-MS. The results obtained in real-time continuous recording confirmed the previous results obtained for ethyl acetate in a discontinuous way while validating the developed methodology. The metabolism of aroma molecules related to dairy products, more relevant to human sensory perception, has been then studied. Ex-vivo experiments conducted with rat OM revealed the formation of volatile metabolites in each case. The relevance of this metabolic process in human

olfaction has yet to be confirmed. The purpose of the present paper is to give an overview of the development conducted in this pioneering domain.

Experimental Methods

All the experiments were conducted with a PTR-Time of Flight-MS instrument (PTR-ToF 8000, Ionicon Analytic, Innsbruck, Austria). The drift tube parameters were fixed as follows: P*drift* 2.3 mbar, T*drift* 110°C (or 80°C), U*drift* 350 V (or 486 V) giving an E/N value of 90 Td or 110 Td, respectively (1Td = 10^{-17} cm².V). Mass spectra were acquired at a scan speed of 0.108 s for the mass range m/z 0-250. The data were expressed in normalized CPS (ncps) relative to the primary ions H_3O^+ and $(H_2O)_2H^+$ that were systematically monitored following their respective ^{18}O isotopologues at m/z 21.022 and m/z 39.032. All the volatile compounds were monitored through their respective protonated molecular ions.

All the chemicals ethyl acetate and the food grade molecules pertaining to the dairy sensory context (pentane-2,3-dione, hexane-2,3-dione, 2-acetoxybutanone) were from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Ex-vivo experiments (conducted with the agreement of the Ethical Committee for Animal Experimentation, Dijon, France): freshly dissected rat olfactory mucosa was placed convolution upwards either in 20 mL headspace vials for discontinuous experiments, or in one branch of a specially designed two independent-way circuit for on-line continuous measurements. The headspace from the vials or from the on-line arrangement was admitted in the instrument with a flow rate fixed at 40 or 60 mL.min^{-1}. Known gaseous concentrations of odorous volatile organic compounds were admitted in the headspace above the olfactory mucosa and different blanks and controls were recorded in the absence of olfactory mucosa, or with enzymes-denatured heated mucosa. All the measurements were done in triplicate.

Results and discussion

Although the developed methodology in headspace vials allowed only discrete sampling and not true real-time monitoring, the results demonstrated for the first time that an odorant molecule (ethyl acetate) can be metabolized by an *ex-vivo* olfactory mucosa within seconds, producing an identified volatile metabolite (ethanol). To access real-time data, a six-way valve whose end was connected to the PTR-MS instrument was implemented to allow odorant delivery in a two-way circuit. A fresh explant of rat olfactory mucosa was placed in the first branch, the second one serving as a control. A known concentration of gaseous ethyl acetate could be delivered continuously to the valve, and the flow passing through either circuit was continuously monitored by the PTR-ToF-MS. Blanks of the headspace above the mucosa in absence of any VOC were recorded using humidified zero-air and was not found different from the control. Heated olfactory mucosa was used to investigate enzymes denaturation and adsorption by the tissue.

Injection of ethyl acetate above the *ex-vivo* rat OM resulted in immediate apparition of ethanol m/z= 47.049 whose concentration increased continuously during recording time, while a significant depletion of ethyl acetate signal at m/z= 89.060 was observed in comparison to control signal. Denaturation of the enzymes by heating the OM resulted in no appearance of ethanol and no depletion of ethyl acetate despite significant adsorption by the tissue (Figure 3).

Injection of the 2,3-diketones in the same conditions resulted in an immediate depletion of the molecular ions at m/z 101.060 (pentane-2,3-dione) and m/z 115.075 (hexane-2,3-dione), with apparition of volatile metabolites whose structure remained to be established. Injection of 2-

acetoxybutanone resulted in the depletion of the molecular ion at m/z 131.070 and the apparition of the odorous diacetyl (butan-2,3-dione) at m/z 87.044.

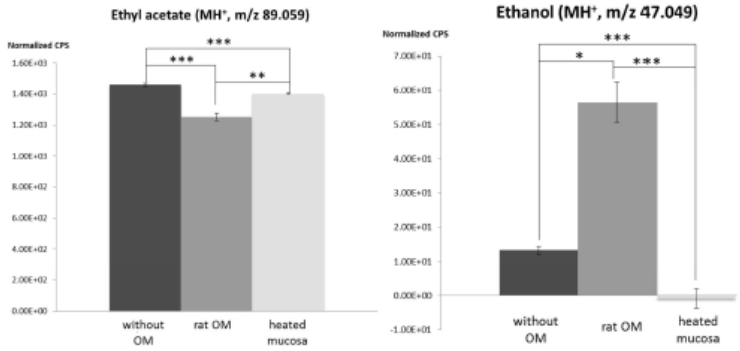

*Figure 3: Production of the volatile metabolite ethanol and concomitant depletion of ethyl acetate when submitted to fresh explant of rat olfactory mucosa. Heated mucosa is an enzymes-denatured control. *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$ (Student's T-test).*

Volatile metabolite formation at olfactory mucosa level seems a general process implying various enzymatic pathways, as proven in the above *ex-vivo* experiments conducted with rat OM. Therefore it appears important to evaluate the significance of such a phenomenon in humans and its potential impact on sensory perception, by confirming the role of the human XMEs in producing odorous volatile metabolites that could have a role in olfaction. The characterization of the metabolites produced from the dairy-evoking aroma compounds will be described.

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PTR-ToF-MS for the study of coffee quality: the role of the time dimension

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Abstract

Coffee represents the gold standard for the application of PTR-MS to food analysis. The advantage of PTR-MS over separation techniques (e.g. GC-MS) becomes particularly evident when the Time-of-Flight mass analyzer is employed. This is mostly due to the possibility to monitor processes at high time resolution. The present work describes the application of PTR-ToF-MS to two stages of the coffee-consumer interaction: (i) coffee preparation in the case of soluble coffee and (ii) drinking of espresso coffee. In the first example, PTR-ToF-MS is used to monitor volatile release during the preparation of different instant coffee blends; the results show that each blend has its characteristic time-dependent signature. In the second example, volatile release, studied by means of nosespace analysis, shows interesting correlations with the data produced by a dynamic technique of sensory analysis (i.e. Temporal Dominance of Sensations or TDS). Overall, the results demonstrate the value of PTR-ToF-MS in describing time-related phenomena.

Introduction

Coffee is nowadays a major commodity, with widespread diffusion worldwide. The headspace of coffee is rich in aroma compounds which are extremely diverse from a chemical point of view. PTR-ToF-MS has already been applied in online monitoring studies, with applications to coffee roasting, drinking and brewing. The present research work aims at studying aroma release at two different stages of the interaction between coffee and consumer (*i.e.* preparation and drinking). The use of a dynamic method of sensory analysis (*i.e.* Temporal Dominance of Sensations or TDS) at the same time with PTR-ToF-MS permitted to obtain an all-round image of the coffee tasting experience, coupling sensory and instrumental information.

Experimental Methods

Above-the-cup coffee aroma was measured by adding hot (85 °C) water to a 600-mg aliquot of instant coffee powder, further details can be found in a previously described procedure [1]. Nosespace analysis was performed as described elsewhere [2]. During nosespace, panelists performed an evaluation of the samples by means of a sensory analysis methodology known as Temporal Dominance of Sensations (TDS). In this technique, the judge is asked to select, among the attributes of a list made of aroma and taste descriptors, the sensation that most catches her/his attention in that given moment. The final result (as in PTR-MS), is a series of time-resolved profiles.

Results and Discussion

When used to monitor the real-time evolution of above-the-cup coffee aroma, PTR-ToF-MS allowed to obtain different signatures according for different blend compositions. A simple method is proposed, based on component proportion, and allowing to predict the evolution in time of volatile release of each coffee blend [1]. In the case study of espresso coffees, the coupling of sensory and instrumental data allowed to pinpoint putative chemical markers of sensory notes [2], with characteristic temporal patterns. Figure 1 shows the example relative to the "roasted" note. Summarizing, the use of PTR-ToF-MS provided data not obtainable by mean of separation techniques. The methodologies developed in this work could find application to the study of other types of food.

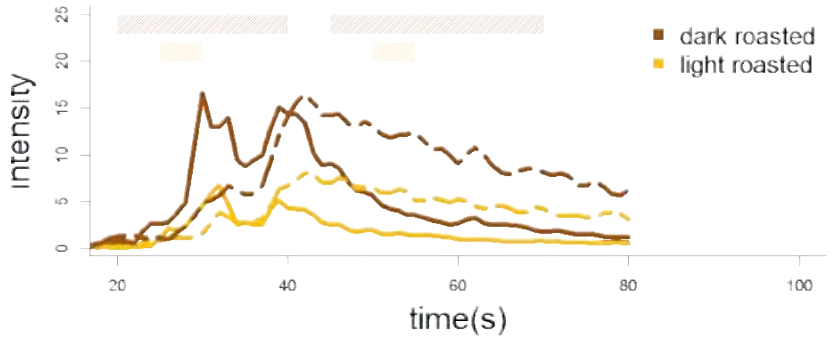

Figure 1: Nosespace analysis by PTR-ToF-MS coupled to TDS. Profiles refer to two putative "roasted" markers while boxes highlight the time dominance of the "roasted" sensory note. Continuous line: m/z 82.065 Th; dashed line: m/z 124.072 Th.

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The use of PTR-MS and consumers to understand how quality changes during food spoilage

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Abstract

Food spoilage results in additional costs for both the food manufacturer and consumer. The objectives of this research were to understand the relationship between microbial numbers and volatile organic compound (VOC) generation; the affect of microorganism composition on off-odour generation; and the relationship between microorganisms, the nature of the odour, its concentration and the subsequent rejection of the product as spoiled. Using a combination of Proton Transfer Reaction Mass Spectrometry (PTR-MS), microbiological methodologies and consumer sensory testing methods it was found that for significant VOC generation to occur in the foods tested a threshold number of microorganisms of around $1x10^7$ CFU/mL was required. Further, consumers only started to detect quality changes at around $5x10^7$ CFU/mL. Importantly spoilage microorganisms differed in the concentration and composition of the VOC they produced, with some microorganisms capable to causing the rejection of the food by consumers at lower total bacterial numbers than others.

Introduction

Food spoilage costs a family of four in the USA an estimated USD 24 000 p.a. [1]. Generally food spoilage is a quality failure not a food safety issue. As such whether the food is suitable for consumption, is generally determined immediately prior to consumption by the consumer using visual and/or odour cues. In contrast the food industry typically uses the number of microorganisms present to determine the end of shelf life, and this measure may have no validated link to sensory changes occurring during spoilage. A lack of understanding of the relationship between microbial numbers and sensory changes in the food product produces a disconnect between the food manufacturer and the consumer. This disconnect results in increased waste due to the unwarranted disposal of food that has been incorrectly classified as spoiled or alternatively it can result in an increased number of consumer complaints due to the sale of food incorrectly classified as being acceptable.

In general there is a lack of knowledge on: the relationship between microbial numbers and off-odour generation; the types and concentration of volatile compounds (odour) microorganism generate; and odour concentration required before consumers will reject a product as spoiled.

Understanding the progression of spoilage and the factors that cause consumers to reject food products and the associated microorganisms responsible for the spoilage will allow food manufacturers to optimise product shelf life which will result in the reduced dumping of unspoiled food, a decrease in the number of consumer complaints and reduced costs to both manufacturers and consumers.

Experimental Methods

Product

A commercially available chilled neutral pH, high water activity food with a shelf life of 14 days (as determined by the manufacturer) was used as a model system for all studies.

Study 1. Changes in the volatile organic compounds (VOC) with increases in microbial numbers.

One batch of product was aseptically repacked into smaller aliquots and stored at 4.5°C until analysis. Three samples withdrawn at each time point on days 3, 6, 9, 10, 13, 15 and 17 after manufacturing and the VOC determined by PTR-MS and microbial numbers determined using standard microbiological plating methods on plate count agar (PCA).

Study 2. Consumer determined end of shelf life.

The consumer rejection threshold was determined by presenting a series of sample pairs (one fresh - control and one aged sample) to consumers and asking them which sample they preferred. The Rejection threshold was the point where consumers preferred the control sample.

The consumer rejection threshold was determined by 55 consumers split into 5 sessions. Product was obtained in a staged manner so that in each session samples could be presented to consumers that had been stored for 3, 7, 14, 16, 18, 20, 21, 22, 23, 24, 25 and 26 days since manufacture. Each consumer was presented with 12 pairs of samples, consisting of one control sample (day 3) and one stored sample selected from each of the days mentioned above. Consumers were asked to select the preferred sample within each pair and to indicate the reason for that choice. For each time point within each session, VOC in the sample headspace were determined by PTR-MS and total microbial numbers were estimated using standard microbiological plating methods on PCA..

Study 3. Inoculation with food spoilage microorganisms

Microbial isolates (10) recovered from the product during study 2 were identified using 16 rRNA sequencing technology (9 isolates) and the API ID 32C test system (1 isolate). The isolates were individually inoculated (about $1x10^5$cfu/mL) into the product. To account for any background microorganism present un-inoculated controls were run. VOC analysis and microbial numbers in triplicate samples were determined by PTR-MS and drop plate (PCA plates), respectively for samples held for 2, 4, 6, 8, 10, 18, 20 and 25 days at 4^oC.

Results and Discussion

A simple relationship between VOC and microbial numbers was not observed over the 25 day shelf-life. VOC concentrations did not increase significantly until microbial numbers reached a threshold of $1x10^7$ (Figure 1). However, once this threshold was reached the rate of the VOC generation increased rapidly. The volatile secondary metabolites detected included ethanol, acetylaldehyde, acetic acid, dimethyl sulfide and butanoic acid.

The shelf-life study demonstrated that relatively high total microbial numbers were required before increases in volatile secondary metabolites could be observed but it did not address at which point the increase in the VOC caused consumers to reject the product and hence the consumer orientated end of shelf life.

In a rejection threshold study it was found that once microbial numbers reached about $5x10^7$ consumer preference shifted markedly in favour of the fresh product (Figure 2).

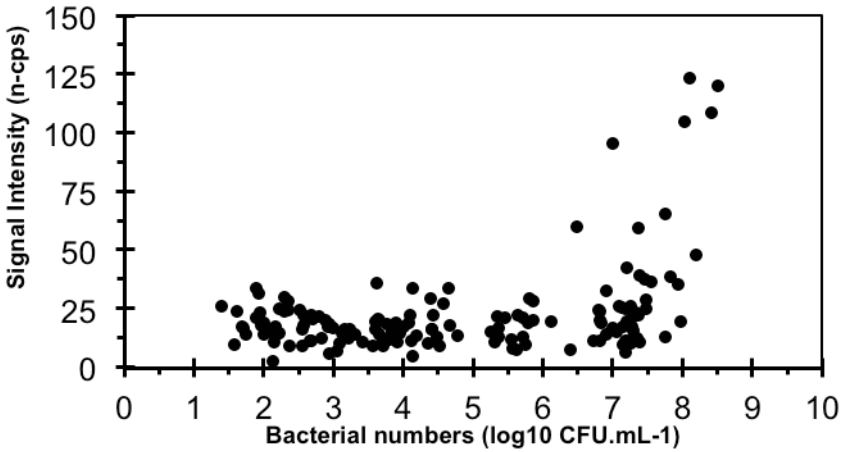

Figure 1: Relationship between bacterial numbers (log10 CFU/mL) and generation of dimethyl sulphide, expressed as normalised counts per second (n-cps).

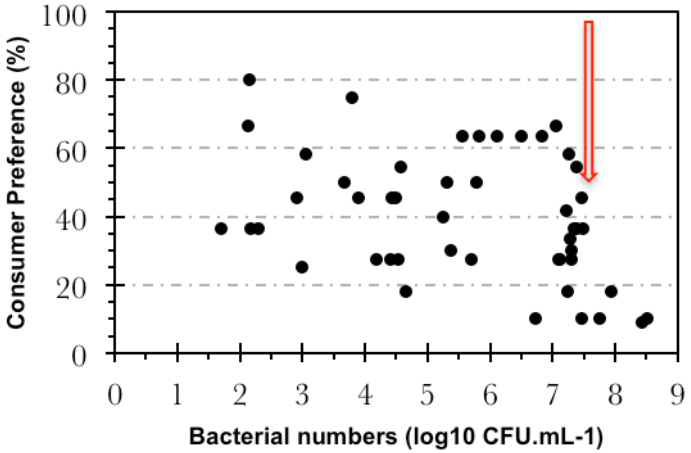

Figure 2: Relationship between bacterial numbers (log10 CFU/mL) and % consumer preference for the aged sample compared to the fresh sample. Each dot represents % consumer in 1 session (total 10 - 12 consumers). The red arrow indicates the rejection threshold.

At this point consumers were describing the product as chemical tasting, foreign flavour, foul and disgusting (Figure 3). The rejection point corresponded to a marked increase in VOC generation,

in particular the generation of methanol, acetaldehyde, ethanol, acetic acid, dimethyl sulfide, butanoic acid and 2-pentanol.

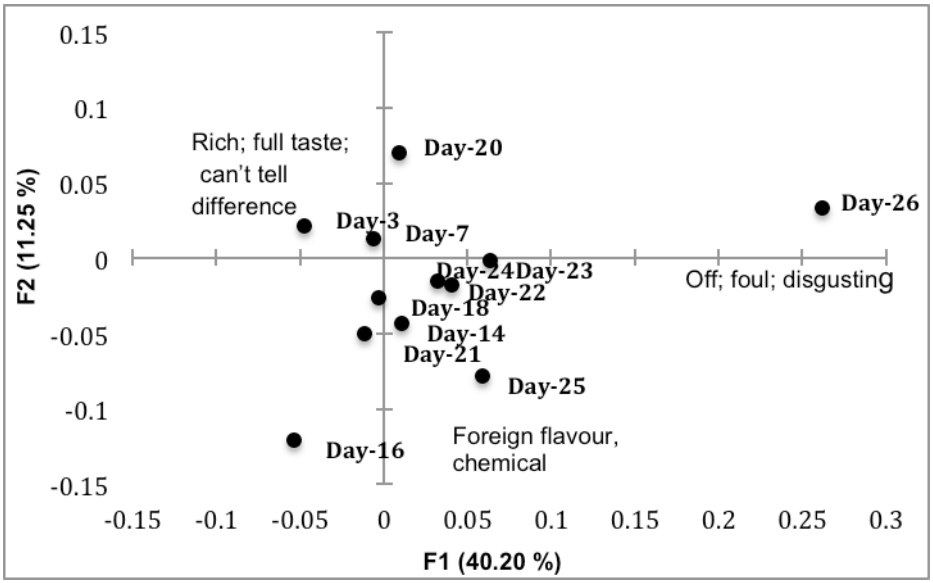

Figure 3: Correspondence analysis showing the relationships between day of storage since manufacture (average of 5 sessions) and summarised free comments.

In the inoculation studies a significant ($p<0.05$) interaction between VOC and microbial isolates were observed indicating that the generation of individual VOC occurred at different rates for each isolate. This data highlights that the rate of spoilage organism is dependent on the species present, the type of VOC produced by the organism and the concentration of the VOC produced. It appears likely that depending upon the composition of microbial population present and their VOC production that spoilage will occur at different total microbial number thresholds.

Conclusions

A threshold number of microorganisms of about $1x10^7$ CFU/mL was required to be present in the food before significant VOC increases occurred and consumers detected quality changes at around $5x10^7$ CFU/mL. In addition it was found some spoilage organisms produced higher VOC concentrations at the same microbial numbers and that the VOC composition generated was also different. Therefore, spoilage microorganisms differ in the concentration and composition of the VOC they produce, with some microorganisms capable to causing the rejection of the food by consumers at lower total bacterial numbers than others. A better understanding of the progression of spoilage and the factors that cause consumers to reject food products and the associated microorganisms responsible for the spoilage will allow food manufacturers to optimise product shelf life

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PTR-ToF-MS Coupling to an Autosampler for Dynamic Headspace Analysis of Coffee

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Abstract

PTR-MS has been used in the field of coffee science for analysis of volatile organic compounds released during roasting or from roasted coffee and coffee brew. While the focus has mostly been on time-resolved analysis of process gases, PTR-MS has also the benefit of very high sensitivity, relative to e.g. gas chromatography. Yet, in order to exploit PTR-MS for static headspace profiling, it is important to develop a robust and reproducible sampling technique. Currently, most of the work is done by manual sampling, and long-term reproducibility in terms of RSD (relative standard deviation) of measurements is typically larger than in GC analysis.

We present the development of an autosampler unit that is coupled to the PTR-ToF-MS instrument. The system is aimed to be used for analysis of headspace over various coffee samples. The two main advantages of an automated system against manual sampling are better reproducibility, and higher sample throughput. Compared to GC-MS the new PTR-ToF-MS coupled to the autosampler has higher sensitivity and throughput. This is achieved by having highly reproducible sample handling procedures and temperature control of the critical elements of the autosampler. The design of the setup enables measuring both static and dynamic headspace properties of a given sample. The coupling with the PTR-ToF-MS is completely automated and the instrument proved to be highly reliable.

The autosampler was validated on coffee samples; dynamic headspace of samples of green coffee beans, roasted coffee beans, roasted and ground coffee and coffee brew was measured. The reproducibility and repeatability is dependent on the sample, sample preparation protocol, and observed mass peak, since roasted coffee is not a stable sample.

Introduction

Volatile organic compounds (VOCs) released from roasted coffee samples are most commonly analyzed using gas chromatography with either direct headspace injection or solid phase micro extraction [1]. Solid phase micro extraction was also used to profile VOCs in the headspace over green coffee beans [2]. These methods are robust and reliable, but are not high-sample throughput and represent only static measurements of headspace.

PTR-MS is an excellent tool for measuring dynamic processes involving release of VOC, such as coffee roasting and gives complimentary results to off-line methods [3]. PTR-ToF-MS is also a suitable tool for off-line analysis of coffee headspace [4], since the high resolution of the time-of-flight mass spectrometer enables separation of isobaric compounds. Dynamic headspace measurement (DHS) of coffee samples is possible with PTR-MS [5]. Manual sampling of off-line measurements by PTR-MS introduces an additional uncertainty compared to automatic handling and sampling. Therefore, coupling the PTR-MS to an autosampler leads to strong improvement towards achieving highly reproducible results. An example of such coupling for high-throughput measurements of coffee samples is described in the literature [6]. The aim of this project was to

develop an autosampling setup for the PTR-ToF-MS in order to improve the reproducibility and increase the throughput.

Experimental Methods

Coffee samples

Coffee samples of Arabica coffee were roasted on a Probatino drum roaster (Probat, Germany) in 1 kg batches to a roast degree of 100 Pt (measured with Colorette 3, Probat). Coffee was ground with a Ditting KR 805 coffee grinder and the brew was prepared by stirring 10 g of ground coffee in 100 mL of water at room temperature for 5 min.

Coupling of the autosampler to the PTR-ToF-MS

A Gerstel MPS autosampler (Gerstel GmbH, Germany) with a custom developed Purge XL module was used for coupling with PTR-ToF-MS. A sample in a 20 mL or a 100 mL glass vial is put into the Purge XL module. The headspace is stripped with a purge flow and a dilution flow (nitrogen) is added to the purge flow to reduce the concentration of the analytes, introduced to the PTR-ToF-MS. For 100 mL vials the flow rates were: purge flow 35 nmL/min, dilution flow 250 nmL/min for green beans, 500 nmL/min for roasted whole beans and brew and 900 nmL/min for roast and ground coffee. 1/16" PEEK capillaries with i.d. 1mm were used, the transfer capillaries and connective pieces were heated to 90 °C, and the incubation temperature in the Purge XL unit was 50 °C.

PTR-ToF-MS settings and data analysis

A PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Austria) was used for detection of VOCs released from coffee. The PTR-ToF-MS settings were as follows: p (drift) = 2.30 mbar, U (drift) = 600 V, T (drift) = 80 °C. Mass spectra were recorded in the mass-to-charge (m/z) range of 0-310 a.m.u. with a time resolution of one second. Mass calibration was performed on $[H_3{}^{18}O]^+$, $[C_3H_7O]^+$ and $[C_6H_9N_2]^+$ ions.

The data was analyzed using the PTR TOF Data Analyzer and R environment (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

The autosampler settings were optimized for highest reproducibility of the experiment and therefore the sample throughput was modest. The vials were put in the Purge XL module for 10 min to incubate the sample and then the headspace was sampled for 15 min. The purge flow was selected based on the volume of the vial such that it enabled to measure static (at the start of an experiment) and dynamic headspace in the same sample measurement. The dilution flow was needed to reduce the concentration of analytes in order to keep the primary ion signal in the PTR-ToF-MS stable.

Validation for repeatability and day-to-day reproducibility of the coupled setup was performed by measuring a sample of benzoic acid in a 100 mL vial. Repeatability of 3 % (RSD, n=3) and reproducibility of 6 % (RSD, n = 3) was measured. Compared to roasted coffee this is a very stable sample and gives a good indication of flow and temperature stability and also PTR-MS performance. In case of roasted coffee, which is intrinsically not a stable sample, sampling protocol, storage and age affect the reproducibility of results.

PTR-MS mass spectra from all four types of coffee samples are presented in Fig. 1. The dominant mass peak of the green beans (Fig. 1a) is at m/z 33 that can be tentatively assigned to methanol. During coffee roasting the beans undergo major changes in composition and a large number of VOCs is formed (Fig 1b). In the roasted coffee beans, the VOCs are trapped inside the pores together with CO_2 that was formed during roasting. Once the roasted beans are ground, rapid degassing occurs and with the gases large amounts of volatiles are released as well (Fig. 1c). When a coffee brew is prepared, a complex mass spectrum can still be observed, but a clear difference in the composition, compared to roast and ground coffee, is seen (Fig. 1d) due to different partitioning of polar and non-polar compounds. Examples of such behaviour are two of the most intense peaks at m/z 59 and m/z 61 that can be tentatively assigned to acetone and acetic acid. Those peaks show different ratio of intensities, acetic acid being higher in intensity in roast and ground coffee compared to acetone, while the relative intensities are reversed in the brew.

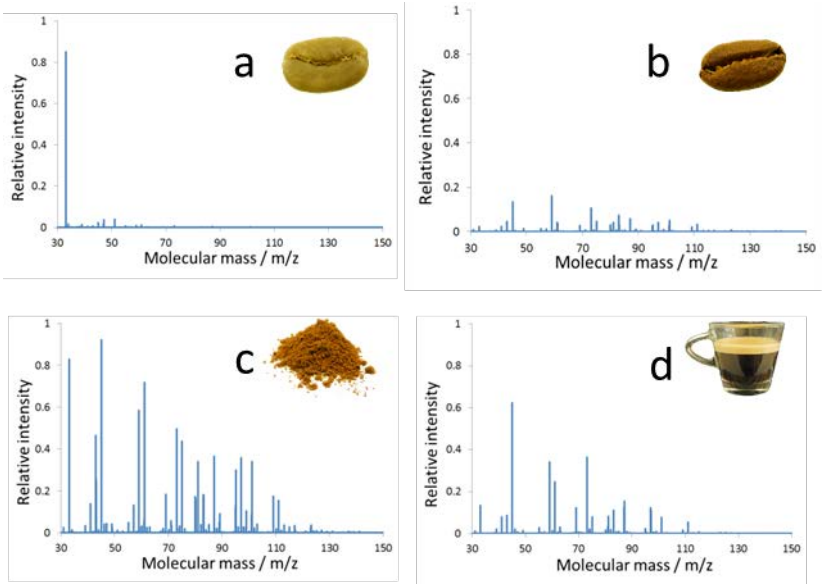

Figure 1: PTR-MS spectra of Arabica coffee green beans (a), whole roasted beans (b), roast and ground beans (c) and coffee brew (d).

Examples of DHS profiles for roast and ground coffee are presented in Fig. 2. In general there are two types of dynamic behavior of components in the headspace above roast and ground coffee (Fig. 2a and 2b), which can be assigned to high-volatiles and less volatile compounds. Highly volatile compounds are depleted from the headspace during measurements, while those with lower volatility remain rather stable in intensity. The compounds acetic acid and acetone mentioned in the previous paragraph have clearly different DHS profiles.

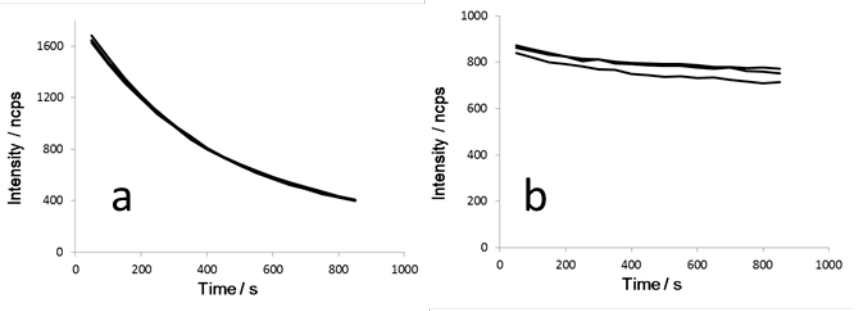

Figure 2: DHS profiles of roast and ground Arabica coffee, m/z 60 (a) and m/z 62 (b).

Conclusion

We have developed a very robust setup of an autosampler coupled to the PTR-MS. First experiments have shown excellent results with high reproducibility and possibility to measure DHS profiles of green beans, roasted beans, roasted and ground coffee and coffee brew. The measurements of coffee samples have revealed that compounds of different volatility or polarity are clearly distinguishable by observing the DHS profiles.

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Extraction Kinetics of Coffee Aroma Compounds during Espresso Extraction: On-line Analysis by PTR-MS

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Introduction

Progress in aroma research has always been linked to the development of new analytical techniques to determine volatile compounds. The introduction of electronic sensors and direct injection mass spectrometry techniques, capable of monitoring dynamic processes in real-time has been the last revolution in aroma analysis [1,2]. Chahan Yeretzian and co-workers, in close collaboration with Werner Lindinger, introduced PTR-MS into coffee research. Since then, PTR-MS has proven its high performance in various applications and fundamental studies such as the monitoring of coffee roasting [3-6], in-vivo aroma release [7-12], origin discrimination [3,13] or measurement of physical constants of aroma compounds [14-16].

The last contribution of Yeretzian's group to coffee aroma research has been the development of a PTR-MS based method to monitor the extraction dynamics of coffee [17,18]. The method consists on sampling volatiles from the coffee flow as it is extracted. This on-line method allows determination of volatile concentration in real time with high time resolution (1s), as a powerful complement to manual sampling and off-line analysis with GC-MS [19].

Here we will present this latest development; the application of PTR-ToF-MS to the study of extraction dynamics on single dose coffee capsules and a semi-automatic coffee machine.

Experimental Methods

Sampling

Volatile compounds were sampled with the custom build setup described on Sánchez et al. 2014, including minor modifications for the case of extraction with a semi-automatic coffee machine. Volatile organic compounds (VOCs) are sampled by "sniffing" by PTR-ToF-MS on the flow of coffee upon leaving the extraction hose. VOCs are diluted with compress air in order to avoid condensation on the sampling tubes and adjust their concentration to the dynamic working range of the mass spectrometer.

PTR-ToF-MS and data processing.

A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used. The diluted sample was introduced via a 90 °C heated sampling line into the drift tube operated at 2.3 mbar, 90 °C and 600 V drift tube voltage, resulting in an E/N value (electric field

strength/gas number density) of 140 Townsend (Td, 1 Td = 10^{-17} cm^2/V.s). PTR-ToF-MS data were recorded by TOFDAQ v.183 data acquisition software (Tofwerk AG, Thun, Switzerland).

Dead time correction, mass calibration and peak extraction and integration were performed using PTR-TOF DATA Analyzer software v4.17 [20]. Duty cycle corrected signals were normalized to 10^6 H_3O^+ primary ions.

Results

Time intensity profiles of aroma compounds during extraction.

Dynamics of extraction were compound dependent (Figure 1). While some compounds were extracted relatively fast, in few seconds, others kept being extracted during the whole extraction time. Therefore, the ratio between compounds was changing over time, affecting the aroma profile of the extracted coffee cup.

Using Hierarchical Cluster Analysis (HCA), compounds were grouped according to their time intensity profiles. The resulting families were formed by compounds sharing similar physicochemical properties and revealing polarity as one of the main properties affecting extraction.

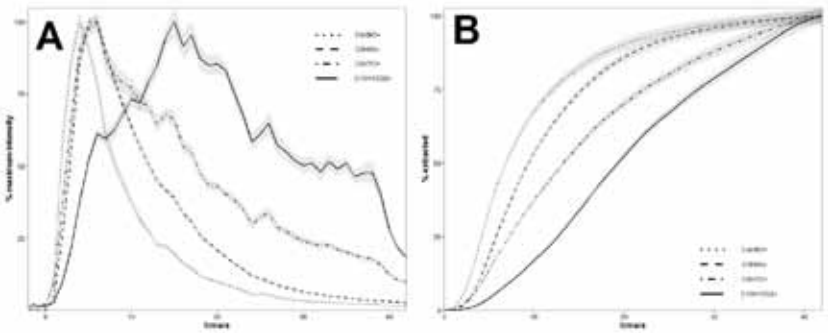

Figure 1: Time intensity profiles in a single dose capsule showing differences in extraction between four compounds (A) and integration of the area under the curve at each time point as a percentage of the total area at the end of the extraction (B). Shaded ribbons show the 95% confidence interval.

Coffee classification.

The dynamic data recorded could also be used for classification of single dose capsules according to coffee type. Principal Component Analysis (PCA) on the area under the curve for 95 ion traces showed grouping of samples according to capsule type (figure 2). HCA also resulted in classification of coffees into six clusters, each cluster corresponding to one of the coffee type.

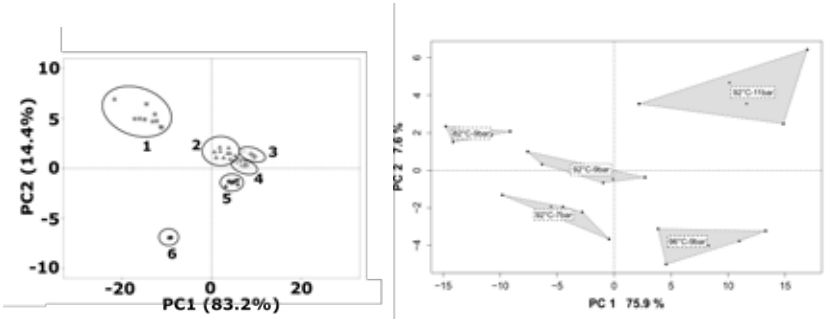

Figure 2: PCA showing separation of different coffee capsule types (left) and extraction conditions (right), using the area under the curve for all the masses detected in each case.

Impact of extraction parameters.

By using a semi-automatic coffee machine, the effect of pressure and temperature on coffee volatile extraction was studied. Figure 2 (right) shows a PCA performed with the area under the curve of the 120 ion traces measured on extracted coffee. Five groups could be differentiated, corresponding to the five different combinations of water pressure and temperature used for extraction. The different temperature-pressure combinations resulted in different extraction profiles of the volatile compounds. Those differences appeared mainly at the last part of the extraction time and were compound dependent, therefore affecting the aroma balance.

Discussion

On-line PTR-ToF-MS analysis of volatiles released from coffee flow in real time is a powerful method to study the kinetics of aroma extraction. The presented method overcomes the problems of previous GC-based approaches: (i) it increases temporal information, from a few data points over the whole extraction time to a one second time resolution; (ii) and it reduces sources of variability, as the time-evolution of each VOC is monitored on-line in a single extraction process and is not a combination of multiple different extracts.

On-line analysis of coffee extraction not only revealed the kinetics of extraction for different volatile compounds, but also allowed us to identify differences among commercial coffee types and evaluate the impact of different parameters (pressure and temperature) that affect extraction dynamics of aroma compounds.

The simplicity, high sensitivity and time resolution of this method makes it a perfect approach to study the effect of any parameter affecting espresso extraction (i.e. water composition, ground size), to get analytical data that helps the barista community or to evaluate coffee machines performance, amongst other potential applications.

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Applications in Medicine and Biotechnology

Exhaled breath diagnostics: potential applications for real-time analysis at organism (*in vivo*) and cellular (*in vitro*) levels of organization

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Abstract

Clinical diagnostics, medical monitoring, and other health-based analytical methods all rely on measuring perturbations from an established norm to detect changes in health state. Blood, breath and urine are the three primary biological media used for clinical diagnosis. Of these, breath sampling is non-invasive, avoids potentially infectious wastes, and can be administered in any time frame. Unlike blood and urine, breath is primarily gas-phase (plus some aerosol) and so can be directly analyzed using real-time instrumentation such as proton transfer reaction mass spectrometry (PTR-MS). In the case of breath, the analytes are aggregates of systemic cellular respiration, in essence, the sum of all "cell breath".

Studies of intact organisms (animal or human) are considered the "holy grail" for health-based research because they encompass the complete systemic response; however, they are also resource and time intensive. Reproducing certain aspects of real-world response and metabolism at the cellular level is an attractive streamlined alternative. As such, an emerging topic of medical research is assaying off-gas products from individual cell-lines *in vitro* as a surrogate for systemic breath measures from animal or human subjects. Recent research has explored bacterial cellular respiration *in vitro* for ultimately detecting pulmonary infections *in vivo*. Other work is exploring human cell-lines under varying stressors including potentially toxic (exogenous) chemicals and their metabolites.

In this presentation, we discuss a series of new experimental designs for linking standard *in vivo* breath tests with their *in vitro* counterparts. In particular, we present *in vitro* concepts for assaying specific metabolic pathways with gas-phase probes, and for monitoring cellular metabolism with flow-through multi-well plates. With these new technologies, we expect that real-time monitoring of cellular respiration will become an important tool and ultimately compete favorably with existing liquid-phase (off-line) analytics.

Gas-phase probe molecules

Standard toxicity testing uses *in vivo* animal tests to assess systemic responses and metabolism for chemicals in commerce (e.g. pesticides, solvents, etc.). To streamline such tests, we have proposed using single cell-lines in a bioreactor scheme wherein we analyze metabolism directly via gas-phase analysis. The analytical signal is a change in metabolite production of a probe molecule in response to different stressors such as external conditions (nutrients, temperature, etc.), exogenous compounds, and chemical metabolites [1].

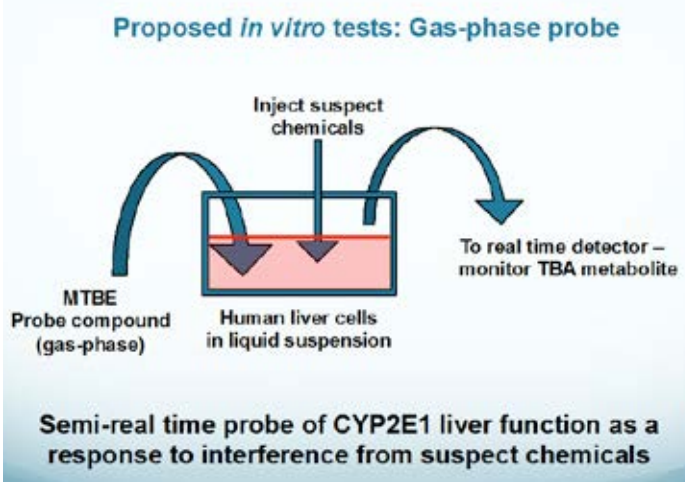

Figure 1: Proposed scheme for testing metabolic response of human cell-lines to external stressors

Dynamic multi-well plate analysis

A follow-on of the bio-reactor model of *in vitro* cellular respiration analysis is to further reduce the scale to the multi-well plate, yet maintain metabolic competency. New technology is now emerging wherein microfluidic channels are used to connect multi-wells to allow metabolite products to flow sequentially from one micro-culture to the next. This will allow different cell-lines, representing different organ systems, to be exposed *in vitro* in a micro scale that simulates systems biology. This new technology has been developed by SciKon Inc., Chapel Hill, NC. The premise is to culture cells in wells independently, and then apply sufficient nutrient/buffer in the source well column to establish micro-channel flow connecting them sequentially. Figure 2 shows a diagram of the multi-well system.

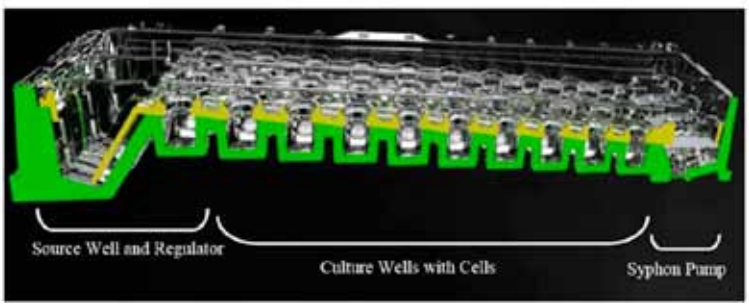

Figure 2: Cross section of SciKon "SciFlow" 96-well plate system. Green fill shows well locations and surfaces. Fluid flows from left to right. The plate is standard size 96-well plate, but has position 1 (left) as a deep source (buffer) reservoir, 10 wells for cell cultures, and position 12 as a fluid sink. Gas phase analysis is amenable to headspace analysis of individual wells [2].

Figure 3 shows a test with blue dye applied to the source wells in rows 1 and 2. Both rows have been partially prefilled with buffer just below the micro-channel level. Sufficient buffer was added to the source well of row 2 to establish flow.

Figure 3: Photographs illustrating scale and micro-channel flow (left to right). Once the source well is primed, the dye is seen to migrate from left to right.

Summary

Both the macro-scale bioreactor and the micro-scale multi-well plate technology have the advantage of maintaining metabolic competency as expected in an intact organism. As such, any breath based analytics application such as PTR-MS is equally amenable to "cell-breath" analysis. In fact, the low flow requirements for PTR-MS are especially favorable for smaller cell-based systems.

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Two decades of breath analysis using PTR-MS: past achievements, current endeavours, and future visions

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Abstract

PTR-MS was launched two decades ago as a standalone tool for volatile trace gas analysis. Although it rapidly emerged as an indispensable apparatus in atmospheric chemistry and food applications, the very first PTR-MS publication focussed on breath analysis, namely on the detection of acetonitrile and benzene in the breath of smokers and non-smokers. In the ensuing period PTR-MS has become a powerful analytical instrument that is in worldwide use for many mainstream and niche research applications relating to trace gas detection. Concurrently, breath analysis as a whole has emerged as a flourishing scientific discipline to cover aspects ranging from physiology, clinical diagnostics, the *in vitro* metabolome, and the human exposome, to name but a few. PTR-MS has played a strong role in contributing to the progress in many aspects of this field over the last 20 years, primarily owing to its ability to perform direct breath-by-breath analysis of exhaled volatiles down to ultra-trace levels, and more recently with the increased wealth of data delivered by PTR-TOFMS. This talk will offer a retrospective of the notable role of PTR-MS in key breath research discoveries, will explore current endeavours, and will look to the horizon of what is to come; a limited selection is presented here in this abstract.

Introduction

Two decades ago, in the year that Crutzen, Molina, and Rowland won the Nobel Prize in Chemistry for their work in atmospheric chemistry on the formation and decomposition of ozone [1], a publication reporting on a new chemical ionisation-based instrument to detect trace amounts of volatile organic compounds (VOCs) in ambient air was published [2]. This newly-introduced technique of proton-transfer-reaction mass spectrometry (PTR-MS) made a decisive step in comparison to its predecessor, the selected ion flow drift tube mass spectrometer (SIFDT-MS), in that unlike the latter, which was developed solely for the study of ion-molecule reaction kinetics and pathways [3], this instrumentation was purposely designed as an applied research apparatus for the dedicated detection of VOCs in ambient air, with atmospheric chemistry, food flavour, and breath analysis initially flagged as the primary areas of application. Indeed several years after its introduction PTR-MS was implicit in gathering data for a large-scale air pollution study, with the research paper reporting on the findings – co-authored by one of the aforementioned Nobel laureates – being the second most cited PTR-MS publications to date (with 399 citations) [4].

As might be expected, besides that paper, the most cited PTR-MS publications are those that initially introduced the technique to the scientific community, outlining its functionality, proposing potential applications, and demonstrating its performance. Three of the four highest cited publications are the well-known Hansel *et al.* 1995 [2] (in fourth place, with 363 citations), and the two Lindinger *et al.* 1998 papers [5,6] (respectively in third and first place, with 375 and 849 citations) (data retrieved from Thomson Reuters on 1/12/15). Although the Hansel *et al.* article from 1995 [2] is widely assumed to be the earliest PTR-MS publication, the first article to report on the new technique (albeit a letter rather than a full research paper) focussed on its

application in breath analysis and was entitled ‘Acetonitrile and benzene in the breath of smokers and non-smokers investigated by proton transfer reaction mass spectrometry (PTR-MS)’, written by the same authors and published in the same journal one month earlier [7]. The paper showed for the first time the washout kinetics, or toxicokinetics, of these two compounds from the body, demonstrating, for example, that acetonitrile requires almost a week after a cessation of smoking before its concentrations diminish to background levels; such kinetics studies were principally achievable due to the on-line capabilities of the PTR-MS instrument.

The early comprehensive articles on PTR-MS equally reported on breath applications, such as the analysis of exhaled trace components in people afflicted with liver cirrhosis, again a comparison of acetonitrile in the breath of smokers versus non-smokers [2], or other varied aspects [6]. Key features of PTR-MS made it highly suited for breath gas analysis: its use of air as a carrier/buffer gas, its relative insensitivity to changes in sample gas humidity, and its rapid response time, together making the non-invasive analysis of breath volatiles accessible. These early proof-of-concept studies established the utility of PTR-MS in breath analysis from the outset.

Past achievements

Human breath contains in excess of 800 VOCs, all of which contribute to the human volatilome [8]. Breath volatiles are either of endogenous (biochemical) origin, or arise due to environmental exposure (exogenous). When embarking on any new scientific discipline it is essential to first establish the status quo from which discoveries can be made. In breath analysis this relates to what is considered to be ‘normal’ in terms of the nature and concentrations of endogenous compounds ubiquitous in human populations, based upon which perturbations or the appearance of unique markers can be established. Understanding the origins and representative concentrations of these endogenous compounds are thus paramount before undertaking a search for biomarkers.

Acetone and isoprene are the most abundant endogenous compounds present in exhaled human breath: the former arises through the decarboxylation of acetoacetate in the liver, whereas the latter is in part related to cholesterol biosynthesis, although the exact mechanisms have not yet been fully elucidated. One of the major contributions of PTR-MS breath analysis to the field to date has been the data gathered on these two compounds, with both being quantified in breath in relation to general physiology, age and gender [9,10], in comparison to blood concentrations [11], or characterised based on compartmental mathematical modelling [12,13] using PTR-MS data. These cumulative findings have increased our understanding of what can be considered ‘unremarkable’ levels from which potential health-related perturbations can be deduced.

Current endeavours

A current major challenge in breath research is the absence of standardised, cross-validated analytical approaches that would enable more direct comparisons between datasets of independent studies: the fact that the manifold studies conducted on lung cancer, for example, have each revealed their own unique (set of) volatile biomarkers that are not comparable to their neighbouring studies is a very blunt indication that this lack of benchmarking is holding back progress in the field. Establishing standardised practices in breath analysis is presently a high priority of the International Association of Breath Research (IABR), and PTR-MS is playing a prominent part in achieving this. There are many aspects for consideration in creating standardised approaches, including past exposure (the exposome), breathing manoeuvres, sampling conditions, and data-mining procedures, to name but a few. The increasing popularity of PTR-MS as a breath research tool throughout the world, notwithstanding its key features for real-time and repeatable analysis, make it an ideal apparatus for conceptualising and testing

standardised breath analysis approaches. A proposed framework for establishing standardised practices was recently published, heavily referencing PTR-MS by way of example on several aspects under consideration [14].

Future visions

A recent study utilised PTR-MS to screen for potential volatile biomarkers in liver cirrhosis patients, with an initial discovery approach identifying limonene as a prospective candidate, and a subsequent targeted approach yielding a high predictive value for the disease based on this marker [15]. The novel aspect of this study was that the authors attributed limonene not to disease-related endogenous production, but rather to its reduced metabolism by the impaired liver; in other words, they utilised a xenobiotic compound and its lack of metabolism by a dysfunctional organ to flag the disease. In particular, it was suggested that future tests to diagnose liver disease via the breath might involve the administration of labelled substrates to follow the metabolism of the target compound as a first screening to discriminate between ordinary kinetics and a perturbed washout; this will likely prove more successful from a negative predictive aspect (i.e., giving an 'all clear') than a positive prediction (i.e., disease diagnosis), whereby the latter will instigate proceeding with further, clinically-established examinations (e.g., blood tests, biopsy). This use of labelled probative substrates has been successfully demonstrated for other diseases [16] and is clearly viable using PTR-MS [17]; thus, it hold high potential as a future routine breath test.

Another promising application for PTR-MS, particularly PTR-TOFMS, is *in vitro* metabolomics and cellular respiration (cf., abstract by Winters, Beauchamp, and Pleil). The high-throughput capabilities offered by PTR-MS make it an ideal tool to record the volatile fingerprints of diverse cell-lines, bacteria, or viruses, as has been demonstrated in several studies (e.g., [18,19]). Generating a library of unique fingerprints might in future allow for patients to be screened for bacterial or virual infection via the breath, again with a negative prediction ruling out specific conditions and a positive prediction providing an impetus for further examinations.

Summary

Overall, both PTR-MS and the field of breath analysis have come a long way over the last two decades. Ongoing technological advancements in PTR-MS – including PTR-TOFMS and the recently-introduced peripheral equipment such as the fastGC and autosampler interfaces – will push breath analysis to new frontiers, with the many novel applications promising exciting discoveries on the horizon.

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Real-time Monitoring of VOC Emissions from Bioprocesses – What you see is not what you get!

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Abstract

Monitoring volatile metabolites in the offgas of fermenters is a promising new method for advanced process monitoring and control. We develop a model to simulate volatile metabolites in a fermentation, which is experimentally tested. We are able to show that measured offgas concentrations and their metabolic production rates do not necessarily correlated directly, which could explain the deficiency of established applications of offgas VOC monitoring in bioprocess control. However, using the developed model we are also able to calculate the relevant production rate from dynamically measured offgas concentrations.

Introduction

Modern biopharmaceutical drugs are produced in microbial fermentation processes. The monitoring of these bioprocesses is becoming increasingly relevant, especially in the manufacturing sector to promote cost-effective production. To date, biotechnical processes are generally monitored using off-line analysis, which is labor and cost intensive, providing results often too late for corrective actions. Consequently, process development is mostly empirically driven only by the control of unspecific parameters, such as pH, pO_2, pCO_2, aeration, temperature and stirrer speed. The product quality is assessed only at the end of the process, the so-called "Quality by Testing" concept. The aim of the FDA's (US Food and Drug Administration) PAT (Process Analytical Technology) initiative is to change this by promoting advanced on-line process monitoring for real-time process control.

Apart from the metabolic end-product CO_2, microorganism in a fermenter produce a broad range metabolites as by-products of growth and production of the actual product. Some of these metabolites are secreted to the fermentation broth and those with sufficient volatility end-up in the off-gas of the fermenter. Consequently, real-time monitoring of these volatile organic compounds (VOCs) will provide information, directly linked to the metabolic activity of the microorganisms. The measurement of compounds in the off-gas is thus a promising, non-invasive approach to easily access highly-relevant process information. PTR-MS in combination with a specialized inlet system has already successfully been used to monitor more than 70 volatile metabolites in the offgas of different bioprocesses with *E. coli* and CHO cells [1].

In contrast to the classical offgas variables CO_2- and O_2-concentration, direct correlations between emitted volatile metabolites and process relevant parameters could not yet be established. In this paper, we discuss the underlying reason and a solution for this shortcoming. Unlike highly volatile CO_2 or O_2, most VOCs have a lower volatility and higher solubility in the fermentation medium. We develop a model, that allows us to simulate the offgas concentration of metabolites with different volatility at a given production rate of the microorganisms. We are able to show that the process relevant production rate and the resulting offgas concentration of a volatile

metabolite are not directly correlated. We develop a method to calculate the production rates from the dynamically measured offgas concentration.

Methods

We simulate the behavior of VOCs in a fermenter to investigate the correlation of the production rate of a VOC and its concentration in the offgas. When VOCs are produced by microorganisms they are released into the fermentation broth and form a solution. From the liquid medium they are emitted into the gas, used for aerating the fermenter. The resulting concentration in the gas depends on the liquid gas partition coefficient, given by the compound specific Henry's law constant (*H*). To define the variables in our model, figure 1 (left) shows a simplified scheme of a fermenter including the aeration gas flow (*F*). The fermenter is filled with an aqueous medium with volume *V*. We assume a production rate (*P*) of a volatile that is emitted by the cells into the fermentation medium. Further, we use the VOC concentration in the liquid (*cl*) and in the gas (*cg*). The total amount of a VOC in the liquid is $cl \cdot V$ and its change in time can be described by a differential equation:

$$\frac{\partial}{\partial t}(V \cdot cl) = P - cg \cdot F \qquad (1).$$

The amount of VOC in the medium is increased by the production rate *P* and it is decreased by the emissions into the gas flow ($cg \cdot F$). The Henry's law constant (*H*) connects the concentration in the gas to the concentration in the liquid as $cl = H \cdot cg$. Now we can write the differential equation as:

$$V \cdot H \cdot \frac{\partial}{\partial t}(cg) = P - cg \cdot F \qquad (2).$$

In a further step, we simulate the head-space concentration, which is equivalent the concentration in the offgas that is measured. When the head-space volume is comparably small in relation to the aeration flow, its concentration quickly approaches *cg*. Therefore, we omit this additional step and assume that *cg* is equivalent to the offgas concentration. We also assume that the interaction (time and surface area) between gas and liquid is sufficient to establish equilibrium, where Henry's law applies. This assumption is supported by the fact that the aeration is designed to form small bubbles (large surface) which have a long residence time in the liquid in order to deliver oxygen efficiently.

Results and Discussion

Simulation

With differential equation (2) we can simulate the VOC concentration numerically using small, discrete time steps. We input a step function for the production rate, which corresponds to a metabolic process becoming active to produce a VOC at constant rate for a defined time, and then becoming inactive again. We simulate three different compounds with high, medium and low Henry's law constant, respectively. The three profiles are depicted in figure 1 (right).

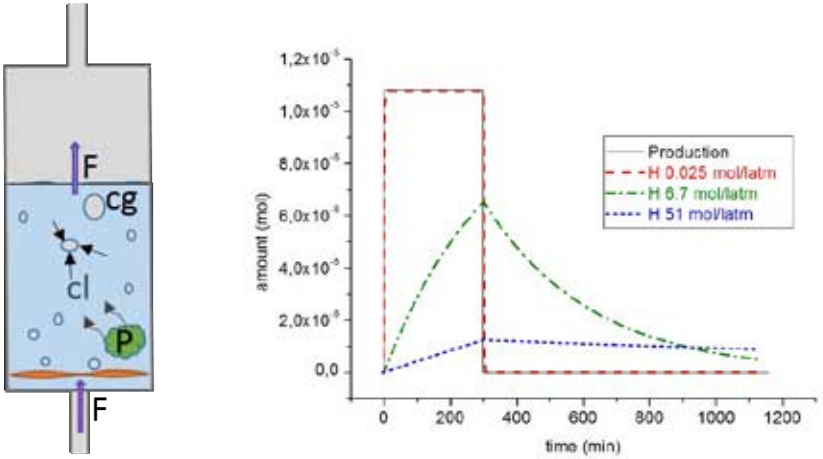

Figure 1: (left) Schematic of a fermenter. (Right) Assumed Production rate and concentrations (cg=H·cl) of three compounds with different volatility: CO_2 with H = 0.025 mol/latm; acetaldehyde with H = 6.7 mol/latm; 1-butanol with H =51 mol/latm. Simulations were performed with parameters typical for a fermenter: Duration 300 min, P = 1.1×10^{-5} mol/min production rate, V = 10 l liquid media, T = 310 K and F = 5.3 l/min aeration.

From figure 1 (right) we see that for the same production rate profile the "measured" gas concentration differs substantially for the three compounds with low (CO_2), medium (acetaldehyde), and high (1-butanol) Henry's law constant. While compounds with high volatility (low Henry's law constant) the gas concentration nearly replicates the production rate profile, compounds with lower volatility first accumulate in the liquid and the resulting gas concentration strongly differs from the actual production rate. Except for compounds with a high volatility, the search for direct correlation between gas concentration and production rate must fail.

Experimental validation

To validate our model experimentally we use a real fermenter and emulate the profile used for the production rate in the simulations. A pump feeds a liquid standard at a controlled rate into the fermenter. The feed is at a constant rate for a defined time and then stopped to resemble the profile of the production rate assumed above. The liquid standard contained several VOCs with a range of Henry's law constants and with relevance in bioprocess monitoring.

When the production rate is zero, the exponential decay of the concentration can be used to measure *H*, the Henry's law constant, directly. We have done this for a range of compounds, namely methanol, acetaldehyde, ethanol, 1-butanol, acetone, methylethylketone and indole. Using a PTR-MS, these tests can be conducted simultaneously. For some compounds, we find excellent agreement between literature and experimentally determined *H*. However, for other compounds or by conducting the same test in a different fermenter, we sometimes find deviations as large as 50%. We attribute this equally to the documented uncertainty in the literature values of *H* as well as small differences in the complex setup of fermenters. Therefore, we recommend to determine the Henry's law constant for each combination of compound and fermenter, which will then cover other calibration factors.

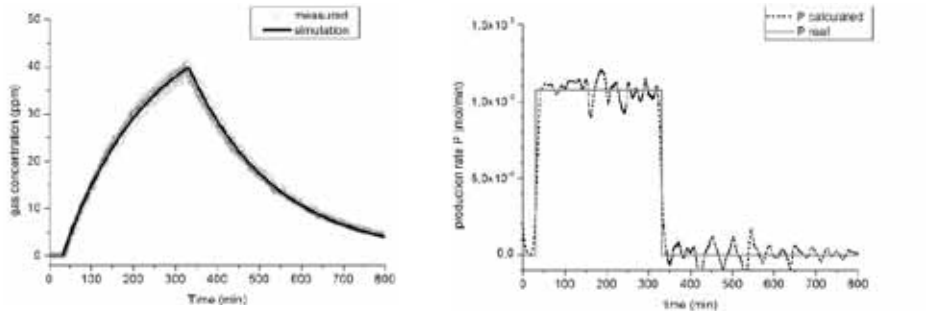

Figure 2: (left) measured and calculated offgas concentration. (right) prediction of the production rate P from the measured data, de-noised using wavelets.

In figure 2 (left) we plot the measured and calculated gas concentration using acetaldehyde as an example. We use the experimentally determined Henry's law constant and find excellent agreement between measured and simulated data.

Calculation of production rates from offgas concentrations

We have shown that the production rate and the gas concentration are not directly correlated. However, with the presented model we are able to gauge the production rate from the dynamically measured concentration in the offgas. This is the key to using offgas VOC measurement for monitoring and control of fermentations. We can easily resolve the production rate P in eq. (2). The variables we need to input, are the measured concentration cg and the change in concentration $d(cg)/dt$, i.e. the change between the current and the last time step (divided by the length of the step). In our numerical simulation, this unsurprisingly allows us a perfect replication of the production rate. However, in a real-life scenario, the measured data is noisy, which particularly affects the slope, $d(cg)/dt$, yielding dreadful results. To overcome this problem we have tested several de-noising procedures. Most promising results have been achieved using wavelets [2]. Figure 2 (right) shows the real production rate and the production rate calculated form the (de-noised) measured offgas concentrations.

Conclusions

The production rate of a volatile metabolite is the relevant parameter for advanced process control based on offgas VOCs. We have developed a model to simulate the gas concentration of a VOC emitted through the offgas of a fermenter for a given production rate. We show that, with the exception of highly volatility VOCs, the production rate is not directly correlated to the offgas concentration. However, applying the developed model, we could successfully calculate the production rate from the measured offgas concentration.

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Monitoring volatile organic compounds in unrestrained mice as a new tool to characterize animal models for human diseases

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Abstract

Proton transfer reaction mass spectrometry (PTR-MS) is widely used in the study of human breath volatile organic compounds (VOCs) with remarkable findings concerning disease specific alterations. However, for many VOCs the metabolic origin and mechanistic association to pathogenic states are not clarified yet. The laboratory mouse as a model organism can complement human studies in these respects. Here, we show the VOC pattern of mice in response to various metabolic challenges and interventions.

Introduction

Disease-oriented research largely depends both on preclinical studies in animals such as the laboratory mouse as well as clinical studies in human patient cohorts. Mouse models are, in contrast to human clinical studies, accessible to genetic modifications and can be analyzed under highly

standardized and controlled conditions. In the German Mouse Clinic at the Helmholtz Center Munich we have established a method to measure volatile organic compounds (VOCs) in un-restrained and non-anaesthetized mice [1]. We use this method for screening mutant mouse lines for alterations in metabolic functions under steady state conditions as well as in response to defined challenges and treatments [2]. In the search for early biomarkers for disease conditions or a specific metabolic status, the online monitoring of volatile organic compounds (VOC) in exhaled breath offers an attractive non-invasive approach addressing both short and long-term responses to challenges and treatments.

Experimental Methods

We induced obesity by feeding a high fat diet to male C57BL/6J mice for 12 weeks (HFD). In addition, we analyzed male C57BL/6J mice carrying a global knock-in mutation in melanocortin-4 receptor (W16X, MC4R-ki). In both experimental groups, the source strengths of 208 volatile organic compounds were analyzed ad libitum fed and after overnight food restriction. Volatiles altered in obese mice were selected using the AUC-RF algorithm. Robustness was confirmed using 5-fold cross-validation and roc curves of real and permutated data. Effects of fasting and obesity intervention were tested further using false discovery rate-controlled mixed effects model. A Gaussian graphical model was employed to identify chemical and metabolic links among the selected volatiles.

Results

In both models for obesity, volatiles relevant for the separation of obese and lean mice were detected (26 in MC4R-ki, 22 in HFD mice, figure 1). Eight volatiles were found to be important in both obesity models. Interestingly, by creating a partial correlation network of the volatile metabolites, the chemical and metabolic origins of several volatiles were identified. HFD-induced obese mice showed an elevation in the ketone body acetone and acrolein, a marker of lipid peroxidation, and several unidentified volatiles. In MC4R-ki mice, several yet-unidentified VOCs were found to be altered. Remarkably, the pheromone (methylthio)methanethiol was found to be reduced, linking metabolic dysfunction and reproduction.

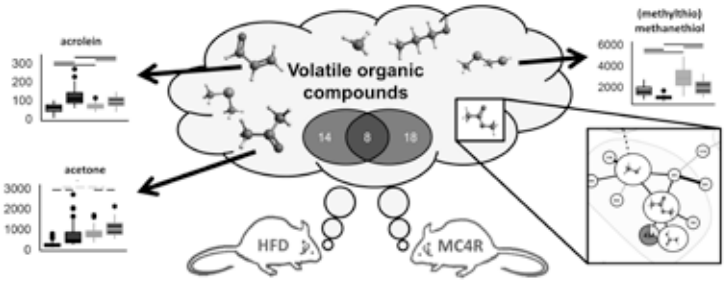

Figure 1: Graphical summary showing a screening for VOCs altered in both a mono-genetic and diet induced obese mouse line.

Discussion

The signature of volatile metabolites can be instrumental to identify and monitor metabolic disease states, as shown in this screening of two obese mouse models. Our findings show the

potential of breath gas analysis to non-invasively assess metabolic alterations for personalized diagnosis. Furthermore, breath gas analysis could aid in the stratification of patients with heterogeneous metabolic phenotypes and risk profiles.

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Advanced bioprocess monitoring: A key to process and systems understanding

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Abstract

Biotechnology with its empirically driven process engineering approaches represents one of the fastest growing and successful industry branches of the last decades. However, if this success story should continue the general accepted rule that comprehensive process understanding represents the key for rational process design and successful implementation of economic processes must not be longer ignored.

The major challenges on the way to process understanding and rational bioprocess engineering are the complexity of biological systems and the limited capabilities of in-situ and real-time monitoring tools. Therefore, improvements in bioprocess monitoring and diagnostic capabilities are mandatory.

The expansion of off-line analytics to -omics techniques and advanced analysis provides a comprehensive data base down to the molecular level. The thus gained physiology relevant process information represents the first pillar of a rational bioprocessing approach.

Control and intervention during the process depend on real time access on relevant key variables but common bioprocess conditions demand a great deal on sensor/analyzer technology. In addition there is a mutual exclusivity of non-invasive signal acquisition and metabolic relevance of the signals. Consequently two different approaches are followed in parallel. The first one is the acquisition of non-specific signals and spectra indirectly related to either the cell population or compounds in the medium. The valuable but “hidden” cell physiology–relevant information is extracted by setting up correlations to off-line acquired bio-analytical data using statistics and chemometrics. Based on this information, data-driven soft-sensor systems have been successfully developed for on-line “monitoring” of complex variables such as cell density or product titer [1].

The alternative approach aims at analyzers for direct detection of physiologically relevant process variables. As the major part of metabolites is enclosed in the cell compartment direct access is very limited. Volatile organic compounds (VOCs) emitted by cells are directly connected to physiologically relevant information. They perfectly match the key requirements of analytes for real-time, non-invasive bioprocess monitoring as they are easily accessible via headspace sampling. Proton transfer reaction–mass spectrometry (PTR-MS) was selected as this technology allows for on-line measurement of VOCs with high sensitivity, down to concentrations in the pptv

range. The thus accessible VOC spectrum represents a host- and process-specific metabolite panel for host cell characterization, efficient process operation, and automation [2].

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Diabetes biomarkers in breath gas, findings, setbacks and perspectives

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Abstract

Currently, cardiovascular, oncologic, neurodegenerative and chronic respiratory diseases as well as diabetes form a severe burden to the countries worldwide. Breath gas analysis (BGA) is supposed to build an efficient, non-invasive diagnostic tool for the detection of these diseases in their early stages. Volatile organic components (VOCs) in the exhaled breath reflect the volatiles composition of the bloodstream and airways and therefore the metabolic status of the whole organism. However, in most cases, there is no one single volatile biomarker indicating a distinct disease. Therefore, an approach to identify a disease (diabetes mellitus) by the response of the organism to a metabolic challenge was used. First results were quite promising but some setbacks are forcing us to rethink the approach.

Introduction

It has been shown, that the analysis of exhaled breath and exhaled breath condensates (EBC) are useful, non-invasive tools for the detection of biomarkers of respiratory diseases like asthma and lung cancer [1]. Due to the intense exchange with the blood stream, exhaled breath does not only reflect the conditions within the airways, but contains also volatile organic compounds (VOCs) from the internal metabolism. Thus, exhaled breath analysis has a strong potential to monitor biomarkers of diseases like diabetes mellitus (DM)[2].

Due to the rapidly increasing prevalence in both, adult and childhood obesity, DM is a worldwide growing problem. Gestational diabetes mellitus (GDM), defined as a glucose intolerance, first detected during pregnancy, is the most frequent metabolic problem during gestation and its prevalence is also increasing across most racial/ethnic groups studied so far [3]. GDM is associated with an increase in perinatal morbidity and mortality. The risk for the development of diabetes postpartum in these women as well as for the development of obesity and diabetes for their offspring in later life is significantly increased [4]. Therefore, screening of GDM is important and, based on an oral glucose tolerance test (oGTT), currently practiced in women considered at risk in many Western countries. The diagnosis of the oGTT test is based on the blood glucose concentration measured just before and repeatedly after the ingestion of 75 mg glucose. A non-invasive test, based on breath gas analysis, could increase the numbers of women screened and subsequently treated, leading to a reduction in perinatal morbidity.

Experimental Methods

In close connection with a GDM screening (75 g glucose oGTT) we performed breath gas analysis by means of a standard proton transfer reaction quadrupole mass spectrometry (PTR-MS)

device (Ionicon GmbH, Innsbruck, Austria). Tested persons breathed at 6 to 10 min. intervals in a buffered end-tidal sampler (Ionicon GmbH) connected to the PTR-MS by a heated flexible PTFE tube. This set-up allowed the controlled measurement of the end-tidal breath fraction. From a broad spectrum of initially 142 signals (m/z 18…174) the responsive signals were selected for further analysis. Two distinct response patterns were observed between 0 and 120 min of the oGTT, a "kinetic" response (19 signals), resulting in an impulsive peak response and a "linear" response (eight signals), with either decreasing or increasing trend. For each type of response, signals were fitted to mathematical equations with a time evolution parameter μ. These parameters as well as the basal and peak levels of the measured signals were used to distinguish between the different patient classes.

Since PTR-MS is not able to identify individual components from given m/z values, additional measurements using needle trap micro extraction (NTME) in combination with thermal desorption and comprehensive two dimensional gas chromatography time of flight mass spectrometry to identify individual VOCs in the exhaled breath were carried out. Using short time sampling of about one to two minutes, we were able to identify several isomers or isobars belonging to one m/z value and, due to the time dependent concentration profile, to assign one isomer or isobar as responsible for the direct measured response within the PTR-MS.

To asses confounding factors, further PTR-MS measurements were performed in conjunction with oral glucose tolerance test. These tests were carried out under a particular oral hygiene. For this purpose, the bacterial coating of the tongue was mechanically scraped and subsequently the oral cavity rinsed for about one minute with an antibacterial agent (chlorhexidine). Further, for some tests, the commonly used oGTT juice was replaced by an aqueous glucose solution (75 g).

Results

With the approach on evaluating the time dependent evolution of the different parameters (m/z) it was possible, in a first study [5] to differentiate the four groups (gestational diabetes (GDM), impaired glucose tolerance (IGT), marginal and the healthy control group (for details see [5])

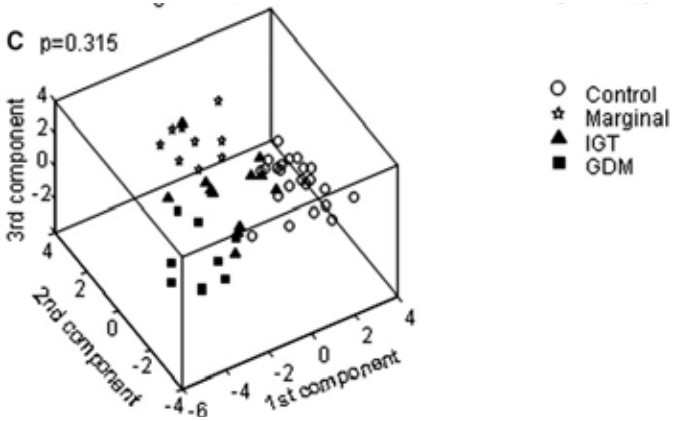

Figure 1: Multivariate analysis of the time dependent evolution dataset associated with the oral glucose tolerance test patients. Only one misclassification(GDM; IGT) (taken from [5])

Unfortunately, in a follow up study on women with previously diagnosed GDM [6], this result could not be repeated. A closer look to the dataset, the identification of individual components representing the m/z values as well as further investigations on confounding factors with healthy (male) volunteers showed that the results are strongly influenced by oral bacteria. The second study on women was performed during autumn and winter time. Besides the unknown hormone status, medication and tobacco smoking, a great number of the patients were influenced by infections of the upper airways. The combination of all these unknowns leads to strong variations in the breath profile, which make a clear classification impossible.

Summary

Since two studies for breath gas biomarkers of diabetes mellitus show different results, we have to critically re-think the analytical approach and the boundary conditions. Although breath analysis seems to be an excellent tool for the detection of diseases in their early states, oral bacteria play an important role. This has to be taken into account in further discussions about biomarkers of diseases as well as for the planning of related studies and the breath sampling.

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Quantification of selected volatile organic compounds (VOCs) in human urine by SPME-GC-SRI-TOF-MS working with NO^+ as the reagent ion

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Abstract

Selective reagent ionization time of flight mass spectrometry with NO^+ as the reagent ion (SRI-TOF-MS(NO^+)) in conjunction with gas chromatography (GC) and head space solid-phase microextraction (HS-SPME) were used to determine selected volatile organic compounds in human urine. While the coupling of the SRI-TOF-MS instrument with a GC column compensated the low selectivity of SRI-TOF-MS and assisted the identification of urinary species, SPME supported the extraction of VOCs of interest from the liquid phase. A total of 16 volatiles (10 ketones, 3 heterocyclic compounds, and 3 sulphur compounds) exhibiting high incidence rates were quantified in the urine of 19 healthy volunteers. Limits of detection (LODs) ranged from 0.08 nmol×L^{-1} for allyl methyl sulfide to 1.0 nmol×L^{-1} for acetone and furan (with RSDs ranging from 5 to 9%). The observed median concentrations ranged from 0.55 nmol×L^{-1} for furan to 11.6 µmol×L^{-1} for acetone and agree reasonably well with the available literature values.

Introduction

Volatile organic compounds (VOCs) emitted by the human organism form a chemical signature capable of providing invaluable information on the physiological status of an individual and, thereby, could serve as powerful biomarkers for medical diagnosis and therapeutic monitoring[1]. They are targeted in different human fluids and tissues including breath, urine, saliva, feces, blood, and sweat; however, noninvasively obtainable ones are the preferable matrices. Urine is a particularly interesting reservoir of disease-related markers. It is readily and noninvasively collectable and may be sampled as often as desired without discomfort for the patient. A wide range of different types of biomarkers can accumulate in urine and subsequently be excreted during urination providing the opportunity to detect changes in cells/tissues metabolism related to a disease development including cancer[2]. A number of recent studies provided evidence that the disease state influences the pattern of urinary VOCs and that the analysis of this pattern could be a valuable diagnostic tool [3-5]. Although the composition of urine volatolome in humans has received a broad attention[2], quantitative data on urinary volatiles are relatively sparse and limited to single compounds, or selected groups of species. The primary goal of this work was to develop a SRI-TOF-MS based method for quantification of VOCs in urine samples and provide reliable reference concentrations for selected urinary species.

Experimental Methods

Head-space solid-phase microextraction of volatiles from urine samples was performed in 20 mL vials. Prior to the extraction vials were evacuated and 0.5 mL of urine, 0.25 mL of Dulbecco's PBS and 1.25 mL of distilled water were transferred into it. Next, a small amount of an anti-foam

agent was added to the vials to reduce foaming. Finally, the pressure in the vials was balanced with high-purity nitrogen.

Solid phase microextraction (SPME) was carried out automatically by inserting a 75 µm Carboxen-PDMS SPME fiber into the vials and exposing it to the head-space gas for 50 minutes at 37°C. Next, the fiber was introduced into the inlet of the gas chromatograph where the volatiles of interest were thermally desorbed at 290°C.

The urine volatiles were separated using a Rt-Q-BOND column (30 m x 0.25 mm, film thickness 8 µm). The column temperature program was as follows: 60°C for 1 min, increase at a rate of 5°C/min to 260°C, constant temperature of 260°C for 13 min. The effluent from the column was introduced into the drift tube of the SRI-TOF-MS instrument via a heated (150°C) empty fused silica tube (1.5m×0.25mm).

An Ionicon Analytik (Innsbruck, Austria) type 8000 SRI-TOF-MS instrument working with NO^+ as the reagent ion was used during analyses. The settings of the ion source were chosen as follows: ion source current 5 mA, source voltage (Us) 20V, source-out voltage (Uso) 70V, and source valve opening 40%. The NO^+/VOCs reactions occurred in the drift tube at a total pressure of 2.3 mbar and a gas temperature of 60ºC. The voltage along the drift section was set to 500V leading to an E/N ratio of approximately 110 Td. The spectral scans of the TOF analyzer ranged from approximately m/z 2.7 to 500 and were acquired in a time of 500 ms. The total duration of a single measurement was 54 minutes, which corresponds to the length of the column temperature program.

The identification of compounds relied on the exact mass measurement of detected parent ions, characteristic reaction mechanisms of VOCs with NO^+, and the comparison of the retention times of peaks of interest with retention times obtained for standard mixtures prepared from pure compounds. Quantification was based on calibration curves obtained from the analyses of urine samples spiked with known amounts of species under scrutiny.

Results and Discussion

Overall 16 compounds were quantified using HS-SPME-GC-SRI-TOF-MS(NO^+) analytical platform. Amongst them there were ten ketones (acetone, 2-butanone, 3-methyl-2-butanone, 2-pentanone, 3-methyl-2-pentanone, 4-methyl-2-pentanone, 2-hexanone, 3-hexanone, 2-heptanone, and 4-heptanone), three volatile sulphur compounds (dimethyl sulfide, allyl methyl sulfide, and methyl propyl sulfide), and three heterocyclic compounds (furan, 2-methylfuran, 3-methylfuran). Limits of detection (LODs) ranged from 0.08 nmol×L^{-1} for allyl methyl sulfide to 1.0 nmol×L^{-1} for acetone and furan. Relative standard deviations (RSDs) varied from 5.0% - 9.2%. The system response was linear within the investigated concentration levels, with coefficients of variation falling within the range of 0.974-0.998.

The observed concentrations ranged from 0.55 nmol×L^{-1} (0.05 nmol×$mmol^{-1}_{creatinine}$) for furan to 11.6 µmol×L^{-1} (1.54 µmol×$mmol^{-1}_{creatinine}$) for acetone considering medians, as shown in Figure 1. The highest concentrations were noted for acetone (11.6 µmol×L^{-1}, or 1.54 µmol×$mmol^{-1}_{creatinine}$), 2-pentanone (294 nmol×L^{-1}, or 30 nmol×$mmol^{-1}_{creatinine}$), 2-butanone (292 nmol×L^{-1}, or 17.7 nmol×$mmol^{-1}_{creatinine}$), and 4-heptanone 167 nmol×L^{-1}, or 15.2 nmol×$mmol^{-1}_{creatinine}$). All species but one (AMS) exhibited incidence rates higher than 90%. The obtained concentration values agree very well with the available literature data and are expected to partially fill the literature data gap with respect to urinary concentrations of volatile organic compounds in healthy individuals.

The coupling of SRI-TOF-MS with gas chromatography improves its analytical power in the context of VOCs analysis in liquid and solid matrices. In combination with one of the sample pre-concentration methods (e.g. solid-phase microextraction, needle trap extraction) it provides the opportunity to detect ultra-low levels of VOCs in human tissues/fluids and reliable identification mechanisms. Moreover, it does not hinder the real-time measurements in SRI-TOF-MS. Consequently, real-time and GC analyses can be performed consecutively using the same analytical system, without additional modifications in the experimental set-up. This feature supports the concept of hybrid volatolomics, an approach combining VOCs profiles obtained for two or more body fluids to improve and complement the chemical information on the physiological status of an individual [6].

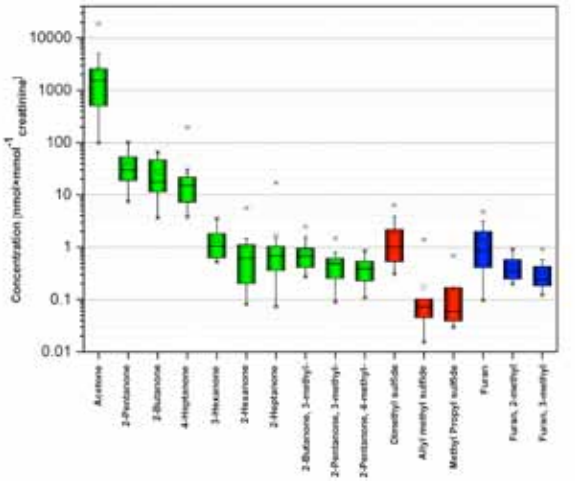

Figure 1: Observed concentrations of VOCs under study in human urine.

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Fast breath analysis using PTR-TOF MS

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Abstract

Breath analysis is an attractive non-invasive method for diagnosis and therapeutic monitoring, especially if the results are available immediately. Phenotypic breath tests currently in clinical validation target 13CO2 as metabolite of isotopically labeled precursors such as 13C-labeled uracil, 13C-labeled dextromethorphan or 13C-labeled pantoprazol. A particularly interesting focus lies on prediction of drug adverse effects and investigation of drug-drug interactions, since the functionality of enzymes concerning drug metabolism might be influenced by various factors such as age, sex, life-style, consumed food, etc, which can be not foreseen only by genetic analysis.

Targeting other molecules than 13CO2 might open up additional possibilities, if the expected target metabolite appears in exhaled air only after administration of the substrate and the analytical device is sensitive enough for its detection in the low ppb (parts per billion) –range.

High sensitivity analytical technologies, such as proton-transfer reaction time-of-flight mass spectrometry enable a sensitive detection of different volatile targets. Besides, they need no time consuming sample preparation, thus allowing a direct on-line and real-time monitoring of exhaled breath.

In a feasibility study we used deuterated 2-propanol (d3-isopropanol) for monitoring the activity of alcohol dehydrogenase (ADH) which was converted to d3-acetone (m/z 62.08). The applied dose was 0.8 mg, leading to a well detectable signal of around 30 ppb (at the peak of response) in exhaled breath. Results and the developed test procedures will be discussed in detail.

Online breath analysis in the clinical environment – New perspectives for PoC monitoring

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Abstract

Volatile organic compounds (VOCs) in exhaled breath can serve as a non-invasive window into physiological and pathophysiological processes in the whole body. VOCs are produced at the cellular level, transported via the bloodstream and exhaled through the lungs in next to no time upon production. An important advantage of breath as a biological fluid is the availability of an essentially unlimited supply for sequential sampling in time frames as short as seconds. This offers optimal conditions for screening purposes or continuous monitoring.

The majority of breath biomarker discovery and identification has been done by means of gas-chromatography coupled with mass spectrometry and in combination with adapted preconcentration techniques such as SPE, SPME or needle-traps. This approach has identified a number of breath biomarkers mirroring metabolic, physiological or pathophysiological processes. Biochemical processes such as dextrose metabolism, lipid peroxidation, cholesterol metabolism, renal failure, liver disease, allograft rejection, lung injury as well as diseases like SIRS, sepsis or cancer can also be traced through breath markers.

While a lab based approach is well suited for biomarker identification, the sample preparation and measurements can be time consuming and breath sampling times are in the range of minutes, thus allowing only punctual investigations of VOC profiles.

The advent of direct mass spectrometric techniques such as PTR-MS have paved the way to overcome these limitations and enable the analysis of breath VOCs without significant delay or the need for additional sample preparation. Breath resolved measurements of VOC profiles by means of PTR have revealed a strong dependency of breath VOCs to important physiological processes such as changes in hemodynamics, compartmental distribution, ventilation or perfusion. An in depth understanding of those effects onto VOC profiles is an important pre-requisite for the clinical interpretation of breath VOC data. Continuous real time VOC profiles may help to gain new insights on physiological monitoring, desease progression and therapeutic monitoring.

This lecture will demonstrate typical examples on the application of real time PTR-ToF-MS for hemodynamic and metabolic monitoring and therapy control (in ventilated patients).

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The effect of growth medium composition on the volatile organic compounds produced by Pseudomonas spp. over time

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Abstract

Temporal changes in the volatile organic compounds (VOCs) produced by 3 Pseudomonas species growing on media containing differing levels of glucose (0.5 or 1%) and egg white protein (0 and 2%) in Vogel's broth (minimal media) at 25 °C over 72 h were analysed using proton transfer reaction mass spectrometry (PTR-MS). Glucose or egg white content influenced the VOC profile specifically for alcohols, carbonyls and sulphur derivatives. A higher level of glucose (1%) induced production of a higher number of compounds (i.e. alcohols, aldehydes, acids, esters, N- and S-containing compounds, hydrocarbons) including the emission of cyclic-based volatiles whereas a lower glucose content (0.5%), resulted in the production of fewer compounds. Egg white supplemented medium produced a notably different array of volatile compounds compared to the solely glucose based medium, including the appearance of a number of sulphur derivatives i.e. methanethiol (m/z 49), S-propyl ethanethioate (m/z 57), dimethyl disulfide and dimethyl sulfone (m/z 95). In the glucose containing media supplemented with egg white, the production of carboxylic acids such as acetic acid (m/z 61) and propanoic acid (m/z 75) significantly ($p < 0.5$) increased. When cells were at the end of their growth phase, a distinctly different fingerprint of the VOCs was identified compared to the early growth phase. Cyclic compounds were produced during the early growth phase (12 and 18 h) while sulphur derivatives were more common during the late growth phase (60 and 72 h). The present work highlights how variation in growth phase, carbon and protein content in the medium can impact on the volatile fingerprint produced by specific bacterial species.

Introduction

Microorganisms from diverse ecosystems can produce a wide range of volatile organic compounds (VOCs). Most VOCs are recognized as being by-products of either primary and/or secondary metabolism formed primarily via oxidation of glucose through various intermediates [1]. Volatiles produced by bacteria are typically either alcohols, acids, esters, alkenes, ketones, benzenoids, pyrazines or terpenes [2]. The underlying biosynthetic pathways that generate the volatiles include aerobic, heterotrophic carbon metabolism, amino-acid catabolism, fermentation, fatty acid degradation, sulphur reduction or terpenoid biosynthesis [3]. Some VOC's are regularly produced by a range of microorganisms, while, other compounds are exclusively released by specific strains [4]. Volatile profiles and their level varies depending on substrate composition, physiological state of the microorganism, the availability of oxygen, the moisture content, pH or temperature [5]. While the appearance of a distinctive volatile compound or profile can be related to the specific metabolic pathway(s) triggered in bacteria [6] there is a lack of fundamental understanding of how VOCs differ in response to different bacterial growth conditions.

Therefore, the aim of the present work was to investigate the influence of varying media composition and growth phase on VOC produced by three Pseudomonas spp.

Experimental Methods

Microbial strains and media composition

Pseudomonas aeruginosa ATCC 27853, P. aeruginosa PA01 and P. fragi were cultured on Vogel's medium comprising 2 g citric acid, 3.5 g NaNH4HPO4.7H2O, 0.2 g MgSO4.7H2O, 10 g K2HPO4 and 5 g of glucose per litre of distilled water. A cell suspension was added to a 100 mL volume of the Vogel's medium to achieve an OD600 of 0.5. The egg white powder was prepared by separating egg white from the yolk, freeze dried and sterilized at 60 °C for 15 min. Egg white powder was used in the present work as it is a relatively odour-free protein source.

PTR–MS operating condition and data validation

The headspace VOC composition of the Vogel's medium was determined using a high sensitivity PTR–MS (IONICON Analytik GmbH, Innsbruck, Austria). The PTR–MS inlet consisted of a 1 m long 1/1600 outer diameter Silco steel TM capillary (Restek Co., Bellefonte PA) heated to 110 °C and with a continuous flowrate of 50 mL per min. The experimental set-up comprised a 1 L sampling bottle fitted with a lid containing two polytetrafluoroethylene (PTFE) tube connection ports for gas inlet and outlet. The sampling bottle and all downstream sampling tubes were placed in an incubator at 25 °C to maintain a constant sample temperature throughout the PTR–MS analysis. For the measurements, the PTR–MS was operated in mass scan mode over a mass range of m/z 20–200 with a dwell time of 100 ms per m/z, which resulted in a cycle time of 36 s. The reaction chamber was operated at a pressure of 2.2 mbar (0.2 kPa), a temperature of 80 °C and with a drift voltage of 600 V. PTR–MS analysis of the empty bottle under dynamic conditions provided a level of VOC contamination within the set-up (i.e. background noise), which was used during data post-processing. Eight cycles of each sample was recorded and the average values from the intermediate cycles of each m/z were subtracted from the background and blank measurements made on the empty bottle and the bottle containing only blank medium (without any cell inoculum). Static headspace measurements were made at 12, 18, 24, 36, 48, 60 and 72 h for 8 cycles (290 s). Off-line SPME–GC–MS analysis of the headspace of a subset of 72-h frozen samples was made in order to aid chemical identification of the VOCs detected by PTR–MS. The volatiles were separated on a ZB-1701 ZebronTM capillary GC column (60 m, 0.25 mm i.d., 0.25 mm film thickness, Phenomenex Inc., Torrance, CA). The GC was coupled to a mass spectrometer (Agilent 5975B VL MSD, Agilent Technologies, Santa Clara, CA) and the MS was operated in electron ionisation (EI) mode at an ionisation energy of 70 eV and a scan range of m/z 30–200. The compounds were tentatively identified based on mass spectra matching with the standard NIST-08 MS library (National Institute of Standards, Gaithersburg, MD).

Statistical analysis

The effects of bacterial strain, glucose level, egg white content and time on the production of VOCs were analysed using a Generalized Linear Model (significance level at $p \leq 0.05$) using SPSS v22. The normalised m/z intensities (n-cps) in triplicate samples were used as dependent variables and the fixed factors were bacterial strain, glucose level, egg white content, time and replicates. The relationships between the significant ($p \leq 0.05$) m/z and the samples were investigated using principal component analysis (PCA) (Unscrambler 10.3, CAMO Software AS, Oslo, Norway)..

Results and Discussion

Volatile organic compounds in the bacterial culture headspace which significantly increased over time are summarized in Table 1.

Table 1 Volatile organic compounds found to increase in the headspace of bacteria over time

Alcohols	Ethanol (*m/z* 47), 1-butanol (*m/z* 75), 2-butanol (*m/z* 75), 2-propanol (*m/z* 61); 1-octen-3-ol (*m/z* 129)
Aldehydes	Acetaldehyde (*m/z* 45), heptanal (*m/z* 115), 2-hexenal (*m/z* 99), benzaldehyde (*m/z* 107)
Ketones	3-Octanone (*m/z* 129), 2-heptanone (*m/z* 115)
Acids	Acetic acid (*m/z* 61), propanoic acid (*m/z* 75), 3-methyl pentanoic acid (*m/z* 117) and Hexanoic acid (*m/z* 117)
Esters	Ethyl butyrate (*m/z* 117), ethyl hexanoate (*m/z* 145), isoprene (*m/z* 69), isobutyl acetate (*m/z* 117)
Aromatic	Pyrrole (*m/z* 68), pyrrolidine (*m/z* 72), dimethyl benzenes (*m/z* 107), 2-aminoacetophenone (*m/z* 136)
Sulfur derivatives	Hydrogen sulphide (*m/z* 35), methane thiol (*m/z* 49), S-propyl ethanethioate (*m/z* 119), dimethylsulfide (*m/z* 63), hydrogen cyanide (*m/z* 28), fluoromethyl methyl disulfide (*m/z* 113)

A variety of classes of volatiles were affected when the glucose content of the medium increased from 0.5 to 1% (Fig 1). Most notable of these were the cyclic-based volatiles along with alcohols, aldehydes, ketones and acids, with the most influential compounds being m/z 97, 123 and 155,

The addition of egg white protein to the growth medium resulted in the production of a different array of VOCs including production of sulphur derivatives i.e. methanethiol (m/z 49), S-propyl ethanethioate (m/z 57), dimethyl disulfide (m/z 95) and dimethylsulfone (m/z 95) and a totally different array of cyclic volatiles such as benzene (m/z 79), trimethyl benzenes (m/z 121) and pyrrole (m/z 68) and pyrrolidine (m/z72).

Interestingly, in the media supplemented with glucose and protein, production of carboxylic acids such as acetic acid (m/z 61) and propanoic acid (m/z 75) significantly ($p < 0.5$) increased. Other compounds found to be significantly($p < 0.5$) produced in egg white containing media were methanol (m/z 33), propene (m/z 43), acetaldehyde (m/z 45), dimethylamine (m/z 46), 1-propanol (m/z 61), 2-butanol (m/z 75), 1-butanol (m/z 75), methyl acetate (m/z 75), terpenes (m/z 137), 2-nonenal (m/z 141), nonanal (m/z 143), and 2-nonanone (m/z 143).

When the cells were at the end of their growth phase (54, 60 and 72 h), the fingerprint of the VOCs was distinctly different compared to in the early growth phase (12 and 18 h) (data not shown).

The headspace VOC's of two species (P. aeruginosa ATCC 27853 and P. fragi) produced very dense and compact data clouds, revealing small variations. In contrast, the data corresponding to the other species (P. aeruginosa PA01) was broadly spread, indicating greater variation exist (data not shown). The VOCs produced by the strain ATCC 27853 differed significantly ($p < 0.5$) from those present in the headspace air of the other two bacteria tested. Similarly two strain (P. aeruginosa PA01 and P. fragi) displayed comparable characteristics in the VOCs (data not shown).

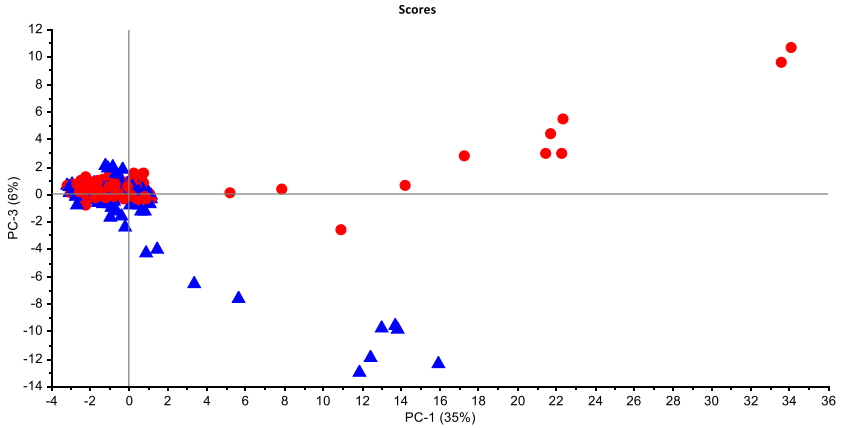

Fig 1: Score plot of the principle components (PC) of scanned m/z detected in the headspace of bacteria. Cells were cultivated in Vogel's broth at 25 °C for 72 h supplemented with glucose (0.5 and 1% w/v) and egg white (0 and 2% w/v). Data represented indicates groupings according to the glucose content (▲ 0.5 or ● 1%).

Conclusion

The present work demonstrated the impact of glucose and egg white on the VOC profile of Pseudomonads over time. Given the wide range of food products spoiled by members of the genus Pseudomonas, the present investigation could also provide insight on the mechanism of spoilage under varying carbon and protein compositions.

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Instruments & Technology and Future Trends

From ppb to ppq: 20 years of PTR-MS History

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Abstract

The first PTR-MS instrument was developed at the University of Innsbruck (UIBK) in the late 90ies. This technique enables monitoring a variety of volatile organic compounds (VOC) present in air in real-time with detection limits as low as a few parts per billion (ppb). In 1998 we founded the spin-off company IONICON Analytik GmbH to provide this technique to a growing user community. Today the most recently developed PTR3-TOF instrument reaches limits of detection in the sub-ppq region. This paper will give an overview of the PTR-MS development in the last 20 years.

Why PTR-MS?

Conventional mass spectrometry (MS) is a well-proven and highly sensitive technique for the identification and detection of organic pollutants. In simple terms, it works by separating organic molecules on the basis of their molecular masses. Molecules entering the mass spectrometer are ionized, usually by electron impact, and are then subjected to electromagnetic fields under whose influence ions with different mass/charge (m/z) ratios will move in different trajectories. Thus the ionized molecules can be separated and individual molecules can be identified. That is the theory. Unfortunately, instead of forming a single ionized species, many molecules break down into smaller fragments, each of which is detected separately. This can result in one compound-giving rise to a complex "mass spectrum". With a mixture of compounds entering the MS detector simultaneously, the final mass spectrum may be so complex that interpretation and quantification become difficult, if not impossible. The traditional solution to this problem has been to separate the compounds with a gas chromatograph (GC) before they are sent to a mass spectrometer. Unfortunately, GCs are inherently slow – a typical separation of just one sample could take 30 minutes – so while GC-MS is fine for analyzing discrete samples or monitoring slowly changing situations, it cannot usually be regarded as a "real time" or on-line technique. PTR-MS can make the GC step unnecessary for many typical VOC analyses and achieve a response time measured in milliseconds. This opens a whole new range of possibilities in environmental monitoring, food science, odor analysis and medical applications.

History of PTR-(Q)-MS

Proton transfer reactions (PTR) for soft chemical ionization of different compounds have been used for many years. In the late 90ies, scientists from the University of Innsbruck (UIBK) reported on the development of Proton Transfer Reaction Mass Spectrometry (PTR-MS); a combination of a PTR drift tube and a quadrupole mass spectrometer, which allows for, in principle, fast detection of volatile organic compounds with concentrations as low as 1 ppb. The method is based on reactions of H_3O^+ ions, which perform non-dissociative proton transfer reactions to most of the common VOC but do not react with any of the components present in

clean air because they all have proton affinities lower than H_2O. The first PTR-MS instruments used a hollow-cathode ion source producing a high density of reactant ions H_3O^+ that are directly transferred to a drift region without mass spectrometric pre-selection. In the drift region an electric field pushes the ions in axial directions allowing for ion molecule reactions at elevated kinetic energies thus suppressing cluster formation. Usually E/N (E being the electrical field strength and N the number gas density) the values are kept at 120 Td, which is a good compromise between avoiding on one hand the formation of too much $H_3O^+(H_2O)_n$ (n= 1,2,3,..) cluster ions which would obscure the quantitative data evaluation and on the other hand breaking up of product ions due to collision induced dissociation (CID). Under these conditions typical count rates of product ions were 1 count per second per ppb. [Hansel et al. 1995, Lindinger et al. 1998 a, b]. Sensitivity, detection limit, gas sampling method and time resolution of PTR-MS have improved significantly since these early days. PTR-MS has found numerous applications in atmospheric science, biology and other scientific fields such as food technology and medicine where the fast monitoring of organic trace compounds is needed. In 1998 the company IONICON was founded as a University spin-off. Within the first ten years (1998 – 2008) IONICON has manufactured more than 100 PTR-MS instruments which are used by Universities as well as renowned company customers distributed all over the world. As exciting as this tool is, the PTR-MS technique did not have the full analytical capability to distinguish between isobaric species these are compounds having the same nominal mass but differ in the atomic composition. The principal reason is that the quadrupole mass spectrometric detection scheme used by PTR-MS does not provide sufficient selectivity (mass resolution) and sensitivity at higher masses.

History of PTR-TOF-MS

Ten years ago, several groups have reported the coupling of a chemical ionization unit to a time of flight mass spectrometer (TOF-MS) [Blake et al., 2004; Ennis et al., 2005; Inomata et al., 2006]. The performance of these first instruments shows a rather poor sensitivity and an insufficient mass resolving power to separate isobaric species. At UIBK a high-resolution proton transfer reaction time of flight mass spectrometer (PTR-TOF) has been developed in collaboration with IONICON in the years from 2005 to 2007. This prototype instrument couples a PTR-ion source (IONICON Analytik GmbH, Austria) and a high mass resolution TOFMS (Tofwerk AG, Switzerland), and was optimized for high ion transmission and mass resolution. A mass resolving power of 6000 (FWHM) was achieved [Graus et al. 2010, Jordan et al 2009]. The high mass resolving power of the PTR-TOF allows for the assignment of exact mass and isotope pattern to individual organic compounds and thus strongly improves the identification capabilities compared to a standard PTR-MS, e.g. we can unambiguously detect hydrocarbons and their oxygenated isobars up to molecular masses of 200 m/z and beyond. The PTR-TOF records full mass spectra from 5 m/z to 500 m/z within a fraction of a second. This represents a substantially improvement of the duty cycle by more than a factor of 1.000 compared to conventional PTR-MS technology! The sensitivity of the Innsbruck PTR-TOF is at least a factor of 100 higher than the sensitivity of TOF instruments reported by other groups reaching limits of detection of ppt levels.

History of IONICON Analytik GmbH

In 1995 a paper was published in International Journal of Mass Spectrometry and Ion Processes entitled „Proton transfer reaction mass spectrometry: on-line trace gas analysis at ppb levels“ by A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel and W. Lindinger. Three of the authors A. Hansel, A. Jordan and W. Lindinger founded together with two other colleagues the University spin off company IONICON Analytik GmbH in 1998 to provide the PTR-MS technology to a growing user community as a fast VOC sensor. Beginning with 1998 the number

of distributed PTR-MS instruments started to grow. To date more than 250 research groups and companies around the world applying this technique in various fields use PTR-MS and PTR-TOF-MS instruments. All these instruments have been manufactured and distributed by IONICON.

The PTR3: an ultra-sensitive PTR-TOF reaching sub ppq levels

Very recently we have developed the PTR3 instrument. This instrument consists of a corona discharge ion source and a novel reaction chamber. The reactant $H_3O^+(H_2O)_n$ (n=1,2,3) cluster ion distribution is controlled via a three-phase radio frequency electrode configuration that allows the decoupling of the ion's axial velocities from the kinetic energies applied to avoid clustering of reactant ions with water molecules present in the sample gas. In contrast to standard drift tubes used in PTR-TOF-MS the ions are transported in axial direction just by the sample gas flow through the reaction chamber thus increasing the reaction time by a factor of 30! The necessary kinetic energy to avoid clustering is established in *radial* direction by a three-phase electrode configuration operated with three sinusoidal waveforms, phase-shifted by 120°. The instrument uses a corona discharge ion source coupled to a contact free inlet system running at high sample gas flow rates of several standard liters through the reaction chamber at 80 mbar. The PTR3 front part is coupled to TOFWERK's newest quadrupole-interfaced Long-TOF mass analyzer. The first prototype has sensitivities of up to 20.000 cps per ppb and a mass resolution of approximately 8.000 m/Δm. The instrument has been successfully tested at CERN for the CLOUD 10 campaign in 2015, where low volatility organic compounds were measured. During pure α-Pinene ozonolysis experiments at low NO_x conditions we observed in total approximately 1.000 peaks in the mass spectrum, including α-Pinene present in the ppb range, first and higher order oxidation products present in the ppt range and highly oxydized α-Pinene monomers and dimers (e.g., $C_{20}H_{30}O_{18}H^+$; m/z = 559.1506 Th) in the low ppq range and even sub-ppq range [Breitenlechner et al. 2016]. The advantage of this new technology based on positive ion chemistry is the capability to measure precursor gases as well as condensing- and even nucleating vapors.

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The PTR3: A novel ultra-high sensitivity PTR-ToF

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Abstract

We have developed and characterized the novel PTR3: contact free sampling and a revolutionary ionization chamber coupled to TOFWERK's latest quadrupole-interfaced Long-ToF mass analyzer. The instrument's new reaction chamber allowing 30-fold longer reaction times and 40-fold higher pressure (3 ms and 80 mbar) compared to classical PTR-MS instruments (0.1 ms and 2 mbar) at comparable collisional energy. We achieved sensitivities of up to 20 000 cps/ppbv at a mass resolution of 8000 m/Δm. The instrument was tested for the first time at the CLOUD (Cosmics Leaving OUtdoor Droplets) experiment at CERN in 2015, where we observed highly oxidized organics from α-Pinene oxidation experiments, including dimers being present at volume mixing ratios in the sub-ppqv regime (0.001 pptv).

Experimental

Inlet and Pressure Regions

The instrument uses an improved inlet system: the core of a laminar 10 slpm inlet flow profile through a 3/8" tube (ID 7.5 mm, Reynolds Number ~ 2000) is sampled through a critical orifice (core-sampling), reducing the pressure to 80 mbar. The flow through the reaction chamber is approx. 2 slpm. Another critical orifice at the end of the reaction chamber reduces the pressure to 5 mbar, allowing coupling to a TOFWERK quadrupole interfaced ToF.

Ionization Process and Watercluster Control

The instrument consists of a corona discharge ion source and a novel reaction chamber. The primary ion cluster distribution is controlled via a three-phase radio frequency electrode configuration allowing – for the first time – decoupling of the ion's axial velocities from the kinetic energies applied to avoid clustering of primary ions with water molecules present in the sample gas. Contrary to standard drift tubes, this allows the ions following the flow through the reaction chamber, rather than being forced to move with velocities on the order of 1000 m/s in axial direction. The necessary kinetic energy to avoid clustering is established in *radial* direction by a three-phase electrode configuration operated with three sinusoidal waveforms, phase-shifted by 120°. The resulting electric field vector in the center of this configuration is *constant* in magnitude and is rotating. We limit the primary ion distribution to $H_3O^+ \cdot (H_2O)_n$ ($n < 4$), therefore sensitivities, e. g., for benzene and toluene are lower than determined by kinetic rates. However, for oxidized organic compounds we find sensitivities in agreement with calculated ones. The described setup allows reaction times independently adjustable from the E/N, however, we limited the reaction time to 3 ms at a sample gas pressure of 80 mbar to avoid secondary reactions and depletion of primary ions in typical outdoor environments.

Results

The first prototype has sensitivities of up to 20 000 cps/ppbv and a mass resolution of approx. 8000 m/Δm. The instrument has been successfully tested at CERN for the CLOUD 10 campaign in 2015, where low volatility organic compounds were measured. During pure α-Pinene ozonolysis experiments (without any OH scavenger) at low NO_x conditions we observed in total approx. 1000 peaks in the mass spectrum, including α-Pinene present in the ppbv range, first and higher order oxidation products present in the pptv range and highly oxydized α-Pinene monomers and dimers (e.g., $C_{20}H_{30}O_{18}H^+$; m/z = 559.1506 Th) in the low ppqv range and even sub-ppqv range.

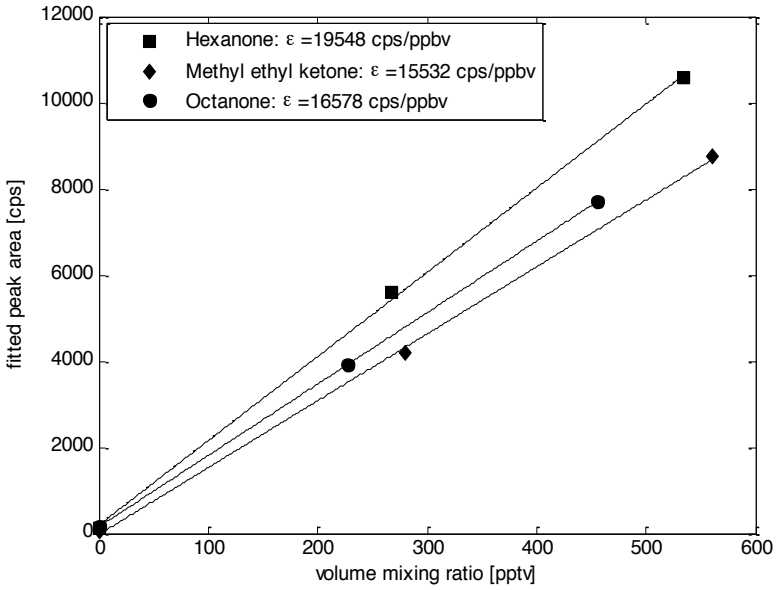

Figure 1: Sensitivities for selected oxygenated VOCs, obtained during the CLOUD 10 campaign. Calibration was done using a dynamically diluted VOC standard (Apel-Riemer Environmental Inc., CO, USA) containing approx. 1 ppmv of the individual VOCs.

Implementation of an RF ion funnel ion guide as a proton transfer reaction chamber

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Introduction

In recent years increasing the sensitivity of PTR-MS apparatus has been an ingeniously pursued topic [1, 2]. Ion funnels have been implemented as common components in mass spectrometry since their initial introduction in the 1990s [3]. The basic tenet of an ion funnel is to use collisions to focus ions at substantially higher pressures than would be allowed with a standard multipole focusing device. The higher pressures mentioned are ~ mbar and therefore an obvious potential exists to exploit the technology for use as a PTR-MS reaction chamber.

Experimental Methods

An ion funnel and corresponding power supply unit have been built at Radboud University based on a design published by Barber et al. [2] and with guidance from Kore Technology Ltd. The funnel is implemented in a proton transfer reaction ion trap mass spectrometer (PIT-MS) [4]. Measurements with the ion funnel are combined with theoretical modeling of ion trajectories using SIMION 8.0.4 to provide a clear indication of the mechanism behind observed sensitivity improvements.

Experimental Funnel

The mechanical design of the funnel consists of a 10 cm long housing into which exist 29 stainless steel plates of equal spacing. The geometry of the plates is shown in Figure 1. Over the first 13 plates, a direct current (dc) only field is applied. Over plates in the tapered section, a dc and radio frequency field (RF) is applied. The final four plates of the funnel are operated with a reduced RF voltage; the reducing factor being approximately 0.6. The dc field produces a constant field gradient in the axial direction of the funnel, with typical voltage over the funnel 40 – 120 V. The RF field component promotes collisions, which in turn promote focusing and is typically 150 – 300 V_{pp} at 820 kHz. The pressure investigated inside the funnel is between the range 1.40 – 2.20 mbar.

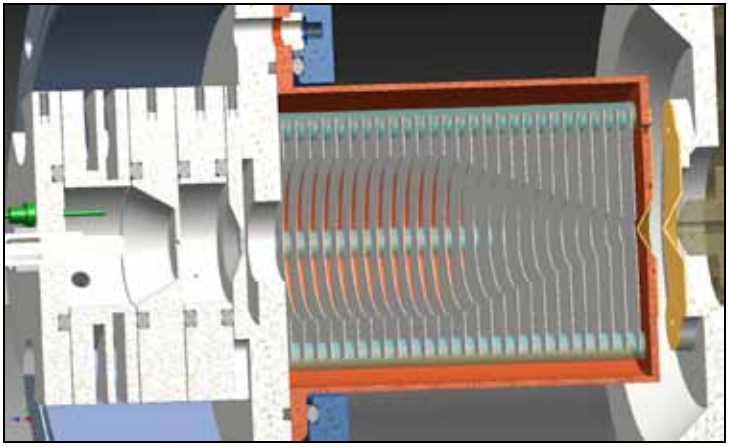

Figure 1: 3-D design diagram showing the ion funnel reaction chamber and ion source in PIT-MS instrument.

The funnel is tested mostly using a 1 ppm calibration mixture in nitrogen of methanol, acetaldehyde, acetone, isoprene, benzene, toluene, o-xylene and α-pinene. This mixture can be further diluted as necessary by mixing with clean nitrogen using calibrated mass flow controllers. The characteristics of the ion funnel are investigated; including the effect of changing pressure and RF and dc voltages on detection sensitivity.

Theoretical Funnel

To study the ion funnel theoretically a model for the funnel is constructed using SIMION; an ion trajectory modeling software. The model allows for the same control of parameters as in the experimental section, while presenting added information, such as ion residence time. By combination of theoretical and experimental study a fuller picture of ion funnel operation is achieved.

Results

Sensitivity

The sensitivity of the ion funnel is shown in Table 1 and compared to the sensitivity achieved with the standard drift tube used at Radboud University and operated with conditions at 119 Td. A significant sensitivity improvement is observed with the funnel, most notably for benzene, toluene and o-xylene.

That a high increase in sensitivity for benzene is observed suggests the funnel allows for a reasonably high purity of reagent ion monomer. Benzene is widely reported as being non-reactive with protonated water clusters [5].

Compound (m/z)	Ion Funnel npA/ ppb/ ms	Drift Tube npA/ ppb/ ms	Ion funnel/ Drift tube
Acetaldehyde (45)	3040	330	9.2
Acetone (59)	46000	1150	40
Isoprene (69)	2520	340	7.3
Benzene (79)	11840	180	65
Toluene (93)	42460	1000	43
o-Xylene (107)	89100	2180	41
α-pinene (137)	39940	1700	23

Table 1: Sensitivity values for ion funnel and conventional drift tube using PIT-MS.

Characteristics

The transmission of the ion funnel was calculated using SIMION and found to be linear with increasing mass of the ion. The measured transmission was found to increase in a non-linear manner, approaching a quadratic increase. This effect is due to the combination of ion funnel and ion trap transmissions. The measured product ion signal at constant analyte concentration (shown in Figure 2) is strongly affected by the dc voltage.

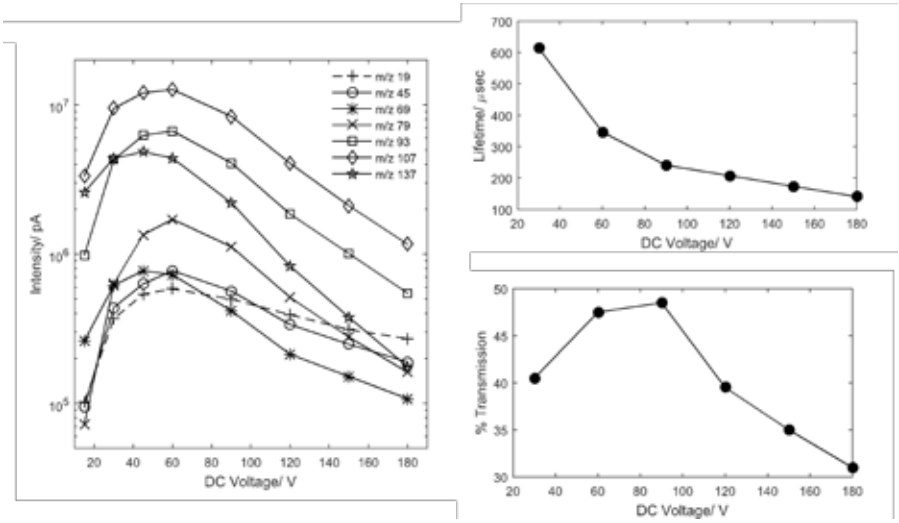

Figure 2: Main figure: Sensitivity of ion funnel as a function of dc voltage for constant pressure and RF voltage. Ions are identified according to m/z value as given in Table 1. Inset upper: Lifetime calculations for H_3O^+ ions in the drift tube. Inset lower: Transmission calculation of m/z 107 as a function of DC voltage.

Theoretical calculations show a twin effect contributing to the ion funnel performance as a function of dc electric field. Firstly, ion focusing is dependent on dc field, but more importantly ion residence time is affected by the dc field. From 60 – 180 V ion focusing and ion residence

time increase as the dc field is reduced. At 60 V the residence time for H_3O^+ ions is calculated to be 350 μs, compared to 60 μs for a low field setting computed with a standard drift tube. Below 60 V overall ion transmission reduces to such a level that the sensitivity begins to decrease.

Discussion

The ion funnel exhibited here shows significant sensitivity improvement over the previously installed conventional drift tube. The major improvements are explained with the help of trajectory modeling using SIMION, showing major gains in ion focusing and ion residence time, while maintaining good reagent ion monomer purity.

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PTR-QiTOF Characterization and Eddy Covariance Measurements

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Abstract

A commercially available PTR-QiTOF was characterized with regards to fragmentation and unintended proton transfer reactions in the quadrupole interface. The settings of the interface could be optimized to keep fragmentation at acceptable levels. Sensitivities of several thousand Hz ppb^{-1} were achieved over continuous measurements. Such sensitivities allow for unprecedented detectability of turbulent VOC fluxes by the PTR-MS method.

Introduction

Proton Transfer Reaction Mass Spectrometry (PTR-MS) has been used in various scientific fields and applied research since its introduction in the 1990ies [1-4]. One particularly challenging scientific application of PTR-MS is the measurement of the surface exchange of volatile organic compounds (VOCs) by the eddy covariance (EC) method. In the past, the need of fast response times led to the introduction of a volume-reduced drift tube that minimized the low-pass filtering of the concentration data [5]. Replacing the quadrupole mass spectrometer (QMS) by a high resolution time-of-flight mass spectrometer (TOF-MS) [6] allowed for the separation and identification of otherwise interfering mass peaks [7, 8] and led to unprecedented ability to measure the turbulent fluxes of numerous VOCs in parallel [9, 10]. The sensitivities of these TOF based PTR-MS instruments had been lower than those of QMA based ones when comparing single mass channels. Even though PTR-TOF outperforms PTR-QMS in terms of sensitivity and limit of detection (LOD) for a typical set of mass channels preselected for sequential analysis by the QMS [11], high sensitivities are crucial for the detectability of VOC fluxes.

The recent introduction of a quadrupole ion-guide between drift tube and TOF-MS (PTR-QiTOF) increased sensitivities of TOF based instruments to levels beyond the sensitivities of the best performing PTR-QMS instruments [12]. Such an instrument should allow for true 10 Hz EC flux determination of all peaks in the TOF spectrum at flux detection limits surpassing those previously achieved by PTR-QMS instruments sampling only a small number of selected ions for virtual disjunct EC analysis [13]. Here we present the results of thorough tests of the performance of a commercially available PTR-QiTOF instrument with respect to concentration and flux measurements in ambient air.

Experimental Methods

The PTR-QiTOF instrument (Ionicon, Austria) was operated in hydronium mode at standard conditions in the drift tube of 112 Townsend. The instrument was set up to sample ambient air from a turbulently purged 3/8" Teflon line. Every seven hours, zero calibrations were performed for 30 minutes providing VOC free air from a continuously purged catalytical converter though a

setup of software controlled solenoid valves. In addition, every other time known quantities of a suite of VOC from a 1ppm calibration gas standard (Apel & Riemer, USA) were added to the VOC free air and dynamically diluted into low ppbv mixing ratios.

Tests for fragmentation of aromatics (ethylbenzene, p-cymene) and monoterpenes (alpha-pinene, limonene, camphene) were performed by purging a buffer volume with VOC free air; individual compounds taken from the headspace of liquid pure standards were allowed – one at a time – to diffuse into the gas stream and mix in the buffer volume. This provided mixing ratios of several tens of ppbv at sufficient stability for the fragmentation test.

Results and Discussion

The fragmentation of the tested monoterpenes and aromatics was relatively insensitive to the electric potential of the sampler, the potential difference between the exit of the quadrupole interface and the amplitude of the quadrupole's RF. A variation of the potential difference between the sampler and the entrance of the quadrupole interface in the range of 0.8V and 2.8V produced a similar change of relative abundance of fragmentation products as a change of E/N in the drift tube in the range of 100 to 124 Townsend. Proton transfer reactions in the quadrupole interface were tested to be likely negligible.

Over a period of two months we performed 100 span calibrations. Calibration factors were between 2780 Hz $ppbv^{-1}$ and 250 Hz $ppbv^{-1}$ for different compounds and at different times. The calibration factors showed a gradual decrease of sensitivities after the initial tuning of the newly installed multi-channel plates (MCP) to 27-40% over 30 days. Readjusting the MCP voltage brought the sensitivities back to their initial values and for the remaining 33 days they varied slowly (relative standard deviation <10%). Three sigma LODs were between 10pptv and 130pptv for all VOCs in the calibration standard for 10Hz data acquisition.

The PTR-QiTOF system was demonstrated to be suitable for EC measurements of fluxes of VOCs from an urban footprint.

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Improving the Sensitivity of Proton Transfer Reaction - Time-of-Flight - Mass Spectrometry (PTR-TOFMS)

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Abstract

In 2009 the first commercial PTR-TOFMS instrument manufactured by IONICON Analytik was a technological milestone with a sensitivity of up to 25 cps/ppbv as well as a mass resolution of over 6000 m/Δm. Meanwhile the need for an even higher level of instrumental performance has continuously become stronger because of more demanding analytical challenges in trace gas detection. Therefore, IONICON started a broad development program in order to meet the users' demands and introduced two powerful measures for sensitivity improvement. First, we launched the novel PTR-QiTOF, which represents our current high-end instrument and is equipped with a Quadrupole ion guide (Qi) between the drift tube and the mass spectrometer. Very recently we developed an ion funnel which can be attached to the drift tube of the more compact PTR-TOF 1000 (PTR-TOF 1000 *ultra*) for improved ion focusing. Both measures boost the instruments' sensitivities by more than one order of magnitude, respectively, and will be presented and discussed here.

Introduction

Blake and Ennis et al. [1,2] were first to publish performance data of Proton Transfer Reaction – Time-Of-Flight – Mass Spectrometry (PTR-TOFMS) prototypes. Their instruments reached sensitivities of 0.17 and 3.7 cps/ppbv, respectively, at a mass resolution of about 1000 m/Δm.
The first commercially available PTR-TOFMS instrument from our company was launched in 2008 and represented a huge step forward regarding instrumental performance. We published results [3] demonstrating that the PTR-TOFMS device could reach a sensitivity of 15-25 cps/ppbv and a mass resolution of over 6000 m/Δm. Soon, PTR-TOFMS became state-of-the-art in many fields of application, where real-time quantification, high sensitivity and high mass resolution were needed (e.g. atmospheric chemistry, environmental research, food and flavor science, etc.). A detailed survey of the various studies performed with PTR-TOFMS has recently published by Ellis and Mayhew [4]. However, the growing importance of this technology also comes with a need for higher instrument performance, particularly concerning the sensitivity. E.g. in applications where time per sample analysis is limited or the sample throughput needs to be very high, sensitivity is directly related to the quality of the data, as the signal-to-noise ratio mainly depends on the obtained count-rates. Here we present two novel instruments, equipped with the latest sensitivity enhancing technologies: the PTR-QiTOF [5] and the PTR-TOF 1000 *ultra*.

Experimental Methods

A detailed description of the PTR-MS technology can be found elsewhere [4]. In established PTR-TOFMS instruments, the ions are transferred via a common lens system from the drift tube

to the TOF spectrometer. In case of the PTR-QiTOF this lens system is replaced by a quadrupole ion guide, which considerably reduces ion losses and additionally improves ion injection conditions [5]. The latter has the effect that the mass resolution gets improved, although no changes are made on the mass spectrometer itself.
The PTR-TOF 1000 *ultra* instrument however, is equipped with an ion funnel adjacent to the drift tube. This ion funnel consists of a series of ring electrodes with gradually decreasing orifice diameters. By applying a RF voltage to the electrodes, the ions get focused, which results in a suppression of ion losses and thus to an increase in the overall sensitivity [6]. The outstanding advantage of this ion funnel is that it is modular, i.e. the funnel can easily be installed in an existing PTR-TOF 1000 instrument, thereby upgrading it to a PTR-TOF 1000 *ultra*. Upgrades to the so-called "ION BOOSTER" funnel technology for other IONICON PTR-MS systems are in development.

Results

The performance data presented here were obtained by introducing a certified calibration gas standard mixture (TO-14A) into the PTR-TOFMS systems. All measurements where performed utilizing H_3O^+ as the reagent ion. For comparison reasons we included data of the compact and affordable PTR-TOF 1000, which is entirely manufactured in-house at IONICON (including the TOF mass spectrometer) and equipped with a conventional transfer lens system. In Figure 1, a comparison of sensitivity data (in cps/ppbv) for the PTR-QiTOF, the PTR-TOF 1000 *ultra* and the PTR-TOF 1000 is displayed. It is noteworthy that the PTR-QiTOF is the instrument with the highest sensitivity, reaching up to 4700 cps/ppbv for dichlorobenzene on *m/z* 147, but the PTR-TOF 1000 *ultra* with >1000 cps/ppbv is within in the same order of magnitude. Importantly, a comparison to the conventional PTR-TOF 1000 reveals that installing an ion funnel, boosts the instrument's sensitivity by a factor of up to 20, while no further changes to the instrument are necessary.

As mentioned in the previous section, the ion guide in the PTR-QiTOF not only improves ion transmission but also the injection conditions. Thus, depending on whether the instrument is optimized to maximum sensitivity or resolution, a mass resolution of up to 10,400 m/Δm can be obtained [5]. This is considerably higher than for a somewhat comparable PTR-TOF 8000 instrument, which is equipped with a conventional transfer lens system and performs at about 5000 m/Δm. The ion funnel also improves the resolution, so that the PTR-TOF 1000 *ultra* reaches a resolution of 2400 m/Δm.

In Figure 2 the beneficial effect of extremely high sensitivity is demonstrated via a measurement that has been performed simultaneously on a PTR-QiTOF and a PTR-TOF 8000 (inlet lines connected via a T-piece to the sampling point): A volunteer swallowed a sip of lemon flavored beer and exhaled via the nose into the inlet line. The figure shows the ion yields on *m/z* 137 on a logarithmic scale. Although both instruments received exactly the same sample for the same duration, the signal-to-noise ratio for the PTR-QiTOF is far superior to the one of the PTR-TOF 8000. This is also reflected in the relative error of the measurement points, which is 5% and 19% per breath cycle for the PTR-QiTOF and the PTR-TOF 8000, respectively.

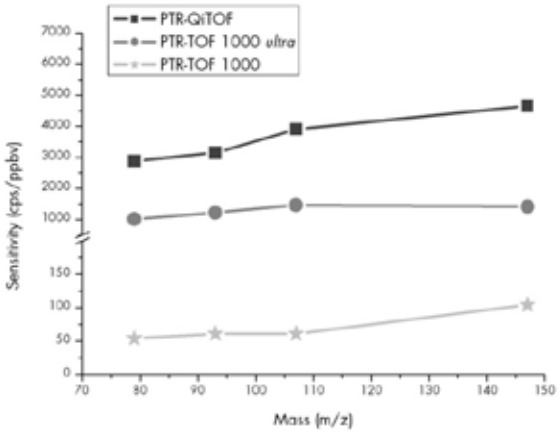

Figure 1: Sensitivity comparison (cps/ppbv) for the latest IONICON PTR-TOFMS instrument (note the gap in the y-axis).

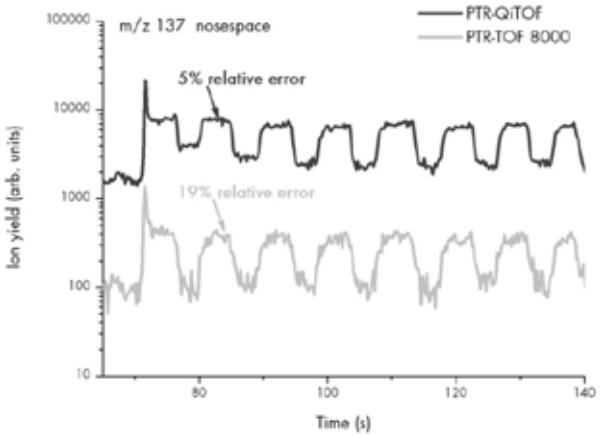

Figure 2: Analysis of m/z 137 in nosespace air after ingestion of lemon flavored beer using the novel PTR-QiTOF and a PTR-TOF 8000 while their inlet lines were connected to the sampling point via a T-piece.

Discussion

We could successfully show that installing an ion guide or an ion funnel between the drift tube and the mass spectrometer of a PTR-TOFMS instrument results in a massive increase in sensitivity. The ion funnel, which can be installed into the compact and cost-effective PTR-

TOF 1000, boosts the instrument's sensitivity to over 1000 cps/ppbv (PTR-TOF 1000 *ultra*). The quadrupole ion guide on the other hand makes the PTR-QiTOF by far the most sensitive commercially available PTR-MS instrument ever, performing at up to 4700 cps/ppbv.

Acknowledgements

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On-line chemical characterization of organic matter in ship engine exhaust by (CHARON-)PTR-ToF-MS

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Abstract

A CHARON PTR-ToF-MS system (Eichler et al., 2015) was used for on-line chemical analysis of organic matter in the gas and particle phase in ship engine exhaust. Test-bench emission measurements from a 1-cylinder, 4-stroke, common-rail marine diesel engine operated with heavy fuel oil (HFO) and marine gas oil (MGO) were conducted at the University of Rostock in the frame of the „WOOd combustion and SHIpping - primary aerosol emissions and secondary aerosol formation potential (WOOSHI)“ project led by Prof. Ralf Zimmermann. Our ongoing analysis has identified and quantified the main gas-phase organics emitted by the engine in the HFO and MGO operation modes. Lubricant oil components were found to dominate the mass spectra of emitted particles. The particles were found to be highly volatile and the measured volatility of individual species agrees well with model predictions.

This work was funded through the PIMMS ITN which is supported by the European Commission's 7th Framework Programme under grant agreement number 287382.

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Contributed Papers (Posters)

Microbial Volatile Organic Compounds (mVOCs) emission by soil

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Abstract

During the last decades, Volatile Organic Compounds (VOCs) became one of the most active topics in the field of environmental pollution. They play a key role in chemistry and composition of the atmosphere. They are precursors of Secondary Organic Aerosols (SOA), which alter radiative transfer in the atmosphere and hence global warming. Additionally VOCs are implied in tropospheric ozone cycle formation [1], [2]. Natural VOCs emissions are estimated to represent 90% of total VOCs emissions [3].

VOCs are emitted by several natural biotic sources like: soil, plants, microorganisms (bacteria and fungi), leaf litter, insects and animals. Besides, interactions between organisms living in soil, as plant-to-plant, plant-to-animal or microbe and microbe-to-microbe interactions, are universally mediated by VOCs, these communications help maintaining the equilibrium of the ecosystem [4]. Emissions are controlled and influenced by abiotic factors such as: temperature, light intensity, water and nutrient availability [5]–[7].

Within this framework new PhD starting focused on VOC emission by soil and interaction with microbes detected by a PTR-TOF-MS technique. The main part of this research concerns the study of the relationship between microorganisms and VOCs production. Hence, the goal of this part is to answer two main questions: (1) why microorganisms produce VOC and what is affecting their production? (2) How can the PTR-TOF-MS technique be used for mVOC soil analysis. Finally, future perspectives are discussed.

Microorganisms and mVOCs production (mVOC?)

Why microorganisms do produce VOCs?

Microorganism's activities are related with carbon's and nitrogen's cycle. Literature shows that bacteria use three major pathways to degrade sugars, preferentially glucose (1) the Embden-Meyerhof pathway; (2) the Entner-Doudoroff pathway; and (3) Heterolactic/homolactic pathway [8]. These three pathways are implied in VOCs production. VOCs are resulting also from secondary metabolism processes. Secondary metabolites are produced only at specific developmental stages or under certain circumstances, for instance, under environmental stress. Intermediates in these processes, such as pyruvate, glyceraldehyde-3-phosphate, lactate and acetate are used as precursors for the biosynthesis of various VOCs [9].

What can affect mVOC production?

Another important topic concerning mVOC is the study of factors? that can influence their production. Difference in soil composition and the variability of soil abiotic parameters, such as nutrients, oxygen and physiological state of microorganisms, affect mVOCs production [5]. Nutrients and oxygen availability depend also on several environmental factors such as: soil texture, soil mixture, temperature, pH and microbial activity [10]. Besides, the availability of oxygen in basic parameters is key in determining the types of VOCs produced as it controls the respiration pathways [11]. In anaerobic conditions, diversity and quantity of VOCs emitted is increased [11].

A database that connects mVOCs emissions, microorganisms and production pathways

Connecting mVOC emissions with microorganisms and their pathways production can be difficult. In literature, for example, we can find a compilation of about 1000 microbial VOCs released from more than 350 bacterial and 80 fungal species. Lemfack and her colleagues (2014) [12] created a database of mVOC (http://bioinformatics.charite.de/mvoc/). The aim was to simplified mVOC research and to summarize in a unique database the main information about these compounds, their pathway formation and associated microorganisms species.

The use of PTR-MS technique for mVOC detection

PTR-MS is the newest technology used to detect VOCs. Major advantages of this technique are its high sensibility (pptv level) and to allow on line VOC measurement of any air gas sample [18] . To obtain a higher resolution PTR-MS can be combined with a time-of-flight (TOF) detector. The PTR-MS was mainly used for the detection of mVOCs in food quality control [15], organic waste decomposition [16], soils and for several other habitats , [21]. Table 1 highlights a non-exhaustive list of studies on microorganisms and their VOC emission detected by PTR-MS.

Table 1. References of in vitro or in vivo VOC production from different bacterial and fungal species and whole microbial communities living in soils or on organic materials detected by the PTR-MS technique.

Organisms investigated	VOC found	Habitat/cultivation media	Source	Year
Escherichia coli ***Shigella flexneri*** ***Salmonella enterica*** ***Candida tropicalis***	Diverse VOCs several unidentified and some identified compounds of low molecular weight <150 u	Complex media	[18]	2008
Pseudomonas spp. ***Enterobacteriaceae*** ***Lactic bacteria*** ***Enterococcus spp.***	Diverse VOCs several unidentified and some identified compounds of low molecular weight <150 u	Air and vacuum packed meat (beef and porck)	[15]	2003
Muscodor albus	Diverse VOCs	Soil grown on potato dextrose agar (PDA)	[19]	2004

Microbial community	Diverse VOCs (31 identified)	Mediterranean soil	[20]	2007
Microbial community	Diverse VOCs (17 identified)	Organic waste	[16]	2006
Microbial community	Diverse VOCs (7 identified)	Temperate soil under different compost load	[21]	2010

Future perspectives

A deeper qualitative and quantitative analysis of soil microbial VOC emitted will be necessary. Thanks to the great progress in analytics technique we have the possibility of analyzing VOCs in soils while discriminating between sources. The aim of the starting PhD it is to determine the possible connections between mVOC emissions profiles and the microorganism diversity and activity in a soil amended with organic waste products. To achieve this several experiments will be performed where the detection of emitted VOCs from soils will be made under controlled conditions for a range of soil samples. In parallel, analysis of soils genetic content and manipulation of genetic diversity of soils will be performed in these experiences.

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How trees respond to stress: VOC emissions during heat waves and drought in Douglas-fir and black locust

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Abstract

Forested ecosystems contribute a substantial amount to the global emissions of volatile organic compounds (VOCs) to the atmosphere. Under a future climate, extreme weather conditions, like heat waves and drought stress, can affect the quantity and quality of VOC emission from trees dramatically. To date, however, there is only limited understanding of VOC emission patterns during prolonged heat and heat-drought stress including post-stress recovery.

In order to evaluate how VOC emissions change during stress, we studied heat and heat-drought stress responses in Douglas-fir and black locust trees under controlled environmental conditions in a greenhouse. Trees in the heat treatments were exposed to repeated heat waves of 14 days at 10° C above ambient temperatures, followed by a recovery period of at least 7 days. In the combined heat-drought treatment irrigation was additionally reduced by 60-80% compared to the control trees. The responses of VOC emissions during heat, heat-drought stress and recovery were compared to a control treatment under ambient temperature. Tree VOC emissions were continuously quantified using a branch chamber set-up (n=3 per treatment and species) connected to a proton-transfer-reaction mass-spectrometer (PTR-MS), and an infrared-gas analyzer to measure branch CO_2 and H_2O gas exchange simultaneously.

Despite partial stomatal closure, VOC emission patterns of black locust and Douglas-fir trees in the heat and heat-drought treatment were significantly higher than under control conditions. Monoterpene emissions from Douglas-fir exposed to heat stress and heat/drought stress were on average by a factor of 10 and 17 higher than for the control trees. Black locust showed three-fold and 7-fold higher emissions of isoprene, respectively. These induced emissions, however, went rapidly back to pre-stress conditions during the recovery. While heat and heat-drought stress decoupled isoprene emissions and CO_2 uptake in black locust, we found a clear relationship between these fluxes before stress and during recovery.

Volatile production in light-struck milk

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Abstract

Milk that is exposed to light undergoes photooxidation that results in light-affected flavour defects. Proton-transfer-reaction mass spectrometry (PTR-MS) is an ideal tool to follow the development of these volatile odour compounds, whose generation and release proceeds rapidly and in some cases transiently. Fresh extended shelf-life (ESL) cow milk (semi-skimmed and whole-milk) was exposed to fluorescent light for up to two days, either at room temperature or under chilled (4 °C) conditions. PTR-MS was used to monitor volatile organic compounds (VOCs) released into the headspace of the milk samples. Two samples were analysed alternately for any one experiment, either with one sample kept dark and the other exposed to light, or with both samples of different milk type under identical light exposure conditions. Sensory aroma profile analysis (APA) was performed on a subset of the samples (chilled whole-milk) exposed to light for different durations (0, 0.25, 1, 6, and 24 h) for comparison with the PTR-MS VOC data. The results shed light on the temporal nature of production of specific light-affected flavour compounds.

Introduction

The phenomenon of light-affected flavour in milk has been studied for almost a century, yet a comprehensive understanding of the exact nature of the processes involved, especially at the early stages of photooxidation, is still lacking. Light-struck milk can develop off-odours within 15 minutes that are characterised by burnt, oxidised, cabbage or mushroom-like odour attributes [1,2]. The odorants causing these impressions arise from secondary volatile products, such as carbonyl and sulphur compounds, which are formed from the breakdown of unsaturated fatty acids, proteins and amino acids [3]. Most scientific studies on the volatile organic compounds (VOCs) associated with light-affected milk have been performed using gas-chromatography mass spectrometry (GC-MS), which delivers a wealth of information on compound identities but cannot be used to follow their rapid production during light exposure. The real-time analytical capability of proton-transfer-reaction mass spectrometry (PTR-MS) offers a unique opportunity to investigate these fast processes in more details, as was conducted in the present study. A full account of these experiments and their outcome has been published in the scientific literature [4,5].

Experimental Methods

PTR-MS Analysis

A high sensitivity proton-transfer-reaction mass spectrometer (hs-PTR-MS; IONICON Analytik GmbH, Innsbruck, Austria) was used to perform on-line analysis of VOCs in the headspace of the milk samples. The PTR-MS instrument was operated at 132 Td *E/N* (drift tube settings: 600 V, 2.2 mbar and 60 °C). The custom-made dual inlet system of the PTR-MS instrument allowed two samples to be measured alternately, with switching between samples occurring every 9 min. Milk samples (400 mL) were poured into 500 mL glass bottles (Schott AG, Mainz, Germany) and each experiment analysed two milks, either cross-over samples (semi-skimmed and whole-milk) or parallel light/dark samples (same milk type in both bottles; one kept dark, the other exposed to light). A fluorescent light (10.6 W/m^2) was used to irradiate the samples. In some experiments both samples were kept in darkness for an initial period of 1 h followed by continuous and prolonged exposure to light. In others, the light was switched on and off at 1-h intervals in order to follow the transient changes induced by light exposure. PTR-MS analysed the samples in mass scan mode, from *m/z* 20-130 at 500 ms dwell time per *m/z*, resulting in a scan time of 55 s. Each sample was analysed for 10 cycles before switching to the alternate sampling, which provided an analysis interval of 9 min.

Sensory analysis

Aroma profile analysis (APA) was performed on a subset of milk samples by a panel comprising nine trained assessors (4 female, 5 male; aged 25-50 years). Specifically, chilled whole-milk samples were prepared and exposed to light for 0, 0.25, 1, 6, and 24 h, with all samples ready for sensory analysis at the same time. The panel evaluated the samples according to orthonasal and retronasal, as well as taste attributes, with intensities ranging from 0 (no perception) to 3 (strong perception at intervals of 0.5. The seventeen attributes that were assessed were: milk-like, cooked milk-like, buttery, fatty, sweaty, cooked cabbage-like, metallic, mushroom-like, plastic-like, hay-like, green/grassy, stable-like (barny), cardboard-like, rubbery, musty/earthy, caramel-like, and overall intensity.

Statistical analysis

The sensory data from the APA assessments on the light-exposed milks were analysed by principal component analysis (PCA) conducted on score loadings.

Results

Several VOCs were observed to emerge or increase under conditions of light exposure of the milk samples in both semi-skimmed and whole-milk samples. In particular, methanethiol (*m/z* 49) increased rapidly, before peaking and subsequently decreasing equally rapidly (cf. figure 1). This behaviour was not observed in the samples kept in the dark. Similarly, acetaldehyde (*m/z* 45) steadily increased immediately after initiating light exposure, but rather than peaking, this compound continued to increase until the moment when the light was switched off (cf. figure 2). Other compounds showed similar patterns, including a series of aldehydes (pentanal, hexanal, heptanal; data not shown).

Although APA revealed differences in the sensory attributes of milk exposed to light for different periods (figure 3), the VOC analysis by PTR-MS did not detect any changes that help explain the sensory result. The 24-h light-exposed milks featured lipid oxidation sensory characteristics (oxidised, rancid, hay-like) and scored higher for total odour and flavour intensities.

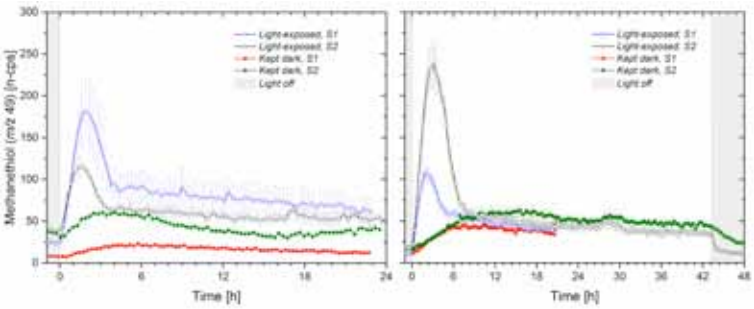

Figure 1: The release of methanethiol (m/z 49) from semi-skimmed (LHS) and whole-milk (RHS) in two sets of samples (S1 and S2) over 24 and 48 h light-exposure.

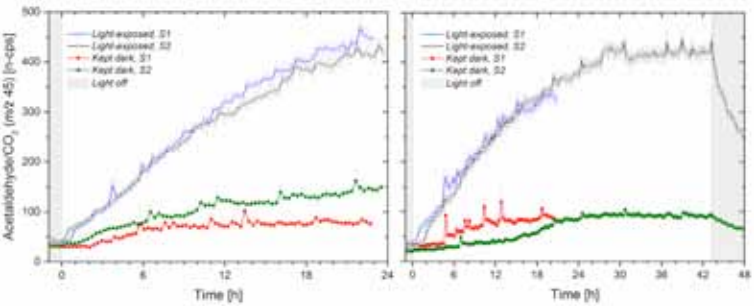

Figure 2: The release of acetaldehyde/CO_2 (m/z 45) from semi-skimmed (LHS) and whole-milk (RHS) in two sets of samples (S1 and S2) over 24 and 48 h light-exposure.

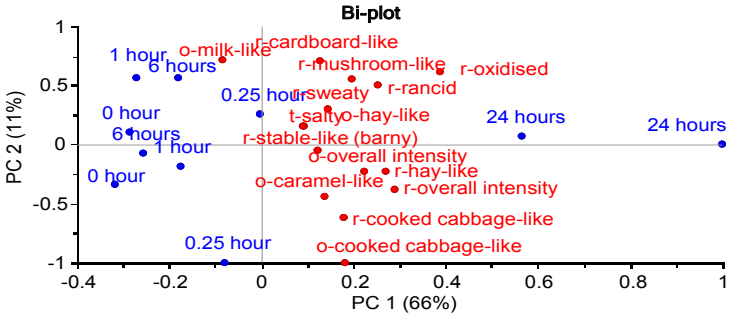

Figure 3: PCA biplots of the APA on chilled whole-milk samples exposed to light for 0, 0.25, 1, 6, and 24 h (duplicates are shown). Attributes significantly ($p<0.05$) discriminating samples are shown in red; o-, orthonasual; r-, retro-nasual.

Conclusions

Exposure of milk to light resulted in the generation of several VOCs, including methanethiol (rapid increase upon light exposure, followed by a similarly rapid decrease), and a series of aldehydes including hexanal, acetaldehyde, pentanal/octanal/nonanal and heptanal (immediate yet steady increase throughout exposure period). These common light-oxidation products are derived from the fat constituents of milk, some of which have previously been associated with the light-affected flavour of milk. The present study demonstrates the utility of PTR-MS in characterising fast oxidation processes, such as photo-oxidation, on sensitive food products such as milk. More in-depth studies of this nature might help to reveal the precise dynamics involved in these reactions, thereby assisting manufacturers to optimise product processing procedures and retailers to adopt suitable point-of-sales conditions.

Acknowledgements

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Fate of isoprene hydroxy hydroperoxide (ISOPOOH) in metal smog chambers under low NO_x conditions

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Abstract

The fate of isoprene hydroxyl peroxides (ISOPOOH) from OH initiated oxidation of isoprene has been investigated in the CLOUD chamber with a SRI-ToF-MS.

Not only intra-instrumental conversion reactions, either on metal or due to instrumental settings [1,2, prove to be a factor that need to be considered when investigating low NO_x isoprene chemistry, but also catalytic surface reactions on the metal walls of the smog chamber must be kept in mind. On contact with metal, ISOOPOOH decomposes to methyl vinyl ketone (MVK), methacrolein (MACR) and formaldehyde which are too volatile to play a role in new particle formation. Due to this catalytic surface reaction, low NO_x chemistry in metal chambers can't fully reproduce atmospheric isoprene photooxidation.

Introduction

Isoprene is the predominant non-methane biogenic volatile organic compound in the atmosphere [3]. Due to its high emission, its chemistry plays an important role in the oxidative cycles of the atmosphere, which are driven by catalytic cycles of hydrogen oxides (HO_x) and nitrogen oxides (NO_x), and is coupled to the formation of secondary organic aerosol (SOA). Urban areas are generally NO_x dominated and chemistry in pristine regions is driven by reaction with HO_x since NO_x concentrations are sufficiently low.

Isoprene shows a high reactivity towards the atmospheric oxidants OH, O_3 and NO_3. Even though emission mostly occurs during daytime, oxidation with NO_3 and the formation of organic nitrates can be an important isoprene sink during the early evening [4,5]. Ozonolysis in turn is a comparatively slow process and leads mainly to MVK, MACR and formaldehyde via the formation and decomposition of two primary ozonides. This reaction also produces OH radicals with a yield of 26% per reaction.

Oxidation with the OH radical is the most important atmospheric oxidation pathway. It occurs across the double bonds and is followed by reaction of the alkyl radical with O_2 to form hydroxyl substituted alkyl peroxy radicals (RO_2). The subsequent reaction of the RO_2 radical is highly NO_x dependent. The two different pathways lead to profoundly different oxidation products in terms of their chemical and physical properties. While high NO_x with OH leads to the breaking of the C_5 carbon backbone and the same main products as ozonolysis, low NO_x photooxidation on the other hand retains the carbon backbone and leads to more oxidised products. RO_2 reaction with HO_2 produces isoprene hydroxyl peroxides and their OH oxidation products isoprene epoxydiols

(IEPOX). These compounds have a lower vapour pressure and can play a role in SOA formation and reactive uptake [6,7,8].

Experimental Methods

Measurements on isoprene oxidation have been carried out at the CLOUD (cosmics leaving outdoor droplets) chamber at CERN, Geneva during the CLOUD 9 (autumn 2014) campaign. The CLOUD experiment allows the study of the formation and evolution of new particles originating from organic precursor gases in an ultraclean and very well controlled environmental chamber. The chamber has a capacity of 26 m³ and is entirely built out of electropolished stainless steel.

Isoprene and its oxidation products from ozonolysis and, to a lesser extent, reaction with OH at different NOx levels and different temperatures were measured by a custom-built metal free SRI-ToF-MS (operated with H_3O^+ and NO^+ as reagent ions) during CLOUD9.

Results

Real-time chamber measurements have been compared to the MCM based chemical box model UWCMv2.2 [9]. The applied model uses the MCM v3.3 [10,11] and is constrained to the measured experimental conditions.

CLOUD9 model simulations generally underpredict the main ozonolysis products MVK, MACR and formaldehyde under HO_x dominated conditions under all experimental settings. Inclusion of a wall conversion reaction of the corresponding isoprene hydroxy hydroperoxide (ISOPOOH) isomers reduces the discrepancy between model and measurement, indicating that catalytic reactions on the metal surface of the CLOUD chamber walls lead to the same C4 oxidation products as the NO_x dominated pathway. This reaction takes place even at temperatures below 273 K.

There are three possible sinks for the ISOPOOH concentration: reaction with OH radicals, chamber dilution and wall loss. The chamber lifetime is 2.8 h at a flow rate of 150 lpm, which is significantly lower than the calculated lifetimes of ISOPBOOH (55.6 h) and ISOPDOOH (24.2 h) at an OH concentration of $1*10^5$ molecules/cm³. OH concentrations are an order of magnitude lower than atmospheric concentrations since the sole OH source was the ozonolysis of isoprene. Considering the functional groups of ISOPOOH a wall loss rate $1.1*10^{-3}$ s^{-1} has been assumed which is equal to a residence time of 15 minutes before wall contact. The fast occurring wall loss makes the catalytic surface conversion the most important sink for ISOPOOH in a metal chamber.

Only at high isoprene and ozone concentration (> 100 ppbv) was it possible to observe a signal of the main ISOPOOH mass (m/z 85.066, $C_5H_9O^+$) in NO^+ reagent ion mode. The signal corresponds to an estimated concentration in the order of 200 pptv which is a factor of 20 lower than the concentration predicted by the model simulation.

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A novel method for electronic cigarette aerosol analysis using Proton Transfer Reaction - Mass Spectrometry

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Abstract

A novel sampling setup for studies on electronic cigarette (e-cigarette) aerosol with Time-Of-Flight based Proton Transfer Reaction-Mass Spectrometry (PTR-MS) instruments is presented. Key features of this setup are a sophisticated sampling interface, which prevents condensation and memory effects and a double-stage dilution system that enables accessing a wide concentration range from <1 ppbv to >1000 ppmv. Furthermore, an exchangeable adapter system allows for mainstream aerosol analysis directly from the mouthpiece of the e-cigarette, as well as breath analysis following the use of e-cigarettes. Thus, the setup is particularly well-suited for retention studies, where compound concentrations in the mainstream aerosol are compared to the concentrations in exhaled breath following inhalation or mouth-hold (no inhalation) scenarios.

Introduction

E-cigarettes are gaining popularity worldwide and already became an alternative to conventional tobacco products. Due to this fact, there is a strong scientific interest in e-cigarette aerosol analysis. E-cigarettes are battery powered devices that convert a liquid (e-liquid) containing a mixture of propylene glycol, glycerol, flavour compounds and (optionally) nicotine into an aerosol, which can be inhaled by the user. So far Gas Chromatography coupled with Mass Spectrometry (GC-MS) has been the established analytical method for e-cigarette aerosol analysis as it provides a very high selectivity and sensitivity. However, GC-MS is very time consuming and samples have to be prepared prior to analysis, which makes real-time measurements impossible. In contrast, with PTR-MS compounds can be quantified online, without the need for any sample preparation, thus making it ideal for puff-by-puff analysis, which has been confirmed in a recent study by us [1] where we investigated the concentration of nicotine in exhaled breath following different inhalation and mouth-hold (no inhalation) topographies of e-cigarette aerosol.

Here, we introduce an advanced setup for the online quantification of the constituents in e-cigarette aerosol. The two major improvements compared to previous setups are: 1) a sophisticated sampling interface that prevents condensation of aerosol and thus, memory effects and building up of contamination and 2) a double-stage dilution system, that enables shifting the dynamic range of PTR-MS instruments to very high concentrations, which are observed in mainstream aerosol (>1000 ppmv).

Experimental Setup

The PTR-MS technique has been described in [2]. For our studies we used a PTR-TOF 8000 (IONICON Analytik GmbH, AT [3]), which is a time-of-flight based PTR-MS instrument. The inlet line as well as drift tube chamber were heated up to 120 °C to avoid condensation. The drift tube pressure was set to 2.3 mbar and drift voltage to 500 V, which corresponds to a reduced electric field of 130 Td (Townsend; 1 Td = 10^{-17} cm^2 V). In order to determine the exact concentrations of the studied compounds, we used a Liquid Calibration Unit (LCU [4], IONICON Analytik GmbH, AT) to calibrate the PTR-TOF 8000 for propylene glycol, glycerol and nicotine.

For testing and evaluating our novel setup we used a commercially available e-cigarette (JAI, Fontem Ventures B.V., NL) with a tank system, a model where the e-liquid can be (re-)filled by the user.

Scenarios the novel e-cigarette aerosol setup has been designed for:

i) Analysis of mainstream aerosol while the e-cigarette is activated via a linear smoking machine (LX1, Borgwaldt, DE) – this is the aerosol an e-cigarette user would inhale. Very high concentrations expected.
ii) Analysis of exhaled breath following mouth-hold (no inhalation) of mainstream aerosol. Medium concentrations expected.
iii) Analysis of exhaled breath following the inhalation of mainstream aerosol. Low concentrations expected.

Results of the Method Development

A schematic drawing of the novel sampling interface is presented in Figure 1: Two types of adapters can be mounted at orifice (A), a funnel type adapter for scenario i) and a disposable mouth-piece for scenarios ii) and iii). Air is constantly drawn through the aluminium housing of the interface via tube which connects the interface with the membrane pump already installed in the PTR-TOF 8000 (H). This constantly flushes the interface and prevents building up of contaminations. The actual sampling happens via a heated (about 170 °C) passivated stainless steel tube (B). In mouth-hold and inhalation scenarios excess breath is ejected via an overflow valve (G).

The double-stage dilution system is shown in Figure 2: The system can be seen as two single stages (stage (A) and (B)) connected in series and the easiest way to explain such a dilution stage is via an example. A mass flow controller (FC), which is connected to a membrane pump generates a controlled suction flow of 200 sccm. A second mass flow controller is connected to a zero-air supply and generates a pressurized flow of 190 sccm. If the suction and the pressurized flows are connected to a T-piece, the remaining port of the T-piece (connected to the sampling interface) will show a suction flow of 10 sccm (200 sccm – 190 sccm). In this case the dilution factor will be 20 (200 sccm / 10 sccm), i.e. the measured concentration in the diluted gas has to be multiplied by 20. If two stages are connected in series, the respective dilution factors have to be multiplied and thus, very high dilution factors (up to 5000) can be reached without the need for excessively high flows.

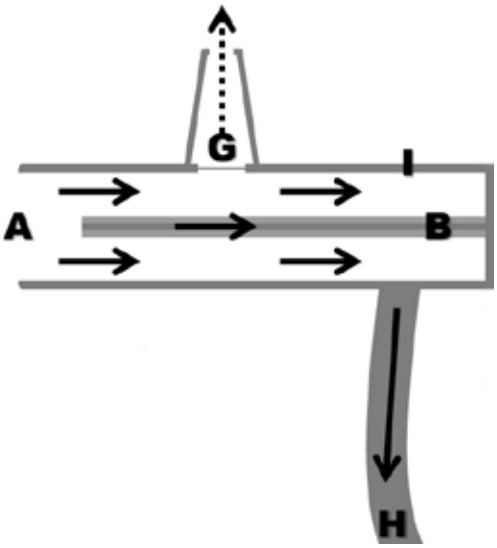

Figure 1: Schematic drawing of the new sampling interface.

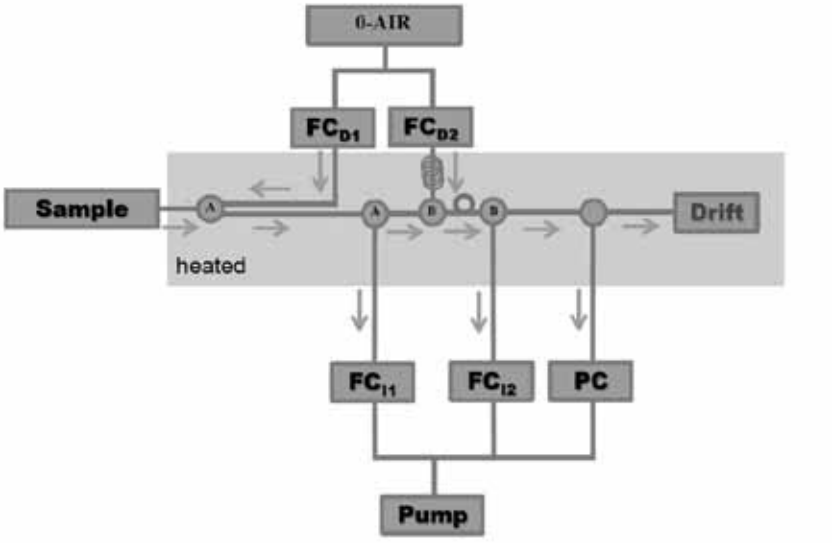

Figure 2: Schematic representation of the double-stage dilution system.

Discussion

We successfully tested the novel sampling interface for e-cigarette aerosol analysis for all three scenarios. No building-up of signal background was observed and the time from the onset of the signal peaks to the beginning of the decrease corresponded well to the puff durations, which are clear indicators that the interface works well and the aerosol is analyzed in real-time.

For scenario i) we measured concentrations between 10^1 and 10^3 ppmv for propylene glycol, glycerol and nicotine. For scenario ii) the concentrations were between 10^0 and 10^2 ppmv and for iii) between 10^{-3} and 10^1 ppmv. With the double-stage dilution system the dynamic range of the PTR-TOF 8000 could be easily adapted to these concentration ranges.

The combination of the sampling interface and the double-stage dilution system will enable a wide variety of puff-by-puff e-cigarette studies and thus make PTR-MS play a key role in the evaluation of the effects of e-cigarette use for users as well as for bystanders.

Acknowledgement

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Changes in urine headspace composition as an effect of strenuous walking

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Introduction

In breath, skin and urine the volatile organinic compound (VOC) profile has been well studied in healthy people. Changes in concentration of targeted VOCs within an individual's profile can indicate changes in metabolism or disease state. These targeted VOCs become biomarkers for particular states, such as acetone for diabetes or nitric oxide for airway inflammation. This study uses PTR-MS and multivariate and univariate statistical techniques to investigate the effect of exercise on VOC headspace composition of urine. Breath acetone was measured concurrently and shown to correlate positively with non-estrified fatty acids and beta-hydroxybutyrate to provide information about fat burning during exercise [1]. We will look at the link between breath and urine VOCs and determine which VOCs in urine are potential biomarkers for the effect of strenuous walking over 4 days.

Experimental Methods

Subjects and Urine Samples

Urine was collected twice a day for 4 days in succession. Each day the participant walked 30, 40 or 50 km (depending on age and gender), samples were collected before and after walking. All participants were taking part in the International Four Days Marches in July 2012, an annual walking event in the Netherlands. A total of 51 participants gave samples, among them were 23 healthy controls (CT), 11 type-1 diabetes mellitus (T1DM) and 17 type-2 diabetes mellitus (T2DM). Urine samples were divided into two vials; one for VOC analysis with PTR-MS, the other for creatinine measurements. A total of 40 ions were measured with PTR-MS, chosen for possible biological significance. Urine samples were transferred at room temperature to a sealed cuvette for measurement. The sample headspace was flushed with 2 l/hr of catalyzed air, with 1.5 l/hr sampled by the PTR-MS and the rest sent to an exhaust. A fixed measurement time was used per cuvette. Intensity values per *m/z* were recorded in cps. These were each normalized to the reagent ion signal and subsequently to creatinine concentration.

Statistical Techniques

Firstly, the data was analyzed with multilevel partial least squares discriminate analysis (M-PLS-DA), a multivariate technique used to identify the ions of significant interest. The identified ions were then analyzed with univariate techniques. Distributions were tested for normality and found to be non-normal so were analyzed using a Wilcoxon signed rank test to judge the effect of exercise and a Friedman test to judge the effect over the 4 days.

Results

Multivariate

Each individual provided samples before and after exercise and thus acted as his/her own control. To study the effect of exercise on the different groups separate models were built for each group across all days. The performance of the models are shown in Table 1 (b).

(a) Performance per day			(b) Performance per group		
Day	AUROC	p-value	Group	AUROC	p-value
1	0.63	0.012	CT	0.87	<0.001
2	0.64	0.066	T1DM	0.70	0.004
3	0.90	<0.001	T2DM	0.81	<0.001
4	0.94	<0.001			

Table 1: Table showing the results from ML-PLS-DA of 33 major ions detected from VOCs in urine headspace.

In each group, the model is able to distinguish a higher significant exercise effect than a randomly permuted dataset. The highest performance is extracted from CT and T2DM as shown by comparing the area under receiver operator curve (AUROC) of each group. The effect of exercise over the course of the 4 days was evaluated by using separate ML-PLS-DA models for each day, the performances of the models are shown in Table 1 (a). A significant exercise effect is extracted from models on day 1, 3 and 4. The discriminating ability is greatest in the day 4 model, followed by day 3. This indicates that the effect of exercise on the VOC headspace profile of urine is more marked on the last 2 days of the marches.

The contribution of each ion to each model is shown in Figure 1. Against each axis 1 minus the p-value is plotted, such that more significant ions appear towards the outer edge of the diagram. Ions at *m/z* 61, 62 and 79 contribute significantly to the model on each of the four days. These ions are all thought to be due to acetic acid. Other ions are significant, but not over all days. Ion signals were observed to be significantly contributing to the model at *m/z* 55, 73, 83, 87 and 117, hypothesized to be from hexanoic acid.

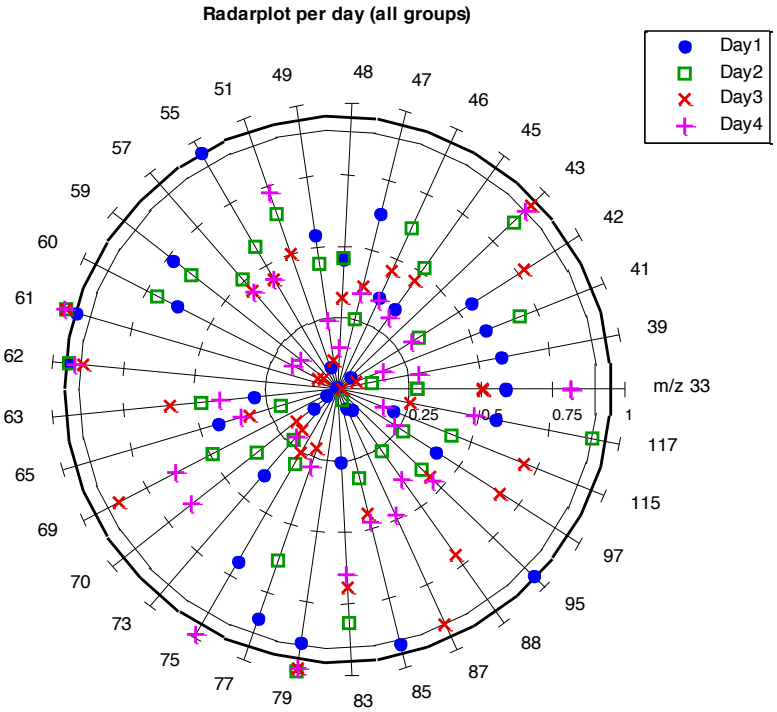

Figure 1: Radar plot per day showing the ions' relative contribution to the M-PLS-DA model before and after exercise. Radial axes represent significance to the model, points lying outside the outermost circle are significant, points lying between the two circles are classed as trend to be significant.

Univariate

Due to the high significance of acetic acid indicated by the multivariate analysis (per group and per day) univariate analysis of *m/z* 61 was undertaken. A box plot of the univariate analysis is shown in Figure 2. Acetic acid is shown to vary significantly with the effect of exercise on each of the 4 days.

Acetone (*m/z* 59) only shows a significant effect of exercise on day 3 and 4. This is in contrast to the complimentary dataset taken with breath, where acetone varied significantly on each day as an effect of exercise.

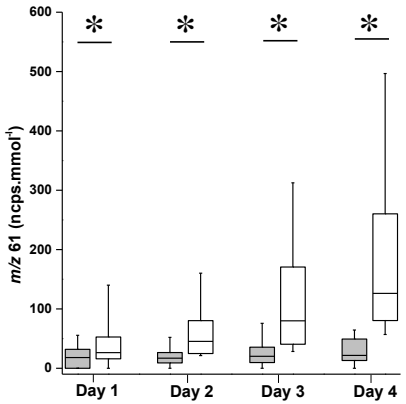

Figure 2: Univariate analysis of acetic acid (*m/z* 61) as a function of days for all groups. Grey – before exercise; White – after exercsie.

Discussion

By using multivariate analysis discrimination between before and after exercise was possible for three out of four days and for all three cohorts. The obvious advantage of multivariate analysis was demonstrated by indicating 12 important ion signals from a total of 33. Acetic acid is shown by univariate analysis to vary significantly with the effect of exercise. Acetate has been linked to the mammalian stress response to hypoxia [3] and increased dietary acetic acid has been shown to stimulate glycogenesis [4]. Univariate analysis of acetone revealed different information compared to breath as a function of exercise. Breath analysis provides a glimpse on the body's metabolism at the moment the sample is collected, whereas urine metabolites will be derived from a longer process. This difference in timing may result in complimentary information being obtained and leading to routine whole body metabolic analysis in future.

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Environmental Science integrated into School

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Abstract

Within the Sparkling Science project AiR (**A**nalysis of Trace Gases **i**n an Inneralpine **R**egion) students from two schools in Salzburg are actively included as young scientists into air quality measurements. During the first field campaign in June 2015 the VOC composition of the outdoor air was monitored punctually at the school with a Switchable-Reagent-Ion-ToF-MS (SRI-ToF-MS) as well as meteorological parameters with a weather station. On one day during the campaign the punctual VOC measurement at the school was extended by collecting air samples at 14 locations in the valley Pinzgau. With the help of easy in use, inexpensive sensors for temperature, humidity, wind speed, NO_2, CO, SO_2 and O_3 concentrations the students take part in collecting and interpreting air data. The project is accompanied by a didactic evaluation.

Introduction

Although volatile organic compounds (VOC) occur in small quantities, they contribute significantly to the air quality. Beside their ability to take part in aerosol particle formation [1], they play an important role in the formation of ground-level ozone [2]. As VOCs are emitted from a variety of anthropogenic and biogenic sources and react in the atmosphere, they show a limited lifetime. Thus, their concentrations differ locally on a large scale. The aim of the Sparkling Science projekt AiR (tracegas Analysis in an inner alpine Region) is to create a unique dataset of the composition and source strengths of VOCs and investigate the spatial representativeness of VOC measurements in a rural inner alpine region. For this aim a collaboration of several institutions from Tyrol and Salzburg carry out measurements at the two participating schools (BORG Mittersill and BG/BRG Zell am See) in the alpine region Pinzgau in Austria. Within a total of four measurement campaigns the schools will become modern air quality monitoring stations equipped with a SRI-ToF-MS (Switchable-Reagent-Ion-ToF-MS) for high sensitivity measurements of VOCs, a weather station, an ozone detector and an air quality monitoring station from the Umweltbundesamt. Students from those schools take part in cutting edge atmospheric science and contribute to collecting data using several different methods.

Experimental Methods

A SRI-ToF-MS [3] was used to measure quantitatively the outdoor concentrations of VOC with high resolution on the top of the roof of the school in Mittersill (Salzburg) and to analyze air samples grabbed with air sampling canisters. Its operation in H_3O^+ and NO^+ reagent ion modes allows possible isomeric separation of certain molecules with different functional groups (e.g. separation of methyl vinyl ketone and metacrolein). Calibration of the SRI-ToF-MS was performed by dynamically diluting a VOC standard gas at ppmv level with scrubbed air. Next to the inlet tube of the SIR-ToF-MS a weather station was placed to monitor temperature, humidity, wind parameters, pressure, net radiation and precipitation. Air from 20 rural and urban locations were sampled by the students with air sampling canisters (SilcoCan®, Restek). These sulfinert treated stainless steel containers are designed for air sampling for trace gas analysis in the sub ppbv range. Each air collection was accompanied by measuring the ambient temperature, humidity and wind speed with a mobile phone weather station (Skywatch Windoo 3) [2] equipped with a multi-directional propeller for wind speed and sensors for temperature, humidity and pressure. The data obtained can be shared and compared with data from other users of the accessory on the cloud of the manufacturer. Furthermore we stored the data on a private server and linked it to a Google Map of the region Pinzgau (http://aited.org/sparkling/map.php).

To provide the students an insight into measuring air quality parameters besides temperature and humidity and handling measured data, the students also collected data with two Air Quality Eggs [1]. With inexpensive sensors the Air Quality Eggs can detect qualitatively the concentrations of NO_2 and CO or O_3 and SO_2. These sensor tools provide easy handling and real time measurements of the air quality but the sensors' accuracy and precision is significantly worse than those of scientific instruments. Furthermore the data can be sent to the cloud at Opensensors.io, an open data service, which both stores and gives free access to the data. The Air Quality Eggs are designed to allow a large number of people to measure the air quality of their surrounding environment.

Results and Discussion

During the first field campaign from May to June 2015 at the BORG Mittersill the outdoor concentrations of VOCs were analyzed. Small groups of students of the BORG Mittersill each assisted the measurements for one day and were introduced into handling the smart phone weather station and the air sampling canisters. With the smart phone weather station the students can playfully train their awareness of their environment but also learn about measuring techniques and measurement errors.

The highlight of the campaign was an air sampling excursion on one day during the campaign. For this purpose 14 groups of students simultaneously grabbed ambient air with the air sampling canisters at the exact same time at 14 locations along the valley axis of Pinzgau, see Figure 2. To smooth possible peak concentrations of VOCs (e.g. a passing car) a capillary with a calculated length was used as an inlet tube for the air sampling canisters. With the capillary inlet it took 10 minutes to fill the evacuated air sampling canister. While filling the canisters the students filled out a protocol to determine the weather conditions with the smart phone weather station, GPS coordinates and special events. Furthermore they had to describe the characteristics of their sampling site. The air samples were analyzed with the SRI-ToF-MS at the school resulting in a snapshot picture of the air composition of the entire valley. For the compounds benzene (detected at its protonated mass m/z79.05), toluene (m/z93.069) and xylene (m/z107.08) the results from all sampling locations are shown in Fig. 1. The concentrations of those compounds reflect the descriptions of the students from their sampling location. For example several cars, trucks and

two diesel locomotives were registered in Hollersbach (canister No. 6), the location with the highest concentrations of benzene, toluene and xylene. Meanwhile the sampling above a meadow in Kaprun (canister No. 14) and at the water falls in Krimml (canister No. 1) show very low concentrations of these compounds.

To further develop the awareness of air pollution and plant stress consequences the students (mostly females) were invited to a Girls in the Lab Week at the Institute of Ion Physics and Applied Physics in Innsbruck with a lot of experiments including the SRI-ToF-MS, a registering balloon equipped with the Air Quality Eggs and a radiosonde, as well as air analysis from air sampling at rural and urban locations. Moreover the students were trained in presenting scientific data and showed their skills and knowledge in a Science Slam on the last day of their visit.

Three more field campaigns are planned in winter and summer in 2016. In winter the students will sample air along the cross-section of the valley to get a snapshot of the air composition in and above an inversion layer. With the Air Quality Eggs the students will generate concentrations profiles of NO_2, CO, SO_2 and O_3 along the cross-section of the valley to get an idea of air quality parameters during winter and summer conditions.

The project is accompanied by a didactic evaluation of changes in motivation, interest and environmental awareness of the students. For this purpose the students full fill questionnaires after every field campaign and several of them are randomly interviewed with a previously defined outline of questions.

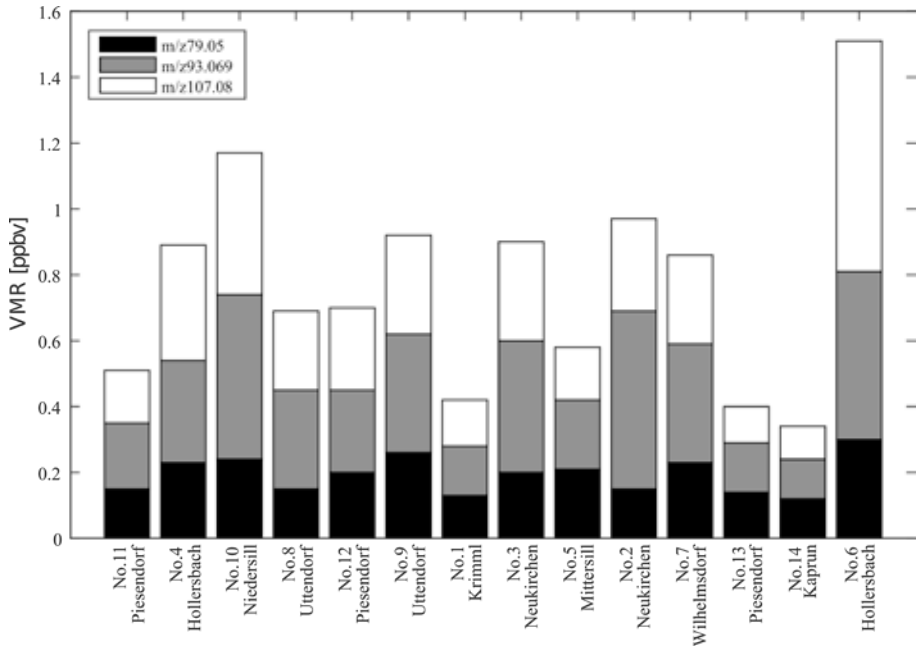

Figure 1: Fig. 1: Volume mixing ratios of benzene (m/z79), toluene (m/z93) and xylene (m/z107) of air samples simultaneously grabbed all over the region Pingzau on June 2015. The highest concentrations were found in Hollersbach where several vehicles passed by. At the two most rural places (a meadow in Kaprun and at the water falls in Krimml) the lowest concentrations of aromates occurred. The numbers of the air samples correspond to the marked loactions in Figure 2.

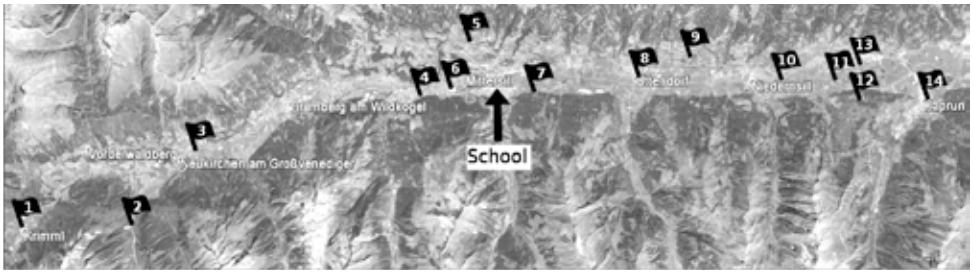

Figure 2: Locations of the 14 grabbed air samples in June 2015 in Pinzgau. In Mittersill (middle of the map) the school is located, where the SRI-ToF-MS was analyzing the VOC composition. Map taken from Google Maps.

Acknowledgements

The authors would like to thank our working group for technical support and for helpful discussions. This project was financially supported by the Bundesministerium für Wissenschaft, Forschung und Wirtschaft (BMWFW) in the frame of the Sparkling Science AIR project (SPA 05-080).

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Plant emission of CO_2 and VOCs

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Abstract

Plant metabolic processes exert a large influence on global climate and air quality through the emission of the greenhouse gas CO2 and volatile organic compounds (VOCs). Despite the enormous importance, processes controlling plant carbon allocation into primary and secondary metabolism, such as respiratory CO2 emission and VOC synthesis, remain unclear.

Our project (VOCO2) develops a novel technological and theoretical basis to couple CO2 fluxes with VOC emissions and establish a mechanistic link between primary and secondary carbon metabolism. This radically new approach uses stable isotope fractionation of central metabolites (glucose, pyruvate) to trace carbon partitioning at metabolic branching points. A unique combination of cutting-edge technology (δ13CO2 laser spectroscopy, high sensitivity PTR-TOF-MS and isotope NMR spectroscopy) will allow an unprecedented assessment of carbon partitioning, bridging scales from sub-molecular to whole-plant and ecosystem processes in an interdisciplinary approach. Innovative positional 13C-labelling will break new ground quantifying real-time sub-molecular carbon investment into VOCs and CO2, enabling mechanistic descriptions of the underlying biochemical pathways coupling anabolic and catabolic processes, particularly the long overlooked link between secondary compound synthesis and CO2 emission in the light. This approach will permit the development of a novel mechanistic leaf model and its integration into a state-of-the-art ecosystem flux model.

Experimental Methods

An unique facility will be created by merging state-of-the-art technology, allowing real-time detection of carbon allocation into respiratory CO2 and VOC emissions in intact plants and their fluxes in atmospheric trace gases. We will combine a highly sensitive proton transfer time-of-flight mass spectrometer (PTR-TOF MS) with a 13CO2 continuous wave Isotope Ratio IR Spectrometer (IRIS). Based on a series of controlled climate chamber experiments with high (e.g. Halimium halimifolium) and low (e.g. Oxalis triangularis) VOC emitting species, the biochemical pathways and interconnection of VOC and CO2 emissions will be identified. Position-specific 13C-labelled metabolites (such as pyruvate) will be used to trace carbon partitioning into the different anabolic and catabolic pathways.

Results and Discussion

Our central hypothesis is that in plants an investment of assimilated carbon to secondary metabolism is associated with the decarboxylation of the naturally 13C-enriched C1 of pyruvate and is therefore associated with detectable emissions of CO2. Specifically, tracing sub-molecular carbon partitioning by position-specific 13C-labelling will allow a new step of quantification carbon fluxes into VOC synthesis and CO2 emission, thus reflecting the relative activity of primary (i.e. respiratory) and secondary (i.e. VOC) metabolism.

Thus, position-specific labelling of metabolites is an innovative approach opening new horizons to trace the fate of every single atom in biochemical cycles. We have shown that leaf feeding of pyruvate-1-13C enables tracing pyruvate C1-decarboxylation reactions in the light and dark. In contrast, leaf feeding with pyruvate-2-13C or pyruvate-3-13C tracked high CO2 release during dark respiration from the TCA cycle. Pyruvate-2-13C labelling also proved that pyruvate feeds biosynthesis and emission of volatile isoprenoids and oxygenated VOCs connecting primary with secondary metabolism by fuelling the PDH bypass, MEP, and mevalonic acid pathways. However, these rapid processes are difficult to assess due to limited sensitivity or time-resolution of measurement techniques. Furthermore, VOCs and 13CO2 have rarely been measured in concert.

Indeed, recent developments of position-specific labelling, PTR-MS and 13C laser spectroscopy will open the door to trace dynamic changes at a high sensitivity and an unprecedented temporal resolution. A first innovative experiment, alternating different positional-labelled pyruvate solutions, yielded a first proof of concept: the labelled C1-position of pyruvate was released as 13CO2 while the 13C from 2,3 positions were emitted as VOC.

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Volatile compounds present in vacuum-packed lamb chilled at 2 °C for 15 days

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Abstract

Red meats such as lamb are often vacuum-packed to extend their shelf-life, which is especially important in the global export market, for instance, for shipping lamb from New Zealand to Europe. In the case of lamb, often a whole leg of lamb is vacuum-packed for transportation, which includes lean meat, fat and bone. The influence of these different parts on the production of volatile organic compounds (VOCs) during storage at 2 °C was followed for up to 15 days using proton-transfer-reaction mass spectrometry (PTR-MS) by creating samples containing different ratios of these individual parts. Solid phase micro-extraction gas chromatography-mass spectrometry (SPME-GC-MS) was additionally used to aid compound identification, and standard microbiological methods were applied to determine bacterial numbers. Slightly higher VOC levels were detected in meat samples packaged with 20 % added fat compared to lean meat alone, which corresponded to higher bacterial numbers of the former at the beginning of the experiments. VOC production was found to predominantly depend on storage time (corresponding to microbial numbers). The presented method could serve as a potential tool for rapid screening of stored vacuum-packed meat samples after transportation to determine the degree of spoilage based on the nature and abundances of VOCs present.

Introduction

Vacuum-packed (VP) lamb can have a shelf-life of up to 12 weeks when packaged in low oxygen permeability packaging and stored at chilled conditions [1]. As its quality decreases at the end of shelf-life, lamb becomes unacceptable to consumers due to the development of off-odours as fat, proteins and carbohydrates breakdown [2,3].

The amount of bone and fat in a packaged sample potentially influences the nature and degree of VOC development in raw lamb meat. For instance, lactic acid bacteria (LAB) have been observed to occur at significantly lower numbers on the fat layer than on lean meat [4], suggesting lower VOC production, and higher amounts of spoilage bacteria (*Serratia* and *Brochothrix spp.*) were present in lamb shoulders containing bone than in shoulders without the bone [1]. Nevertheless, systematic studies on these effects are scarce.

This study addressed this issue by investigating the VOCs produced in vacuum-packed lamb containing different ratios of protein (lean meat), fat and bone stored at 2 °C over 15 days using proton-transfer-reaction mass spectrometry (PTR-MS), with concurrent microbial analyses.

Experimental Methods

Lamb samples

Six 2 kg legs of lamb were deboned, with the bones then cut into ~1 cm pieces. The fat was removed from the remaining meat, and both the lean meat and fat were minced separately. The minced meat and fat, and pieces of bone were vacuum-packed in multi-layer vacuum pouches at different ratios, with duplicate samples made for each configuration. The samples were stored at 2 °C and duplicates were sampled (microbial analysis, pH measurement, and PTR-MS analysis) 1, 7, 10 and 14 days after packaging.

Lean meat was packaged either on its own, with 10 or 20 % minced fat, with 1 or 2 pieces of bone, or a combination of all three (e.g., 10 % fat, 2 pieces of bone). The total mass of meat and fat was maintained at 50 g.

PTR-MS analysis

VOCs in the headspace of the vacuum-packed samples were determined using a high sensitivity proton-transfer-reaction mass spectrometer (hs-PTR-MS, IONICON Analytik GmbH, Innsbruck, Austria). The PTR-MS 1 m long, 1/16" outer diameter Silcosteel™ capillary (Restek Co., Bellefonte, PA, USA) inlet was heated to 110 °C and had a steady sampling flow of 52-53 mL/min. Samples were individually placed inside a glass sampling bottle (500 mL), which was flushed with instrument grade synthetic air and placed in an incubator at 30±2 °C throughout the PTR-MS analysis. Sample bottles were capped and allowed to equilibrate for 15 min prior to sampling. Sampling was then performed at a reduced synthetic air flow of 50 mL/min for 8 cycles The PTR-MS analyses were performed in mass scan mode: *m/z* 20-160 at a dwell time of 500 ms per *m/z* and resulting cycle time of 70 s. The PTR-MS drift tube settings were 600 V, 2.2 mbar, and 70 °C (*E/N* = 136 Td. The measurements were carried out on duplicate samples on each sampling day. Representative duplicate samples (frozen after PTR-MS analysis and stored at −80 °C) were later analysed by SPME-GC-MS (Agilent 6890N coupled with Agilent 5975B VL MSD, Agilent Technologies, Santa Clara, CA, USA). All data were processed as previously reported [5].

Bacterial analysis

Microorganism numbers in each sample were determined as follows: the minced lamb (25 g) was diluted 1:9 with 0.1 % peptone water in a stomacher bag and mixed at room temperature for 60 s. The resulting suspension was diluted (1:9 v/v) in 0.1 % peptone water and three dilutions were spread-plated (0.1 mL) in triplicate onto both plate count agar (PCA) and *Lactobacilli* MRS agar. The PCA plates were incubated aerobically while the MRS agar plates were incubated anaerobically at 25 °C for 48 h. Total microbial (TMN) and LAB numbers were determined.

Statistical analysis

The effects of storage time, fat and bone content on the VOC production were analysed using a GLM two-way ANOVA with two-way interactions. The normalised *m/z* intensities [n-cps] in duplicate samples were used as dependent variables and the fixed factors were storage time, bone content, fat content and replicates. The relationships between the significant ($p \leq 0.05$) *m/z* and the samples were investigated using principal component analysis (PCA) (Unscrambler 10.3,

CAMO Software Inc, Norway) and the *m/z* were standardised using 1/standard deviation. In this way the correlation between different *m/z* and the samples could be observed.

Results

The main VOCs that showed a significant increase over time were ethanol (*m/z* 47), acetone (*m/z* 59), 1-propanol/acetic acid (*m/z* 61), 2-butanone (*m/z* 73) and ethyl acetate /3-methyl-butanol /1-pentanol/3-hydroxy-2-butanone (*m/z* 89) and other alcohol fragments (*m/z* 41, 43) (tentative identifications) (Figure 1, LHS).

Storage time, fat level, and bone level had a significant effect on 63, 20, and six *m/z*, respectively. In many cases a threshold level of TMN or LAB numbers was required before VOC levels significantly increased. Acetaldehyde (*m/z* 45), ethanol (*m/z* 47) and acetic acid/1-propanol (*m/z* 61), for example, increased at LAB numbers of ~8 log colony forming units (CFU)/g, with further more pronounced increases at 8.5 CFU/g. By comparison, *m/z* 87 (potentially a number of compounds including 3-methyl-butanal, 2,3-butanedione, pentanal and 1-pentene-3-ol) started to increase only once LAB numbers reached 8.5 log CFU/g.

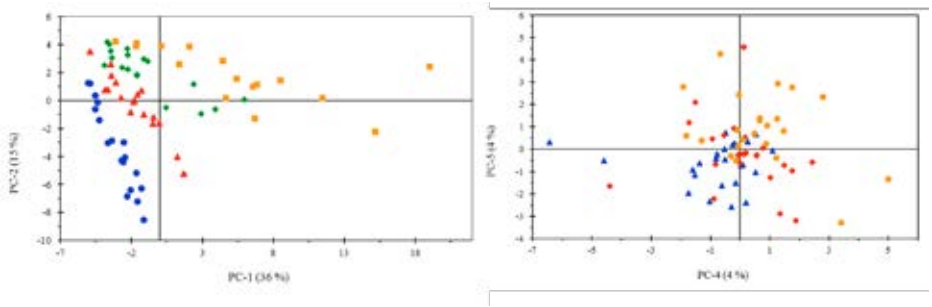

Figure 1: Score plot of the principle components (PC) for lamb stored at 2 °C for up to 15 days, with groupings according to day (LHS): ● = day 1, ▲ = day 7, ◆ = day 10, ■ = day 14, and fat content (RHS): ▲ = 0 % fat, ◆ = 10 % fat, ● = 20 % fat.

A slight increase in initial microbial numbers was observed in samples containing 20 % fat, with corresponding significantly ($p \leq 0.05$) higher levels of acetaldehyde (*m/z* 45), 2-propenal and fragments of carboxylic acids and alcohols (*m/z* 57), 2-butanone (*m/z* 73) and VOCs associated with fragments of alcohols (e.g., *m/z* 41, 55). Acetone (*m/z* 59) was the only compound found to occur at a higher concentration in samples containing bone (Figure 1, RHS).

Discussion

The current experiments suggest that the presence of 20 % added fat or added bone leads to an increase in the number of microorganisms, especially non-LAB species, as well an increase in VOC production (20 % added fat samples) or the production of specific VOC (samples containing bone). VOC production was observed to increase dramatically once bacterial numbers reached a threshold number, hence storage time (to reflect microbial growth) had the greatest influence on VOCs detected in vacuum-packed minced lamb stored at 2 °C. The presence of added fat and bone did not lead to the production of fat or bone-specific VOCs, but did promote either an overall higher production of VOCs (20 % fat) or higher production of some VOCs (added bone), which can be attributed to slightly higher initial microbial numbers and differences in the composition of the microbial populations.

Further studies are required to generate more conclusive evidence for a species-specific or substrate mix-specific production of VOCs in red meat and to translate these initial findings into commercial use for determining the shelf-life of vacuum-packed red meat, such as lamb.

Acknowledgements

The Royal Society of New Zealand and the German Federal Office for Agriculture and Food (BLE) are gratefully acknowledged for their financial support of these studies.

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Analysis of drugs with GC-PTR-TOFMS

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Abstract

Proton Transfer Reaction – Time of Flight – Mass Spectrometry (PTR-TOFMS) is a well-established technique that allows for online quantification of VOCs (Volatile Organic Compounds) down to lowest concentrations (ppt levels and below). Here we ascertain the capability of coupling PTR-TOFMS with Gas Chromatography (GC) with the aim to detect drug compounds or more general, Semi Volatile Organic Compounds (SVOCs). Target molecules are controlled drugs pharmaceuticals, and metabolites thereof in biological samples, which were analyzed to demonstrate the protruded power for the qualitative and quantitative analysis using a GC-PTR-TOFMS system.

Introduction

For standardized analysis of drug compounds LC (Liquid Chromatography)-MS or GC-MS are well established techniques in fields like forensic analysis, environmental analysis, clinical chemistry, therapeutic drug monitoring and metabolic profiling. Besides, there is a need for an analytical method with high sensitivity and selectivity, especially in cases when only minute amounts of sample are available. Therefore, research activities are indented to optimize the complete analytical process like optimization of chromatographic separation and improvement of mass spectrometric detection. Coupling GC to a PTR-TOFMS is not new and already well described [1], but mostly used as a complementary method for the unambiguous identification of VOCs. Here a GC-PTR-TOF-MS detector is utilized for the analysis of SVOCs.

Experimental Method

Different concentrations ranging from 0.001 to 10 mg/l of a calibration standard containing a series of typical drug compounds like valproic acid, nicotine, caffeine, morphine, codeine, methadone, diazepam and others were analysed as well as real biological samples. For comparison, an Agilent GC-EI (Electron Ionisation)-MS system with an auto-sampling unit optimized for standardized analysis of drug compounds was used. The GC section of the GC-EI-MS system was connected to an IONICON PTR-TOF 1000 analyser and operated under identical conditions. For the connection, a 2m long, passivated transfer capillary (passivated TSP-FS, phenyl methyl, heated to a maximum temperature of 130°C) was used. To minimize retardation effects this capillary was positioned directly into the inlet drift ring of the PTR-TOFMS (connection to the GC column was accomplished via a passivated glass liner). The PTR-TOF 1000 was operated under the following conditions: 2.3 mbar drift tube pressure, 120 °C temperature, H_3O^+ mode and 600 V drift voltage. Since the GC column provided only 2 sccm of sample gas, N_2 was introduced into the PTR-TOFMS instrument as makeup gas. The acquisition of the PTR-TOF 1000 was triggered by sample injection onto the GC column.

Results

Six replicate runs of injecting a drug standard mixture and analysing via PTR-TOFMS show identical results and indicate high reproducibility of this method. Figure 1 shows the signal response of the respective [MH^+] peaks of valproic acid ($C_8H_{17}O_2^+$), nicotine ($C_{10}H_{15}N_2^+$), methadone ($C_{21}H_{28}NO^+$) and diazepam ($C_{16}H_{14}ClN_2O^+$) versus retention time. Soft ionization by using H_3O^+ results in low fragmentation of ionized compounds and clearly enhances mass related sensitivity. For most of the compounds tested (in total 31), only the protonated molecular ion [MH^+] and its natural isotopes could be found (data not shown). The high sensitivity of the PTR-TOFMS method reveals a much increased detection efficiency by a factor ten to hundred in comparison to the standard EI-MS method (data not shown).

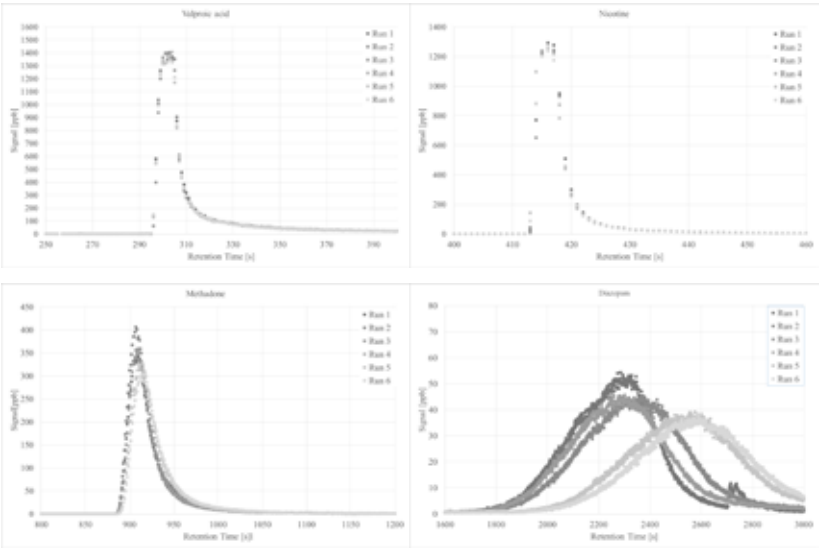

Figure 1. Chromatograms of valproic acid, nicotine, methadone and diazepam for six identical runs with GC-PTR-TOF. Signal of the protonated molecular ion [MH^+] found for those compounds are plotted in [ppb] versus retention time in [s].

In comparison to standard GC-EI-MS some compounds show much higher retention times and induce partly massive peak broadening. We attribute this effect to the transfer system. A long transfer capillary with 2m length and maximum temperature of 140°C causes additional retardation of the analytes as a result of the SVOCs "sticky" nature. Minimizing length (and eventually the stationary phase) as well as increasing the temperature of the capillary may considerably improve the results.

Discussion

The suitability of a PTR-TOFMS instrument coupled with a GC system was ascertained for a range of drug compounds routinely analysed in forensic toxicology. The ability to detect semi volatile compounds down to lowest concentrations and (due to) soft ionisation comprising low

fragmentation makes this technique a very powerful tool. In comparison to a conventional GC-EI-MS system we can achieve lower detection levels by a factor of ten to hundred.

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Influence of ozone on VOCs in silicon lined air sampling canisters

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Abstract

In order to support the Sparkling Science Program, where an ambient air canister measurement campaign is planned, the performance of silicon lined canisters is investigated with an advanced SRI-ToF-MS. Although a special focus was directed towards testing different cleaning procedures, it is not possible to remove all impurities, especially light oxygenated organic compounds are problematic. In addition storage stabilities of reactive VOCs are evaluated. It turns out that in order to reach increased stability of reactive VOC in SilcoCans when sampling air an ozone scrubber is mandatory. This is achieved by preconditioning the canister with nitrogen monoxide.

Introduction

The emission of a variety of "pollutant" gases (e.g. nitrogen oxides (NO_x), volatile organic compounds (VOCs)) is caused by anthropogenic and biogenic sources. VOCs are essential components of tropospheric chemistry. Their oxidation, especially in urban areas, leads to the production of tropospheric ozone. Tropospheric ozone concentration has doubled over the last century and it is known to have adverse effects on human health, vegetation (e.g., crops) and materials. VOCs are precursor gases for secondary organic aerosols (SOA) and new particle formation, which leads to direct and indirect radiative forcing and therefore influence our climate.[1],[2],[3]

Various techniques have already been developed for the monitoring of VOCs in the environment. Real time analytical instruments allow the simultaneous sampling and analysis of ambient air and are therefore generally used to study the temporal variation of compounds. Gas Chromatography (GC) and Proton Transfer Reaction-Mass Spectrometry (PTR-MS) techniques have been successfully used during field campaigns for the measurement of a variety of VOCs. However, they require heavy and expensive equipment, and are therefore difficult to set up during field campaigns. [4]

As an alternative, offline measurement methods can be used, by sampling with canister or sorbent cartridges. The canister method collects air samples directly in evacuated stainless steel canisters. There are two common canister types on the market, which are electro polished SUMMA canisters or silicon lined canisters (SilcoCan). These passivation methods reduce the active sites of the surface and make them more inert. Numerous studies have evaluated storage stabilities of several VOCs in air canisters under various conditions.[5]

In this work two cleaning methods for SilcoCans, namely one with high ozonized, humidified purified air treatment and the other one with humidified nitrogen are investigated. The canisters are spiked with atmospherically relevant concentrations of a calibration standard gas mixture (Apel Riemer) including alkene, oxygenated, and aromatic hydrocarbon compounds. The storage stabilities under influence of ozone with representative atmospheric conditions (45% relative

humidity, 35° Celsius) are observed. An approach is taken to scrub ozone by preconditioning canisters with nitrogen oxide (NO).

Experimental Methods

Instruments

Canister analysis is carried out using an advanced SRI-ToF-MS, which is described in more detail elsewhere. [6] The E/N is 130 Td and the inlet line is a 40 cm long polyether ether ketone (PEEK) line, with an inner diameter of 1/32'', to gain the maximum canister measurement time of approximately 5 minutes to ensure constant drift pressure of 2.3 mbar. The filling setup is depicted in figure 1, where the ozone, calibration gas and NO concentrations are adjustable. During canister filling a total flow of 2.5 slpm is used. A PEEK capillary is placed before the canister valve as a flow reducer, to ensure that no laboratory air is sucked into the canister through the overflow. To measure a canister the capillary is removed and a direct connection via a 1/8'' Teflon line is established by the stopcock valve.

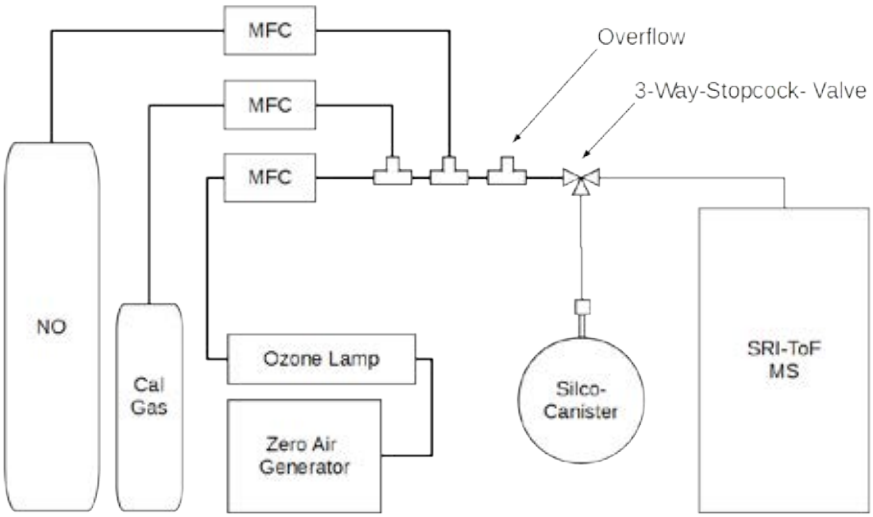

Figure 1: Schematic experimental canister filling and measuring setup. The filling is achieved if the 3 way stopcock valve is open in all directions and for the analysis of the canister the valve connects only the canister with the advanced SRI-ToF-MS.

Canister Cleaning Methods

The following two sections give a short description of the ozone cleaning method, which is based on the CLOUD chamber cleaning method and the nitrogen cleaning method, which is the recommended method by the distributor.

Ozone Cleaning Method

The canister valve is replaced by an inert T piece, where a 1/8'' Teflon line goes through to the SilcoCan. It is purged with humidified, ozonized zero air and it is heated up to 60° Celsius in a temperature controlled oven. The ozone concentration as well as the duration time is varied up to 500 ppb and up to 24 hours, respectively. After the cleaning cycle, the canister is evacuated and filled with zero air, which is analyzed to determine the background concertation.

Nitrogen Cleaning Method

The canister is evacuated to 1 mbar and afterwards filled up to 2 bar with humidified nitrogen. Pressurization, approximately for 20 minutes, will dilute the impurities and the moist air will hydrolyze them. The impurities are removed by re-evacuating the canister for 1 hour. This cleaning cycle is repeated up to 6 times and is always heated to 60° Celsius. Finally the canister is filled with zero air to evaluate the background concentration.

Results

Comparison of the analyzed zero air of both cleaning methods show no big differences. The measured canister backgrounds have significant impurities, which are dominated by light C1-C3 oxygenated organic compounds. However, when filling cans with ozonized zero air at relevant atmospheric concentrations (25–100 ppbv O_3), the ozone cleaned canisters show higher concentrations of oxygenated VOCs.

Primarily alpha pinene loss is observed, if the SilcoCan is filled with calibration standard gas mixture and ozonized zero air at concentrations of around 2 ppbv for each compound and 70 ppbv O_3. After 20 min a loss of 35% of the injected alpha pinene is measured, and after 40 min as well as 60 min a loss of 85% is measured. Due to the ozonized zero air, increased concentrations of previously mentioned oxygenated VOCs are observed. Alkene and aromatic hydrocarbon compounds concentrations remain unaffected.

One approach to stop the loss of alpha pinene is to precondition the canister with a sufficient NO concentration. The simulation with the master chemical mechanism program (MCM) suggests a NO concentration higher than the sum of O_3 and NO_2 concentration, which is in the range of 80 ppbv to 200 ppbv for ambient air [7]. If preconditioning the canister with NO is done, concentrations have to be up scaled due to wall losses. In the first attempt 80 ppmv and 8 ppmv NO is mixed to the gas flow, which is injected to the canister. The measurement result shows that the ozone reaction with alpha pinene is suppressed, but still an increase of the oxygenated VOCs is observed. The same result appears if NO concentrations (120, 30 and 20 ppmv) are injected to the canister before filling it with the ozonized calibration gas mixture.

Discussion

Good agreements are achieved with storage stabilities of alkenes and aromatic compounds presented in the literature, which are commonly measured by GC/MS systems [5]. The ozone cleaning method points out that O_3 is responsible for increasing concentrations of oxygenated VOCs, which are normally stable end products by ozonolysis reactions. This indicates that impurities are still present after various cleaning cycles with either nitrogen or ozonized zero air. Due to the increase of light C1-C3 oxygenated organic compounds during canister background measurements, which could come from chemical adsorption, it is not recommended to use these results, which is also supported by E. Hunter Daughtery et al. [4].

The measurement of the ozonized calibration gas mixture is in accordance to simulation results of (MCM). In the gas mixture the only alkenes are isoprene and alpha pinene and therefore mainly alpha pinene loss is observed due to its greater reaction constant with O_3 off $8.4*10^{17}$ $cm^3mol^{-1}s^{-1}$ [2]. Based on this result, it is important to use an ozone scrubber when filling the canister.

The first results of the NO method show a loss of isoprene, whereas alpha pinene stays constant. This is due to a significantly higher water concentration inside the canister because of the cleaning process. After changing the cleaning process so that the last two cycles are without humidification, all non-oxygenated organic compounds stay constant.

Acknowledgements

The authors would like to thank our working group for technical support and for helpful discussions. This project was financially supported by the Bundesministerium für Wissenschaft, Forschung und Wirtschaft (BMWFW) in the frame of the Sparkling Science AIR (SPA 05-080) project.

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Methanol emissions above and beneath an alpine meadow

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Abstract

Methanol exchange above and beneath a managed mountain grassland was investigated, in July and August 2015, using a PTR-Quad-MS. Additionally, PTR-TOF-MS measurements were conducted at the same field site, in July and August 2014, and methanol data from both summers were compared. Our goal was to investigate a possible coupling between methanol flux above the canopy and within the soil, to characterize if below-ground activities, of microbial or plant origin, add or remove methanol in the atmosphere.

Introduction

Approximately 40% of the Earth's land surface is covered by grassland, as for example tropical savannah, tundra, and managed temperate meadows [1]. Biogenic Volatile Organic Compounds (BVOCs) are emitted by a variety of plants [2], including grass species. Above grassland methanol is often the most abundant BVOC within the troposphere [3]. Approximately two-thirds of the atmospheric methanol is due to living plant emissions, for example through demethylation of pectin during plant cell wall expansion [4], which occurs both in the plant's green parts as well as in roots and rhizomes. Abiotic and biotic factors can influence methanol emissions. Abiotic factors, such as solar irradiance, precipitation, soil temperature, and soil moisture can affect methanol emissions either directly or indirectly through boosting the activity of soil microorganisms. Microorganisms, for example bacteria and fungi as part of their microbial metabolic pathways in the decomposition of plant litter [5], play an important role in methanol production. Methanol is not only emitted within soils, but can also be degraded or consumed by microorganisms as a carbon source [6]. Therefore, soils can act as either a source or a sink to the overall atmospheric methanol budget. Leaf level emissions of methanol within a controlled laboratory environment have been investigated [7], but scaling up these emissions to an ecosystem level leads to a discrepancy between the expected and measured. Methanol flux measurements over the course of 2 years were conducted above grassland in the Stubai Valley and correlated with biotic and abiotic factors, as PAR and air temperature, which accounted for about 47-70% of the methanol flux [8]. To close the gap between field and laboratory results studies of methanol exchange of leaf and soil with the atmosphere are needed.

So far, not many studies have been conducted to investigate the interaction between soils and atmosphere outside the laboratory.

Experimental Methods

Methanol emissions, above and beneath a managed mountain grassland were investigated, in July and August 2015. A PTR-Quad-MS was used to quantify methanol emissions of the meadow, close to Neustift (47°07' N, 11°19' E) in the Stubai Valley. Methanol was sampled above the grassland on a 3 m high tower and directly on the soil's surface, within the grass canopy (0 cm). Beneath the soil, the sampling depth was at -5 cm, within the grass roots, and at -20 cm. The soil layer is approximately 1 m deep. The first 0.1 cm consist of an organic layer, followed by 2 cm with an organic volume fraction of about 14%, the rest is sandy loam. About 80% of the roots are concentrated in the upper 13 cm, just 20% reach down to 50 cm [9].
Through a 10 m semipermeable line at each depth, a flow of about 700 ml zero air per minute transported below-ground BVOCs to our instrument. The sampling time was 30 min for flux measurements on the tower, and 8 min for 0, -5, and -20 cm respectively.
Additional to methanol concentrations, a vast amount of environmental data was recorded, for example air temperature and relative humidity, soil water content, soil temperature, precipitation, Photosynthetic Active Radiation (PAR), surface temperature, wind direction, and wind velocity. Additionally, a PTR-TOF-MS was employed, in July and August 2014, to quantify BVOC emissions at the same location and the same sampling depths at 0, -5, and -20 cm. We used these PTR-TOF-MS measurements to compare concentrations beneath the soil and within the grass canopy over the course of two summers.

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Eddy-covariance flux measurements in a forest using PTR-ToF-MS, PTR-Q-MS, FIS and other instrumentations

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Abstract

A field campaign in a small forest in Ispra, Italy, was performed by JRC-Ispra, Italy, together with University of Innsbruck, Austria, in 2013, using PTR-ToF-MS (Proton Transfer Reaction – Time-off-Flight - Mass Spectrometry), PTR-Q-MS (Proton Transfer Reaction – Quadrupole - Mass Spectrometry), FIS (Fast Isoprene Sensor) and several other instrumentations. One of the main goals of this investigation was to compare the three techniques and to compare the results that could be extracted from each of them, focusing on the VOC-flux values that could be measured, the number of VOC's identified/measured, and finally if any oxidation products from primary emitted VOC could be identified/measured. Another goal was to look at the uptake of ozone by forest ecosystems (ozone deposition), which is attributed to both stomatal and non-stomatal pathways. The collected data were used to calculate daily and seasonal changes in ozone fluxes and also partitioning of total ozone fluxes between stomatal and non-stomatal sinks. Finally, additional results are still being extracted from the campaign.

Introduction

The forest flux station 'IT-Isp' is situated in a small and unmanaged forest inside the JRC-Ispra, situated in North Italy (location: 45.8127 N, 8.6338 E). The forest consists mostly of deciduous trees (Quercus robur L. (80%), Alnus glutinosa L. (10%), Populus alba L. (5%) and Carpinus betulus L. (3%)) with an average leaf area index of 4.2 m2/m2. It consists of a self-standing 36 m high tower with two platforms at 18 m and 36 m located at 45.8127 N, 8.6340 E. Infrastructural details: The instrumentation is placed in a 6m x 3m container with air conditioning, approx. 15 kW electrical power, local TCP/IP network to tower top with gateway to JRC network, data acquisition PCs and Campbell data loggers. The monitoring station is operated since 2012.

Experimental Methods

The main analytical techniques mainly applied here were two types of PTR-MS and one FIS. The PTR-MS technique for on-line detection of VOCs, which was developed at the University of Innsbruck, Austria, has been thoroughly reviewed elsewhere, see e.g. ref. [1,2]. Examples of the use of PTR-ToF-MS and PTR-Q-MS can e.g. be found in ref. [3,4,5]. The technique FIS and examples of the use can e.g. be found in ref. [6]. In addition, several other measurements were also performed during the campaign. *On tower top of tower measurements /sampling on top of tower:* NOx concentrations (Thermo); CO_2 & H_2O fluxes: Licor 7200 (and also the CO_2/H_2O vertical profile); O_3 fluxes; Sonic anemometer: Gill HS-100 (highly accurate vertical flow

analysis, HS-100 monitors wind speeds of 0-45 m/s and has an update rate of 100 Hz); temperature, RH, air pressure and precipitation; net radiation (measures the difference between downward/incoming and upward/outgoing radiation from Earth); Photosynthetically Active Radiation (PAR), total, diffuse (measures the spectral range (wave band) of solar radiation from 400 to 700 nanometers that photosynthetic organisms are able to use in the process of photosynthesis); fraction of Absorbed PAR, FaPAR (the Photosynthetically Active Radiation spectral region that is absorbed by a photosynthetic organism). *On the ground close to tower measurements:* Soil heat flux; soil temperature and water content profiles; ground water level; NO/NO_2 soil fluxes: automated dynamic chamber system; hemispheric photography for LAI (used to calculate solar radiation transmitted through (or intercepted by) plant canopies, as well as to estimate aspects of canopy structure such as Leaf Area Index (LAI)); litter collection (biomass) in the forest; soil respiration (CO_2 emissions from the soil). A nearby EMEP/GAW station (within 200 m) measures many other pollutants/parameters (for a complete list of the additional measurements, see ref. [7]).

Results and Discussion

Findings with PTR-ToF-MS / PTR-Q-MS / FIS:

Figure 1 shows that isoprene fluxes could easily be measured by PTR-ToF-MS. In addition, several other VOC's were identified/measured in high enough concentrations, so that the fluxes could be measured (protonated masses): 31.017 CH3O Formaldehyde; 33.034 CH5O Methanol; 42.033 C2H4N Acetonitrile; 43.017 C2H3O Acetic Acid fragment + interferences; 43.051 C3H7 Typical fragment; 45.034 C2H5O Acetaldehyde; 47.052 C2H7O Ethanol; 57.049 C2H5N2 C3H4O Instrument background + e.g. acrolein; 59.050 C3H7O Acetone / propanal; 61.030 C2H5O2 Acetic acid / methyl formate; 63.014 C2H6S DMS; 69.069 C5H9 Isoprene; 71.054 C4H7O MVK / MACR / croton-aldehyde; 73.034 C3H5O2 Propionic acid e.g.; 73.071 C4H9O Methyl-ethyl-ketone / butanal; 79.051 C6H7 Benzene; 87.056 C5H10O Unknown;; 89.055 C4H9O2 Unknown; 93.068 C7H9 Toluene; 107.078 C8H11 C8-alkylbenzenes; 121.097 C9H13 C9-alkylbenzenes; 137.145 C10H17 Monoterpene. Finally it should be mentioned, that several oxidation products from primary emitted VOC could clearly be identified, e.g. MVK/MeCr from isoprene. Figure 1 also shows that isoprene fluxes could also easily be measured with PTR-Q-MS. In addition, several other VOC's were identified/measured: Potential interesting masses identified by PTR-Q-MS with a significant count/value during the summer campaign (protonated masses): mass 33 (methanol), mass 42 (acetonitrile), mass 43 (acetic acid and fragments/interferences), mass 45 (acetaldehyde), mass 59 (acetone), mass 61 (acetic-acid/methyl-formate), mass 73 (propionic-acid/methyl-ethyl-ketone/butanal), mass 87 (unknown), mass 89 (unknown), mass 93 (toluene), mass 107 (C8-alkyle-benzene), mass 137 (monoterpenes). Finally, Figure 1 also shows that isoprene fluxes could also easily be measured with FIS. FIS is only measuring isoprene.

Some overall findings from this section:

During the summer campaign in 2013, isoprene concentrations were measured up to 16 ppb. In addition, isoprene fluxes up to 85 nmole m^{-2} s^{-1} were measured. During a winter campaign in 2013 in the same forest, no isoprene fluxes were observed (measured with PTR-Q-MS). It should be mentioned, that isoprene-flux-measurements were possible with all 3 instruments, but up to 30 % differences were observed (see Figure 1). Table 1 show that about 20-30 VOC's were clearly identified by PTR-ToF-MS and fluxes can be achieved/measured for most of them. Using PTR-Q-MS much less VOC's could be clearly identified and it was only possible to achieve fluxes (from biogenic compounds) for isoprene. It could also be seen in Table 1 that with PTR-ToF-MS several oxidation products from primary emitted VOC could clearly be identified, e.g. MVK/MeCr from isoprene.

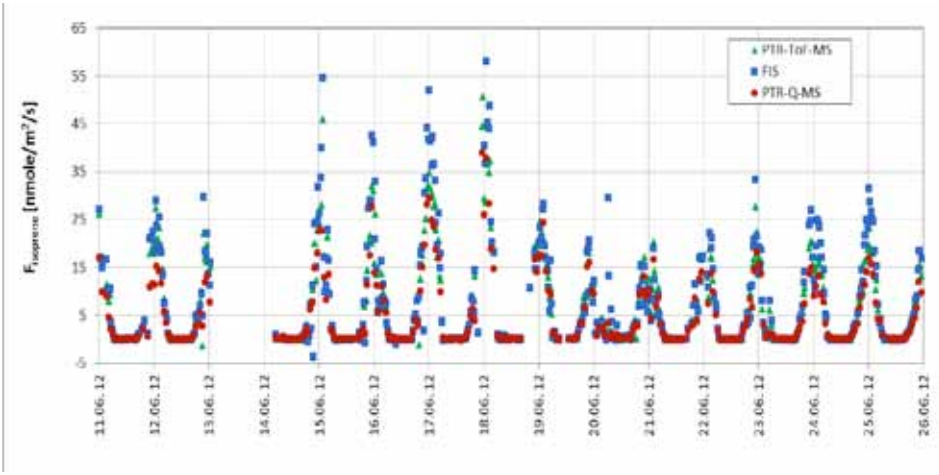

Figure 1: Isoprene fluxes measured with all 3 instruments during summer 2013. Explanation to x-axis: E.g. "18.06.12" stands for 18.06.2013 at 12:00 GMT/UTC.

Uptake of ozone (ozone deposition):

Figure 2 shows the daily averages of ozone concentrations/fluxes during summer 2013. High levels of ozone concentration were observed (up to 118 ppb as a peak value with a 30 min. average) during the measurement period of the campaign, and with ozone fluxes reaching up to -40 nmol m-2 s-1 (peak value with a 30 min. average). Total ozone fluxes varied diurnally with maximum values at midday and minimum values close to zero at night (Fig. 3). Stomatal ozone fluxes resulted in minor part of the total ozone flux over the studied forest ecosystem (23%). The measurements have shown that the ozone deposition is for the major part due to the non-stomatal sinks, however the ratio of the stomatal to total ozone flux was subject to seasonal and diurnal changes.

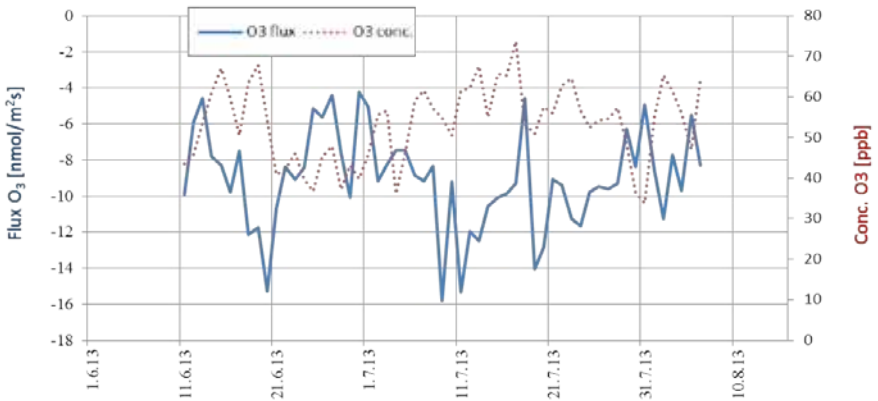

Figure 2: Daily averages of ozone fluxes and concentrations during summer 2013.

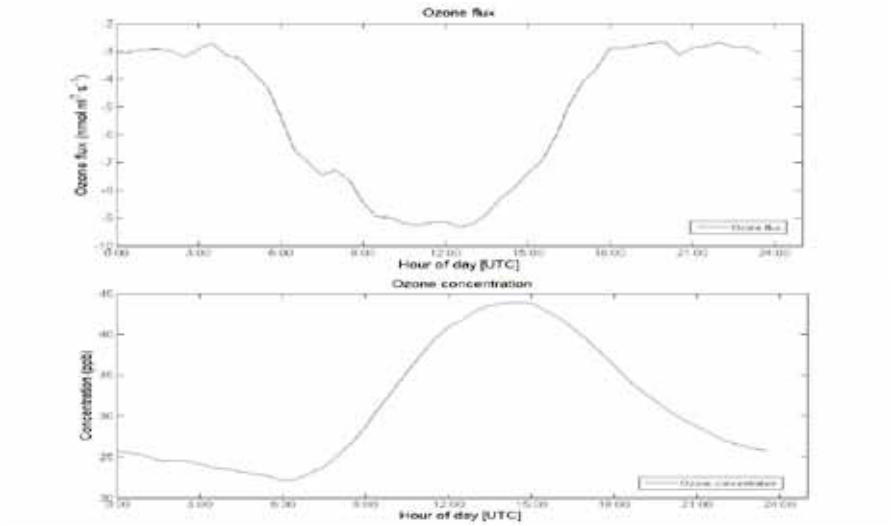

Figure 3: Diurnal cycle of ozone flux and ozone concentration (average summer 2013).

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fastGC PTR-TOFMS: Extremely High Sensitivity and Real-Time Analysis

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Abstract

We introduce a novel fastGC add-on for PTR-TOFMS instruments. The fastGC column is integrated into a sophisticated inlet system, which allows for rapid, software controlled switching between the established real-time direct injection mode and chromatographic separation of isomers within about one minute. We demonstrate the separation power of this novel setup by identifying six different monoterpenes in a blend, which in traditional PTR-TOFMS all appear on *m/z* 137.13 and are therefore not distinguishable.

So far a user had to decide between the gold standard selectivity of GC-MS and the real-time quantification capability of PTR-TOFMS. With fastGC PTR-TOFMS the unique advantages of both technologies are merged, which will be of vital importance for all fields which are dealing with complex matrices and various isomers (e.g. plant biology, food sciences, etc.).

Introduction

Although Proton Transfer Reaction – Time-Of-Flight – Mass Spectrometry (PTR-TOFMS) has unique advantages, such as extremely high sensitivity and online quantification capability, substance identification and separation is limited to information about the exact *m/z*. Thus, particularly isobaric compounds, which by definition possess identical masses, cannot be distinguished. To overcome this problem, already in the early 2000's the coupling of Gas Chromatography (GC) and PTR-MS has been suggested. However, this came at the cost of losing real-time capability. Recently, several near real-time methods to increase selectivity have been introduced, such as using information on E/N (reduced electric field) dependence [2], switching reagent ions [3] or a combination of both [4]. These methods are fast and easy to apply, but cannot reach the gold standard selectivity provided by GC. An important step to compliment the speed of PTR-MS with the selectivity of GC was the introduction of a multi-capillary-column PTR-MS system in 2013 [5]. Here we present the next development stage of this approach, namely a PTR-TOFMS instrument with an integrated fastGC inlet system. This fastGC PTR-TOFMS instrument combines the outstanding advantages of both technologies, real-time quantification in direct injection mode and extremely high selectivity in fastGC mode.

Experimental Methods

All IONICON PTR-TOFMS instruments can be equipped with the fastGC addons, from the compact and cost-effective PTR-TOF 1000 up to the ultra sensitive PTR-QiTOF with up to 4500 cps/ppbv. For the studies presented here, we utilized a PTR-TOF 8000. The installed nonpolar fastGC column has a length of 6 m and can be resistively heated up to 350°C with a maximum heating rate of about 30°C/s. Nitrogen is used as a carrier and make-up gas. The inlet system is equipped with a set of electronically controlled valves that allow for software controlled switching between the established direct injection and the novel fastGC mode. In fastGC mode, a sample loop gets filled with the gas entering the instrument's inlet line. Subsequently, the content of the sample loop is injected into the fastGC column. Typically, spectral runs are completed within about 1 min, which is when the instrument can be switched back to direct injection mode or is ready for the next spectral run.

Results

For a first test of the fastGC PTR-TOF 8000 we introduced the headspace above minute amounts of Manuka tea and spruce resin into the instrument in direct injection mode and focused our attention on the detection of protonated monoterpenes ($C_{10}H_{16}.H^+$; *m/z* 137.13). As expected, for both samples an abundant mass spectral peak at the corresponding *m/z* appeared, indicating the presence of at least one monoterpene (upper graph in Figure 1). However, in direct injection mode it is impossible to identify the various isomers that could contribute to this mass spectral peak and only the sum concentration can be quantified. After switching the instrument to fastGC mode and performing a spectral run within less than a minute, the situation changes drastically. In the lower graphs of Figure 1 it can be seen that indeed several monoterpenes are present in both samples, at considerably different relative concentrations. Please note that the x-axis in the lower graphs does not represent m/z anymore, but the retention time in the fastGC column.

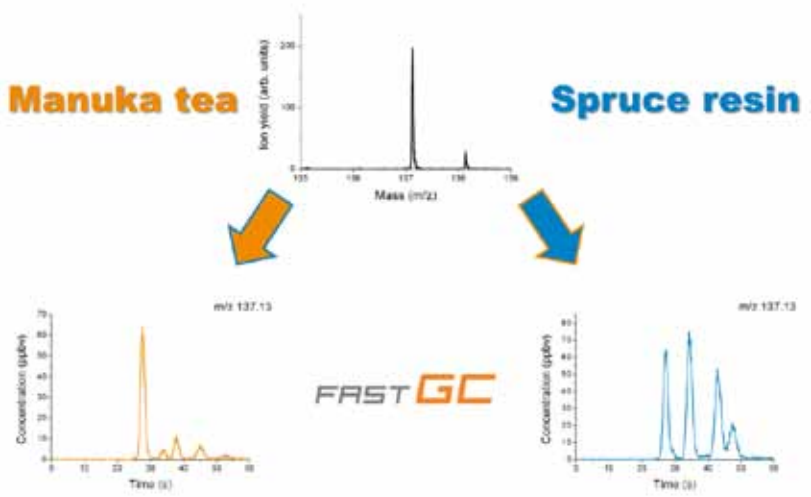

Figure 1: (upper) Mass spectrum around m/z 137 (monoterpenes) for both, manuka tea and spruce resin; (lower) fastGC chromatograms of m/z 137 for manuka tea (left) and spruce resin (right).

After this first proof-of-concept we decided to perform an extensive study on the separability of monoterpenes. We purchased six of the most relevant monoterpenes in plant and biological research, α-pinene, camphene, β-pinene, myrcene, 3-carene and R-limonene, and investigated their retention times. Afterwards, we created a mixture of all six monotepenes to check if we can distinguish them in a blend, which would be crucial for a real life sample. The result is displayed in Figure 2. Within less than 70 s all isomers are clearly separated in the chromatogram and can be quantified independently. As a next step we collected some plant samples from the suburban area of Innsbruck and investigated them with the fastGC PTR-TOF 8000. As a result we found that all of the main peaks in the GC spectra could be assigned to one of the six investigated standards [7], although not all monoterpenes were present in all samples and the relative abundances varied.

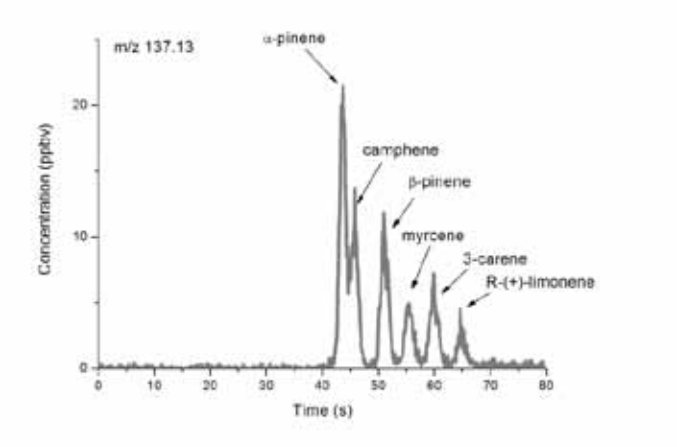

Figure 2: fastGC chromatogram of m/z 137 for a monoterpene standard mixture.

Discussion

We show that the fastGC inlet system is a valuable add-on for PTR-TOFMS instruments. Not only that spectral runs can be performed very rapidly, usually within about 1 min, but the real-time quantification capability is fully preserved, when the instrument is being operated in direct injection mode. Thus, depending on the dynamics within the isomers, by continuously measuring a sample in direct injection mode and switching to the fastGC mode e.g. once every 10 min, the unique advantages of PTR-TOFMS and GC-MS can be combined.

The fastGC mode offers an additional advantage for PTR-MS measurements, namely the separation of compounds with sample concentrations so high, that the direct inlet PTR-MS analysis would be derogated. Ethanol is such a compound of high abundance in wine head space samples, which would normally require a dilution of the sample. In fastGC mode, the ethanol elutes the column early and the analysis of other compounds at later times thus remains unaffected [7].

Acknowledgements

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The effect of pine weevils on VOC emissions from Scots pine

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Abstract

Introduction

Plants emit a large variety of volatile organic compounds (VOCs) into the atmosphere [1]. Environmental factors, both biotic and abiotic, impact the emission rates and chemical composition of VOCs released from vegetation [2]. Emissions of plant volatiles depend strongly on physiological conditions, and changes in these conditions could rapidly induce emissions or alter emission dynamics [3]. One class of VOCs, called herbivore induced plant volatiles (HIPVs), are produced by plants in response to herbivory. The primary function of these volatiles is to provide plants with direct and indirect herbivore protection against herbivores by repelling herbivores from attack, deterring feeding, or attracting natural enemies of actively feeding herbivores [2]. Many VOCs are produced and emitted by intact plants, called constitutive emissions. In contrast, HIPVs refer to the specific VOCs that are produced after herbivore damage, some of which may be constitutively emitted in lower quantities prior to attack. HIPVs can be classified into three groups based on their chemical structure [3]. The most commonly emitted compounds are the terpenoids. The most basic terpenoid is isoprene, a five-carbon compound with two double bonds. The other terpenoids are constructed from five-carbon isoprene units and include monoterpenes (two isoprene units, C10) and sesquiterpenes (three isoprene units, C15). There are some other variations in this terpenoid structure including homoterpenes (C11 and C16 compounds) and oxygenated versions of all these structures. The second group of compounds are the Green Leaf Volatiles (GLVs). These are six-carbon alcohols, aldehydes, and acetates synthesized from damaged cell membranes. The third group of compounds contain an aromatic ring and include methyl salicylate and indole.

HIPV emission rates have rarely been measured using proton transfer reaction-mass spectrometry (PTR-MS)—an analytical technique that enables rapid and continuous monitoring of plant emissions before and after herbivore damage. The benefits of this technique have been demonstrated in two previous studies. For example, Maja *et al*. studied the responses of silver birch VOC emissions during herbivore exposure [4], and Schaub *et al.* investigated the release of HIPVs from branches of hybrid aspen during infestation of larvae [5]. Results from these studies highlighted the added benefits of better temporal resolution of HIPV emissions measurements relative to previous techniques. Maja *et al.* noticed that the temporal dynamics of GLV emissions were strongly dependent on the feeding activity of herbivores [4]. Similar observation was made by Schaub *et al.* who observed that herbivore feeding induced GLV emissions to occur at uneven

time intervals [5]. These insights into GLV emission dynamics would be missed with lower time-resolution sampling techniques. However, the overall duration of these experiments was short (≤ 2 days), and more long-term measurements of plant responses to herbivory would be beneficial for improving current estimates of HIPV emission rates and temporal dynamics.

The aim of this study was to measure and quantitatively describe HIPV emission dynamics from Scots pine (*Pinus sylvestris*) after Scots pine saplings were exposed to bark borer herbivory. VOC emissions were monitored continuously before and after treatment by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS). To supplement the PTR-ToF-MS measurements, cartridge samples were collected and analyzed offline via thermodesorption-gas chromatography-mass spectrometry (TD-GC-MS). PTR-ToF-MS measurements were used to monitor the rapid changes of different classes of VOCs, and TD-GC-MS measurements were used to calculate emission rates of individual monoterpene and sesquiterpene isomers. To investigate bark borer impacts on HIPV emission dynamics, a time-series of monoterpene and sesquiterpene emission rates analyzed from PTR-ToF-MS data will be shown. Furthermore, emission profiles from GC analysis will be presented to identify specific compounds that were induced by herbivore feeding. A sample of the PTR-ToF-MS fragmentation patterns of specific terpenoid isomers identified from GC samples will also be shown.

Experimental Methods

To study HIPV emission dynamics, 7-year old Scots pine saplings were exposed to four bark feeding-pine weevils (*Hylobius abietis*) in the laboratory for two days. Four sets of experiments were conducted that involved two trees each, one control tree and one treatment tree for each experiment. Each tree was placed inside a 40 x 100 cm Teflon® bags, herein referred to as the plant enclosure. Purified air was continuously flushed through the plant enclosures at a flow rate of 3.0-4.0 l min^{-1}, and additional PAR light for the trees was provided from 6 A.M. to 6 P.M to simulate a natural diel cycle.

The emissions from both trees were continuously measured by PTR-ToF-MS (PTR-TOF 8000, Ionicon Analytik, Austria) using an automated valve switching system. PTR-ToF-MS was operated under controlled conditions (2.3 mbar drift tube pressure, 600 V drift tube voltage, 130 Td E/N, and 60 °C temperature), and sample air was introduced into PTR drift tube via a 1.5 m length of heated (50 °C) Teflon® tubing, and a 1.5 m length of heated (60 °C) PEEK tubing at a flow rate of 100 ml min^{-1}. PTR-ToF-MS was calibrated for transmission using the calibration gas standard containing 8 aromatic compounds with a mixing ratios of ~100 ppbv (BOC, United Kingdom). In addition to PTR-ToF-MS, duplicate cartridge samples (Tenax TA/Carbograph 5TD material, MARKES international, United Kingdom) were collected from each plant enclosure twice per day, once in the morning and once in the afternoon. The sampling time was 15-20 minutes for each sample with an air flow of ~200 ml min^{-1} through the sample tube. The trapped compounds were desorbed from the sample tube with a thermal desorption unit (TD, Perkin-Elmer ATD 400 Automatic Thermal Desorption system, USA) and analyzed by GC-MS (Hewlett Packard, GC 6890, MSD 5973, USA). The results were used to 1) quantify emission rates of individual compounds and 2) to account for fragmentation patterns and improve quantitation with the PTR-ToF-MS.

Prior to herbivore exposure, plants were acclimated to laboratory conditions for a minimum of 24 hours in order to reach stable baseline VOC emission rates. In each experiment, the bark of treatment plant was exposed to four weevils for two days. Herbivore exposure was conducted by placing the herbivores inside a mesh enclosure that was attached to the tree trunk. Empty mesh

enclosures were attached to the bark of control trees to account for any change in emissions due to handling the plant during treatment application. After pine weevils were removed, post-treatment monitoring was continued for 3-14 days to see how long the emissions remained elevated. The post-treatment monitoring range represents different durations for different experiments.

Results and Discussion

Four sets of experiments were performed to study the effect of pine weevils on VOC emissions from Scots pine. These results showed that the most dramatic change in emissions caused by pine weevils was observed for monoterpene emissions. Other compounds, like sesquiterpenes, had elevated emissions after treatment as well, but to a lesser extent than monoterpenes. Figure 1 shows the change in monoterpene concentration in the plant enclosure due to pine weevil feeding during one of the experiments. Pine weevil feeding led to at least a 50-fold increase in monoterpene concentration in every experiment. As highlighted in Figure 1, the damage caused by pine weevils affected monoterpene emissions for multiple days after the treatment period; monoterpene concentrations stayed elevated above pre-treatment values and control tree values for more than 5 days after pine weevils were removed.

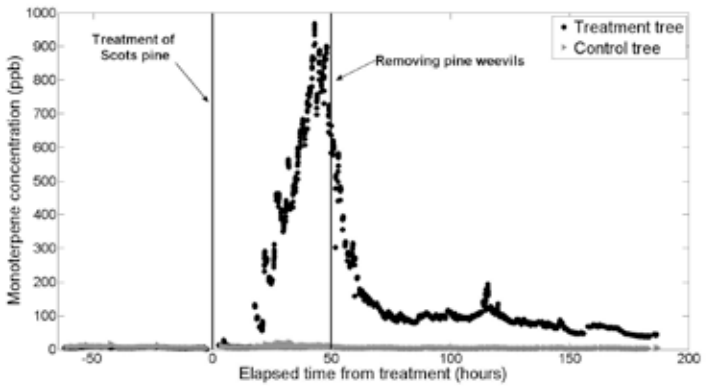

Figure 1: The change in concentration of monoterpenes (m/z 81 + m/z 137) due to pine weevil feeding, monitored by PTR-ToF-MS. Black circles are data points measured from treated Scots pine, and grey triangles are the data points measured from control Scots pine. At time 0 the treatment was started.

For preliminary analysis, monoterpene concentration has been calculated from PTR-ToF-MS data by summing up the concentration of its compound peak (m/z 137) and the concentration of its main fragment peak (m/z 81). However, GC-MS analysis showed that in different experiments different monoterpenes were emitted by Scots pines with different quantities, and it is known that some monoterpenes are fragmenting more than others depending on PTR settings [6]. For this reason, we will develop a new calibration system for PTR-ToF-MS, where the fragmentation of different monoterpenes and sesquiterpenes can be measured. These fragmentation patterns of monoterpenes and sesquiterpenes inside PTR-ToF-MS will be used to more accurately calculate

monoterpene and sesquiterpene emissions by Scots pines. Expected fragmentation patterns in the PTR-ToF-MS will be determined from the specific chemical profile measured from the GC-MS cartridge sample analysis.

The preliminary results demonstrate that pine weevils had a large impact on VOC emissions emitted by Scots pine, and especially for emissions of monoterpenes. The results also illustrate that it took days before the tree recovered from the herbivore damage after pine weevils were removed. This highlights the importance of conducting experiments with a long enough post-treatment duration to determine plant recovery time. These early results clearly show that herbivores have a significant effect on plant VOC emissions, and suggest that herbivore outbreaks could impact climate by influencing the formation of new secondary organic aerosol precursors emitted by vegetation.

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Acknowledgments

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Vehicle dependent characterization of the VOC emissions from mobile sources: Aircraft, ships and motor vehicles

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Abstract

Volatile organic compound (VOC) emissions from mobile sources, including aircraft, ships and motor vehicles, are a significant contributor to global anthropogenic VOC emission. Such emissions are particularly important to urban and regional atmospheric chemistry 1. Characterization of mobile VOC emissions is particularly important to understand their impact on health, air quality and climate. In this work, VOC emissions of about 200 compounds from aircraft engines, ship engines and motor vehicles were measured by a high resolution proton-transfer-reaction mass spectrometer (PTR-ToF-MS). The engine operation mode dependent emission factors (EF) (figure 1) and the exhaust chemical compositions (figure 2) were determined. The dependence of VOC EFs and the exhaust chemical composition (e.g. fraction of aromatics, aliphatics, carbonyls, acids) on engine type and operation mode and on the fuel type will be discussed.

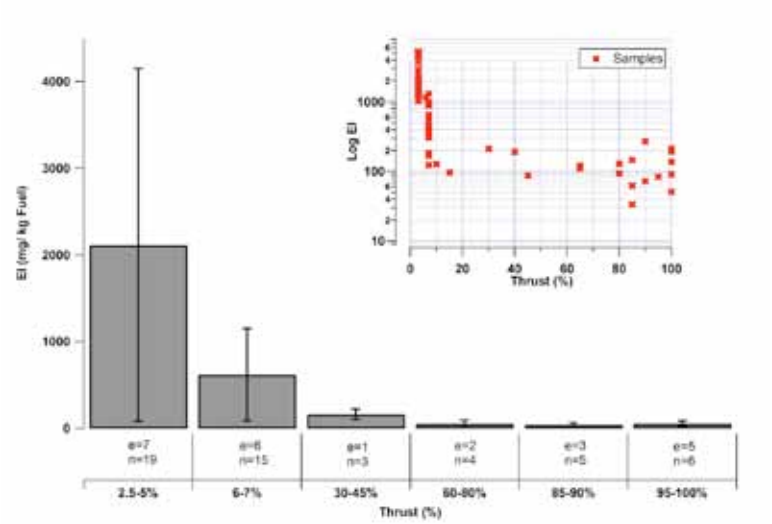

Figure 1 Bottom: Mean total VOC emission index (mg/kg fuel) as a function of thrust. "e" is the number of aircraft jet engines tested and "n" is the total number of tests at the designated thrust level. Top-right: VOC EIs for individual measurements as a function of thrust

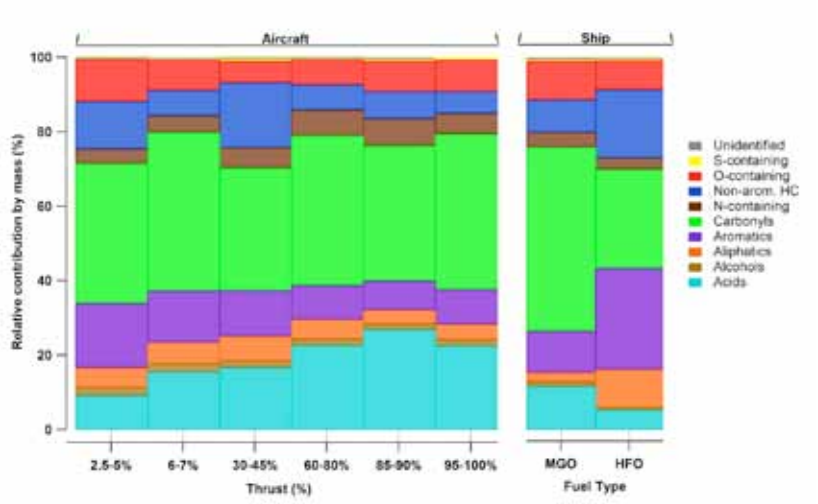

Figure 2 Relative contribution (%) from the functional groups are shown as a function of aircraft jet engine thrust (left). Functional group mass fractions for two typical fuel type used for ships are on right: Marine Gas Oil (MGO) and Heavy Fuel Oil (HFO)

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Characterization of gas phase cooking emissions using advanced online and offline techniques

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Abstract

Cooking processes are one of the main sources of volatile organic compounds (VOCs) and particulate matter (PM) in non-smoking households and restaurants. These emissions can have adverse effects on public health. For example, Ko et al (2000) reported a direct link between cooking emissions and lung cancer in non-smoking Chinese women. However the chemical and physical properties of gas and particle phase emissions from cooking are mostly unknown.

The approach adopted for our study involved laboratory quantification of VOC emission factors from the main cooking processes. Primary emissions from deep frying, vegetable boiling, vegetable frying and meat cooking using different oils, meats and vegetables were analyzed under controlled conditions after ~100 times dilution. A high resolution proton transfer time-of-flight mass spectrometer (PTR-ToF-MS) and a two dimensional gas chromatography time-of-flight mass spectrometer (GC×GC-ToF-MS) were used to quantify the VOC emissions and to investigate their chemical composition.

The VOC emissions from frying consisted mainly of saturated and unsaturated aldehydes, formed as a result of fragmentation of the fatty acids present in the vegetable cooking oils (Fig.1). Vegetable cooking was associated with significant VOC emissions composed mainly of alcohols and considerable amounts of sulfur species (Fig.2). Gas phase composition as well as emission factors from all cooking styles and products will be presented.

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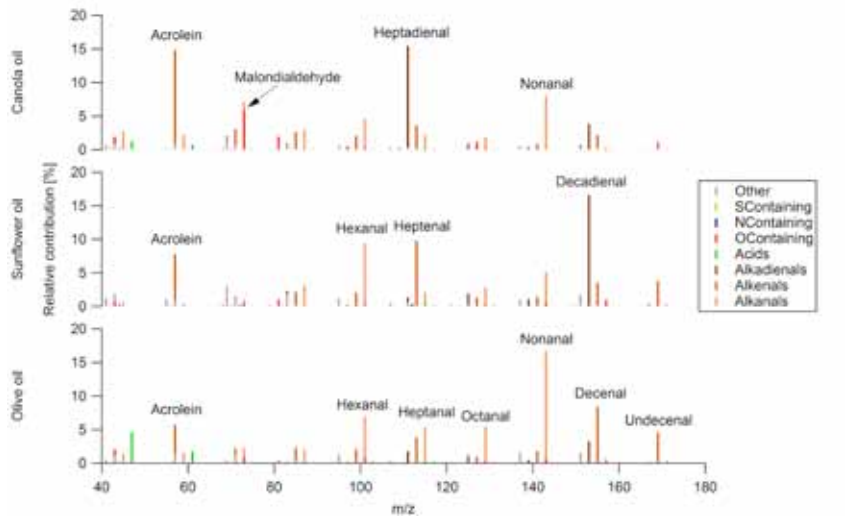

Figure 1: Mass spectra of gas phase emissions from three different cooking oils, measured using the PTR-ToF-MS..

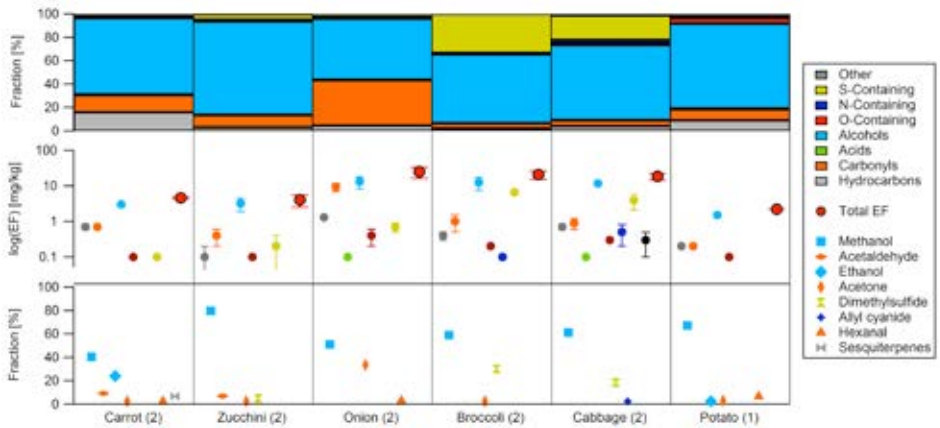

Figure 2: Chemical composition, emission factors and most abundant compounds for the boiling of six different vegetables

References

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Impact of blueberry consumption on volatile organic compounds in breath and saliva: a pilot study

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Abstract

The aim of this study was to test the potential impact of blueberry polyphenol on the volatile organic compounds (VOCs) in breath and saliva via Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) analysis. Fourteen healthy volunteers were recruited (7 M / 7 F; mean age 31.2 years, range 18 – 52 years, and with body mass index BMI 22.5 ± 2.0 kg/m^2), in this cross-over, single blind and randomized controlled study. Each subject consumed 200 g of fresh blueberry per day for one intervention week and had habitual diet (without blueberry) for the control week. Real-time breath analysis was performed on fasting subjects using a buffered end-tidal on-line sampler directly coupled to a PTR-ToF-MS, while collected saliva was analysed later with automated injection on PTR-ToF-MS. Overall, the volatile profile in both breath and saliva are very similar between intervention and control groups. Nonetheless, a few differences for some VOCs were found in both saliva and breath.

Keywords: VOCs, saliva, breath, indole, microbiota

Introduction

The relationship between breath composition and health has been known for many centuries. The metabolomics research focused on volatile organic compounds (VOCs) in breath, feces, urine, blood, saliva and skin is strongly emerging due to its non-invasive approach and potentiality to find early disease biomarker (Kusano M. et al, 2013; De Lacy CB. et al, 2014). Due to the high sensitivity for VOC measurements, a fast response time and direct analysis without sample pre-treatment, Proton Transfer Reaction – Mass Spectrometry (PTR-ToF-MS) has been repeatedly applied for both single and real-time measurements of VOCs in breath (Herbig, J. et al, 2008).

Few studies were performed to evaluate the correlation of VOCs between breath and saliva upon dietary interventions, which potentially influence the gut microbiota or directly impact the metabolism, thus influencing the breath composition (Ajibola, O. A. et al, 2013). Some human studies showed that polyphenols from fresh blueberries physiologically act as dietary fibre, being fermented by gut microbiota.

In this study it was hypothesized that blueberries might influence the intestinal microbiota and trigger production of new gut- or metabolism-derived VOCs that might change composition of VOCs in breath and saliva. Thus a randomized cross-over intervention trial was carried out and the analysis of VOCs in both breath and saliva by means of PTR-ToF-MS was performed.

Experimental Methods

Subjects

The recruitment was performed among the students and researchers of the Fondazione Edmund Mach (FEM, San Michele All'Adige, Trento, Italy). The selected subjects were healthy, without undergoing any medication or drug therapy. They were advised not to vary their physical activity during all the period of the study, and to avoid sport activity the day before the experimental days.

Study design

The design and protocols of this study were approved by the Ethics Committee of University of Naples "Federico II". The study had a cross-over, single blind, randomized design. Once enrolled in the study participants were asked to reduce polyphenols as much as possible in their diets for one week (baseline, BL). Afterwards, subjects were randomized for the intervention (INT) or control (CT) week and they switched on the other arm after one week. During the intervention week subjects included in their diets 200g/day of fresh blueberries. During the control week subjects were advised to keep their own diets as in baseline week.

At the end of each week fasting participants reached the research center for the analysis of VOCs in the breath, and for collection of saliva samples and dietary data.

Breath VOCs analysis by PTR-ToF-MS

Real time breath analysis was performed using a buffered end-tidal (BET) on-line sampler (Jens Herbig et al, 2008) coupled to a Proton Transfer Reaction Time-of-Flight Mass Spectrometer 8000 (PTR-ToF-MS 8000, Ionicon Analytik GmbH, Innsbruck, Austria). Every subject was required to sit in front of the interface and to breathe normally room air. After a short time, the subject was given a single exhalation in a disposable mouthpiece (with a sputum trap), which was connected to the BET system. Fractions of exhaled gas, collected through the BET system, were drawn directly to the drift tube of the PTR-ToF-MS as an on-line detection, recording system of the volatile organic compound spectra. Instrumental conditions for the proton transfer reaction were the following: Inlet temperature 110 °C, drift voltage 500 V, drift temperature 80 °C and drift pressure 2.4 mbar resulting in an E/N value of 120 Townsend (1Td = 10-17 cm^2 V^{-1} s^{-1}). Sampling was performed with a flow rate of 40 sccm. The mass resolution ($m/\Delta m$) was at least 3500 (Aprea et al., 2012). Considering the fragmentation of the VOCs, as well as effects of multi precursor ions, $H3O^+$ mode was applied in all tests, and these data were used for further analysis.

Saliva VOCs analysis by PTR-ToF-MS

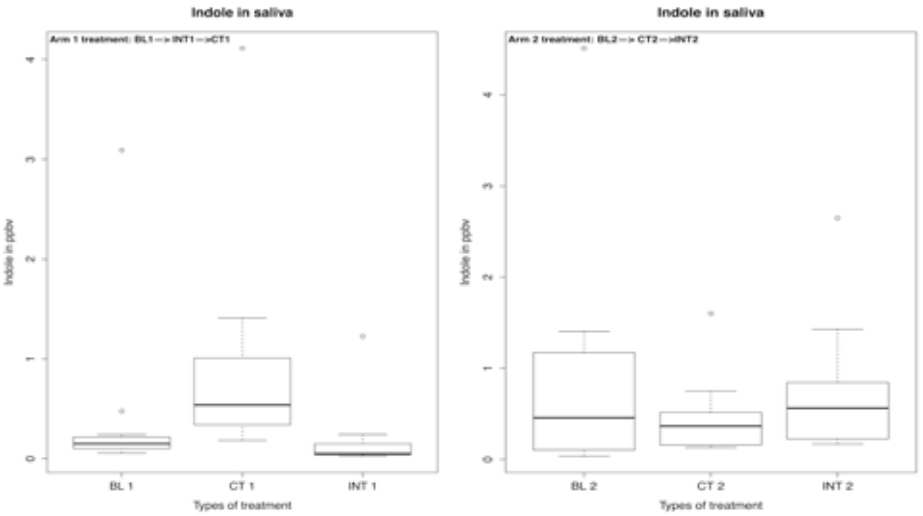
Saliva VOCs were immediately quantified by HeadSpace PTR-ToF-MS whereas the rest of each sample (~1 mL) was immediately frozen. Part of that was re-analysed by HeadSpace PTR-ToF-MS after 6 hours of freezing and part was stored until HeadSpace GC-MS for further VOCs identification which is not discussed here. Injections were performed by using a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany). Its syringe was connected to the inlet of PTR-ToF-MS together with the clean air generated by a gas calibration unit (GCU, Ionicon Analytik GmbH, Innsbruck, Austria), which was used as zero air generator. Test cycle for each vial consisted of flushing the headspace of the sample with clean air for 1 min at 43 sccm, then incubation for 30 min at 37 °C and finally measurement. Analysis order was randomised to avoid possible systematic memory effects. The sample headspace was measured by direct injection into the PTR-ToF-MS drift tube via a heated (110 °C) peek inlet. Each sample was measured for 30 s, at an acquisition rate of one spectrum per second.

Data analysis

Spectra data acquisition was performed through the software TOF-DAQ (Tofwerk AG, Switzerland), with a mass range of 10 – 400 Th and stored in format of HDF5 for data storage for further data analysis. Signal distortions derived from detector dead time were corrected before mass calibration, peak detection and area extraction were performed through a cumulative peak fitting, as in the procedure described by Cappellin et al. (2011). Internal calibration was based on two peaks: 1) m/z = 21.0221 ($H_3{}^{18}O^+$) and 2) m/z = 29.9974 (NO^+), which were always present in PTR-MS spectra.

Results

The breath VOC dataset consisted of 724 mass peaks and the saliva VOC dataset generated 574 mass peaks. Data were filtered by comparing blank and human specimens by Wilcoxon test ($p<0.05$). A second filtering was performed considering VOCs of intervention and control group in saliva and in breath by Wilcoxon. Upon Bonferroni correction's post hoc comparison test, with respect to its trace level p-value, no significant differences were found among pre-filtered dataset. Setting significance at $p=0.05$, 22 mass peaks of saliva VOCs and 39 mass peaks of breath were finally selected. Among the selected peaks, the one with ms118.0683 ($p=0.036$) was identified as indole (confirmed by GC-MS).



(a) *(b)*

Figure 1: Boxplots of salivary indole. (a) in arm 1 group whose treatment order was INT and CT. (b) in arm 2 group whose treatment order was CT and INT.

Discussion

Data analysis allowed us to extract from a huge amount of data some peaks that might be influenced by the dietary treatments applied in this study. Among these peaks indole was identified. Indole was previously reported in both mouth air and saliva from both healthy subjects

and subjects with severe periodontitis (Kostelc, J. G., *et al*., 1980). Moreover, indole was known as a bacteria metabolite produced from the tryptophan and showing ability to modulate the secretion of glucagon-like peptide-1 (GLP-1) from immortalized and primary mouse colonic L cells. Thus indole is a signaling molecule by which gut microbiota communicate with L cells and influence host metabolism (Chimerel C., *et al*., 2014). Previous studies showed that blueberry polyphenols (proanthocyanidins) had prebiotic effect and others demonstrated that a prebiotic effect could be associated with increased GLP-1 (Neyrinck, A. M., et al, 2013).

In this study mean variation of salivary indole between INT and CT week was dependent from the treatment order (Figure 1). This finding might be due to individual dietary habits as well as to the composition of individual gut microbiota at baseline that might have affected the individual susceptibility to a one-week dietary treatment.

The protocol adopted in the present study did not allow us to evaluate the baseline composition and the variations of the gut microbiota or the gut peptides over the study. However, finding of indole is worth of notice and further studies with longer intervention time are warranted to demonstrate in humans the link of breath and saliva indole with gut microbiota, metabolism of polyphenols and individual health status.

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Chemical signatures of freshly distilled French brandies according to their origin and comparison with sensory data

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Abstract

Sixteen French brandies originating from three different growth areas of a limited geographic zone, freshly distilled in the same distillery, were analyzed combining sensory and chemical approaches. A free sorting task conducted by a panel of 30 trained assessors allowed the discrimination of the spirits according to their origin to be done. The chemical composition of the brandies headspace using PTR-MS was analyzed in an approach using protonated ethanol as reagent ion while limiting ions fragmentation. Multivariate analyses of these headspace data allowed a clear separation of the spirits according to their growth area to be assessed. Compared to sensory evaluation, the PTR-MS generated data appeared more powerful for the discrimination task. A PLS-DA model allowed predicting the growth area of origin of freshly distilled French brandies, within the limited geographical zone, with the significant variables identified.

Introduction

Spirits under investigation are brandies made from white wine distillates, produced in a limited zone in the South West of France. Even before their ageing in oak barrels, freshly distilled spirits exhibit a strong aromatic character with highly developed fruity and floral notes that evade ethanol perception despite their high alcohol content (~70% v/v). Sensorial differences can be perceived between freshly distilled brandies of the same production area in terms of vintage effect (heat, sunshine, rain) – which will determine grape quality – and in terms of distillation effect (habits of distillers, distilled volumes, regularity of pot still washing). The objective of this work was to try to discriminate brandies freshly distilled in the same distillery according to their initial wine growth area. The total zone of brandy production was previously delimitated in several growth area, according to a soil characterization, assumed to produce spirits with perceptible sensory differences. Actually, century-old cellar master reports indicate that the sensory aromatic properties of brandies depend on the growth area of their initial wines, even in a limited geographical zone and as early as in freshly distilled spirits. To confirm this cellar master statement, the current study assessed the headspace characterization of freshly distilled French brandies, according to their growth area of origin within a limited geographic production area, using a direct-injection mass spectrometry technique (PTR-MS) in comparison to sensory data obtained in a free sorting task.

For alcoholic beverages, however, the ethanol content is generally an obstacle for the reliability of the MS analyses, the primary reactant ions being significantly depleted to the profit of ethanol-related ions. Former studies on alcoholic beverages conducted with atmospheric pressure chemical ionization mass spectrometry or PTR-MS showed the possibility of substituting

protonated ethanol for the hydronium reagent ions to assure compound ionization [1, 2], at the expense of sensitivity and/or large fragmentation issues. A recent work conducted in our group on wine (13% v/v ethanol content) demonstrated the possibility to use ethanol chemical ionization with modified PTR-MS operating conditions that allowed limiting the fragmentation of volatile organic compound protonated molecular ions [3]. Therefore, the present study also aimed at adapting this recent work to spirits with higher ethanol content than wine.

Experimental Methods

Sixteen freshly distilled French brandies originating from three different growth areas (named A, B, and C) of a limited geographic zone of less than 65.000 ha and processed in the same distillery were used.

Sensory evaluation: a free sorting task was realized by a panel of 30 trained assessors familiarized with alcoholic beverages. A multiple correspondence analysis (MCA) was conducted for data treatment.

Chemical analysis: headspace analyses were accomplished by direct-injection mass spectrometry using a PTR-ToF-MS instrument (PTR-ToF 8000, Ionicon Analytik, Innsbruck, Austria). Parameters of the PTR-MS instrument were adjusted from a previous work conducted on wine that allowed ethanol chemical ionization conditions while minimizing the protonated molecular ions fragmentation [3] using the following conditions: P*drift* = 2.31 mbar; T*drift* = 353K; U*drift* = 336 V resulting in E/N = 80 Td, with a Udx value fixed at 27 V. Headspace analysis was conducted by first connecting a reference vial containing a water-ethanol mixture of the same ethanol content than the samples (diluted to 20% v/v). This allowed calibration of the instrument using ethanol-containing ions always present in the headspace, and background signal recording. Then, the intake was switched to the sample headspace using three-way valves to adjust the desired flow way. The ethanol content was fixed at 20% v/v after optimization for sensitivity, as higher ethanol content resulted in significant decrease of reagent ions intensity. The mass spectra were acquired in duplicates at 1.08 s/scan with an m/z range from 0 to 250. All the spectra were background subtracted and the abundances of 73 variables (ions) could be followed. These variables were used in multivariate analyses: Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA) with 3 Y variables (growth area).

Results and discussion

The result of a multiple correspondence analysis (MCA) conducted on the sensory data obtained in a free sorting task aimed at categorizing the 16 spirits samples is shown in Figure 1. Except for one C outlier, the MCA plot clearly distinguished the brandies according to their growth area. Therefore, for these 16 samples, sensory evaluation using a free sorting task with a panel of trained assessors was able to classify the samples according to their origin.

Using the PTR-MS with ethanol chemical ionization conditions as described above, 73 ions originating from the samples headspace could be followed with reliable intensities. After background subtraction, these variables (ions intensities) were submitted to a PCA analysis. The sample scores of the first two dimensions are plotted in Figure 2. The first plane of this PCA explained 71% of the total variance. The groups formed by samples of each growth area were clearly differentiated, samples B being opposed to the others on the first axis (explaining 48% of the variance), the second axis (23% of total variance) allowing the separation of samples A and C.

A PLS-DA was conducted on the same ions intensities (X: 73 variables) in order to establish a model allowing the distinction of the growth areas (Y: 3 variables).

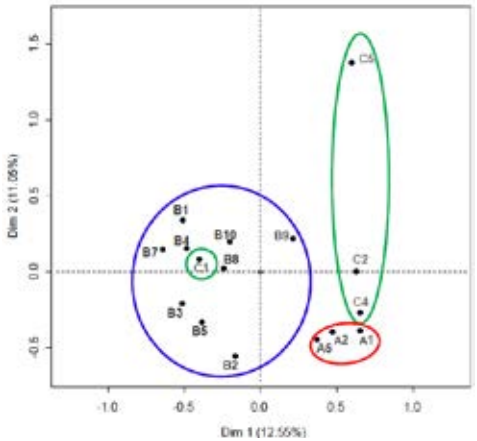

Figure 1: Multiple correspondence analysis (MCA) of the data generated by the panel in a free sorting task (16 French brandies at 40% ethanol content (v/v) originating from 3 different growth areas A, B, and C, freshly distilled in the same distillery).

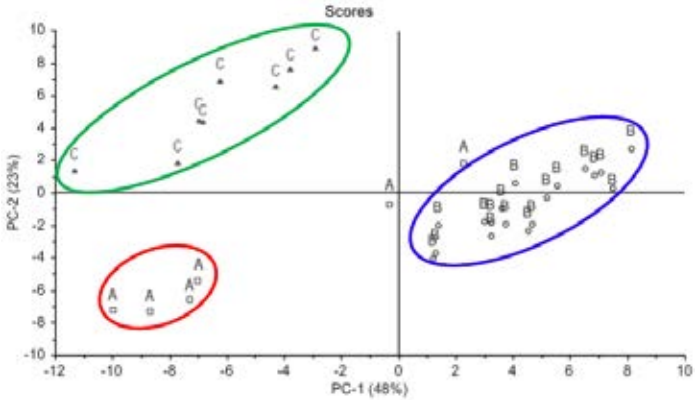

Figure 2: Principal component analysis (PCA) of the headspace data obtained with ethanol chemical ionization PTR-MS of the 16 freshly distilled French brandies diluted at 20% ethanol (v/v) content (two replicates).

The PLS-DA plot (two first factors) shows clearly that the spirits under investigation could be separated according to their origin (Figure 3, left), mostly along the first component that carries the major part of the explained variance (47% for X and 51% for Y). This first component makes growth area B distinct from the A and C groups. The second component explained a significant part of the variance (24% for X and 29% for Y) making groups A and C distinguishable. The model was found robust after validation by leave-one-out cross validation.

The first component could be explained by a large number of variables collinear with the axis (Figure 3, right), meaning it could be a 'concentration' axis. Thus, the growth area B seems more concentrated in most of the variables. The second axis was explained by some variables more

present in the growth area C, while growth area A appeared less concentrated in almost all the variables. Some variables inside the model were found significant according to the leave-one-out cross validation. These ions are the most important in the model, and therefore their intensities could be used to predict the growth area of an unknown sample.

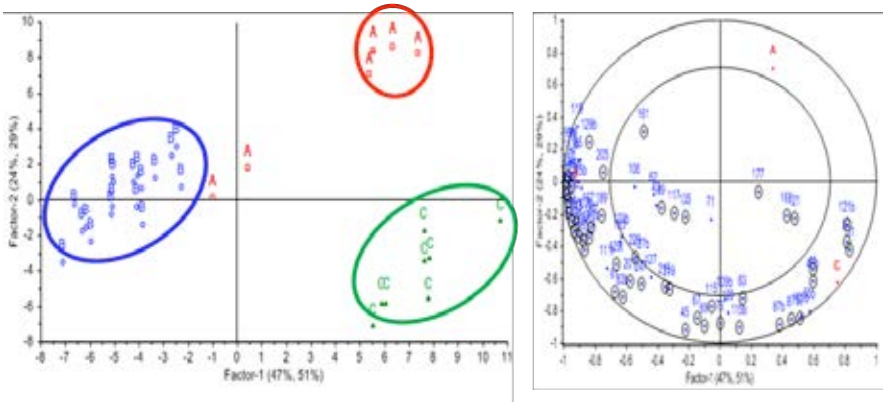

Figure 3: Partial least square-discriminant analysis (PLS-DA) of the headspace data obtained with ethanol chemical ionization PTR-MS of the 16 freshly distilled French brandies diluted at 20% ethanol (v/v) content (two replicates).

It has been shown that using PTR-MS with ethanol chemical ionization conditions it is possible to analyze brandy headspace, using a reference vial at the same ethanol content than the samples allowing formation of the reactant ions, calibration of the instrument and spectra background subtraction. Working at 20% v/v ethanol content, it was possible to discriminate 16 French brandies freshly distilled in the same distillery according to their growth area within a limited geographic zone. Compared to sensory evaluation conducted with a free sorting protocol, the PTR-MS generated data appeared more powerful for the discrimination task.

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Microbial Volatile Organic Compounds (mVOCs) emission by soil

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Abstract

Volatile organic compounds (VOCs) have a central role in environmental pollution. In particular, biogenic volatile organic compounds (bVOCs) contribute 90% to global VOCs emissions. One of the most important sources of bVOCs are microorganisms. The aim of this bibliography research is to start studying the relationship between microorganisms and VOCs production. To achieve this purpose we begin answering two main questions: firstly, why microorganisms produce VOCs and what can affect their production; secondly, how the PTR-TOF-MS technique can be used for microbial VOC soil analysis. Literature shows that VOC production depends on the sugar degradation pathway, with emissions affected by soil temperature, soil moisture, nutrient and oxygen availability and physiological state of microorganisms. In order to detect VOC emissions a PTR-MS technique will be used. The PTR-TOF-MS is one of the newest and more sensible techniques that allow microbial VOC detection. Also a non-exhaustive summary of VOC production from bacteria and fungi detected by the PTR-MS technique is shown. Finally, future perspectives are discussed.

Introduction

During the last decades, Volatile Organic Compounds (VOCs) became one of the most active topics in the field of environmental pollution. They play a key role in chemistry and composition of the atmosphere. They are precursors of Secondary Organic Aerosols (SOA), which alter radiative transfer in the atmosphere and hence global warming. Additionally VOCs are implied in tropospheric ozone cycle formation [1], [2]. Natural VOCs emissions are estimated to represent 90% of total VOCs emissions [3].

VOCs are emitted by several natural biotic sources like: soil, plants, microorganisms (bacteria and fungi), leaf litter, insects and animals. Besides, interactions between organisms living in soil, as plant-to-plant, plant-to-animal or microbe and microbe-to-microbe interactions, are universally mediated by VOCs, these communications help maintaining the equilibrium of the ecosystem [4]. Emissions are controlled and influenced by abiotic factors such as: temperature, light intensity, water and nutrient availability [5]–[7].

Within this framework new PhD starting focused on VOC emission by soil and interaction with microbes detected by a PTR-TOF-MS technique. The main part of this research concerns the

study of the relationship between microorganisms and VOCs production. Hence, the goal of this part is to answer two main questions: (1) why microorganisms produce VOC and what is affecting their production? (2) How can the PTR-TOF-MS technique be used for mVOC soil analysis. Finally, future perspectives are discussed.

Microorganisms and mVOCs production (mVOC?)

Why microorganisms do produce VOCs?

Microorganism's activities are related with carbon's and nitrogen's cycle. Literature shows that bacteria use three major pathways to degrade sugars, preferentially glucose (1) the Embden-Meyerhof pathway; (2) the Entner-Doudoroff pathway; and (3) Heterolactic/homolactic pathway [8]. These three pathways are implied in VOCs production. VOCs are resulting also from secondary metabolism processes. Secondary metabolites are produced only at specific developmental stages or under certain circumstances, for instance, under environmental stress. Intermediates in these processes, such as pyruvate, glyceraldehyde-3-phosphate, lactate and acetate are used as precursors for the biosynthesis of various VOCs [9].

What can affect mVOC production?

Another important topic concerning mVOC is the study of factors? That can influence their production. Difference in soil composition and the variability of soil abiotic parameters, such as nutrients, oxygen and physiological state of microorganisms, affect mVOCs production [5]. Nutrients and oxygen availability depend also on several environmental factors such as: soil texture, soil mixture, temperature, pH and microbial activity [10]. Besides, the availability of oxygen in basic parameters is key in determining the types of VOCs produced as it controls the respiration pathways [11]. In anaerobic conditions, diversity and quantity of VOCs emitted is increased [11].

A database that connects mVOCs emissions, microorganisms and production pathways

Connecting mVOC emissions with microorganisms and their pathways production can be difficult. In literature, for example, we can find a compilation of about 1000 microbial VOCs released from more than 350 bacterial and 80 fungal species. Lemfack and her colleagues (2014) [12] created a database of mVOC (http://bioinformatics.charite.de/mvoc/). The aim was to simplified mVOC research and to summarize in a unique database the main information about these compounds, their pathway formation and associated microorganisms species.

The use of PTR-MS technique for mVOC detection

PTR-MS is the newest technology used to detect VOCs. Major advantages of this technique are its high sensibility (pptv level) and to allow on line VOC measurement of any air gas sample [18] . To obtain a higher resolution PTR-MS can be combined with a time-of-flight (TOF) detector. The PTR-MS was mainly used for the detection of mVOCs in food quality control [15], organic waste decomposition [16], soils and for several other habitats , [21]. Table 1 highlights a non-exhaustive list of studies on microorganisms and their VOC emission detected by PTR-MS.

Table 1. References of in vitro or in vivo VOC production from different bacterial and fungal species and whole microbial communities living in soils or on organic materials detected by the PTR-MS technique.

Organisms investigated	VOC found	Habitat/cultivation media	Source	Year
Escherichia coli ***Shigella flexneri*** ***Salmonella enterica*** ***Candida tropicalis***	Diverse VOCs several unidentified and some identified compounds of low molecular weight <150 u	Complex media	[18]	2008
Pseudomonas spp. ***Enterobacteriaceae*** ***Lactic bacteria*** ***Enterococcus spp.***	Diverse VOCs several unidentified and some identified compounds of low molecular weight <150 u	Air and vacuum packed meat (beef and porck)	[15]	2003
Muscodor albus	Diverse VOCs	Soil grown on potato dextrose agar (PDA)	[19]	2004
Microbial community	Diverse VOCs (31 identified)	Mediterranean soil	[20]	2007
Microbial community	Diverse VOCs (17 identified)	Organic waste	[16]	2006
Microbial community	Diverse VOCs (7 identified)	Temperate soil under different compost load	[21]	2010

Future perspectives

A deeper qualitative and quantitative analysis of soil microbial VOC emitted will be necessary. Thanks to the great progress in analytics technique we have the possibility of analyzing VOCs in soils while discriminating between sources. The aim of the starting PhD it is to determine the possible connections between mVOC emissions profiles and the microorganism diversity and activity in a soil amended with organic waste products. To achieve this several experiments will be performed where the detection of emitted VOCs from soils will be made under controlled conditions for a range of soil samples. In parallel, analysis of soils genetic content and manipulation of genetic diversity of soils will be performed in these experiences.

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Measurements of isoprene peroxide with a new generation PTR-TOF-MS

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Abstract

Isoprene (2-methyl-1,3-butadiene, C_5H_8) is emitted to the atmosphere by deciduous plants with global emissions of approximately 535 Tg C per yr [1]. In rural areas (low NO_x conditions), isoprene oxidation leads to hydroxyhydroperoxides (ISOPOOH) with a yield of 70% [2]. Isoprene peroxide (ISOPOOH) is difficult to detect. Cleavage of the weak peroxy bond can be catalyzed by metallic surfaces and as a consequence either methyl vinyl ketone (MVK) or methacrolein (MACR) is formed. Regarding measurements of isoprene oxidation products in the atmosphere the catalytic reaction of ISOPOOH with metallic surfaces leads to an observational bias. The new generation of SRI-TOF-MS (Selective Reagent Ionisation-Time of Flight-Mass Spectrometer) is able to detect ISOPOOH. Metallic surfaces were replaced by conductive PEEK.

Introduction

Atmospheric oxidation products of volatile organic compounds (VOC) are precursors of secondary organic aerosols (SOA) and contribute to new particle formation, which affect climate and environment [3]. Isoprene is emitted to the atmosphere by many plants. It contributes one third of global non-methane emissions [1]. Photooxidation of isoprene yields a peroxy radical. In urban regions this peroxy radical reacts with NO and forms methyl vinyl ketone (MVK) or methacrolein (MACR). In regions without anthropogenic influences the peroxy radical predominantly reacts with HO_2. The second reaction pathway is leading to ISOPOOH and as a consequence IEPOX, a product of ISOPOOH and OH (figure 1). Globally, the reaction with HO_2 dominates [3].

Homolytic cleavage of the weak peroxy bond can be catalyzed by metals inside the measuring instrument and ISOPOOH reacts to MVK or MACR. Hence, ISOPOOH is detected as its urban counterparts and distorted detection generates an observational bias towards the urban, high-NO_X products [4]. ISOPOOH was first measured without fragmentation or reaction using the CF_3O^- CIMS [5].

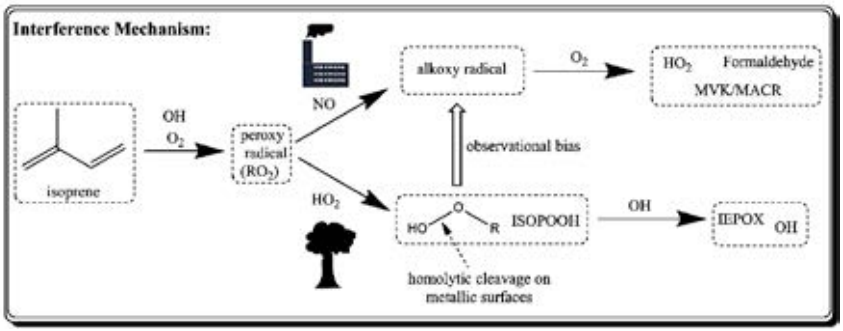

Figure 1: Photo-oxidation of isoprene yields a peroxy radical. In urban regions the peroxy radical generates an alkoxy radical, which leads to the formation of MVK/MACR, formaldehyde and HO_2. In pristine regions the peroxy radical reacts with HO_2 to form ISOPOOH. Even at room temperature, metallic surfaces catalytically cleave the weak peroxy bond forming alkoxy radicals. This leads to an observational bias. [4]

Experimental Methods

The Institute for Ion Physics and Applied Physics in Innsbruck has developed an advanced version of the Selective Reagent Ionisation-Time of Flight-Mass Spectrometer (SRI-TOF-MS). The most important details of the advanced SRI-TOF-MS regarding ISOPOOH measurements are the high flow pumping architecture and the new metal-free drift tube, consisting of PTFE and PEEK. [6]

Two experiments were realized separately:

Coldtrap experiments:

A PTFE line is directed into a cold ethanol bath (233° K). Less volatile compounds such as ISOPOOH stream through the PTFE line and are cryogenically trapped, while solvents with high vapor pressure pass the cold section of the line. After the trapping procedure, which takes 20 hours, the cold trap is heated up over two days and trapped compounds with lower volatility start to re-evaporate. This way, the compounds of the sample air can be sorted by their volatility.

LCU experiments:

The Liquid Calibration Unit (LCU) [7] allows measurements of samples with constant flow. The measurements are realized by alternately using a PTFE- and a metallic line, on which the catalytic cleavage occurs. Switching between the two different lines gives further indications and simplifies analysis. Signals that disappear after switching to the metallic line are attributed to ISOPOOH.

A nuclear magnetic resonance (NMR) analysis confirmed the purity of the ISOPOOH samples for the measurements with ISOPOOH.

Results and Discussion

The coldtrap setup allows a detailed partitioning of the sample. While the coldtrap temperature increases, different compounds evaporate at different times according to their vapor pressure. Most of the solvents pass the low temperature trap without freezing. The solvents of the ISOPOOH sample, hexane, diethyl ether and ethyl acetate have their maximum of occurrence at an early stage of the coldtrap experiment. In contrast, ISOPOOH masses arise when the cold trap is heated up. This method also reveals contaminations in the sample. The time trends of the different signals allow to seperate solutions, contaminations and most importantly ISOPOOH.

The LCU is able to generate a constant ISOPOOH flow. The flow is flushed into the advanced SRI-TOF-MS. After switching from a PTFE line to a metal line (353° Kelvin), the mass contribution of ISOPOOH changes significantly. Masses associated with ISOPOOH decrease, while MVK (or MACR) increases.

Detected ions, that are linked to ISOPOOH are $C_5H_7O^+$, $C_5H_9O_2^+$ in H_3O-Mode and $C_5H_9O^+$ in NO-Mode. These masses can be used as markers for isoprene peroxide in the atmosphere. Further investigations are needed to improve our understanding of the isoprene oxidation process. The advanced SRI-TOF-MS can play an important role in investigating remaining open questions on this topic.

Acknowledgements

We are thankful for the fantastic support by our colleagues Eva Canaval, Dr. Martin Breitenlechner, Lukas Fischer, Markus Hainer, Lukas Hausberger, Irina Herdlinger-Blatt, Dr. Gerhard Steiner and Anne-Kathrin Bernhammer. We would also like to thank Dr. Holger Kopacka for the NMR analysis of our samples.

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Continuous PTR-ToF measurements of breath VOC profiles mirror (patho)physiological effects and therapeutic interventions in mechanically ventilated patients

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Abstract

In this pilot study we monitored exhaled volatile organic compounds (VOC) and their relation to (patho)physiological effects and therapeutic interventions in ventilated patients. VOC profiles from mechanically ventilated patients after open heart surgery were assessed by means of breath resolved PTR-ToF-MS analysis. Electrical impedance tomography (EIT) was used to detect atelectasis and to monitor recruitment maneuvers consisting of a stepwise increase of inspiratory pressure (Pinsp.) and positive end-expiratory pressure (PEEP). Physiological effects (e.g. change of cardiac output), interventions (change in ventilation) and consecutive effects were mirrored by changes of VOC concentrations. Combination of real-time breath analysis and ventilation imaging by means of EIT can be used to gain complementary information on patients' status. In a perspective, these techniques could be employed for non-invasive control of therapeutic ventilation strategies.

Introduction

Disturbances in ventilation/perfusion ratios due to the occurrence of atelectasis are frequent problems in mechanical ventilation. Global parameters of gas exchange determined by blood gas analysis cannot differentiate causes sufficiently. Breath gas analysis providing additional data on pathophysiology represents a promising alternative. The aim of this study was to investigate the effect of recruitment maneuvers and changes in ventilation on concentrations of volatile organic compounds (VOC) in breath.

Experimental Methods

Eleven mechanically ventilated adult patients were enrolled into the study. All patients underwent open heart cardiac surgery and a defined pulmonary recruitment maneuver with stepwise increase of inspiratory pressure (Pinsp.) and positive end-expiratory pressure (PEEP). All patients were connected to pressure-controlled ventilation and supplied with monitoring of ECG, blood pressure (invasive), oxygen saturation, cardiac output and Electrical Impedance Tomography (EIT)-device (PulmoVista®) to visualize dynamic changes in ventilation distribution within defined areas of the lung and to monitor the therapeutic interventions of the recruitment maneuvers.

Breath sampling was done using a 6 m long heated silcosteel transferline that was connected to the respiratory system via a sterilized T-piece located at the end of the endotracheal tube. The drift tube of the PTR-ToF 8000 (Ionicon) was operated at 2.3 mbar (75°C) resulting in a E/N ratio of 138 Td. Time resolution was 200 ms to enable phase resolved measurement of breath cycles. The mass scale was calibrated every 60s. The files were processed by a Matlab-based algorithm,

separating alveolar and inspiratory breath phases. VOC intensities of alveolar phases of each minute file were averaged for final analysis.

Results

Continuous, phase resolved real time monitoring of breath profiles in mechanically ventilated patients could be realized by means of the adapted setup. Out of 300 detectable peaks in the spectrum we selected 8 mass traces reflecting potential compounds of interest. The concentration profiles of these compounds showed a characteristic behavior during and after the therapeutic intervention (figure 1).

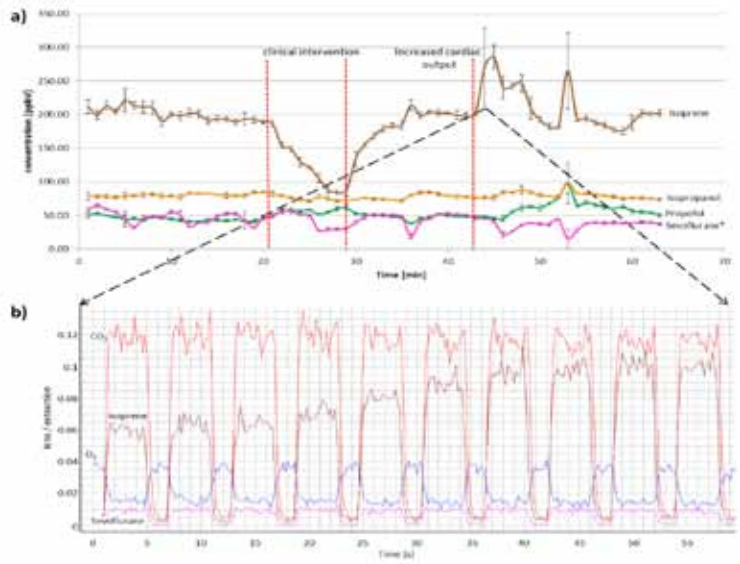

Figure 1: a) Time – concentration profiles of CO2, isoprene, sevoflurane and O2 in a mechanically ventilated patient before, during and after recruitment. b) Phase resolved profiles (tres= 200 ms) of sixty seconds (representing a single time point in profile a)) . Reprinted with permission from: Trefz et al. Anal Chem 85(21):10321-9. Copyright (2013) American Chemical Society).

Patients showing ventilation in dorsal lung areas (EIT data) with an improvement of more than 50 % were defined as Responder (n=4), and Nonresponder (n=7), if less than 50 % improvement.

Most VOCs declined during the recruitment maneuver in both groups but came back into the range of their initial intensities (figure 2). Differences in normalized concentrations indicate recruitment induced changes in ventilation/ perfusion ratios of the lung.

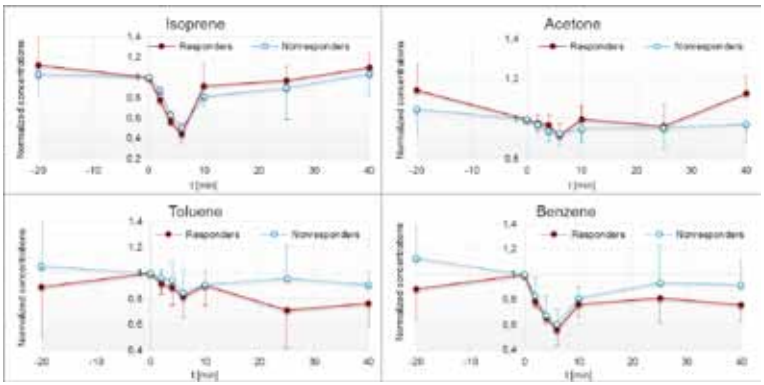

Figure 2: Normalized Data (Start of recruitment maneuver) of selected substances: Isoprene and acetone (endogenous) and benzene and toluene (exogenous).

Conclusion

Continuous VOC monitoring by means of PTR-ToF-MS enables immediate recognition of (patho)physiological and therapeutic effects in ICU patients. Combination of real time breath gas analysis and ventilation imaging by means of EIT can be used to gain complementary information on patients` status. For interpretation of breath biomarkers, respiratory and hemodynamic parameters have to be taken into account.

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In-vivo aroma persistence. Real-time monitoring of volatiles in-nose and in-mouth.

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Abstract

Persistence of aroma compounds in breath after swallowing results in prolonged sensation. We have studied the persistence of compounds both in-nose and in-mouth after administration of volatiles in gas phase to five different panelists and breathing according to four different protocols. The physicochemical properties of the compounds, mainly polarity and vapor pressure, controlled the interactions of the volatiles with the mucosa and were therefore responsible of the different persistence between compounds. The use of different breathing protocols allowed us to study the differences between nasal and oral mucosa in volatile absorption and desorption back in the breath flow. Measured intensity in-mouth was always higher, suggesting higher absorption in the oral cavity. Only the more polar compounds presented differences in persistence between the breathing protocols.

Introduction

Aroma sensation does not end after swallowing the food product. Some aroma compounds remain in breath for several seconds, or even minutes, producing the sensation commonly referred as “aftertaste”. Persistence of aroma in breath depends mainly on three different kinds of factors: physicochemical properties of the aroma compounds, physiological characteristics of the consumer and composition of the food matrix.

Several authors have studied aroma persistence after consuming a water solution containing an aroma cocktail. The use of aqueous solutions allowed them to reduce food matrix effects to a minimum and relate persistence with the physicochemical properties of the compounds. Polarity, partition coefficient air/water and vapor pressure were found to be the main drivers of persistence[1]–[4]. Compounds with high water solubility and low vapor pressure are more likely to partition into the mucus, therefore having higher persistence over time. Also, after swallowing a beverage, some rests of the fluid can be retained on the mouth or the throat, acting as aroma reservoirs that keep releasing volatiles overtime contributing to persistence[5]. This effect has been observed for aqueous solutions and it has been described as one of the main contributions to persistence. In fact, Hodgson et al. observed no persistence for isoamylacetate when the compound was swallowed in gas phase but prolonged persistence was observed when it was swallowed as an aqueous solution[6].

In this work, we have studied aroma persistence using volatiles in gas phase, therefore eliminating any matrix effect and the possibility of having solution debris that keep releasing volatiles. By using four different breathing protocols and monitoring volatile intensity on exhaled breath by PTR-MS, we have studied the differences in aroma persistence between the nasal and oral cavities.

Experimental Methods

Aromatized air preparation

An aroma cocktail containing 2-heptanone, guaiacol, 4-methylguaiacol, ethyloctanoate, ethylbutyrate, 2,5-dimethylpyrazine, isoamylacetate and phenylethylalcohol was prepared. Some droplets were introduced in a Tedlar bag full of compressed air and vaporized at 60°C for 3 hours. 500 mL of the aromatized air were administered by a gas tight syringe to 5 different panelists. Four different breathing protocols where used by each panelist.

Breathing Protocols

Four protocols were selected (figure 1). **M1**: volatiles were injected in the mouth and inhaled, then air was exhaled by the mouth and inhaled by the nose for each breath; **M2**: volatiles were injected in mouth and then air was both exhaled and inhaled by the nose while mouth was kept completely closed; **N1**: volatiles were injected in the nose and then air was exhaled by the nose and inhaled by mouth; **N2**: volatiles were injected in the nose and then air was both inhaled and exhaled by the mouth while nose was closed with a clip. For all protocols, the breath tempo was defined as 3 seconds inhalation and 3 seconds exhalation and was marked with a metronome. Each breathing protocol was performed in triplicate for each of the 5 panelists.

PTR-MS analysis

Exhaled volatiles were sampled from nose via commercial N.A.S.E (Ionicon Analitik GmbH, Austria). For mouth sampling, a custom build setup heated at 90°C was used. Volatile analysis was performed with a commercial PTR-MS (Ionicon Analitik GmbH, Austria). PTR conditions were as follow: 160°C inlet temperature, 80°C drift tube temperature, 2.2mbar drift tube pressure, 495V drift voltage; yielding a E/N value of 120 Td. The same dwell time (50ms) was used for all the m/z measured: 71 (isoamylacetate), 105 (phenylethylalcohol), 109 (2,5-dimethylpyrazine), 115(2-heptanone), 117 (ethylbutyrate), 125 (guaiacol), 139 (4-methylguaiacol), 173 (Ethyloctanoate).

Results

Intensity of the selected volatiles in breath was monitored by PTR-MS, and averaged for each exhalation during the first two minutes after administration of the aromatized air. The first exhalation showed high intensity as it contains the dead volume of the respiratory tract that was filled with aromatized air. This first exhalation was discarded and the rest were fitted to a power curve $y=ax^{-b}$ where b represents the decay rate.

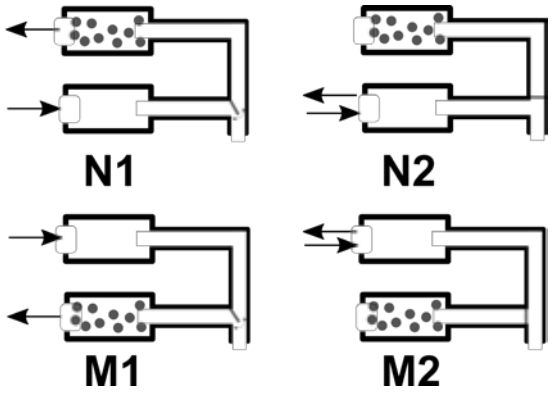

Figure 1: Breathing protocols. Dots represent the volatiles that were injected into the cavities. Arrows show the breathing pattern (inhalation/exhalation).

Differences in persistence between compounds.

Aroma persistence in gas phase was dependent on the physicochemical characteristics of the compounds (Figure 2). The most polar compounds (2,5-dimethylpyrazine, guaiacol, 4-methylguaiacol and phenylethylalcohol) presented low decay rates and therefore longer persistence. Compounds with lower polarity had lower persistence in breath, with isoamylacetate having the higher decay rate and followed by ethylbutyrate, 2-heptanone and ethyloctanoate. These differences were higher than those between panelists or protocols.

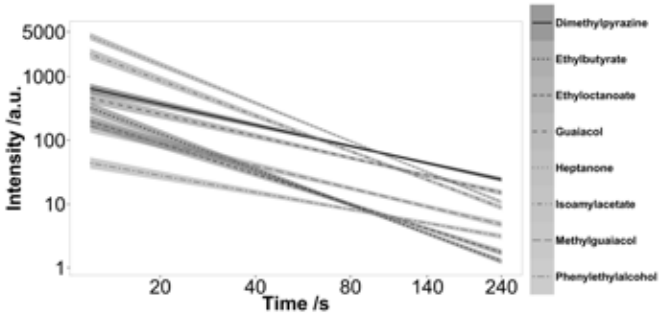

Figure 2: Decay curves for the eight aroma compounds. The slope of the curve represents the decay rate (b). Higher slope means lower persistence in breath.

Differences in persistence between breathing protocols.

The four breathing protocols showed differences in measured intensity and decay rate. Breathing maneuver M1 resulted in the highest intensity for all the aroma compounds and panelists. One panelist did also exhibit high intensity for the four most polar compounds during N1 protocol, but the rest of panelists did not show any significant difference in the intensity measured for protocols N1, N2 and M2. Regarding decay rate, differences were dependent on the compound and panelist.

In general, the lower polarity compounds (ethylbutyrate, ethyloctanoate, 2-heptanone and isoamylacetate) did not present significant differences on decay rate among the protocols. The rest of compounds (2,5-dimethylpyrazine, guaiacol, 4-methylguaiacol and phenylethylalcohol) had higher decay rates for N1 protocol, although differences were not always significant.

Discussion

Persistence of aroma compounds in breath mainly depends on the polarity and the vapor pressure of the volatile compounds. This behavior has been previously reported for aqueous solutions and reflects the partitioning of volatiles in breath with the mucosa. The different composition of the nasal and oral mucosa resulted in different retention of volatiles in the two cavities. As a general trend, higher intensity of the compounds could be measured in mouth, indicating higher concentrations absorbed and desorbed in this cavity. Furthermore, the decay rate of the polar compounds in-nose was slightly faster, indicating lower persistence.

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Volatiles microbial spoilage markers in milk

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Abstract

The shelf-life of fresh, pasteurised milk is limited by the growth and activities of bacteria that either survive the pasteurisation process or arise from post-pasteurisation contamination. Volatile organic compounds (VOCs) generated by microbial spoilage potentially offer clues as to the degree of degradation of a product and whether or not it is still fit for human consumption. Proton-transfer-reaction mass spectrometry (PTR-MS) was used to analyse a series of milk samples of different fat composition to relate VOCs with the number of bacteria present. The findings indicate that below a threshold number of bacteria the milk is still suitable for use, but above which the abundances of VOCs released increase rapidly, causing flavour defects.

Introduction

Milk is an animal-based food product that is highly susceptible to microbial spoilage that renders it unsuitable for human consumption. The shelf-life of milk is dictated by the presence of micro-organisms such as Gram-positive psychrotolerant, endspore-forming bacteria that survive pasteurisation and grow at typical refrigeration temperatures, or by Gram-negative bacteria that contaminate the milk post-pasteurisation. This latter group, which includes *Pseudomonas* spp., is considered the predominant cause of spoilage of fresh, pasteurised milk [1]. Presently, milk shelf-life estimations are based on global data gathered from typical spoilage processes, rather than being specific to the actual product in the milk carton. Intelligent packaging is currently being sought by many food manufacturers, to help extend the shelf-life of their products, and with the implementation of food freshness indicators (FFIs), to monitor and react to the status of the product inside the packaging. The identification and use of volatile spoilage markers for such intelligent systems is desirable as they will provide a more accurate indication of how much longer a product will still be suitable for consumption, which will thereby ultimately contribute to reducing food waste.

The study presented here investigated volatile organic compounds (VOCs) arising from fresh, pasteurised milk during bacterial spoilage and how these related to the bacterial load in the milk. Proton-transfer-reaction mass spectrometry (PTR-MS) was used to detect VOCs in the headspace of three types of milk with different bacterial loads over a 25-day period. Solid-phase micro-extraction gas chromatography-mass spectrometry (SPME-GC-MS) analysis was performed on a subset of samples to aid and confirm compound identification. A full account of these experiments and their outcome has been published in the scientific literature [2].

Experimental Methods

Milk samples

Three types of fresh, pasteurised milk (FPM) were used for the present experiments: trim (0.25-0.40 % fat), lite (1.40-1.50 % fat) and full-cream (3.18-3.28 % fat). Half of each batch was treated with 10 ppm_v sodium azide (NaN_3), a bacteriostatic agent used to supress bacterial growth. Aliquots of each sample were transferred into 50 mL sterile glass brown bottles that were capped and placed under refrigeration at 4.5 °C for up to 15 days. A complete triplicate subset of each milk type, both treated and untreated with NaN_3, were removed from the fridge on each experimental day and allowed to warm to room temperature by placement in a heated water bath prior to PTR-MS analysis.

Analysis

The number of bacteria in three replicated samples of each milk type incubated in the presence or absence of NaN_3 was determined on each sampling day by spread plating triplicate dilutions onto plate counts agar plates that were subsequently incubated at 25°C for 72 h.

A high sensitivity proton-transfer-reaction mass spectrometer (hs-PTR-MS; IONICON Analytik GmbH, Innsbruck, Austria) was used to analyse the headspace of the milk samples for constituent VOCs. The PTR-MS instrument was operated at 136 Td *E/N* (drift tube settings: 600 V, 2.2 mbar and 70 °C). Milk samples were transferred into 500 mL glass bottles (Schott Duran, Mitterteich, Germany) for analysis. After flushing the headspace with instrument grade synthetic air via an active charcoal filter, the samples were left to equilibrate before performing static headspace analysis. The PTR-MS instrument was set to measure in mass scan mode over the range *m/z* 20-200 at a dwell time of 100 ms per *m/z*. Each analysis took 90 s.

Gas chromatography-mass spectrometry (GC-MS) analyses of a subset of samples were made using solid-phase micro-extraction (SPME) to aid identification of the PTR-MS *m/z* signals.

Results

In the absence of sodium azide (NaN_3) the number of bacteria detected in the FPM increased with increasing storage time to varying levels across the three types of FPM (data not shown). Differences in bacterial numbers were likely due to differences in the levels and type of post-pasteurization contamination. In the presence of sodium azide bacterial numbers remained relatively constant over time. Thirty-eight *m/z* detected from the headspace above the FPM samples significantly ($p \leq 0.05$) differentiated between milks with or without NaN_3. The VOC concentrations in the FCM headspace remain largely unchanged until the number of bacteria reached a threshold number. Interestingly, not all VOCs started to change in concentration at the same threshold number of bacteria and the rate of change at the inflexion point varied from compound to compound. As total number of bacteria reached 1×10^8 CFU mL^{-1}, *m/z* 61 (acetic acid and/or 1-propanol) or *m/z* 89 (Acetoin (3-Hydroxybutanone), Ethyl acetate, Butanoic acid, 1-Pentanol, 3-Methyl-1-butanol) that started to rapidly increase (data not shown). Acetaldehyde (*m/z* 45), ethanol (*m/z* 47), dimethyl sulphide (*m/z* 63, data not shown) and 2-pentanone, 3-methylbutanal, pentanal and/or 2,3-butanedione (*m/z* 87) all started to increase once bacterial numbers exceeded 1×10^8 CFU mL^{-1} (Figures 1–3).

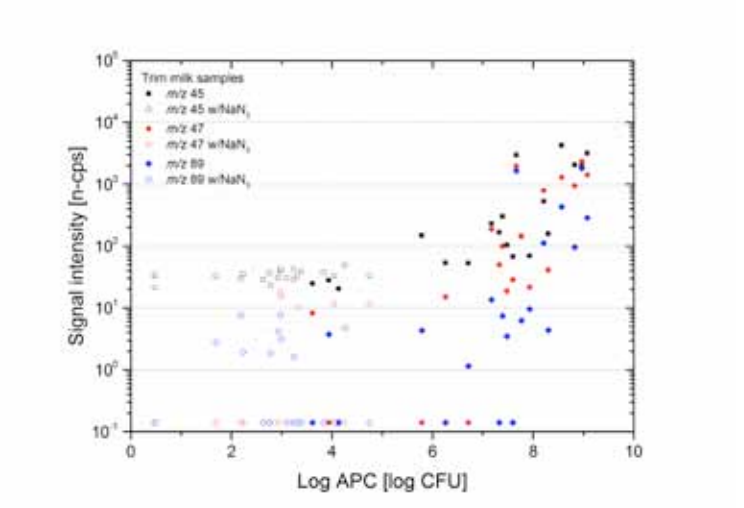

Figure 1. Plot of normalised signal intensity (ncps) versus log colony forming units of signals at m/z 45, m/z 47, and m/z 89, tentatively identified as acetaldehyde/CO2, ethanol, and acetoin, respectively, for the trim milk samples.

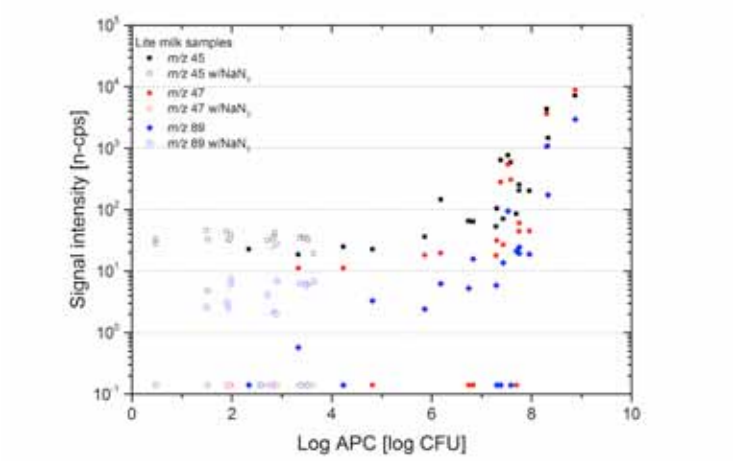

Figure 2. Plot of normalised signal intensity (ncps) versus log colony forming units of signals at m/z 45, m/z 47, and m/z 89, tentatively identified as acetaldehyde/CO2, ethanol, and acetoin, respectively, for the lite milk samples.

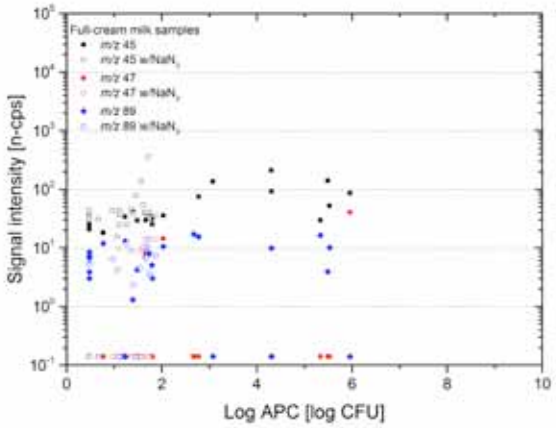

Figure 3. Plot of normalised signal intensity (ncps) versus log colony forming units of signals at m/z 45, m/z 47, and m/z 89, tentatively identified as acetaldehyde/CO2, ethanol, and acetoin, respectively, for the full cream milk samples.

Conclusions

The PTR-MS data reveal a clear link between the concentrations of VOCs in the milk headspace and the bacterial load in the liquid milk. A particularly interesting finding of these studies is that several VOCs, which could potentially act as volatile spoilage markers, remain at relatively steady levels when bacterial numbers are low, but increase to high amounts after a threshold number of colony forming units has been surpassed. This indicates that there is a narrow margin in bacterial numbers below which the milk will not exhibit a characteristic spoilage odour, but above which this odour will be present. These data demonstrate a proof-of-principle in potentially using volatiles present in the milk headspace to estimate the degree of spoilage. Further comprehensive studies would be required to fully characterise the nature of this relationship, but the use of non-destructive gas sampling to determine current shelf-life has promising applications, both along the production line and at the point-of-sale.

Acknowledgements

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Glyoxal measurement with a proton transfer reaction time of flight mass spectrometer (PTR-TOF-MS)

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Abstract

Here we present a characterization and the calibration of glyoxal measurement with a PTR-TOF-MS in detail. In order to calibrate the PTR-TOF-MS for glyoxal, the mixing ratio of glyoxal emitted from the prepared sample trap was measured with an UV absorption spectrometer in parallel to a PTR-TOF-MS. Extensive fragmentation of glyoxal to formaldehyde was observed. The obtained sensitivity ranges between 0.8 ncps/ppb for the dry calibration and 0.2 ncps/ppb for the humid calibration. The detection limit ranges from 250 ppt for the calibration with the dry dilution flow up to 2 ppb for the calibration with the greatest humidity. The calibration demonstrates that it is difficult to measure glyoxal with PTR-TOF-MS due to the low sensitivity and associated high detection limit. This limits the use of PTR-TOF-MS for ambient measurements since the atmospheric abundance of glyoxal in rural regions is typically in the range between 10 to 100 ppt.

Comparison of VOCs concentrations at urban and background site in southern China

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Abstract

VOCs concentrations were measured by PTR-MS at urban supersite in Shenzhen and background atmospheric monitoring station in Wuzhi mountain both in Southern China. In this study we compared variations of biogenic and anthropogenic VOCs concentrations at these two measurement stations both during spring period. The diurnal variations show the contributed sources of VOCs were complicated in Shenzhen but dominated by biogenic source in Wuzhi mountain. The emission ratios for VOCs species to CO demonstrates great discrepancy in two sites.

Introduction

Volatile organic compounds (VOCs) are central constituents in the troposphere, playing significant roles in the formation of ozone and secondary organic aerosols (SOA). Shenzhen is one of the megacities in China, with the total residential population of more than 10 million and the civilian vehicle fleet over 2.5 million. As the highest peak in Hainan region, Wuzhi mountain (altitude 953 m) is away from the city and anthropogenic pollution sources. The different dominant emission sources of VOCs resulted in variations of concentrations and compositions which should be concerned. Online measurements of ambient VOCs concentrations were performed at this urban city and background site in southern China both during spring period so as to compare the diurnal behavior and the different sources contribution. Previous studies have shown that vehicle exhausts, solvent evaporation and industrial activities are the major sources of VOCs in Shenzhen but rare studies have conducted in Wuzhi mountain.

Experimental Methods

In this study, a proton transfer reaction mass spectrometer (PTR-MS) was deployed at the urban site in University Town of Shenzhen (22.60N, 113.97E) from March 5th to April 5th, 2013, while the observation campaign conducted at the background site in Wuzhi mountain (18.83N, 109.48E) was from March 15th to April 15th, 2015. The PTR-MS was run at the standard mode with drift pressure at 2.24 mbar and voltage at 550 V (equivalent to E/N=124 Td). The background checks were conducted after every 300 scan cycles of ambient measurements with a Pt catalytic converter at 365℃. The PTR-MS measurements were calibrated using a gas standard every 5—7 days. The sensitivities for all measured VOCs species were steady throughout these two campaign (RSD<10%).

Results and discussion

The biogenic VOCs measured in urban and background sites both exhibit a distinct diurnal pattern (Fig 1). Mixing ratios of biogenic VOCs (except monoterpene in Shenzhen) increase early in the morning and rise steadily until achieving a peak around 15:00-16:00 (LT). Rapid decrease soon after sunset is observed, followed by a more gradual decline during the night. The peak concentration of isoprene and monoterpene in Wuzhi mountain were two to three times higher

than Shenzhen due to more abundant vegetation. It should be mentioned that the diurnal profile of oxygenated VOCs measured in Wuzhi mountain are similar to isoprene and monoterpene, indicating obvious contribution of biogenic emission. While the oxygenated species in Shenzhen have two peak concentrations in the morning (09:00-10:00 LT) and the late afternoon (16:00-18:00 LT) which corresponding to the morning and evening rush hours. Aromatics, as an abundant group of primary anthropogenic NMHCs, performed similar daily variations in Shenzhen: stable during the night, decreasing after sunrise, dropped to minimum in afternoon (14:00-15:00 LT) and then rise again in the night. But the mixing ratio of aromatics in Wuzhi mountain show unchanged during the whole day, suggesting much lower emission of anthropogenic primary source.

Emission ratios for hydrocarbons versus CO from measurements made in Shenzhen and Wuzhi mountain are shown in Fig 2 for comparison purposes. For toluene and C8 aromatics, Shenzhen emission ratios to CO are approximately a factor of 8—9 larger than corresponding values in Wuzhi mountain. Besides, emission ratios of aromatics in Shenzhen are significantly higher than Beijing (Yuan et al., 2012) and Mexico City (Bon et al., 2011). Difference of aromatics probably due to the higher emissions from industrial solvent use in the Shenzhen campaign. Comparison of emission ratios of selected OVOC species in Shenzhen and Wuzhi mountain (this study), Beijing (Yuan et al., 2012), Mexico City (Bon et al., 2011) and New England in 2004 (Warneke et al., 2007) are shown in Fig 3. Some large discrepancies can be found among these campaigns. The emission ratios of the selected four OVOCs in Shenzhen were much higher than those reported in other regions, while the emission ratios of acetaldehyde, acetone and MEK obtained from Wuzhi mountain generally fall in the ranges of reported values from the several field campaigns in Beijing, the New England and Mexico City. The abnormal high emission ratios for OVOCs in Shenzhen indicated unusual source attribution, which should be discussed further.

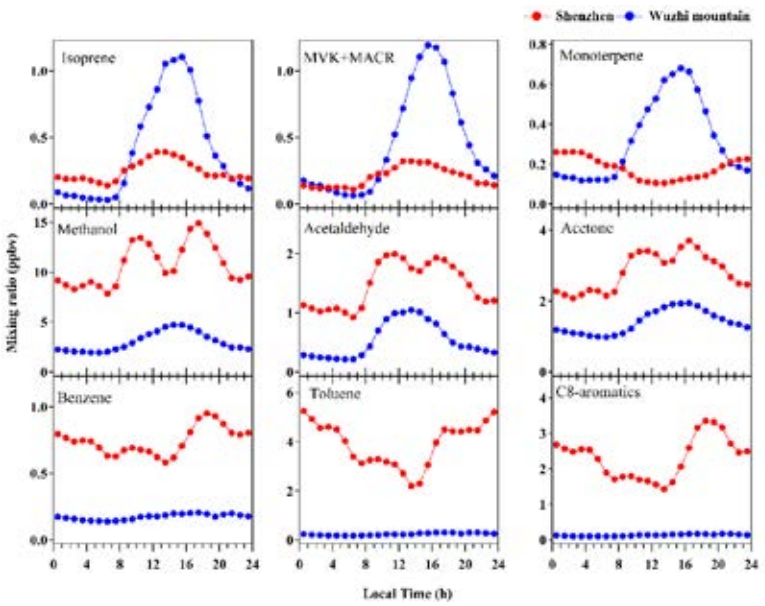

Figure 1 Diurnal variations for selected VOCs in urban and background sites

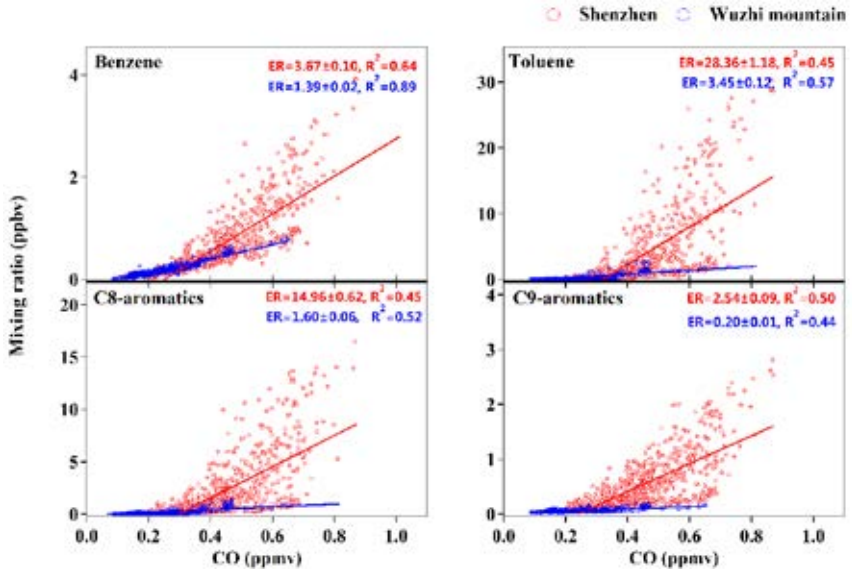

Figure 2 The emission ratio of NMHCs to CO in urban and background sites

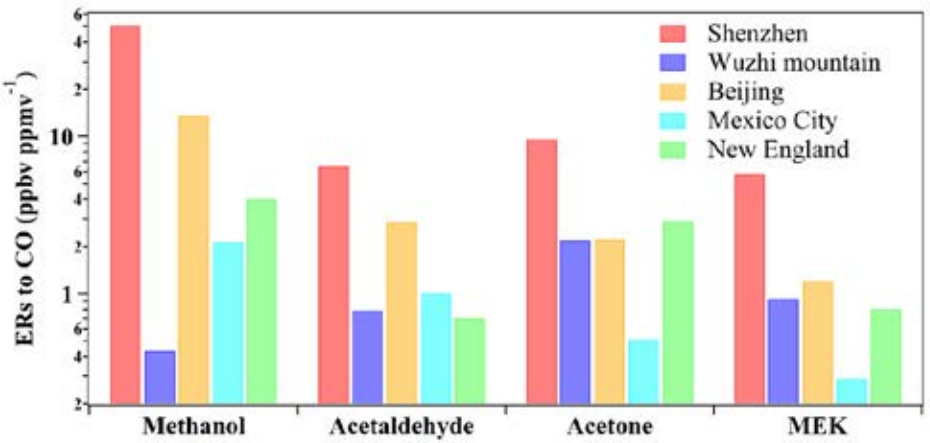

Figure 3 The emission ratio of OVOCs to CO in Shenzhen, Wuzhi mountain, Beijing, Mexico City and New England

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Improved sampling technique for volatiles in the mouth cavity

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Abstract

Nose- and mouth-space measurement of the exhalation stream are well-established techniques in food science and medical analysis. For sensory analysis where volatiles present in the mouth or nasal cavity are the analytes the process of exhaling is effectively diluting the sample. In this work we present the results from a newly developed method termed "direct mouth-space sampling" (dMoS-sampling) combining an active sucking out of sample from the mouth while breathing through the nose. The measurements conducted by this method showed an increase in signal areas of 5-fold and 20-fold compared to traditional mouth-space and nose-space sampling, respectively.

Introduction

Mouth- and nose-space analysis have been used abundantly in research in the last decades for medical purposes as well as for sensory analysis. All of these sampling methods have been using the analysis of the respiratory flow to determine the content of volatiles present in the lungs, the mouth or the nasal cavity. With regard to sensory analysis this means that the compounds of interest that are accumulating in the mouth and nasal cavity get diluted by the respiratory flow, leading to low signal intensities and only a limited number of flavor active compounds that can be monitored The goal of this work was to increase the signal intensity from volatile aroma compounds present in the mouth cavity, so that volatile compounds responsible for aroma during the actual drinking as well as the lingering smell after ingestion can be measured with increased sensitivity.

Experimental Methods: In-vivo on-line analysis by PTR-ToF-MS of volatile compounds from coffee

Filter coffee extraction procedure:

The extraction was conducted with 250 +/-0.1 g of water at 93 +/-0.5 °C with a total extraction time of 2.5 min. 15.0 +/-0.1 g of a pure Arabica coffee (Rushashi, Rwanda) was ground using a Ditting KR805. The extract had a weight of 220 +/- 5 g. The concentration of dissolved solids (TDS) was at 1.25 - 1.3 % (m/m) corresponding to an extraction yield of 18.6 +/-0.1 % (m/m).

Sampling methods

Three different sampling methods were tested:

1. Nose-space by indirect sampling of the exhalation stream (with nose-piece): iNoS-sampling
2. Indirect mouth-space: Sampling of the exhalation stream with a Buffered End-Tidal breath sampling inlet (BET) by Ionicon: iMoS-sampling
3. Direct mouth-space: Drawing sample gas directly from the mouth cavity while breathing through the nose - Mouthpiece from BET (non re-breathing mouthpieces) coupled to a sampling and dilution lance [1]: dMoS-sampling

Figure 1: Direct Mouth-Space (DMOS) sampling setup: BET mouthpiece coupled to the heated sampling and dilution lance

Data aquisition and processing

Mass spectra were recorded in 1 s intervals for a mass range of 15-200 m/z. The data processing and peak fitting was conducted at a time resolution of 5 s with the PTR-ToF Data Analyzer [1] since this significantly improved the number of identified peaks and their reproducibility.

Results

To assess the differences between the three different sampling methods the signal intensities a range of coffees have been recorded. In this work we will show exemplary data for one coffee only since the differences between the different sampling methods were of the same magnitude for all coffees. Figure 2 shows two series of graphs. The upper series depicts the intensities for m/z 153.055 and m/z 153.091, tentatively identified as Vanillin and Ethylguaiacol, respectively, which are representative for the lowest intensity signals that were still discernable from the background. The lower series shows the intensities of m/z 73.065, m/z 87.080 and m/z 101.0S60, tentatively identified as 2-Methylpropanal, 2-/3-Methylbutanal and 2,3-Pentadione, respectively, which are representative for the signals with high intensities.

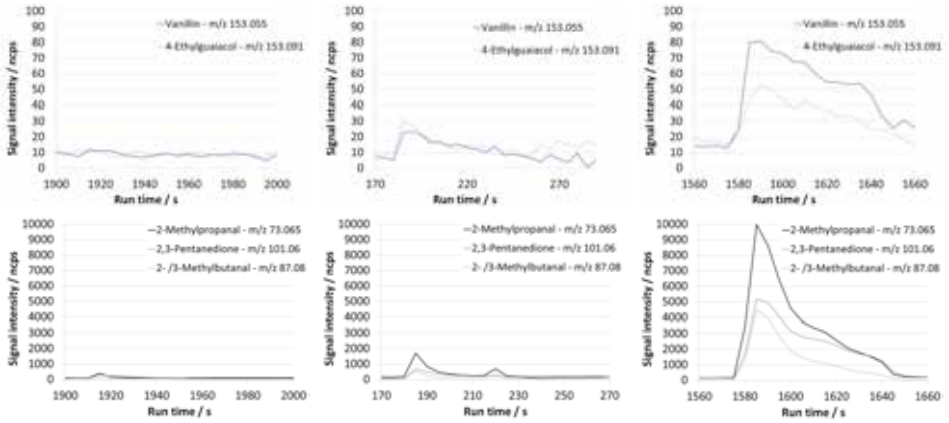

Figure 2: Comparison of signal intensities for three different sampling methods: Left panels: nose-space; Middle panels: BET mouth-space; Right panels: direct mouth-space

In summary the signal area of our new direct mouth-space sampling method were at least a factor of 5 higher, compared the indirect mouth-space sampling and a factor of at least 20 higher compared to nose-space sampling.

Discussion

The results from the comparison of the three different sampling methods have shown a massive improvement in signal intensity of the newly developed direct mouth-space sampling compared to the other two (conventional) sampling methods. The improvement in signal intensity can be directly linked to the fact that this approach uses a direct sampling of the volatiles present in the mouth-cavity instead of indirectly sampling them in the exhalation stream. It therefore enables to increase the sensitivity of the measurement under otherwise equal conditions such as time resolution and a given measurement setup. Thereby the dMoS method enables a dynamic measurement of volatiles during the aftertaste or lingering phase following the ingestion of food or drinks.

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Automated Measurement and Evaluation Software – An Approach for Enhanced Isomer Separation

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Abstract

We present a mathematical concept for separation of isomeric compounds. We exploit the altered product ion ratios of a compound when different reaction conditions are applied. While the principle is not new, we here present a structured and general approach, integrated in a software solution.

Introduction

With the introduction of PTR-TOFMS instruments, several shortcomings of the quadrupole based versions have been overcome. A TOF acquires a full spectrum containing several hundred compounds in a split second and the high mass resolution allows for the separation of isobaric compounds, due to their slight difference in exact mass. Isomers, compounds with identical chemical composition, have the same exact mass and are hence not separated. Several methods have been proposed to add further separation to distinguish isomers in PTR-MS, such as a recently introduced fastGC hardware add-on, see [1] and references therein. In this paper, we focus on strategies that can be implemented in software to separate isomers. This is possible by utilizing the different reaction products of a compound when reaction parameters are altered. One example is the use of different ion chemistry by switching regent ions (SRI) [2]. As an example, while in H_3O^+ ionization the reaction products of propanal and acetone are isomers, their products are on a different m/z when using NO^+ and can easily be distinguished. Another method is the ionization at different E/N settings. This changes the collisional energy and can promote or suppress fragmentation. Two isomers will likely differ in their fragmentation pattern [1], which can then be used to gauge their individual contributions. While the data interpretation of the above example with SRI is apparent when no other compounds are involved, the E/N example already gives an idea that data interpretation will involve calculations.

Methods

To introduce the math behind, we use a realistic example of two monoterpene isomers ($C_{10}H_{16}$), alpha-pinene (AP) and camphene (CA). Their signals overlap on their molecular ion at m137 [3] as well as on their main fragment at m81. The product ion ratios can be determined for each compound independently, e.g. in a head-space measurement using the pure compounds. At standard operating conditions [3] we have measured a fragmentation ratio (m137 / m81) for alpha-pinene to be 30% / 70% and for camphene to be 45% / 55%, respectively [1]. Knowing these ratios, the signal on m137 and m81 can be predicted.

m137	=	30%	AP	+	45%	CA
m81	=	70%	AP	+	55%	CA

Where AP and CA are the (unknown) contributions for alpha-pinene and camphene, respectively. This is a system of linear equations, that can also be written as

$$\begin{pmatrix} m137 \\ m81 \end{pmatrix} = \begin{pmatrix} 0.30 & 0.45 \\ 0.70 & 0.55 \end{pmatrix} \begin{pmatrix} AP \\ CA \end{pmatrix} \quad \text{or in a general form as} \quad m = A \cdot c$$

Having identified this as a system of linear equations, a range of mathematical tools becomes available. We exemplify some of those tools that are useful for the underlying problem. Most importantly, we get the solution to the system of linear equations: The matrix A is directly available, when the fragmentation is known and nearly every mathematical software can easily calculate its *inverse* (A^{-1}). By multiplying both sides with the inverse we get

$$c = A^{-1} \cdot m \quad \text{and, explicitly this is} \quad \begin{pmatrix} AP \\ CA \end{pmatrix} = \begin{pmatrix} -3.37 & 3.00 \\ 4.67 & -2.00 \end{pmatrix} \begin{pmatrix} m137 \\ m81 \end{pmatrix}$$

Now, the unknown contributions c can be calculated from the measured data on m137 and m81. To account for a compound dependent sensitivity, one more step is needed: we suggest to multiply the separated contributions by a calibration factor to convert them to calibrated concentrations. This also implies that to establish a separation it is not necessary to normalize the all product ion signals of a compound to a total sum 100%. Thus, measured signals from a head-space measurement of the single compound can be directly used to establish the mathematical separation. Once established, a calibration measurement can be performed, even with a mixed standard containing both compounds.

The inverse can only be calculated when the system of linear equations has exactly one solution. For this we need as many measured variables (m137, m81) as we have unknowns (AP, CA). Strictly, also the equations must be independent. The same example for three monoterpenes with only two measured variables cannot be solved. The solution is to add information by determining the fragmentation ratio and measure the sample at another E/N ratio. Now in principle measuring the variables m137_600V, m81_600V, as before, and additionally m137_400V, would suffice to calculate the concentrations of a mixture of three monoterpenes. Somewhat unfulfilling is the fact that the variable m81_400V is available but not used, since this would lead to an overdetermined system of linear equations, which has no longer a unique solution. In simple terms, with the excess supply of measured variables there are several ways to calculate one concentration, with slightly different results due to measurement noise. This can elegantly by overcome by an *ordinary least squares* approach, which allows using as many variables as available and still gives a simple solution:

$$c = (A^t \cdot A)^{-1} \cdot A^t \cdot m$$

The matrix $(A^t \cdot A)^{-1} \cdot A^t$, the so-called *pseudo inverse*, can be determined and then be used to calculate the unknown contributions c from the many more measured variables m. For an overdetermined system this solution gives the concentrations that best "fit" the measured data, with a minimum of quadratic deviations. In general this approach works not only for variables acquired at different E/N setting, but also by measurements with different pre-cursor ions, or changes in any other parameter, or combinations thereof. Moreover, "simple" compounds, e.g. compounds that do not fragment can be treated in the same matter.

Results and discussion

To implement this approach, IONICON Analytik has created an optional software for Automated Measurement and Evaluation (AME). This software allows defining different measurement steps with individual settings, such as different E/N. These steps are automatically executed for a predefined duration. It is possible to omit parts of the data to account for stabilization after a

change in settings, to average the remaining data, and to subtract measurements that are marked as background. After the completion of a full cycle the data of all steps can be used in a matrix to calculate concentrations, which are also displayed and automatically exported. For demonstration we measure a BTEX standard; an acronym for a standard mixture of Benzene, Toluene, Ethylbenzene, and Xylenes, which are common indicators of gasoline contamination. BTEX standards are widely used but pose a problem in PTR-MS, since ethylbenzene is an isomer to xylene and its fragment overlaps with benzene in PTR. As outlined above, the normal measurement of these three VOCs Benzene, Xylene and Ethylbenzene with only two m/z, m79 and m107, would not be possible. The toluene measurement is undisturbed at m/z 93 and is therefore not shown.

	Ethylbenzene	**Benzene**	**Xylene**
79_400V	0.055	0.247	0.000
107_400V	0.544	0.000	0.625
79_500V	0.111	0.575	0.000
107_500V	0.642	0.000	0.729
79_600V	0.614	0.840	0.000
107_600V	0.493	0.000	0.888

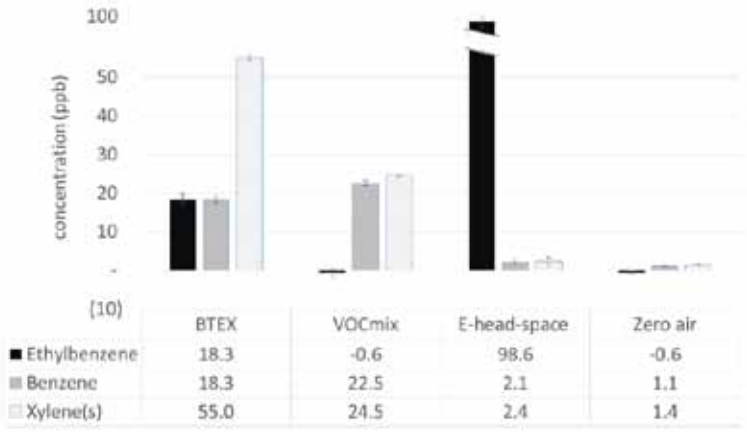
In the above table, we list the data for three E/N settings measured using a stable concentration of each compound. The system of linear equations (and consequently A) can be directly inferred. We use Octave 4.0.1 to calculate the pseudo inverse $(A^t \cdot A)^{-1} \cdot A^t$ for the solution. The numbers of the pseudo inverse (including the calibration factors, see below) are entered into the AME software.

	Calib. factor	**79 _400V**	**107 _400V**	**79 _500V**	**107 _500V**	**79 _600V**	**107 _600V**
Ethylbenzene	*1.42* ×	-0.682	0.770	-1.728	0.963	1.382	-1.332
Benzene	*1.04* ×	0.593	-0.416	1.457	-0.520	0.019	0.720
Xylene	*1.14* ×	0.497	-0.196	1.259	-0.276	-1.007	1.490

In the AME software, we have defined three measurement steps for 400V, 500V, and 600V, which are automatically repeated. We measure several samples of different composition to calibrate and check the residual cross-sensitivity of the method.

	BTEX diluted 1:5 to	**VOCmix diluted 1:50 to**	**E-head-space**
Ethylbenzene	18.3 ppb	0.0 ppb	high ppb
Benzene	18.3 ppb	19.4 ppb	0.0 ppb
Xylene(s)	55.0 ppb	20.0 ppb	0.0 ppb

BTEX is contained in the aromatic subset of the TO14 standard, which has been used to determine the *Calibration factors* for E, B and X. VOCmix is a 15 compound VOC standard containing B and X. This measurement is used to check the cross-sensitivity of B and X to E. E-head-space is sample where Ethylbenzene head-space has been injected into a clean bag to measure the cross-sensitivity to B and X. In addition, a zero-air measurement with nominally 0 ppb has been measured.



	BTEX	VOCmix	E-head-space	Zero air
Ethylbenzene	18.3	-0.6	98.6	-0.6
Benzene	18.3	22.5	2.1	1.1
Xylene(s)	55.0	24.5	2.4	1.4

Figure 1: Measured data (in ppb) from the measurement of different samples detailed above. Plotted are average values and their standard deviations.

In figure 1 we summarizes the results from the measurements performed with the described method. The absolute quantification is within the specifications of the two standards. We also see that cross-sensitivity, with < 5%, is sufficiently low in all cases. Slightly negative values can occur due to the calculations and inaccuracies in the measured fragmentation ratios. This leads directly to a limitation of this method. While, in normal measurements any aspect that leads to a change in the product ion ratios will affect the quantification of the respective compound, here it can also affect the results of compounds connected though the calculations. It is therefore paramount to ensure that product ions ratios are accurately determined and stay constant. Further investigation of the stability of the product ion ratios are necessary to determine the re-calibration intervals.

Conclusion

We have presented a mathematical method to combine all information provided by various measurement modes of a PTR-MS, which can be used to separate isomeric compounds. The implementation in software for automated measurement and data evaluation is one step further towards an autonomous monitoring solution with integrated advanced data evaluation.

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[3] To improve readability, we will refer to the mass-to-charge ratios as „m137“ instead of „m/z 137 Th“. Depending on the context, „m137“ can also refer to the signal measured on m/z 137 Th. Standard conditions were E/N 140 Td, labelled “_600V”, in reference to the drift voltage.

BVOC Fluxes from a Bioenergy Maize Plantations - From Flowering to Senescence

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Abstract

Germany, and other countries as well, intends to largely increase the plantation area for bioenergy production in the near future. Potential plant species that are likely to be intensively cultivated are corn, energy grasses, and woody crops, which might emit biogenic volatile organic compounds (BVOC) at higher rates than common agricultural species. Due to the reactivity of some BVOCs with oxidizing atmospheric compounds, future land-cover changes and the extension of planation area might affect air quality and the formation of secondary organic aerosols with potential consequences for the local climate.

In the current project, we measured the BVOC fluxes from maize plants on an agricultural site in Northeastern Germany using automated large chambers (5.625 m^3) and online detection of BVOCs by PTR-MS. BVOC fluxes were continuously followed for 7 weeks, from the flowering of the plants to the senescence stage. The highest fluxes were observed for acetaldehyde, ethanol, monoterpenes, and isoprene during grain development and ripening. After the ripening process mainly monoterpenes and isoprene still show significant emission rates.

Introduction

In order to fulfill its bioeconomic goals, Germany intents to increase the fraction of renewable energy sources up to 80% by 2050. Maize, together with other crops such as rapeseed, energy grasses or poplar and willow coppices, will thus be an important landscape component in the future. Currently, maize (*Zea mays*) is the most common bioenergy crop produced on approximately 2.3 Mio ha, 25 % of it used for bioenergy production. Due to the envisaged land cover changes, potential environmental issues might arise. Besides possible negative impacts on soils and water quality by intensive cultivation, air quality and local climate might be affected by emissions of biogenic volatile organic compounds (BVOCs). To assess the sustainability of the bioenergy production, it is imperative to evaluate the impact of BVOCs from large bioenergy plantation to local climate. However, the BVOC emission potentials of many agricultural crops, including maize, are still largely unknown. Despite few investigations [1,2], comprehensive analysis of BVOC emissions and their respective temporal variability are still unknown. In the present project, we studied BVOC fluxes and their phenological and environmental (i.e., light and temperature) controls from a maize field in northeastern Germany.

Experimental Methods

Site description of the maize field site and experimental set up of gas exchange analysis

The field experiment was conducted at the CarboZALF site in Dedelow, Brandenburg, northeastern Germany. The site is equipped with instruments to observe fluxes of CO_2, CH_4, and H_2O, meteorological parameters (temperature, radiation, wind, rel. humidity, atm. pressure) and soil moisture. Leaf area index (LAI) and biomass were determined at the beginning, middle, and end of the field experiment. Maize was seeded in May 2015 and harvested in September 2015. Measurements were performed from 24th July to 16th September (two days after harvesting). We used two transparent automatic polycarbonate chambers with a size of 1.5m x 1.5m x 2.5m each [3]. One fan per chamber ensured an homogeneous air mixing in the chamber headspace. In order to minimize the impact of the chambers on the grown of the plants and ensure ambient environmental conditions, chambers were kept open for 80% of the time (i.e. 48 min hr^{-1}). Quantification of BVOC fluxes were performed online using proton transfer reaction mass spectrometer (PTR-MS; Ionicon Analytik GmbH, Austria) [4] by measuring the linear changes in BVOC concentrations during the closure time of the chambers.

A 3/2 way PTFE valve of each automatic chamber switched between drawing air to the PTR-MS inlet when the chamber was closed (2 l min^{-1} near the PTR-MS inlet) and drawing air with a similar volume when the chamber was opened for flushing the tube. The PTR-MS was connected to the chambers with around 18 m ¼" PTFE tubes each and some T-pieces and fittings all in PTFE. To avoid condensations, the tubings were isolated and heated. Identification of BVOC emission pattern i.e. mono- and sesquiterpenes was achieved offline by collecting 8.4 l of air in glass tubes containing polydimethylsiloxane foam (Gerstel, Mülheim an der Ruhr, Germany) at a flow rate of 150 ml min^{-1} for 56 min. The sample tubes were sent to EUS (Germany) and stored at 0 °C prior to GC-MS analysis. The identification and quantification of different BVOCs was achieved by thermo-desorption (Gerstel) and Gas Chromatography MS (GC-MS; GC type: 7890A; MS type: 5975C; both from Agilent Technologies, Palo Alto, CA, USA), as described in [6].

Data Processing

We calculated the fluxes as the slope of a simple linear regression, fitted to the data points obtained during sampling from a closed chamber. By using the volume and base area of the chamber, the molar volume of the air within the headspace, the number of carbon atoms of the observed components, and the LAI, we scaled the emission rates to nmol C s^{-1} m^{-2} leaf area.

Results and Discussion

Upon closing the chamber, we observed increasing (emission) or decreasing (deposition) concentrations of many BVOC (e.g., Fig. 1 depict the increasing concentrations of sesquiterpenes and the diurnal emission pattern). The magnitude of this effect depends on the daily time. Highest emission rates were observed on sunny days around 1 PM (radiation optimum) for isoprene, monoterpenes, and sesquiterpenes, whereas no significant emission rates were observed during night time. When the chamber opened, the calculated emission rates were always zero.

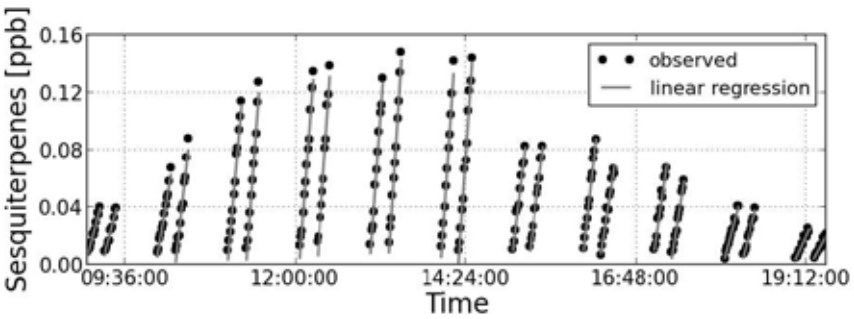
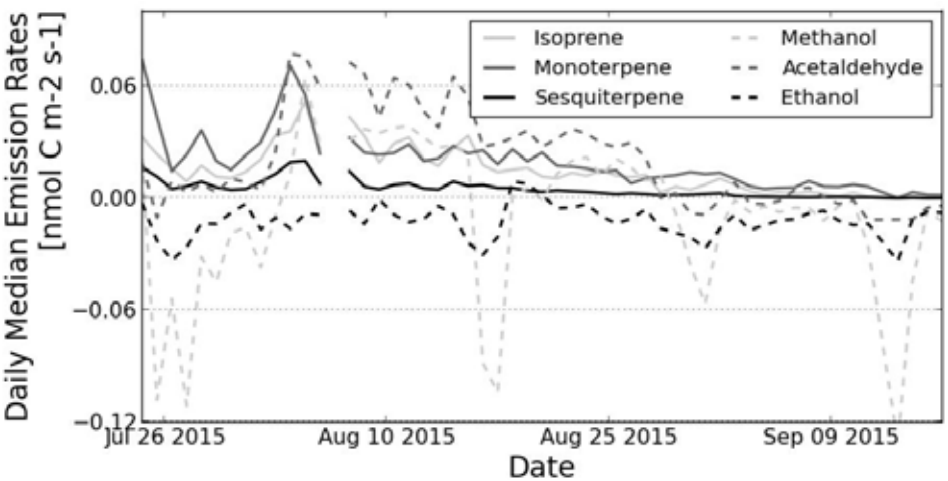


Figure 1: Example of increasing sesquiterpene concentrations during one day of measurement. Emissions originate from 21 maize plants within a transparent automatic chamber (V = 5.625 m3) on the 7th August 2015 in Dedelow (black dots). Linear regressions (gray lines) fitting to the observations were calculated to derive canopy emission rates.

Fig. 2 shows first results of daily (24h) median emission rates over the entire experimental period for six volatile compounds. Generally, methanol exhibits the largest variability with emissions up to 0.06 nmol C m^{-2} s^{-1} and periodically deposition fluxes with more than 0.1 nmol C m^{-2} s^{-1} for a few days. During the entire experimental period, ethanol showed deposition fluxes at around 0.02 nmol C m^{-2} s^{-1}. All components except ethanol show an initial increase in emission up to 0.06 nmol C m^{-2} s^{-1} at around beginning of August. This period is characterized by the beginning of the grain development. Subsequently, during the period of late fruit development and ripening, the emission rates decreased, with highest values for acetaldehyde, mean values for monoterpenes, isoprene, and methanol, and relatively low values for sesquiterpenes. These results differ from [1], where methanol shows highest emissions throughout the observed period but agree on the magnitude of emissions. After the end of fruit ripening, monoterpene emissions dominated over the other compounds.



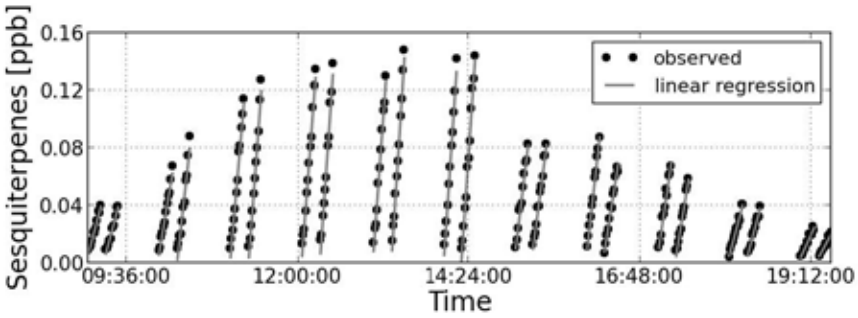


Figure 2: Daily median BVOC fluxes for isoprene (m/z 69, light gray line), monoterpenes (m/z 137, medium gray line), sesquiterpenes (m/z 205, black line), methanol (m/z 33, dashed light gray line), acetaldehyde (m/z 45, dash medium gray line), and ethanol (m/z 47, dashed black line).

Conclusion and Outlook

Here we demonstrate that the setup of our automatic whole canopy chamber system coupled to a PTR-MS instrument can be used for determining emission and deposition fluxes for a multitude of BVOCs. Highest emissions were found during grain development for acetaldehyde, methanol, monoterpenes, and isoprene. Generally, deposition was observed for ethanol throughout the entire measurement period. These preliminary results demonstrate that the fluxes of volatile compounds released from maize plants are significant and highly variable in time and strongly depended on plant development i.e. fruit ripening, much higher than we assumed previously. Compared to other biomass plants the BVOC fluxes of maize, particularly of volatile isoprenoids, are considerable smaller compared to high isoprene emitting woody plants such as poplar [5] and thus may have a lower potential impact on local air chemistry and local climate.

Further analysis will focus on the characterization of the composition and magnitude of BVOC emissions during distinct phenological stages of plant development as well as in relation to the meteorological conditions and soil properties. In 2016 and 2017, we will also extend our analysis to two additional bioenergy crops (rapeseed and energy grass) cultivated adjacent to the maize fields. In laboratory experiments we will additionally characterize the light and temperature dependencies of photosynthesis and BVOC emissions of all three species. The obtained data will be used for the parameterization and evaluation of a detailed ecosystem model. Using this ecosystem model implemented in a regional climate model, the impacts of BVOC emissions on air chemistry under different land use scenarios can be finally and properly examined.

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Application of PTR-MS for characterizing dimethyl sulfide photocatalytic oxidation: Kinetic and reaction pathway study

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Abstract

This study investigated the photocatalytic oxidation of dimethyl sulfide (DMS) in a photocatalytic reactor at low concentrations and short residence time (0.19 s). The removal of DMS in the reactor was assessed at five different concentrations (20 - 130 ppbv) and at nine UV lamp configurations (3.8 - 12.7 mW cm^{-2}). The gas concentration at inlet and outlet was monitored by an online Proton-Transfer-Reaction Mass Spectrometry (PTR-MS). The results showed that more than 70% of DMS was removed at different conditions. The data were fitted well with the Langmuir-Hinshelwood kinetic model ($R^2 > 0.99$). PTR-MS is for the first time demonstrated to be useful for determining Langmuir-Hinshelwood parameters for the highly volatile compound with low adsorption. Furthermore, PTR-MS was used to identify the by-products with the assistance of GC/MS (gas chromatography and mass spectrometry), and their formations was traced by adjusting initial odorant concentrations. Dimethyl sulfoxide, dimethyl sulfone, methanethiol, formic acid were identified by both PTR-MS and GC/MS, and m/z 31, 33, 77, 79, 95, 111 and 127 were also found as by-products by PTR-MS. Therefore, a plausible photocatalytic oxidation mechanism of DMS is proposed based on the by-products detected and possible intermediates formed.

Introduction

Dimethyl sulfide (DMS) is mostly emitted from livestock facilities and some industrial areas such as chemical plants, oil refineries and sewage treatments [1, 2], and may cause serious environmental and health concerns because of its low odor threshold values [3]. Large efforts have been put into developing abatement solutions for sulfur compounds over the past few decades, and photocatalytic oxidation (PCO) is a promising technology for air purification [4, 5]. Online proton-transfer-reaction mass spectrometry (PTR-MS) is a promising online method with high precision (due to high sensitivity and the lack of offline sampling) and with the additional advantages of high sampling frequency. PTR-MS has been proved to be a useful tool for time-resolved quantitative measurement of emissions of a wide range of volatile organic compounds, including odorants [1]. Therefore, degradation kinetics can be precisely determined based on measurement of online PTR-MS and the by-products from the reactor could be identified when initial concentration was low. The study is focused on 1) investigating the effect of initial concentration, UV intensity on the PCO of DMS in a honeycomb monolith photocatalytic reactor under realistic conditions (low initial concentrations and short residence time); 2) deriving degradation kinetics constants of DMS under different driving conditions based on Langmuir-Hinshelwood model; 3) identifying the PCO by-products of DMS using both online PTR-MS and GC/MS.

Experimental Methods

A scheme of the experimental setup is shown in *Figure 1*. Mainly, the reactor consists of one TiO_2 (Anatase) coated ceramic filter (Hokuei, Japan) and ten 9-W blacklight blue UV lamps (emission maximum at 368 nm, UV output of 1.4 W, FPX9BLB, Sankyo, Japan) parallel on both sides of the filter with a distance of 2 cm to the filter. A speed controllable exhaust fan (RS160, Ruck, Germany) was installed at the end of the outlet to provide a stable air stream (air flow rate - 8.7 m^3 h^{-1}, residence time - 0.19 s). DMS (AGA A/S, Denmark) was added into the air stream and concentration was controlled between 20 and 160 ppbv.

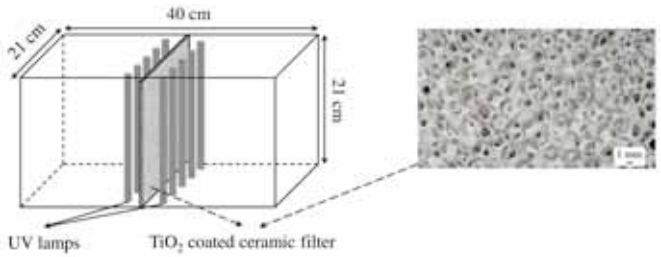

Figure 1: Overview of the photocatalytic reactor and the TiO_2 coated ceramic filter

All the experiments were carried out at room temperature (24.0 ± 0.6 °C) with the relative humidity of 35 - 45%. The concentrations of gases were continuously measured with a High-Sensitivity PTR-MS (Ionicon Analytik, Innsbruck, Austria) equipped with a quadrupole mass filter. The PTR-MS was operated under standard ion drift tube conditions. For assessing the effect of the photocatalytic reactor, the measurements were carried out in multiple ion detection (MID) mode (mass-to-charge ratio (m/z) 63 for DMS), and the concentration of DMS was calculated based on the calculated proton transfer rate constant (2.11×10^9) and on the calibration with a standard [1]. For identifying by-products, a full-scan between m/z 21-150 was performed afterwards by PTR-MS, and the gas samples were also simultaneously collected into thermal desorption tubes for GC/MS analysis. The UV intensity was measured by a UV light meter for UVA/UVB measurement (Mode 5085, PeakTech, Germany) and the UV intensity was set between 12.7 and 3.8 mW cm^{-2} by turning on different numbers of UV lamps.

Results and Discussion

Figure 2 presents the performance of DMS removal in PCO reactor. *Figure 2-A* shows the PTR-MS measurement of DMS concentrations at inlet and outlet when the UV intensity was 12.7 mW cm^{-2}, and *Figure 2-B* was the removal efficiency of DMS at different UV intensities when the lowest and highest concentrations were applied. More than 70% of DMS could be effectively removed, and the maximal difference of removal efficiency at the same UV intensity but different initial concentrations was only 6.7%. Therefore, the UV intensity did not appear to be limiting for the removal of DMS in this study.

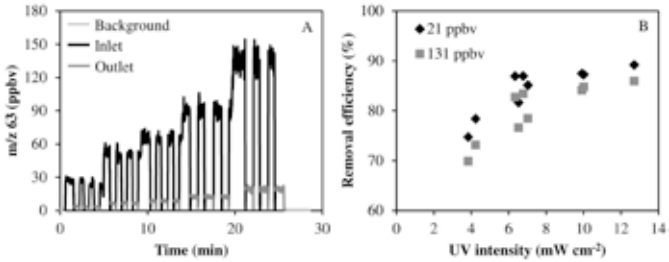

Figure 2: PTR-MS measurements of dimethyl sulfide (A - Concentration at different locations, UV intensity - 12.7 mW cm^{-2};B - Removal efficiency at different UV intensities)

The Langmuir-Hinshelwood (L-H) kinetic model (*Equation 1*) is mostly used to characterize the flow reactor at steady state [6], and the model could be reorganized and integrated to *Equation 2*. Furthermore, based on the measurements at inlet and outlet, a linear correlation was obtained by plotting $(\ln(C_i/C_o)/(C_i-C_o))$ versus $\tau/(C_i-C_o)$ and the two constants, Langmuir adsorption constant K and reaction rate constant k were determined from the intercept and the slope (*Figure 3*).

$$r = \frac{kKC}{1+KC} \qquad \textit{Equation 1}$$

$$\frac{\ln(C_i/C_o)}{C_i - C_o} = kK \quad \frac{\tau}{C_i - C_o} - K \qquad \textit{Equation 2}$$

where r (mmol m^{-3} s^{-1}) is the reaction rate, K (m^3 mmol^{-1}) is the Langmuir adsorption constant, C (mmol m^{-3}) is the concentration of target compounds near the catalyst surface, k (mmol m^{-3}s^{-1}) is the reaction rate constant, τ (s) is the residence time, and C_i and C_o (mmol m^{-3}) are the concentrations of inlet and outlet at the steady-state.

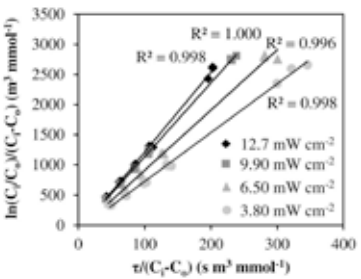

Figure 3: Example of Langmuir-Hinshelwood kinetic model

Five different initial concentrations of DMS were applied in the PCO reactor and the concentrations of the by-products from the outlet were expected to be increased with the increase of the initial concentration. In this way, it is possible to eliminate the interference from ambient air when identifying the by-products. *Figure 4* shows the relation between removed DMS and the production of the by-products measured by PTR-MS.

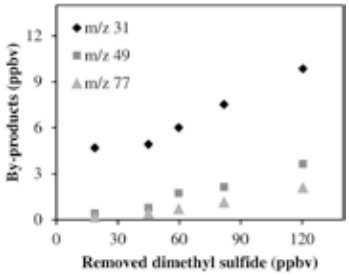

Figure 4: Relations between removed dimethyl sulfide and the production of by-products measured by PTR-MS at different initial concentrations

A similar approach was used for analysing the GC/MS results, as only compounds, for which the integrated area at the same retention time increased with the increase of initial concentrations, were considered as by-products. Table 1 shows the summary of the identified by-products from PTR-MS coupled with GC/MS.

Table 1: Mass-to-charge ratio of possible by-products

Mass-to-charge ratio	*possible by-products*
31	Formaldehyde
33	Methanol
47	Formic acid (confirmed by GC/MS)
49	Methanethiol (confirmed by GC/MS)
77	S-methyl thioformate
79	Dimethyl sulfoxide (confirmed by GC/MS)
	Methylthiomethanol
95	Dimethyl sulfone (confirmed by GC/MS)
111	S-methyl methanethiosulfinate
127	S-methyl methanethiosulfonate

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High throughput analysis of volatile compound release from single beans to investigate coffee origin and roasting

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Abstract

In this study, an experimental protocol was developed based on proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) for the rapid analysis of volatile compounds released by single coffee beans at different stages of roasting. The headspace volatile profiles of roasted single coffee beans (*Coffeea arabica*) from different geographical origins were monitored offline and the differences in aroma formations were investigated [1]. Results are in agreement with previous findings for commercial monoorigin coffees.

Introduction

Aroma is one of the most important attributes to evaluate coffee quality. Green coffee beans are naturally poor in aroma and have no desirable flavor. When roasted, the non-odorous green coffee components are transformed into a highly aromatic mixture that contains hundreds of volatile organic compounds (VOCs) with diverse odor properties. From a technological point of view, industrial roasting requires processing very large amount of coffee beans, however to have a deeper understanding of aroma production in the smallest element of coffee roasting, one should have a closer look into VOC formation at individual coffee bean level. This study represents, a practical application of PTR-ToF-MS to monitor the VOC formation in single coffee beans during roasting by means of offline headspace profiling which provides a comparison of different coffee origins.

Experimental

Green coffee beans (100% *Coffeea arabica*) from Brasil (2 batches), Guatemala (2 batches) and Ethiopia (2 batches) were roasted at 190°C by using a laboratory scale oven with ventilation. One green coffee bean was put in an open glass vial (22 ml). Open vials containing the green coffee beans were then placed on the metal oven tray with grids in 3 rows and 6 cloumns (3 coffee origins x 3 replicates x 2 batches = 18 coffee beans). At every 1 minute a new set of 18 coffee beans were prepared and the coffee beans were roasted up to 25 min. This resulted in preparation of a large sample-set of 468 coffee beans (3 origins x 2 batches x 3 replicates x 26 time points).

After roasting of all coffee beans were completed, the vials were cooled down to room temperature and closed. Weight losses (%) were calculated for each roasted coffee bean by measuring the bean weight before and after roasting. The headspace volatile compounds of roasted coffee beans were analyzed with a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) in an automated manner by using a multipurpose autosampler according to the methodology described in [2] by appying the same PTR-ToF-MS conditions.

Results and Discussion

Roasted coffee beans showed similar weight loss (%) curves over time however according to one-way ANOVA (p<0.05) results, significant changes were observed in weight losses (%) over the time between different coffee origins. Two different regions were observed in the weight loss curves which can be described with a primary linear phase (the first 14 min) and a plateau region (after 14th min). Plotting mass peak concentrations against time allowed visualization of VOCs with different release behaviours and as well as the different VOCs evolutions among different coffee origins. Significant increases were observed in the concentrations of heat induced volatile compounds. Depending on the phase of roasting (described above), some volatile compounds revealed earlier release (*i.e.* methyl pyrrole at *m/z* 82.065 around 6th min); at the same time some volatile compounds were released later *(i.e.* pentanedione at *m/z* 101.060 around 10th min). We also observed clear origin signatures, which are in agreement with previous findings [2, 3], in particular with higher release of monoterpenes detected at *m/z* 137.135 in Ethiopian coffee.

In conclusion, direct, fast and rapid volatile detection with PTR-ToF-MS allowed the aroma profiling of single coffee beans and gave an insight into volatile compound formation. Since the formation pathways of the volatile compounds in coffee are quite diverse and need different activation energies; the highest increase exhibited by volatile compounds may differ depending on the process conditions and the composition of green beans which is highly affected by the geographical origin (reflecting genetics, agronomical practices and post-harvest processing).

Acknowledgements

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OH reactivity at a Mediterranean coastal site

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Abstract

Total OH reactivity, the total first order loss rate of OH in ambient air directly provides the total reactive gases loading in air. We measured the total OH reactivity at a coastal receptor site located in the western Mediterranean basin during summer 2013. Here we examine the reactive gases budget by comparing the measured total OH reactivity with the OH reactivity calculated from the measured reactive gases. The measured total OH reactivity at the site varied between the instrumental LoD (3 s^{-1}) to a maximum of 17±6 s^{-1} and was on average 5±4 s^{-1}. During the period between 23-30/07 we observed a significant fraction of missing OH reactivity (56% on average). A set of tools, as air masses origins and trace gases concentration were helpful to demonstrate the influence that secondary originated products have on the missing OH reactivity. Box model simulations considering the oxidation of the primary biogenic compounds helped identifying the classes of unmeasured reactive species.

Introduction

Photo-oxidation reactions are the most efficient cleansing process occurring in the atmosphere, and constitute an important sink for Volatile Organic Compounds and other reactive atmospheric gases. Most of these reactions are initiated by the ·OH radical. Therefore, total OH reactivity, the first order rate loss of OH in the atmosphere, represents a top down measure of all reactive molecules existing in ambient air. Comparing the total OH sink to the sink with OH of the identified molecules permits determining the level of knowledge of the reactive chemical composition of air. The missing OH reactivity, the fraction of OH reactivity not explained by simultaneous measurements of reactive gases, has been associated so far to unmeasured compounds either primary emitted, either secondary generated, or both (Di Carlo et al., 2004, Noelscher et al., 2012a, Kim et al., 2011).

The Mediterranean basin is characterized by strong local anthropogenic and biogenic emissions and enhanced photochemistry due to the high temperature and intense solar radiation during summertime. Additionally, during summertime, the weather system is dominated from west by the Azores anticyclone and from east by a low pressure monsoon system. In this way, the average air flow is driven from north to south, exposing the basin to air masses coming from European

mega cities and industrialized areas. Reactive locally emitted and transported gases result in a very complex chemistry.

Climate model predictions indicate that the Mediterranean will face unique impacts of climate change. Results show that the region will face higher temperatures and extended drought stress periods, which will affect the strength and type of emissions further impacting air quality and climate (Giorgi and Lionello, 2008). Besides this, it is proved that the Mediterranean lacks of observations, and joint international efforts are needed (Mellouki and Ravishankara, 2007).

In this context, we measured the total OH reactivity at a receptor coastal site in the Mediterranean basin during summer 2013 within the ChArMEx project (Chemistry and Aerosols in a Mediterranean Experiment). Total OH reactivity was measured with the Comparative Reactivity Method during 16/07/2013-05/08/2013 at the monitoring station of Ersa, in the northern cape of the island Corsica, France. The field site was chosen for being: (i) free from local anthropogenic pollutants; (ii) exposed to air masses of different origin, including air masses enriched in processed compounds transported from continental areas. Total OH reactivity was then compared with the OH reactivity calculated from the concentration of the measured chemical species to examine the reactive gases budget at the site.

Experimental Methods

We carried out measurements of total OH reactivity using a Comparative Reactivity Method instrument assembled in our laboratory (CRM-LSCE from Laboratoire des Sciences du Climat et de l' Environnement, see Zannoni et al., (2015)). In brief, the Comparative Reactivity Method (CRM) is based on the concept of producing a competition for in-situ generated OH radicals, between a reactive reference compound, in our case pyrrole (C_4H_5N), and ambient reactive gases (Sinha et al., 2008). This is reproduced by introducing a known amount of pyrrole diluted in zero air and N_2 in a flow reactor coupled to a Proton Transfer Reaction-Mass Spectrometer (PTR-MS). Pyrrole is chosen as reference compound for its well characterized kinetics of reaction with OH (Dillon et al., 2012), for not being present in the atmosphere at normal conditions, and for being detectable at the protonated *m/z* 68 ($C_4H_5NH^+$) with a PTR-MS without any interference. A Proton Transfer Reaction-Mass Spectrometer is the detector of choice for its real-time measurements capabilities and robustness over time (Nölscher et al., 2012).

A usual experiment with a CRM includes in order: monitoring of C0 wet/dry, followed by C1 dry, C2 wet, and C3 ambient. With C0, C1, C2, C3 being in order the concentration of pyrrole detected with the PTR-MS: after injection (C0), when photolysis reaches the equilibrium (C1), after reaction with OH (C2), when ambient air is injected and the competition for OH radicals can start (C3). Alternated switches between C2 (background pyrrole in zero air) and C3 (pyrrole in ambient air) result in pyrrole signal modulations which are used to derive total OH reactivity values from the following equation:

$$R_{air} = \frac{(C3 - C2)}{(C1 - C3)} \cdot k_{pyrrole+OH} \cdot C1$$

With $k_{pyrrole+OH}$ being the rate constant of reaction between pyrrole and OH= $(1.20\pm0.16)\times10^{-10}$ cm^3 $molecule^{-1}$ s^{-1} (Dillon et al., 2012).

A set of additional instruments were deployed on the field for measuring the concentration of reactive gases: a Proton transfer reaction time of flight mass spectrometer, a gas chromatography flame ionization detector, a gas chromatography coupled to mass spectrometry, Hantzsch method analyser and NO_x analyser. A total of 69 compounds were measured, inorganic and organic molecules, among the latter also anthropogenic, biogenic and oxygenated volatiles.

Results

The total OH reactivity measured over three weeks at the site (16/07/2013- 05/08/2013) was maximum 17±6 s^{-1} and was on average 5±4 s^{-1}. It had a characteristic diurnal profile and increased when also temperature was higher.

We examined the contribution to the OH reactivity given by the measured compounds. The largest contribution to the OH reactivity came from those compounds with biogenic origin, although only 7 species were monitored from the total 69 measured compounds. Among such species, a monoterpene, i.e. a-terpinene and isoprene were dominant.

The comparison between total measured and calculated OH reactivity highlighted periods when the air masses composition was fully understood and others where a fraction of reactive compounds was not measured (i.e. missing OH reactivity). Indeed, when the site was receiving air masses from more polluted areas as the south of France and the North of Italy, hence more characterized by anthropogenic emissions, no missing reactivity was reported. In contrast, when the air masses were coming from other sectors, or local drivers as temperature and radiation were influencing the chemistry largest missing OH reactivity was observed (56% on average). We identified that both primary biogenic molecules and secondary molecules generated from the oxidation of biogenic species could have caused this missing OH reactivity.

Discussion

The total OH reactivity measured during summer 2013 at a receptor site located in the northern cape of Corsica varied from the LoD of our instrument to 17±6 s^{-1}. This is a high value of reactivity if we consider that the site was chosen for being a receptor site. Indeed, such value is comparable in magnitude to the reactivity reported from boreal and temperate ecosystems, whereas in such studies the measurements were collected inside a point source of reactive gases. Comparisons with the calculated reactivity show that compounds with biogenic origin had the largest impact in terms of reactivity at the site. A missing fraction of OH reactivity up to 56 % was reported in a period characterized by higher temperature and large BVOCs emissions. Preliminary results from a 0 D chemical model focusing on the oxidation of isoprene, limonene and a-pinene highlighted the classes of compounds that might have caused this missing reactivity fraction.

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Adding complexity to in-vivo measurements: Impact of protocol, type of aroma compound and matrix phase (gas or liquid) in flavour persistence during consumption

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Introduction

Perception of flavor persists after swallowing the food product. This effect commonly known as after taste (or finishing) is a key element in sensory evaluation and depends mainly on the physicochemical characteristics of the volatiles, the food matrix and human physiology. To reduce the impact of food matrix, some authors have studied persistence of aroma compounds in breath using aqueous solutions (1). They found longer persistence for those compounds with low $K_{a/w}$ values (2) and low vapor pressure (3) but interestingly, the volume or concentration of the sample had no effect on persistence. In this study, we have studied persistence in gas systems, to completely eliminate interactions with the food product, and compared these with liquid systems where volatiles were administrated in an aqueous solution as well as in an oil in water emulsion. In the particular case of the gas phase systems, the aroma cocktail containing several compounds with different physicochemical characteristics was prepared and volatilized in a Tedlar bag. A defined amount of this aromatized air was then injected in either the nose or the mouth of a panelist and the exhaled volatiles were monitored by PTR-MS. In both, liquid and gas-phase systems four different breathing protocols were designed (Figure 1) and tested on five different panelists. Real-time measurements were converted into standardized breath profiles by preprocessing, and 10 kinetic parameters were derived. Next to this, panel performance was evaluated per experimental protocol and food product. Comprehensive information about panel performance, individual panelists, studied products and systems, aroma compounds, kinetic parameters and persistence-curves were fitted.

Experimental Methods

In the gas-phase study an aroma cocktail containing several compounds with different physicochemical characteristics was prepared. Some droplets were introduced in a Tedlar bag full of compressed air and vaporized at 60°C for 3 hours. 500 mL of the aromatized air were administered by a gas thigh syringe to 5 different panelists by 4 different breathing protocols. Some droplets were introduced in a Tedlar bag full of compressed air and vaporized at 60°C for 3 hours. 500 mL of the aromatized air were administered by a gas thigh syringe to 5 different panelists by 4 different breathing protocols.

Four protocols were selected (figure 1). M1: volatiles were injected in the mouth and inhaled, then air was exhaled by the mouth and inhaled by the nose for each breath; M2: volatiles were injected in mouth and then air was both exhaled and inhaled by the nose while mouth was completely closed; N1: volatiles were injected in the nose and then air was exhaled by the nose

and inhaled by mouth; N2: volatiles were injected in the nose and then air was both inhaled and exhaled by the mouth while nose was closed with a clip. For all protocols, the breath tempo was defined as 3 seconds inhalation and 3 seconds exhalation and was marked with a metronome during the experiments. Each breathing protocol was performed in triplicate for each of the 5 panelists.
Exhaled volatiles were sampled from nose via commercial N.A.S.E (Ionicon Analitik GmbH, Austria). For mouth sampling, a custom build setup heated at 90°C was used. Volatile analysis was performed with a commercial PTR-MS (Ionicon Analitik GmbH, Austria).

The used liquid phase in this study was milk and water and was spiked with an aroma cocktail at adequate level. This aroma cocktail containing several compounds with different physicochemical characteristics and was prepared once. The spiked milk and water solutions were prepared fresh daily.
Results were obtained using four breath sampling protocols with two spiked tasting solutions; milk and aqueous solution. Measurements of nasally exhaled breath are made with a high sensitive PTR-QMS. The method of breath sampling uses the commercial N.A.S.E (Ionicon Analitik GmbH, Austria). The first three protocols investigate the effect of breathing control on measured aroma release reproducibility: a free protocol is used as a baseline; a further protocol fixes the swallowing time; a third protocol fixes the swallowing time and the breathing rate. The fourth protocol investigates the effect of multiple swallowing on total aroma release. A study of eight subjects, repeating each protocol five times for both solutions produced a large data set, which was analyzed using multivariate techniques and a data pre-processing strategy. (4)

Results

The extent of retro nasal aroma release is a physiological feature that can be individually characterized. Subject differences in oral processing parameters, such as salivary flow rate, nasal anatomy, bite size, and eating speed are (partly) responsible for this. Bite size and duration of oral processing can be controlled in an experimental protocol by standardized amount of product taken, fixed time of mastication before the first swallow and secondary swallows. This was the case in our studies, in gas and in liquid systems, in which 4 levels of protocol controls were compared. As expected and shown in these studies, the panel is heterogeneous in relation to the experimental protocol. Each panelist has different levels of individual repeatability dependent of experimental protocol and considered kinetic parameters. The latter are grouped and correspond mainly to two independent aspects of aroma release: initial release of volatiles including first swallow (AUC_{total}, $Area_{max}$, C_{max}) and the persistence of the volatiles in breath ($T_{+/-20}$ and T_{max}). This is in agreement with the findings of Hodgson et al (2). Additionally, it gives a new insight into the relationship between kinetic parameters and the differences observed for different protocols and panelists.

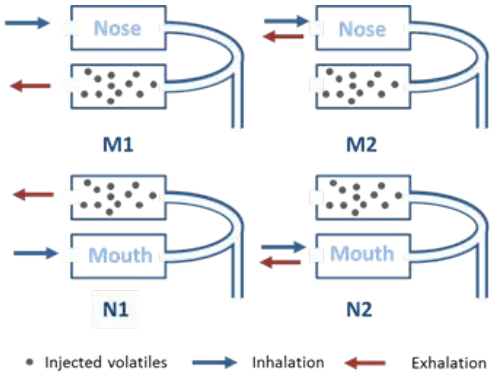

Figure 1: Breathing manouvers in gas-phase systems.

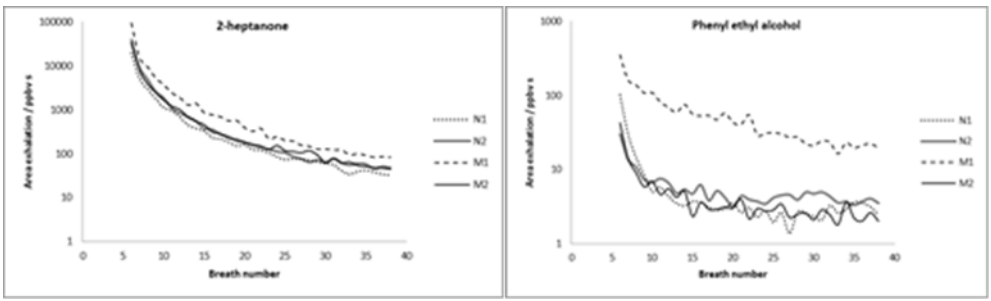

Figure 2: Differences between manuevers for two compounds admnistrated in the gas-phase.

Discussion

In this study, we have used volatiles in the gas phase to completely eliminate interactions with the food product and compared it to two common food systems, aqueous and oil in water emulsion. The results showed that persistence was compound dependent and differences among compounds were always larger than differences between panelists independently of the breathing protocol and if in gas or in liquid systems. The longest persistence was measured when volatiles were injected in the mouth. These results indicate that the interaction between aroma compounds and oral mucosa is higher than with nasal mucosa adding more complexity to oral food processing. Volatiles with lower K_{aw} were more persistent in the mouth that in the nasal cavity, suggesting that the higher water content of the oral cavity is responsible of the increased persistence.

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Recent insights from PTR-ToF-MS deployments in NASA airborne studies

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Abstract

A series of NASA's recent airborne studies have seen the deployment of innovative PTR-ToF-MS instruments.

In 2013 and 2014, a compact PTR-ToF-MS instrument [1] was installed onboard the NASA P3B Airborne Laboratory during three DISCOVER-AQ missions (http://discover-aq.larc.nasa.gov/) in California, Texas and Colorado to study the vertical and horizontal distribution of air pollutants. In one occasion, a plume emanating from a small forest understory fire (Figure 1) was studied in unprecedented detail at a spatio-temporal resolution of 10 m or 0.1 sec. The plume was sampled at its origin for deriving emission factors and followed ~13.6 km downwind for observing chemical changes during the first hour of atmospheric aging. Downwind plume chemistry was investigated using the observations and a 0-D photochemical box model simulation based on a near-explicit chemical mechanism (MCM v3.3) [2].

Figure 1: NASA P-3B front camera frame showing the forest understory fire and the emanating biomass burning plume.

In 2015, a high-sensitivity PTR-ToF-MS (Ionicon Analytik GmbH, Innsbruck, Austria) instrument was deployed onboard a NASA C-130 aircraft in the frame of the *North Atlantic Aerosols and Marine Ecosystems Study* (NAAMES, http://naames.larc.nasa.gov/) to measure

organic trace gases in the atmosphere over the Northern Atlantic. This instrument was equipped with a hexapole ion extraction unit to improve its sensitivity (up to 700 cps/ppbv) and its mass resolving power (up to 3000). 1-second detection limits down to 15 pptV made the detection of low-level organic traces in the remote atmosphere possible.

This presentation will summarize our recent contributions to NASA's DISCOVER-AQ and NAAMES missions and show the performance of state-of-the-art airborne PTR-ToF-MS instruments.

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