

Investigation of Tobacco Pyrolysis Gases and Puff-by-puff Resolved Cigarette Smoke by Single Photon Ionisation (SPI) – Time-of-flight Mass Spectrometry (TOFMS)*

by

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SUMMARY

The work presented deals with the application of Single Photon Ionisation – Time-of-Flight Mass Spectrometry (SPI-TOFMS) for the investigation of tobacco smoke. SPI-TOFMS is a modern analytical technique, which enables the simultaneous analysis of a large number of organic species in complex gas mixtures in real time. The paper is a summary of a PhD thesis (1) and seven research articles, which were recently published in various scientific journals (2-8). Consequently, more detailed information on particular aspects can be found in there.

The experimental part covers two different approaches, and therefore, it is divided into two sub-sections. In the first one, the SPI-TOFMS is coupled to a pyrolysis furnace. The objective is to examine the thermal behaviour of tobacco under various controlled conditions. In so doing, three tobacco types (Virginia, Oriental, and Burley) were pyrolysed in two reaction gas compositions (nitrogen and synthetic air) and seven different furnace temperatures (400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C, and 1000 °C). Results can help to unravel the complex formation and decomposition reactions taking place when tobacco is heated. In the second part the SPI-TOFMS is connected to a cigarette smoking machine in order to investigate the behaviour of cigarette smoke constituents on a puff-by-puff basis. The work incorporates the comparison of whole smoke and gas phase of cigarette smoke, a puff-resolved quantification of several hazardous smoke constituents, and the overall chemical characterisation of the individual smoking puffs. In addition, a critical consideration of the prevailing smoking procedure is given when applied to single puff analysis. A further study examines the influence of five different cigarette lighting devices (gas lighter, electric lighter, candle, match, and burning zone of another cigarette) on the chemical composition of the first puff. [Beitr. Tabakforsch. Int. 23 (2009) 203–226]

ZUSAMMENFASSUNG

Die hier vorgestellte Arbeit behandelt die Anwendung der Einzelphotonen-Ionisation (engl.: Single Photon Ionisation, SPI) – Flugzeitmassenspektrometrie (engl.: time-of-flight mass spectrometry, TOFMS) zur Untersuchung organischer Komponenten in Tabakrauch. SPI-TOFMS ist eine Echtzeit-Messmethode, die eine gleichzeitige Untersuchung einer Vielzahl von organischen Spezies in komplexen Gasmischungen ermöglicht. Der Artikel ist eine Zusammenfassung einer Dissertation sowie sieben damit verbundenen Publikationen, welche in den letzten Jahren in verschiedenen wissenschaftlichen Fachzeitschriften veröffentlicht wurden. Detailliertere Informationen hinsichtlich einzelner Aspekte können daher in den zugrunde liegenden Artikeln bzw. der Dissertationsschrift gefunden werden.

Grundsätzlich werden zwei Ansätze vorgestellt. Im ersten Teil wird die Kopplung des SPI-TOFMS an einen Pyrolyseofen beschrieben. Zweck dieses Aufbaus ist die Untersuchung des thermischen Verhaltes von Tabak bei verschiedenen kontrollierten Bedingungen. Im Rahmen dieser Studie wurden drei Tabaksorten (Virginia, Burley und Orienttabak) in zwei Reaktionsgasen (Stickstoff und synthetische Luft) und sieben verschiedenen Ofentemperaturen (400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C und 1000 °C) pyrolysiert und charakterisiert. Die erzielten Ergebnisse können behilflich sein um die komplexen Bildungs- und Abbaumechanismen von Substanzen aufzuklären, die bei der thermischen Zersetzung von Tabak vorkommen.

Der zweite Teil behandelt die Kopplung des SPI-TOFMS an eine Rauchmaschine zur zugaufgelösten Messung von Rauchinhaltsstoffen. Der Fokus liegt dabei auf folgenden Teilaspekten: Gegenüberstellung von Gesamtrauch und Gasphase, zugaufgelöste Quantifizierung ausgewählter Schadstoffe und eine allgemeine chemische Charakterisierung der einzelnen Zigarettenzüge. Des Weiteren wird

die Anwendung der vorherrschenden Rauchprotokolle zur Analyse ganzer Zigaretten auf die Messung einzelner Zigarettenzüge diskutiert. In einer weiteren Studie wird der Einfluß von fünf verschiedenen Zigarettenanzündemethoden (Gasfeuerzeug, elektrischer Anzünder, Kerze, Zündholz und Glut einer anderen Zigarette) auf die chemische Zusammensetzung des ersten Zigarettenzugs untersucht. [Beitr. Tabakforsch. Int. 23 (2009) 203–226]

RESUME

Cette étude présente l'application de l'ionisation de photons singuliers (SPI) couplée à la spectrométrie de masse à temps de vol (TOFMS) pour l'examen de la fumée de tabac. Le système SPI-TOFMS est une technique analytique moderne, permettant l'analyse simultanée d'un grand nombre de composants organiques dans des mélanges complexes de gaz en temps réel. L'étude est un résumé d'une thèse de doctorat (1) et sept essais scientifiques publiés récemment dans divers journaux scientifiques (2–8). Ainsi, plus d'informations détaillées peuvent être trouvées dans cette littérature.

Dans ce travail, deux approches sont présentées et ainsi la partie expérimentale est divisée dans deux sections. Dans la première section la SPI-TOFMS est couplée à un four pyrolytique. Le but est d'examiner le comportement thermique de la fumée de tabac sous diverses conditions contrôlées. Ainsi trois types de tabac (Virginie, Oriental et Burley) ont été pyrolysés dans deux atmosphères gazeuses (azotes et l'air synthétique) et sous sept températures différentes de four (400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C, et 1000 °C). Les résultats obtenus peuvent aider à démontrer les réactions complexes de formations et de décomposition surgissant quand le tabac est chauffé. Dans la deuxième section le système SPI-TOFMS est connecté à une machine à fumer pour examiner le comportement des composants de la fumée de cigarette bouffée par bouffée. L'étude comporte la comparaison de la fumée complète avec la phase gazeuse de la fumée de cigarette, la quantification par bouffée de plusieurs composants dangereux de la fumée et la caractérisation globale de bouffées individuelles. En plus, une considération critique de la procédure de fumage dominante est donnée dans le cas de l'application à l'analyse d'une seule bouffée. Une étude supplémentaire examine l'influence de cinq différents dispositifs d'allumage (à gaz, électrique, chandelle, allumette, et zone brûlante d'une autre cigarette) sur la composition chimique de la première bouffée. [Beitr. Tabakforsch. Int. 23 (2009) 203–226]

INTRODUCTION

In tobacco smoke more than 4,800 components have been identified so far (9). Advances in analytical techniques let assume that more than 10,000 substances are present (10), whereas estimations of the number of unidentified species have been as high as 100,000 (11).

In general tobacco smoke is composed of a gas phase and a particulate phase whereas many substances are partitioned between these two phases. The gas phase is comprised of

approximately 400 to 500 individual compounds (12) and about 300 can be classified as semi-volatiles (13). The greater number of smoke constituents appears in the particulate fraction (14). All these facts result in a highly dynamic matrix having a continuously changing chemical composition (9).

Chemical analysis of cigarette smoke is mostly done by application of conventional off-line techniques. These methods can alter the smoke composition during sampling and analytical processing, such as separation, trapping, and derivatisation. In addition, the results obtained are usually for total yields in smoke from whole cigarettes. As a consequence, short fluctuations and variations in concentration during the smoking process are usually not resolved. However, this information is essential in order to understand the complex and often interrelated formation and decay mechanisms of various smoke constituents and to evaluate the real impact of toxic and cancerogenic species on human smokers. However, the analytical task of simultaneously analysing a wide range of compounds at trace levels combined with a high time-resolution is very difficult to achieve. In recent times Single Photon Ionisation – Time-of-flight Mass Spectrometry (SPI-TOFMS) has proven to be well suited for this purpose. In so doing the method has been successfully applied to the analysis and characterisation of organic species in all kind of fields, e.g. waste incineration (15), steel recycling (16), exhaust gases (17, 18), and coffee roasting (19).

When dealing with mass spectrometry ionisation of the molecules of interest is required. This ionisation can be carried out by several different ionisation methods. Electron impact (EI) ionisation is one of the standard techniques in this field. However, for sufficient yields, electron energies are required which lead to massive fragmentation of most organic substances. In the case of highly complex samples this results in superposition of mother ions and fragment ions, making identification of individual compounds difficult or even impossible. Consequently, for these samples soft ionisation techniques are required which cause no or only little fragmentation of the analytes. In this context the single photon ionisation uses vacuum ultraviolet (VUV) photons instead of electrons for ionisation of the target molecules. A VUV photon with the energy E_{ph} is absorbed by the molecule and the molecule is ionised if E_{ph} exceeds its ionisation potential (IP). Therefore, main advantage of SPI is the low transfer of excess energy beyond the required ionisation energy. This results in low or even no fragmentation of the target molecules. The thus generated mother ions are detected in a time-of-flight mass spectrometer. Main advantage of TOFMS compared to other commonly used types such as quadrupol or sectorfield mass spectrometry, is that a wide mass range of species can be detected at the same time. Subsequently, a fast and comprehensive analysis of highly complex mixtures can be achieved. Information about the generation of VUV photons by a two-fold frequency-tripling of a Nd:YAG laser and detailed technical facts of the instrument such as detection limits, mass resolution, linearity etc. can be found in (3, 8, 20).

When a pulsed Nd:YAG laser system is used for VUV generation (here with a repetition rate of 10 Hz) the photons have a wavelength of 118 nm, which is equivalent to the

Table 1. Assignment of observed masses to most possible chemical compounds in tobacco pyrolysate and cigarette smoke (1)

<i>m/z</i>	Most possible compounds	<i>m/z</i>	Most possible compounds
67	Pyrrole	105	Vinylpyridine
68	Furan, Isoprene, 1,3-Pentadiene, Cyclopentene	106	Xylene, Ethylbenzene, Benzaldehyde
69	Pyrroline	107	Ethylpyridine, Methylbenzeneamine, 3-Pyridinecarboxaldehyde
70	2-Butenal, Methyl vinyl ketone, Methylbutene, Pentene, Butenone, 2-Methyl-2-Propenal	108	Anisol, Dimethylpyridine, Methylphenol
71	Pyrrolidine	109	3-Methoxypyridine
72	2-Methylpropenal, 2-Butanone, Butanal	110	Dihydroxybenzene, 2-Acetylfuran, Methylfurfural
74	Water-eliminated glycerol (C ₃ H ₆ O ₂), Tetrahydrofuran	112	Acetylcyclopentane, 2-Hydroxy-3-methyl-2-cyclopenten-1-one
77	Unidentified fragment	116	Indene
78	Benzene	117	Indole, Methylbenzonitrile
79	Pyridine	118	Indane, Methylstyrene, Benzofuran
80	Pyrazine	119	Indoline, Aminostyrol, Methylvinylpyridin
81	Methyl pyrrole	120	Methylethylbenzene, Trimethylbenzene
82	Methylfuran, Methylcyclopentene, Cyclohexene, 2-Cyclopenten-1-one	121	Dimethylaniline, Acetylpyridine, Ethyl-methylpyridine
83	Pentanenitrile, 3-Methylbutanenitrile	122	Benzoic acid, Ethylphenol, Hydroxybenzaldehyde,
84	Nicotine fragment, Cyclopentanone, Dimethylbutene, Hexene, 3-Methyl-3-buten-2-one	124	Dihydroxymethylbenzene, Guaiacol
85	Methylpyrrolidine, Piperidine	126	5-Hydroxymethylfurfural
86	Methylbutanal, 3-Methyl-2-butanone, Pentanone, 2,3-Butanedione	128	Naphthalene
92	Toluene	129	Quinoline, Isoquinoline
93	Aniline, Methylpyridine	130	Methylindene
94	Phenol, 2-Vinylfuran	132	Methylbenzofuran, 1-Indanone, Isopropenyltoluene
95	Pyridinol, Ethylpyrrol, Dimethylpyrrol	134	Isopropyltoluene
96	Dimethylfuran, Furfural	136	Limonene, Methoxybenzaldehyde, 2-Ethyl-5-methylphenol
97	Carbohydrate fragment, Hexanenitrile, Methylpentanenitrile	142	Methyl naphthalene
98	Furanmethanol, Methyl-2-pentenal, 5-Methyl-2(5 <i>H</i>)-furanone, 3-Methyl-2(5 <i>H</i>)-furanone, 1,3-Cyclopentanedione	146	Myosmine
102	Phenylacetylene	152	Vanillin, 4-ethyl-2-methoxyphenol, 3-Methoxy-2-hydroxybenzaldehyde, Acenaphthylene
103	Benzonitrile	153	Naphthalenecarbonitrile
104	Styrene, 3-Pyridinecarbonitrile	154	Dimethoxyphenol, Vinylnaphthalene
		156	Bipyridine, Dimethylnaphthalene
		162	Nicotine, Anabasine

energy of 10.49 eV. Subsequently, all molecules featuring an IP below 10.49 eV are ionised. Hence, many background gases such as N₂ (IP = 15.58 eV), O₂ (IP = 12.06 eV), CO₂ (IP = 13.77 eV), and H₂O (IP = 12.62 eV) are not detected as their IP values are above this threshold. Therefore, the detector is not oversaturated since these compounds have very high concentrations in smoke and ambient air. Compounds addressable by SPI are a wide range of aliphatic and aromatic hydrocarbons as well as heterocyclic and carbonylic species, provided that their IP is below 10.49 eV. Regarding tobacco smoke, a definite assignment of all observed masses to chemical compounds and sources is not possible due to the large number of species present and the complexity of possible reaction pathways. Table 1 account for the most likely options according to (1) and references therein. Data processing is done by LabView (National Instruments, USA), based on purpose-written software. Quantification, if wanted, is carried out by performing external calibration with commercial standard gases or standard gas generators such as permeation tubes or syringe pump dosage systems.

EXPERIMENTAL – PYROLYSIS EXPERIMENTS

In pyrolysis (Py) studies many experimental conditions can be adjusted and controlled. This can give insights into the complex chemistry of many smoke constituents by

revealing the preferred formation and decomposition conditions. In the past, a wide range of pyrolysis studies on tobacco have been undertaken. A detailed discussion can be found in (21, 22) and references therein. However, in recent years, application of soft ionisation methods, e.g. molecular beam mass spectrometry (23), field ionisation mass spectrometry (24–26), and resonance enhanced multiphoton mass spectrometry (27, 28), coupled to pyrolysis devices has been of growing interest mainly due to the complexity of the investigated samples.

The results presented here can be found in more detail in (1, 2, 5)

Experimental set-up of pyrolysis experiments

The schematic illustration (Figure 1) of the Py-SPI-TOFMS system demonstrates the experimental setup. A rotary furnace (Carbolite GmbH, Germany) was used for the pyrolysis experiments. Inside the furnace, a quartz glass tube (length 140 cm; 13 mm i.d.) was inserted. At the outlet of the furnace the quartz tube was connected to a filter holder containing a Cambridge filter to separate particles from the gas stream. That was followed by a glass T-piece connected to a transfer line containing a deactivated fused silica capillary (0.32 mm i.d.). The capillary was placed orthogonally to the main gas flow inside the T-piece, extracting a small portion of the pyrolysis gas (length: 1.5 m; retention time: < 0.7 s). At the other end of the transfer

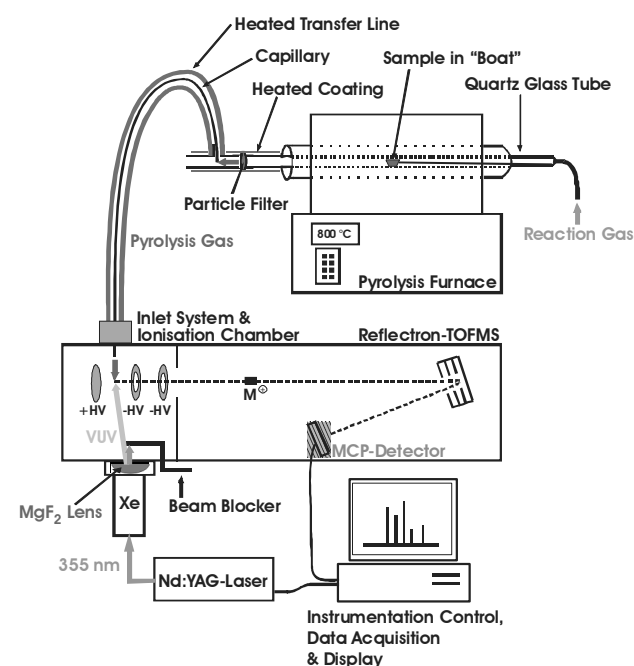


Figure 1. Illustration of the experimental set-up of the Single Photon Ionisation-Time-of-Flight Mass Spectrometry SPI-TOFMS system coupled to the pyrolysis furnace

line the capillary is housed inside a heated, hollow inlet needle reaching into the ionisation chamber of the SPI-TOFMS. All devices between the furnace and the transfer line such as quartz tube, Cambridge filter holder, T-piece and all connection parts were wrapped with heating mats which were constantly heated to 220 °C to prevent condensation and memory effects. The transfer line was also heated to 220 °C. In total, the furnace temperature was set to seven fixed isothermic temperatures of 400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C, and 1000 °C, respectively. Two measurement series with different reaction gases, synthetic air (80 % nitrogen, 20 % oxygen) and nitrogen respectively, were carried out, whereby a flow rate of 0.5 L/min was adjusted. The samples were placed in a quartz “boat” and pushed into the middle of the tube by a wire connected to the boat. Simultaneously the data acquisition was started. Tobacco samples have been taken from three different tobacco types: Burley, Virginia, and Oriental. Cigarettes containing these tobacco types were stored for several days under controlled conditions of 22 °C and 60 % humidity. Furthermore, these pure tobacco type cigarettes were analysed for nicotine, total sugar content, and reducing sugar content by conventional analytical methods. Results are summarised in Table 2. Before being pyrolysed the cigarette wrapper was removed and 50 mg of

tobacco was placed in the quartz glass boat. One benefit of the furnace used compared to commercially-available pyrolyser devices is that no further treatment of the sample such as grinding is necessary. In total, three replicates were measured for each tobacco type, temperature and gas composition. These results were used to account for temperature dependence and reaction gas composition dependence.

A further series of measurements dealt with the characterisation and discrimination of the three tobacco types by statistical methods. Here the furnace temperature was set to 800 °C. This pyrolysis temperature was chosen because it roughly resembles the conditions in a burning cigarette but, in principle, any temperature could have been adjusted which leads to thermal decomposition. Furthermore, in contrast to the first measurement series, the number of analysed replicates for the statistical approach was increased to ten measurements. In addition, these pyrolysis experiments were performed in nitrogen, but any other reaction gas composition could have been applied. All other experimental conditions were kept unchanged.

The recorded time-of-flight spectra were converted into mass spectra. Data acquisition was adjusted to average five mass spectra in order to get two complete mass spectra every second by using a 10 Hz laser. Depending on temperature and reaction gas the pyrolysis signals lasted for up to 80 seconds. Since time-resolution did not matter in this experiment, the recorded spectra were added up resulting in a single sum spectrum for each measurement, which is demonstrated for tobacco pyrolysis in nitrogen at 800 °C in Figures 2A to C. In so doing, Figure 2A illustrates the online recorded time profile of the occurring pyrolysis process and Figure 2B describes the resulting sum spectrum after the adding-up procedure. The three replicates of the first and the ten replicates of the second measurement series, respectively, were used to calculate mean and standard deviation of each applied condition which is exemplified in Figure 2C.

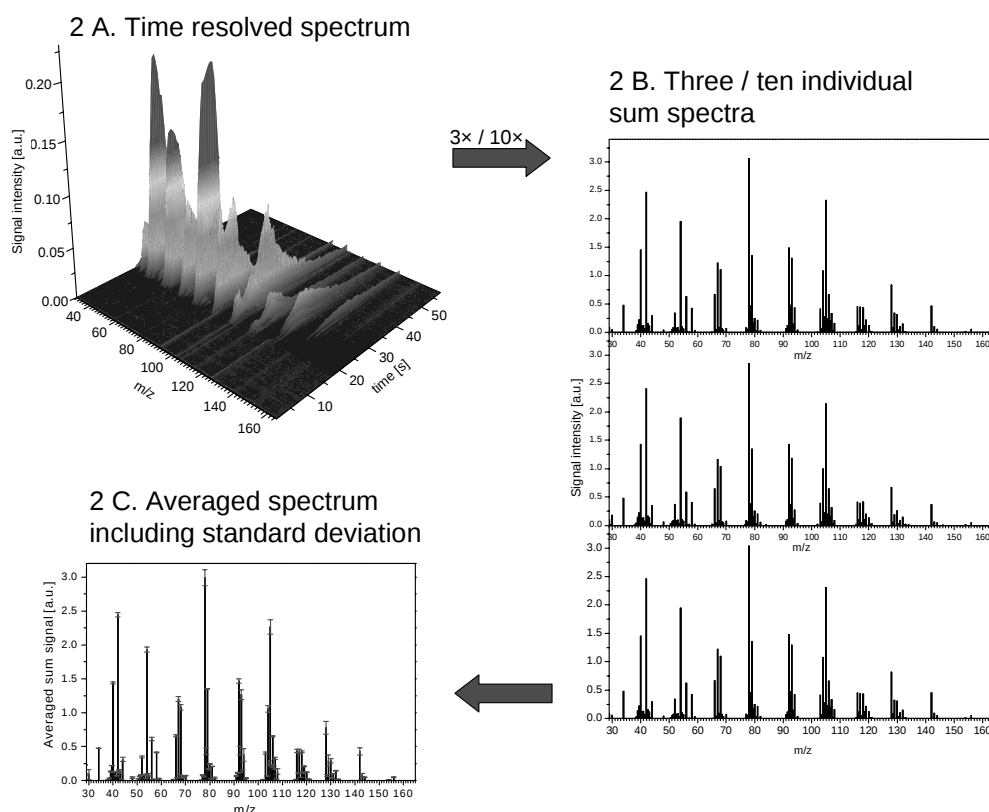
RESULTS AND DISCUSSION – PYROLYSIS EXPERIMENTS

Influence of temperature and reaction gas composition

The occurrence of pyrolysis products strongly depends on pyrolysis conditions, mainly temperature and reaction gas composition. Figure 3A exhibits the total averaged signal SPI mass spectra for pyrolysis temperatures of 400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C, and 1000 °C for the tested burley tobacco in nitrogen. To the naked eye little or no differences of the three tobacco types could be observed. Subsequently, the following interpretation is

Table 2. Characteristics of pure tobacco type cigarettes (NFDPM: nicotine-free dry particulate matter; DWB: dry weight basis)

Property	Virginia	Burley	Oriental
Nicotine [% DWB]	2.80	2.63	1.14
Reducing sugar [% DWB]	12.2	0.5	11.2
Total sugar [% DWB]	13.2	0.1	12.5
Tobacco weight [mg/cig]	650	560	742
Nicotine [mg/cig]	1.28	0.94	0.70



Figures 2A, B, and C. Conversion of time-resolved recorded mass spectra into averaged sum spectra including standard deviation

generally valid for all tobacco types with only slight fluctuations. A detailed characterisation and distinction of the individual tobacco types is given later.

The change of the overall pattern of the mass spectra can be clearly seen in Figure 3A. However, the standard deviations are disregarded in Figure 3A in order to keep the illustration simple. Therefore, small peaks and changes in signal intensities should not be overrated. Scaling of the y-axes (signal intensity) of the individual temperatures is variable because they are adjusted to the highest peak in the corresponding spectrum to enable comparison of the patterns. Absolute signal intensities for different temperatures are discussed later for selected compounds. The same spectra at the different temperatures but in synthetic air are presented in Figure 3B with the same y-axes scaling which makes it possible to compare the patterns of the same temperatures but different reaction gases.

The less severe pyrolysis conditions of 400 °C yield a great variety of species. Besides tobacco components (e.g. nicotine 162 *m/z* and water-eliminated glycerol 74 *m/z*) decomposition products from thermal degradation of carbohydrates such as cellulose, lignin and sugars (e.g. *m/z* 43, 44, 56, 58, 72, 82, 86, 96, 98, and 110) as well as proteins and amino acids (e.g. *m/z* 67, 69, 79, 81, and 93) are present. Signal intensities in synthetic air are mostly higher, especially for 42 *m/z* (propene), 56 *m/z* (2-propenal, butene, 2-methylpropene), 58 *m/z* (primarily acetone), 68 *m/z* (isoprene, furan), and 70 *m/z* (crotonaldehyde amongst others). Obviously thermal degradation takes place to a greater extent when oxygen is present at this low temperature. This

is supported by higher yields of 48 *m/z* (methanethiol), 162 *m/z* (nicotine), and 74 *m/z* (water-eliminated glycerol) in nitrogen which survive in a non-oxidative atmosphere.

At 500 °C the overall pattern is similar to 400 °C. In synthetic air hardly any differences can be observed. Remarkable are the rise of 68 *m/z* (isoprene/furan) and the decrease of 74 *m/z* (water-eliminated glycerol) in nitrogen.

By increasing the temperature to 600 °C the diversity of observed masses is still evident in nitrogen. Thermal degradation products rise, simultaneously 162 *m/z* (nicotine) is decreasing. In synthetic air, higher molecular mass signals are vanishing, 162 *m/z* (nicotine) is not detected. Simultaneously unsaturated and oxygen-containing compounds further increase, especially 42 *m/z* (propene) 44 *m/z* (acetaldehyde), 54 *m/z* (butadiene), 56 *m/z* (2-propenal, butene, 2-methylpropene), and 58 *m/z* (acetone).

At 700 °C, the most striking feature is the immense rise of vinylpyridine (105 *m/z*) in both atmospheres, which is reported as a decomposition product of nicotine and other nitrogen-containing compounds under severe pyrolysis conditions (29, 30). This nicotine decomposition includes rupture of the pyrrolidine and the pyridine ring resulting in pyridines (e.g. vinylpyridine, 105 *m/z*), aryl nitriles (e.g. benzonitrile, 103 *m/z*), and aromatic hydrocarbons (e.g. toluene, 92 *m/z*). SCHMELTZ *et al.* suggested several pathways and identified more than 38 compounds in the nicotine pyrolyzate (31), demonstrating the complexity of the cracking and pyrosynthetic reactions occurring. 68 *m/z* (isoprene/furan) is still abundant in nitrogen whereas it does not play a great role in air anymore. Signal intensities in the

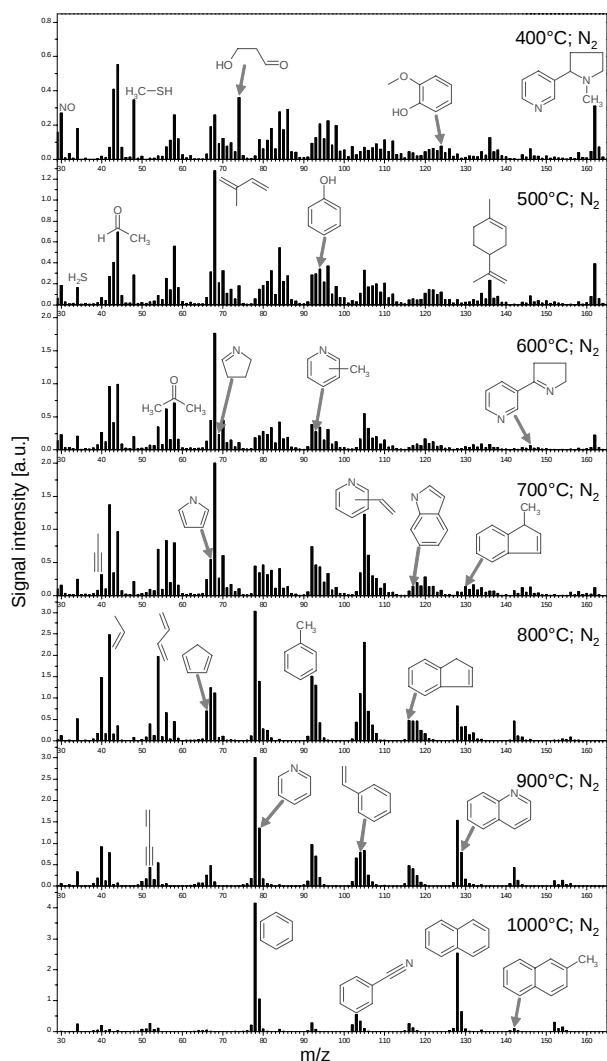


Figure 3A. SPI sum spectra of Burley tobacco at 400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C and 1000 °C in nitrogen

latter atmosphere become generally lower, indicating rising conversion of compounds into substances not accessible by SPI-TOFMS, e.g. CO and CO₂.

When increasing the temperature to 800 °C, the pattern changes. In nitrogen, 68 *m/z* (isoprene/furan) also loses its supremacy and 162 *m/z* (nicotine) vanished. 78 *m/z* (benzene) becomes the prevailing peak in both atmospheres. At this relatively severe temperature thermal degradation of carbohydrates such as cellulose, lignin and sugars as well as proteins, alkaloids and amino acids with subsequent pyrosynthesis of thermally more stable compounds occurs. Subsequently the spectra shift towards unsaturated components such as 40 *m/z* (propyne), 42 *m/z* (propene), and 54 *m/z* (butadiene) and homologous series of methylated benzenes ($78 + (n \times 14)$; $n = 0,1,2,\dots$) and naphthalenes ($128 \text{ } m/z + (n \times 14)$; $n = 0,1,2,\dots$) as well as other substituted aromatic species e.g. 104 *m/z* (styrene), 105 *m/z* (vinylpyridine), 116 *m/z* (indene), and 118 *m/z* (indane). In contrast, oxygen-containing compounds show the opposite behaviour and decrease (*m/z* 44, 56, 58, 70, 82, 86, 96). Apart from signal intensity hardly any differences between air and nitrogen atmosphere can be observed.

The general trend continues at 900 °C. Predominantly unsaturated species are visible, first and foremost 78 *m/z* (ben-

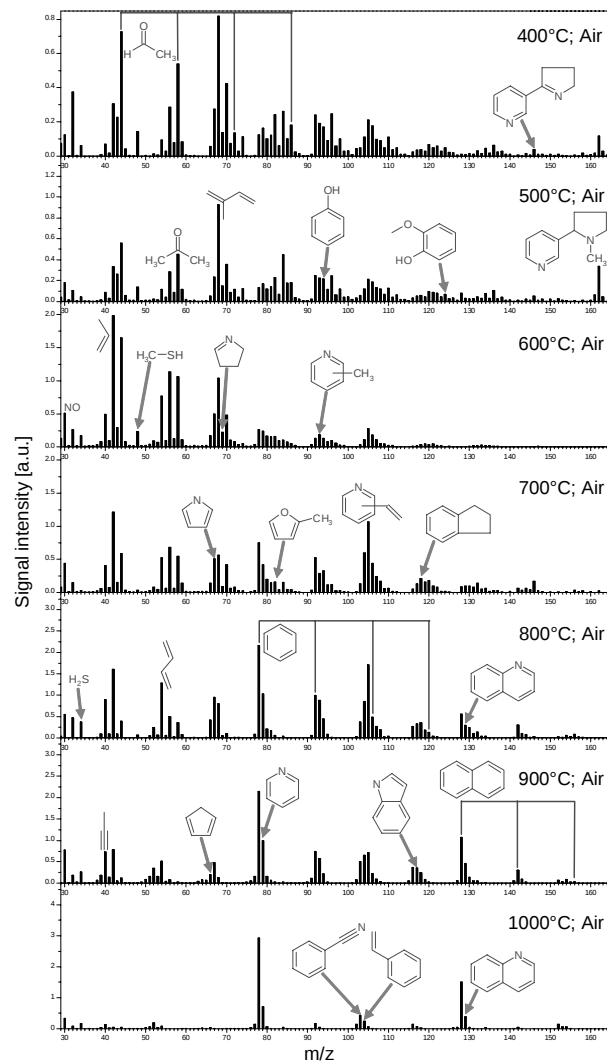


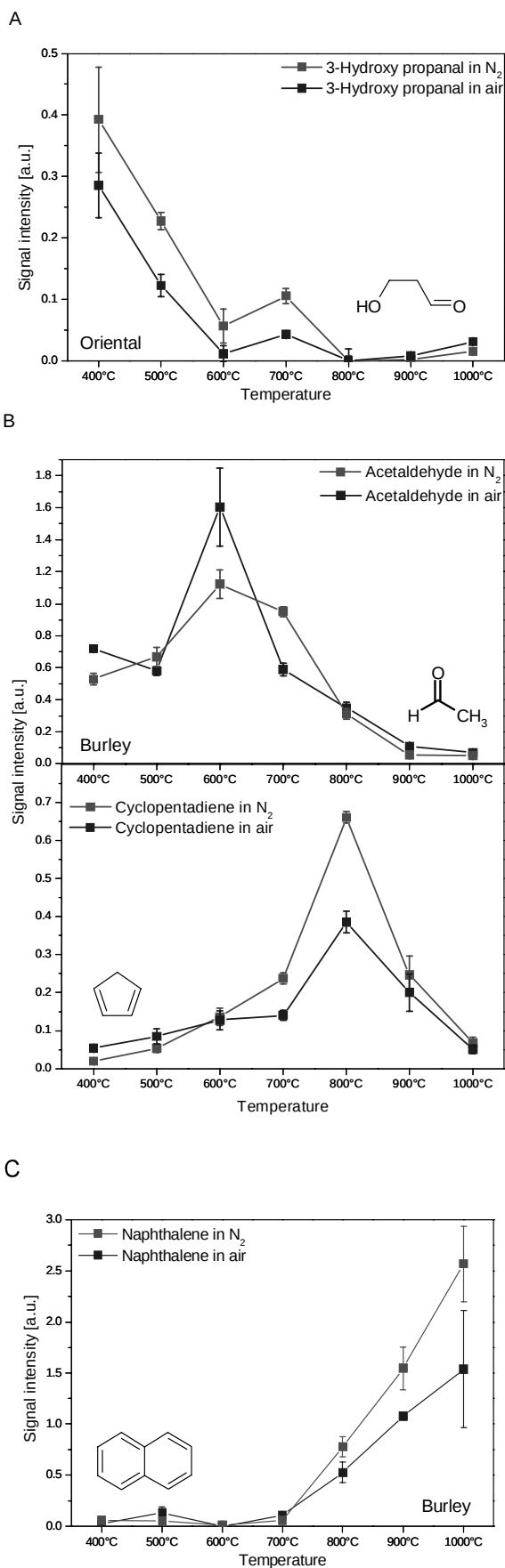
Figure 3B. SPI sum spectra of Burley tobacco at 400 °C, 500 °C, 600 °C, 700 °C, 800 °C, 900 °C and 1000 °C in synthetic air

zene) and 128 m/z (naphthalene). Substituted aromatic chemicals decrease such as 92 m/z (toluene) and 105 m/z (vinylpyridine). The occurrence of 30 m/z (NO) in nitrogen is depleted whereas in air, it still exists.

Finally, pyrolysis at 1000 °C results in dramatic changes to the pattern of the spectra. Almost all masses are gone or only have little signal intensities. The spectra in both atmospheres are dominated by 78 *m/z* (benzene) and 128 *m/z* (naphthalene) together with a few smaller peaks, e.g. 92 *m/z* (toluene), 103 *m/z* (benzonitrile), and 104 *m/z* (styrene). The spectrum at 1000 °C clearly shows that at such high temperatures only the most stable aromatic compounds prevail.

EVANS and MILNE (32) illustrated the nature of the products as a function of pyrolysis temperature. Moderate temperatures lead to so-called primary components, which are substantially free of gas phase cracking products. Under more severe conditions these substances undergo subsequent thermal degradation resulting in secondary components. Tertiary components are generated by further decomposition and pyrosynthesis of the secondary compounds.

At the high temperatures applied in the pyrolysis experiments, glycerol (1,2,3-trihydroxypropane) immediately



Figures 4A, B, and C. Behaviour of primary (A), secondary (B), and tertiary components (C) as a function of temperature from the pyrolysis of tobacco in nitrogen and air by means of selected compounds and tobacco types

eliminates water and finally most likely leads to acrolein. The water-eliminated intermediate product of glycerol, 3-hydroxy propanal ($C_3H_6O_2$, 74 m/z), exhibits the typical behaviour of a primary component which is shown in Figure 4A. In both reaction gases, nitrogen and air, the highest yields can be observed for the lowest applied pyrolysis temperature of 400 °C. With increasing temperature, their signals tend to decline due to thermal degradation up to almost complete decomposition at 800 °C.

The afore-mentioned behaviour of oxygen-containing and unsaturated compounds for different temperatures is again illustrated in Figure 4B by means of acetaldehyde (44 m/z) and cyclopentadiene (66 m/z) in nitrogen and air, respectively. Both substances show a rise and decline in signal intensity within the temperature range but have their maxima at different temperatures. This behaviour results in their classification as secondary components. The formation of these species depends mainly on prior decomposition reactions. In the case of the tested burley tobacco, acetaldehyde clearly exhibited the behaviour of a secondary component, having one formation maximum. Higher yields were detected in air as shown. However, the pyrolysis of the tested Virginia tobacco in air lead to two maxima (400 °C and 600 °C). In addition to unsaturated and oxygen-containing compounds, certain aromatics e.g. indene (116 m/z) and indole (117 m/z) as well as substituted aromatic compounds e.g. alkylated benzenes (92 m/z and 106 m/z), can also be classified as secondary components.

In contrast, the group of tertiary components mainly consists of thermally stable aromatic compounds without side chains and a few substituted aromatic species e.g. benzonitrile (103 m/z). Figure 4C demonstrates their thermal behaviour by means of naphthalene (128 m/z).

The results achieved enable an unambiguous classification of a wide range of tobacco smoke substances into either primary, secondary or tertiary compounds which is illustrated in Table 3. Components not listed either showed behaviour of more than one class or differences between tobacco types occurred as explained for acetaldehyde earlier. The reasons for this can originate from different sources for the same compound, e.g. distillation from the tobacco into the smoke (primary component) and formation via pyrolysis/pyrosynthesis reactions (secondary or tertiary product) in similar measures.

This group assignment makes it possible to draw conclusions on the formation and decomposition mechanisms of the detected species in smoke and provides an insight into the complex tobacco chemistry.

Discrimination of tobacco types by sum parameters

Besides genetic characteristics of the tobacco plants, soil types and curing processes strongly influence the character of the tobacco leaf, and therefore, play a key role in chemical composition of generated smoke and pyrolysis gas.

Burley tobacco is a light air-cured tobacco, usually grown on heavier-textured, more fertile soils where nitrogen-fertilisation with ammonium nitrate and urea is common. During the long curing process lasting several weeks chemical and microbiological reactions take place. Thereby carbohydrates are depleted to a great extent via metabolism

Table 3. Classification of observed tobacco masses as primary, secondary or tertiary component

<i>m/z</i>	Compounds identified in tobacco smoke and pyrolysis gas	Classification
34	H ₂ S	Secondary
40	Propyne	Secondary
42	Propene	Secondary
43	Carbohydrate fragment: C ₃ H ₇ ⁺ , C ₂ H ₃ O ⁺	Primary
48	Methanethiol	Primary
54	1,3-Butadiene, 1-Butyne	Secondary
56	2-Propenal, Butene, 2-Methylpropene	Secondary
66	Cyclopentadiene	Secondary
67	Pyrrole	Secondary
74	3-Hydroxy propanal, Tetrahydrofuran	Primary
77	Fragment	Tertiary
78	Benzene	Tertiary
80	Pyrazine	Secondary
85	Methylpyrrolidine, Piperidine	Primary
86	Methylbutanal, 3-Methyl-2-butanone, Pentanone, 2,3-Butanedione	Primary
92	Toluene	Secondary
93	Aniline, Methylpyridine	Secondary
98	Furanmethanol, Methyl-2-pentenal, 5-Methyl-2(5 <i>H</i>)-furanone, 3-Methyl-2(5 <i>H</i>)-furanone, 1,3-Cyclopentanedione	Primary
103	Benzonitrile	Tertiary
104	Styrene, 3-Pyridinecarbonitrile	Secondary
105	Vinylpyridine	Secondary
112	Acetylcyclopentane, 2-Hydroxy-3-methyl-2-cyclopenten-1-one	Primary
118	Indane, Methyl styrene, Benzofuran	Secondary
119	Indoline, Aminostyrol, Methylvinylpyridin	Secondary
128	Naphthalene	Tertiary
129	Quinoline, Isoquinoline	Tertiary
130	Methylindene	Secondary
142	Methyl naphthalene	Secondary
152	Vanillin, 4-ethyl-2-methoxyphenol, 3-Methoxy-2-hydroxybenzaldehyde, Acenaphthylene	Tertiary
162	Nicotine, Anabasine	Primary

of the plant cells resulting in cured leaves with relatively low sugar contents. Consequently, characteristic chemical components are nitrogen-containing species such as proteins, alkaloids, amino acids, and nitrates (33, 34). Figure 5A illustrates an averaged sum spectrum including standard deviation of the pyrolysis of burley tobacco in nitrogen at 800 °C.

Virginia is typically grown on infertile, sandy soils with low organic matter and nitrogen reserves often leading to very light-bodied smooth tobaccos. During flue-curing, degradation of the leaves' chlorophyll takes place and most carbohydrates are converted to simple sugars (34, 35). Figure 5B shows a typical sum spectrum of the pyrolysis of Virginia tobacco in nitrogen at 800 °C.

Oriental tobacco resembles Virginia but it often grows on rocky soils, which are low in nutrients. Oriental tobacco possesses significant amounts of sugars and lesser amounts of protein and amino acids (36). The averaged sum spectrum in Figure 5C gives the result for the pyrolysis of Oriental tobacco in nitrogen at 800 °C.

First differences of the three tobacco types tested in these experiments can be determined by opposing single characteristic masses as shown in Figure 6A and 6B. Figure 6A shows the content of nitrogenous substances by means of 17 *m/z* (NH₃) formed during pyrolysis at 800 °C in nitrogen and air. The columns represent averaged sum signals of the three replicates and are normalised to the highest occurring peak for easier comparison.

The ammonia content of the pyrolysis gases from burley tobacco is more than twice as high as from Virginia and

Oriental, whereas the amounts of ammonia from the latter two are about the same. This behaviour suggests a higher nitrogen-content of the tested burley tobacco. Oxidative pyrolysis conditions by application of air in the pyrolysis atmosphere results in partial decomposition of NH₃ for all tobacco types.

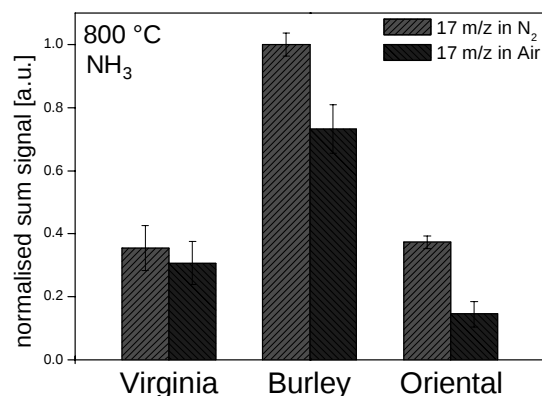
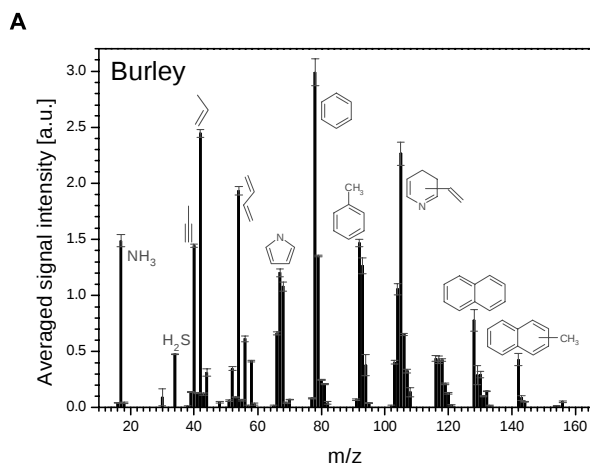
In contrast to burley, the pyrolysis products of Oriental and Virginia show an abundance of peaks assigned to carbohydrate-derived products. Figure 6B illustrates the different contents of 96 *m/z*, which is most likely attributable to furfural and dimethylfuran, pyrolysed at 400 °C. These compounds are known to be decomposition products of cellulose and used as indicators for carbohydrates in tobacco smoke (23).

The amount of carbohydrates increases in the following order: Burley < Oriental < Virginia. The presence of oxygen in the pyrolysis atmosphere might enhance the decomposition reaction of carbohydrates, but the error bars do not allow further interpretations on that.

However, the simple comparison of individual key compounds leads to the assumption that various compound classes, mainly carbohydrates and nitrogen-containing substances, yield specific signal intensities for the three different tobacco types.

Discrimination of tobacco types by statistical methods

Differences between the tested tobacco samples can result in different mass spectral information, which, however, might not be unambiguous to the naked eye. Therefore,



Figures 6A. Comparison of the content of nitrogen-containing substances from pyrolysis of Virginia, burley and Oriental tobacco at 800 °C in nitrogen and air by means of NH₃ (17 *m/z*) formed

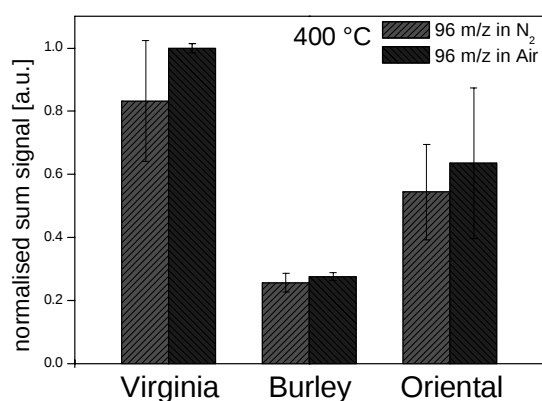
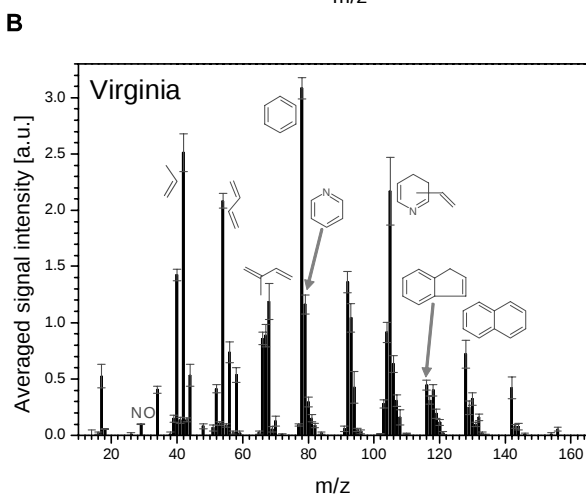
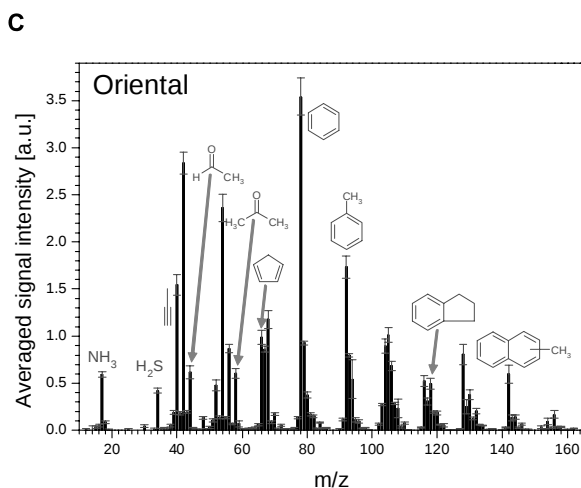


Figure 6B. Comparison of carbohydrate content for Virginia, Burley, and Oriental tobacco pyrolysed at 400 °C in nitrogen and air by means of furfural/dimethylfuran (96 *m/z*) formed



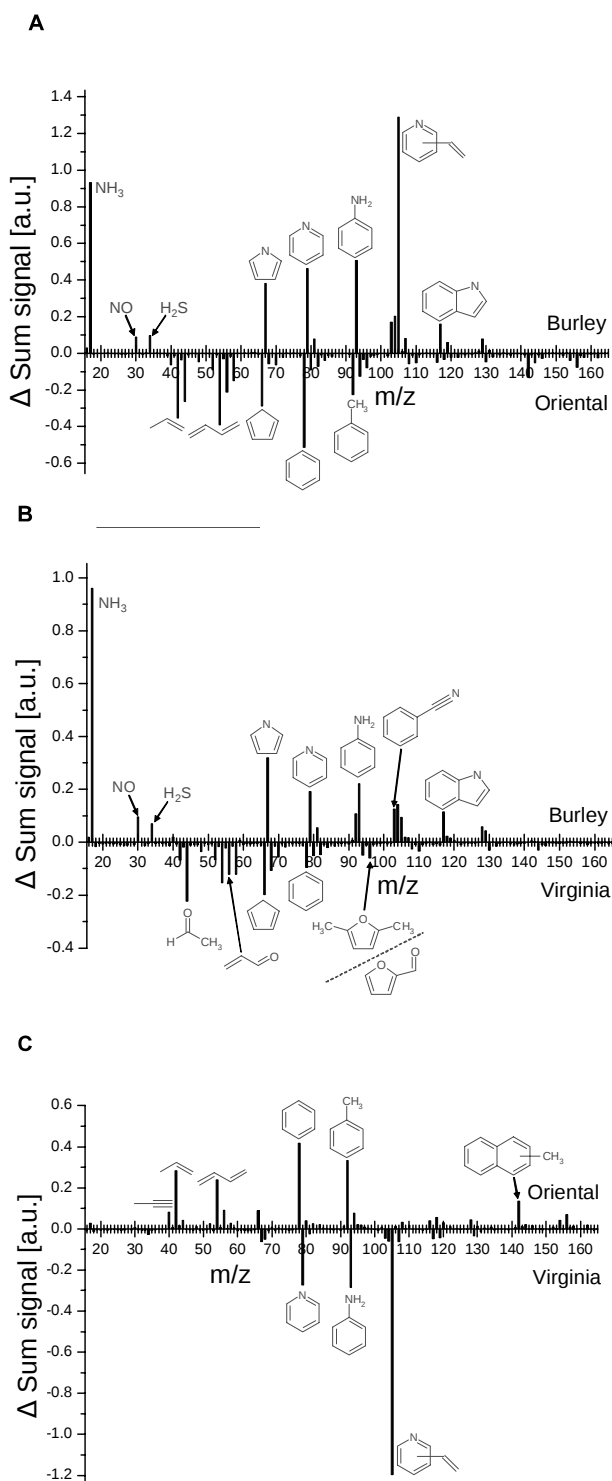
Figures 5A, B, and C. Averaged sum spectrum including standard deviation of the pyrolysis of burley (Fig. 5A), Virginia (Fig. 5B), and Oriental (Fig. 5C) tobacco in nitrogen at 800 °C

data processing methods are required that allow the identification of the most distinctive features of the samples. Therefore, a second series of pyrolysis measurements was carried out. To achieve a greater reliability of the results ten replicates of each tobacco type in nitrogen at 800 °C were pyrolysed.

A simple way of verifying differences in the tobaccos' complete mass spectra is to subtract an average spectrum of one type of tobacco from an average spectrum of the other

type, leading to three difference spectra (Burley – Oriental, Burley – Virginia, and Oriental – Virginia), which are shown in Figures 7A, B, and C respectively.

The difference spectrum between Burley and Oriental tobacco (Figure 7A) illustrates that the tested burley exhibits higher signal intensities for e.g. *m/z* 105, 17, 93, 79, 67, 104, 103, 117, 34, 30. These masses can almost entirely be assigned to nitrogen-containing compounds. In the final browning stage of burley curing, reactions cease as the leaves die. Consequently, the characteristic chemical components are nitrogen-containing species such as proteins, alkaloids, amino acids, and nitrates. These compounds act as precursors of numerous tobacco smoke components such as ammonia, pyridines, amines, nitrosamines, pyrroles, pyrazines, indoles, nitriles, and hydrogen cyanide (33, 34). As a result, concentrations of these compounds in the burley pyrolysis gas are higher than in the Oriental and Virginia pyrolysis gases, which is also illustrated in Figure 7B. Differences between the tested burley and Virginia are not as striking as between burley and Oriental but are still visible. Again, the burley tobacco pyrolysis gas exhibits high amounts of nitrogenous species (*m/z* 17, 67, 93, 79, 104, 103, 117, 30, 105, etc.) but is deficient in masses such as *m/z* 44, 66, 54, 58, 56, 68, 78, 42, etc. compared to the tested Virginia. Similar masses in slightly different order of signal intensity characterise the Oriental tobacco pyrolysis gas compared to burley (*m/z* 78, 54, 42, 66, 44, 92, 56, 58,



Figures 7A, B, and C. Difference spectra between Burley and Oriental (A), burley and Virginia (B) and Oriental and Virginia (C) in nitrogen at 800 °C

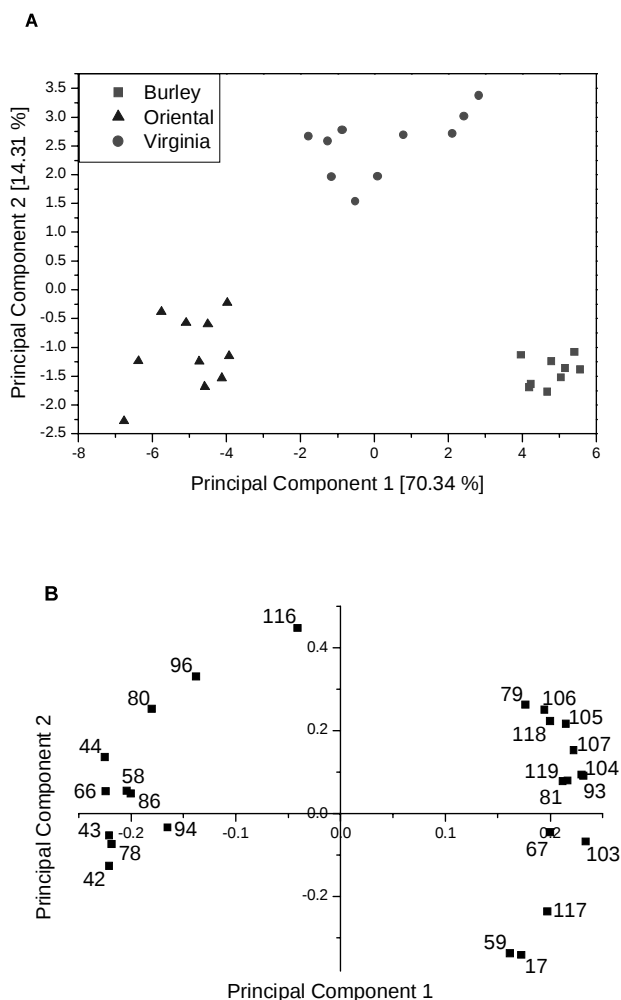
142, 94, etc.) and can mainly be identified as unsaturated hydrocarbons, some of them containing oxygen. An explanation is that Oriental tobacco possesses significant amounts of sugars and lesser amounts of protein and amino acids, resulting in typical masses for carbohydrate breakdown (23, 36, 37). In turn, when Virginia is compared to burley, typical masses for carbohydrate decomposition prevail but if compared to Oriental nitrogenous species play

a more important role demonstrated in Figure 7C. As a result, in this experiment the pyrolysis gas compositions of burley and Oriental are very characteristic and differ quite a lot. In contrast, the tested Virginia tobacco features properties of both other types.

In general, modern analysis techniques enable sensitive and sophisticated investigations of all sorts of samples. At the same time the immense amount of data obtained often requires pruning datasets in order to focus on the most relevant features. In this way, chemometrics has gained great influence in modern analytical chemistry. In this context Principal Component Analysis (PCA) is one common method of choice. It basically seeks to reduce the dimensionality of a dataset consisting of a large number of interrelated variables, while retaining as much of the present variation as possible. This is achieved by transformation to a new set of variables, the Principal Components (PCs), which are uncorrelated and ordered so that the first few components contain most of the variation of the entire original data set. The PCA is based on the covariance matrix of the entire data set. The eigenvectors of the covariance matrix are the so-called loading vectors (which project the original data to the new space spanned by the Principal Components) and the respective eigenvalues represent the fraction of the variance explained by the Principal Component. Often a projection of the original data spanned by the first two PCs is sufficient. The outcome of PCA is mostly depicted by two two-dimensional plots, the loading-plot and the score-plot. The loading-plot visualises the influence of the original variables on the respective Principal Components, the scores are the projected data in the lower dimensional subspace defined by the PCs.

The first step of processing the dataset was performed by normalisation to total ion signal and autoscaling. Autoscaling is often useful when variables span different ranges in order to make the variables of equal importance. It is carried out by mean centring the data, i.e. subtracting the mean and subsequent variance scaling, i.e. division by the standard deviation to make the data independent of scaling. Pre-selection of relevant masses was done by calculating the Fisher-Ratios. The pair-wise Fisher-Ratio between any two classes is defined as the ratio of between-class scatter and within-class scatter. The best features in descending order of the Fisher-Ratios can then be selected for the classification task (38, 39). This Fisher criterion can be extended to multi-class problems enabling the simultaneous distinction between several groups (39, 40). Masses featuring the twenty-five highest Fisher-Ratios were incorporated into the PCA calculations. These masses were in descending order of the Fisher-Ratio value 105 m/z , 17 m/z , 103 m/z , 106 m/z , 104 m/z , 93 m/z , 79 m/z , 44 m/z , 107 m/z , 59 m/z , 81 m/z , 43 m/z , 66 m/z , 67 m/z , 117 m/z , 96 m/z , 80 m/z , 42 m/z , 94 m/z , 119 m/z , 78 m/z , 118 m/z , 86 m/z , 58 m/z , and 116 m/z (1, 5).

The score plot proves that a clear distinction between the three tobacco types can be achieved by Py-SPI-TOFMS which is illustrated in Figure 8A. Burley and Oriental are clearly separated on the first PC (explaining 70.34% of the total variance), while Virginia is separated from the other two tobacco types along the second PC (14.31% of total variance). The loadings (Figure 8B) reveal the influence of the variables on the Principal Components in conjunction with the scoreplot on each tobacco type. Consequently, all



Figures 8A and B. Score (A) and loading (B) plots by using the m/z featuring the twenty

twenty five masses can be divided into two large groups either mainly influencing Oriental or burley tobacco. Nitrogenous compounds typical for burley have a strong influence on the first Principal Component towards positive values, whereas unsaturated carbohydrates show strong influence on negative values along the axis of the first PC. Regarding this, the first Principal Component mainly describes the differentiation between burley and Oriental and is dominated by the appearance of nitrogen-containing species and carbohydrates. Therefore it is also related to cultivation conditions as explained beforehand. Virginia tobacco is affected solely on the second PC by components of both groups whereby some masses have a greater impact (e.g. m/z 116, 79, 96, 80, 106, 118, 105) than others (e.g. m/z 59, 17, 117).

As a consequence PCA enables the discrimination of all three tobacco types and confirms the revelation that Virginia shows features of burley and Oriental tobacco. This difficulty in classification of Virginia can also be seen in the small fraction of total variance explained by the second PC compared to PC1 (14.31% to 70.34%).

Interestingly, a similar distinction of the classes can be achieved by only using the three masses exhibiting the highest Fisher-Ratios, m/z 105 (vinylpyridine), 17 (ammonia), and 103 (benzonitrile). More details on that can be found in (1, 5).

CONCLUSION – PYROLYSIS EXPERIMENTS

The coupling of SPI-TOFMS to a pyrolysis furnace enabled the comprehensive characterisation of many tobacco smoke constituents, regarding the influence of temperature and reaction gas composition. In this context, by spanning a temperature range between 400 °C and 1000 °C in steps of $\Delta 100$ °C and applying two different gas compositions, pure nitrogen and synthetic air, the preferred formation conditions of many individual compounds could be identified as well as the overall change of the pattern was observed. Moreover, the classification of several substances in primary, secondary, and tertiary components was carried out. With respect to the three tested tobacco types Virginia, Oriental, and burley, differences in composition of the pyrolysis gas were clearly illustrated. In this way the principle and possibility of discriminating between these three tobacco types by PCA was demonstrated.

EXPERIMENTAL – SMOKING EXPERIMENTS

Puff-by-puff resolved measurements of cigarette smoke

First investigations on the analysis within single cigarette puffs were carried out by VILCINS (41) and CESCHINI *et al.* (42). Over the years several techniques have been applied for single puff or puff-by-puff characterisation such as Fourier transform infrared spectroscopy (FTIR) (43, 44) and quantum cascade infrared laser spectroscopy (45–47) by PARRISH and co-workers as well as quad quantum cascade laser spectrometry by BAREN *et al.* (48). PLUNKETT *et al.* used a dual infrared tuneable diode laser system (46, 49) whereas GC-MS was applied by THOMAS *et al.* (50), LI *et al.* (51), and WAGNER *et al.* (52). In addition CROOKS *et al.* developed a technique for determining intrapuff nicotine yields (53).

Limiting factors for most of these methods are either the low time-resolution or the fact that only a few substances can be analysed simultaneously. Moreover, in order to gain analytical information of smoke components relevant for human smokers, it is important to investigate relatively fresh smoke (ca. one to two seconds old), rather than smoke that has aged over a few minutes (54). In addition, the analysis technique must not interfere too much with the combustion and pyrolysis processes occurring in the cigarette. In this context, an inherent problem is the fact that besides the composition, the partition between the gas phase and particulate phase can also change continuously and is strongly influenced by time, temperature and dilution of smoke (9). In practice, investigations of the effects of cigarette smoke constituents have generally concentrated on the particulate phase of smoke (12). However, knowledge about the phase affiliation of smoke components is important because gas phase and particulate matter have very different deposition characteristics in the human respiratory tract, be it oral, pharyngeal, bronchial, or alveolar. Consequently, the focus of this study is on whole smoke and gas phase investigations. Main goal of the method is a fast and comprehensive analysis which interferes as little as possible with the complex smoke matrix.

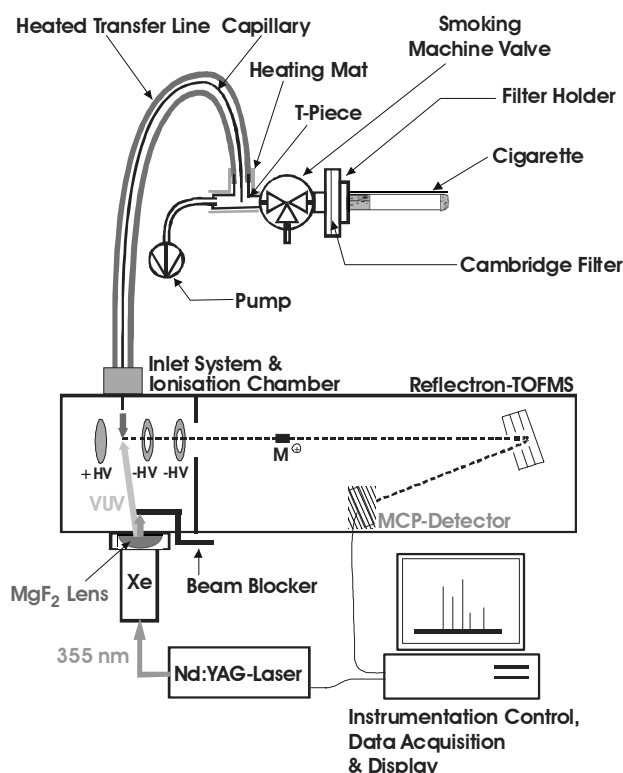


Figure 9. Illustration of the experimental set-up of the smoking machine coupled to the SPI-TOFMS

Experimental set-up of smoking experiments

The smoking machine and the SPI-TOFMS instrument were connected by the heated transfer line containing the deactivated capillary (Figure 9), similar to the experimental set-up described for the pyrolysis experiments. The smoking machine used was a custom-made smoking machine based on a commercial Borgwaldt single port smoking machine. The main reason for the modifications was the fact that the commercial machine was insufficient for real-time measurements since the smoke was collected and mixed in a piston before being pumped to any analytical device. In addition to that, the smoking machine caused much greater memory effects due to its higher dead volume. A detailed description of the modified smoking machine can be found in (8). During analysis a small portion of the smoke (flow: 8 mL/min) was sucked in the ionisation chamber. The T-piece was wrapped by a heating mat and adjusted to ca. 80 °C. The transfer line was heated to 220 °C.

Concerning sample cigarettes, the main focus was on 2R4F Kentucky reference cigarettes whereby only mainstream smoke, smoked under International Standard (ISO) smoking conditions, was investigated. Usually several cleaning puffs without cigarette were taken in between the actual smoking puffs. This procedure was necessary in order to remove any remaining smoke in the smoking machine and will be discussed in detail in the section “Drawbacks of the smoking regime”.

The first test series dealt with the qualitative and quantitative comparison of whole smoke and gas phase. The cigarettes were lit with a Borgwaldt electric lighter. Gas phase measurements were carried out by incorporating a

Cambridge filter pad in the cigarette holder. For whole smoke measurements the Cambridge filter pad was removed. In total three replicates were measured. In contrast to the pyrolysis experiments, three (not five) successive single laser shot mass spectra were averaged (time resolution 3.3 Hz) to improve signal stability. This holds for all following measurements. A detailed interpretation of these results can be found (1, 4, 8).

A further measurement series dealt with a qualitative statistical approach to characterise and discriminate between the individual cigarette puffs by means of their chemical patterns. The cigarettes were also lit with a Borgwaldt electric lighter and measurements of whole smoke only were carried out. Here, only puffs one to eight were considered for the statistical evaluation. In total, ten replicates were measured and the corresponding mean and standard deviation were calculated. Forty one mass signals, which could be clearly detected in the mass spectra, were selected for further analysis. The signal intensities per puff (with and without cleaning puffs) were normalised to the corresponding total ion signal of the puff. This eliminates influences of absolute mass signal values and enables the investigation of changing chemical patterns. Prior to the principal component analysis autoscaling of the data set was carried out similar as described in the pyrolysis experiment. The resultant puff-resolved data sets of the ten measurements were used to perform a PCA. These outcome of this experiment is also covered in (6).

A last test series was performed in order to investigate the influence of different cigarette lighting devices on the chemical composition of the first cigarette puff. Investigated lighting devices were a Borgwaldt electric lighter, a match, a commercial propane/butane gas lighter, a candle, and the burning zone of another cigarette. In the latter case a first cigarette was lit properly and the burning zone of this cigarette was used to light the actual sample cigarette whereby both cigarettes were kept in an angle of approximately 180 °C. For all measurements only the first cigarette puff was recorded and ten replicates were carried out for each lighting device. Normalisation was carried out by normalising the absolute signals to the corresponding total ion signal of the puff. This eliminates influences of varying masses of burnt tobacco. These results can be found in more detail in (7).

For all test series the recorded time-of-flight spectra were converted to mass spectra the same way as described for the pyrolysis experiments. For puff-by-puff evaluation, the time-resolved signal intensities of each individual smoking puff were added. Finally, mean and standard deviation of the corresponding replicates were calculated for each puff. The processing from the time-resolved mass spectra to the puff-resolved yields is demonstrated by means of acetaldehyde in Figure 10.

Besides qualitative evaluation, a few substances, which are considered to be relevant for smoking related diseases, were selected for a quantitative interpretation. These target compounds were chosen according to their accessibility by SPI-TOFMS as well as possible relevance for the human smoker and expected yield in tobacco smoke. The substances studied quantitatively were nitric oxide, acetaldehyde, butadiene, acetone, isoprene, benzene, toluene, and the C2-benzene derivatives ethyl benzene and xylene. The latter two are given as the sum of both since the TOFMS used

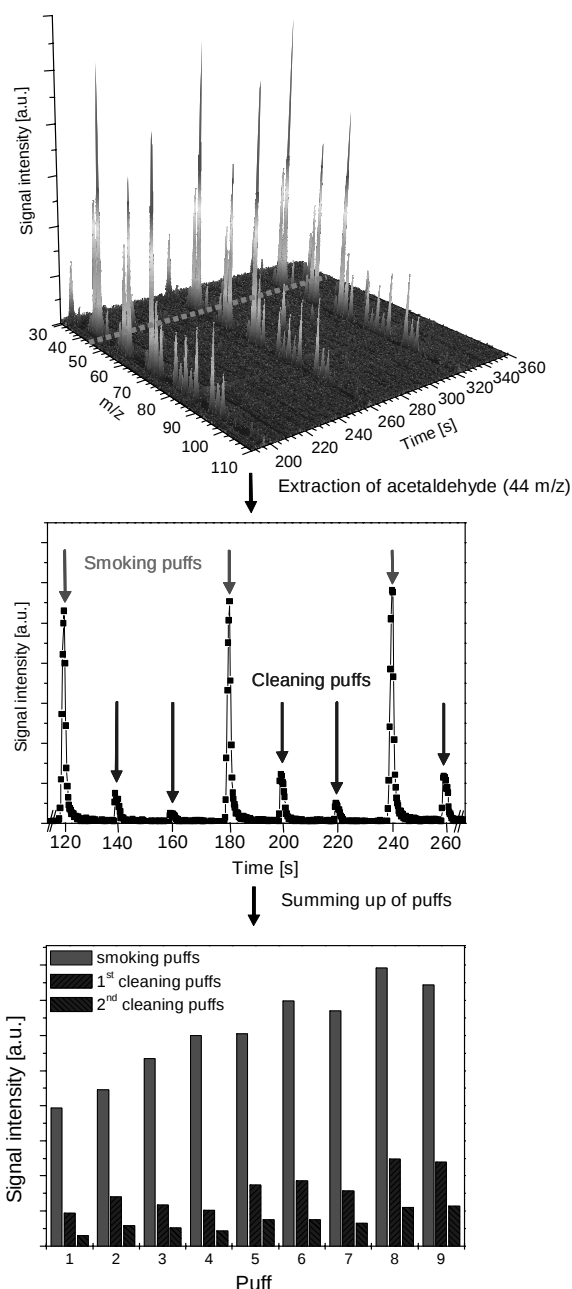


Figure 10. Processing of time-resolved mass spectra to puff-by-puff resolved yields exemplarily shown for acetaldehyde

does not allow separation between them. Some quantitative information might be influenced by small amounts of isobaric species i.e. compounds with the same or very similar molecular weights. A detailed discussion on that can be found in (1). Calibration of the instrument was performed by applying commercial standard gases (Linde AG, Unterschleißheim, Germany; Air Liquide, Gröbenzell, Germany).

RESULTS AND DISCUSSION – SMOKING EXPERIMENTS

For the first evaluation of the composition of whole cigarette mainstream smoke and the gas phase, spectra from the

smoking of a complete 2R4F research cigarette with and without a Cambridge filter pad were summed up (over all puffs) in order to compare the different signal patterns. The summed signal intensities of the gas phase and whole smoke are illustrated in Figure 11. In general, signal intensities of almost all masses are higher for whole smoke compared to the vapour phase. In addition, whole smoke results in a greater variety of masses, especially in the mass region higher than 70 m/z . The assignment of masses observed in cigarette smoke to the most likely compounds is given in Table 1. Good to see is the occurrence of nicotine (162 m/z) in whole smoke whereas in the cigarette vapour phase, nicotine is non-existent.

Drawbacks of the smoking regime

When contemplating puff-by-puff resolved smoking profiles for the first time one must consider massive memory effects and the contamination on the following puffs, even for the modified smoking machine with the reduced dead volume. This effect was much more pronounced for gas phase measurements. Subsequently, several cleaning puffs were taken with no cigarette present in between the actual cigarette smoking puffs until the signals of the cleaning puffs declined to zero. In general, two cleaning puffs were necessary for this purpose for whole smoke. Gas phase measurements required up to five cleaning puffs. These signals were added together and compared to the smoking puffs. Figure 12B shows the yields for acetaldehyde (44 m/z) in the successive unfiltered smoking puffs and subsequent cleaning puffs of a 2R4F research cigarette. To a first approximation, contamination and memory effects for each puff amounted for about one third of the corresponding smoking puff intensity. For whole smoke this will mostly depend on the type of smoking-machine used. However, the summed cleaning puffs of the gas phase measurements with a Cambridge filter pad present resulted in even higher signal intensities than for the smoking puff, especially for the earlier puffs (Figure 12A).

This must be due mainly to desorption effects from the Cambridge filter. In the literature no report about this effect could be found, therefore, it is possible that previous puff-by-puff resolved studies have not considered this problem. However, PARRISH *et al.* (43) described the influence of several Cambridge filter parameters on smoke composition and trapping efficiencies. This desorption effect must also occur in total yield investigations from entire cigarettes but, since in this case signals of all puffs are summed up, not as crucial as for single puff characterisation. Subsequently, the definition of “single puff” is problematical since it is not obvious if the subsequent contamination levels should be added to the yield of the previous puff. If this is not the case, thorough cleaning or filter changing after every puff will be required. Otherwise, this contamination level is added automatically to the subsequent smoking puff, which consequently affects this result. Therefore for single puff evaluations, two approaches are possible, which are illustrated in Figure 13: either contemplation of the smoking puffs only without cleaning puffs (approach B’), or adding together smoking and corresponding (subsequent) cleaning puffs (approach A’). The former method results in a mismatch when all single puff yields are added together and compared to the conventional total yield measurements

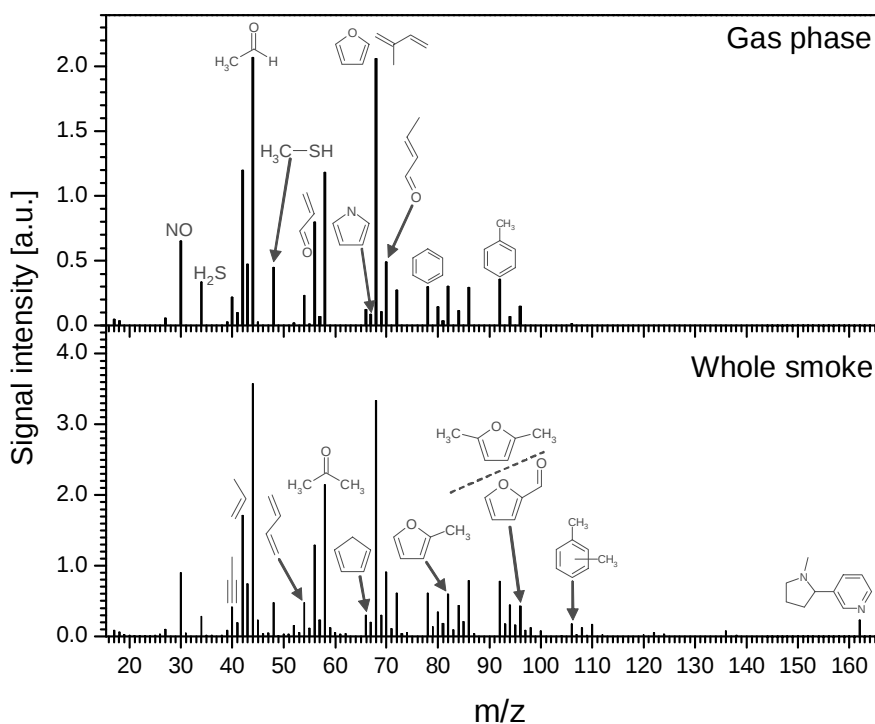


Figure 11. Comparison of gas phase and whole smoke by opposing two sum spectra of a completely smoked 2R4F cigarette

A

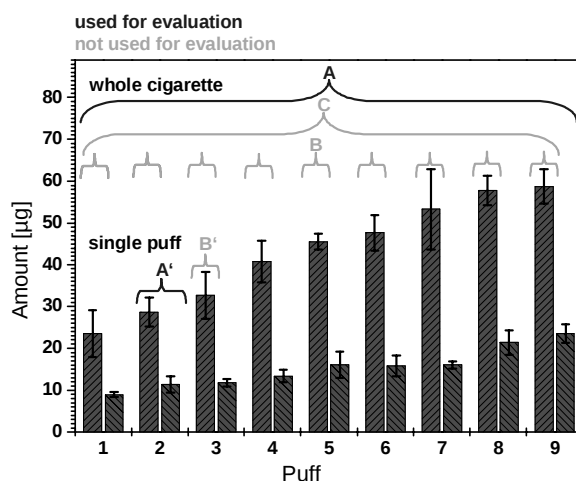
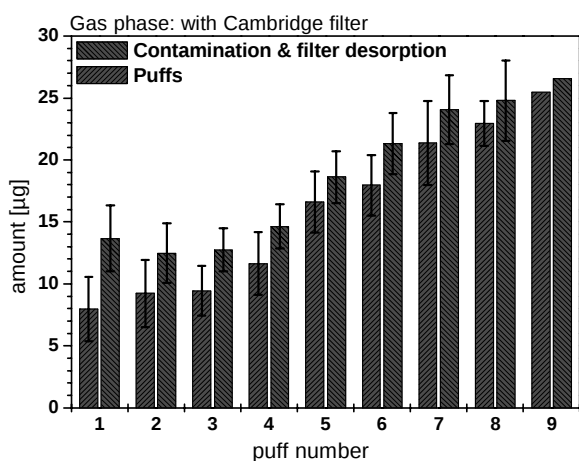
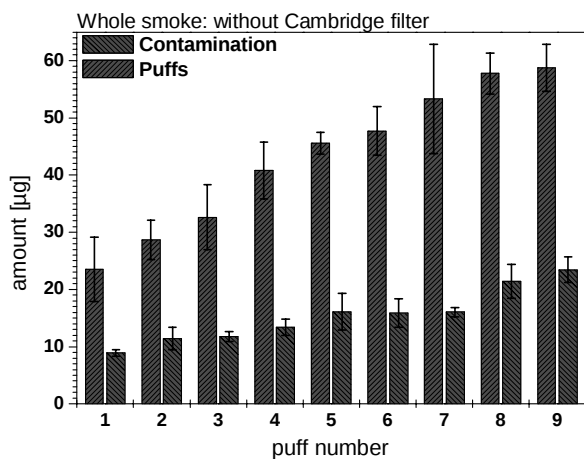


Figure 13. Illustration of the three different approaches (A, B, and C) for the definition of 'whole cigarette' and the two approaches for the definition of 'single puff' (A' and B')

B



Figures 12A and B. Successive smoking and cleaning puffs of the gas phase (Fig. 12A) and whole smoke (Fig. 12B) of the 2R4F cigarette, shown for acetaldehyde (44 m/z)

since it will result in smaller total amounts (approach B). The latter approach (A') makes the results comparable to published data dealing with total amounts of smoke compounds. Secondly, the levels of components in both the smoking puffs and the cleaning puffs are relevant to the human smoker. However, especially for gas phase analysis, some particulate matter held back by the filter might evaporate to the smoke stream of the following puffs and wrongly contributes to the gas phase. This source of error is also relevant for conventional total yield measurements. Regarding this work the puffs are defined as smoking puff plus subsequent cleaning puffs, mainly because both fractions affect the human smoker.

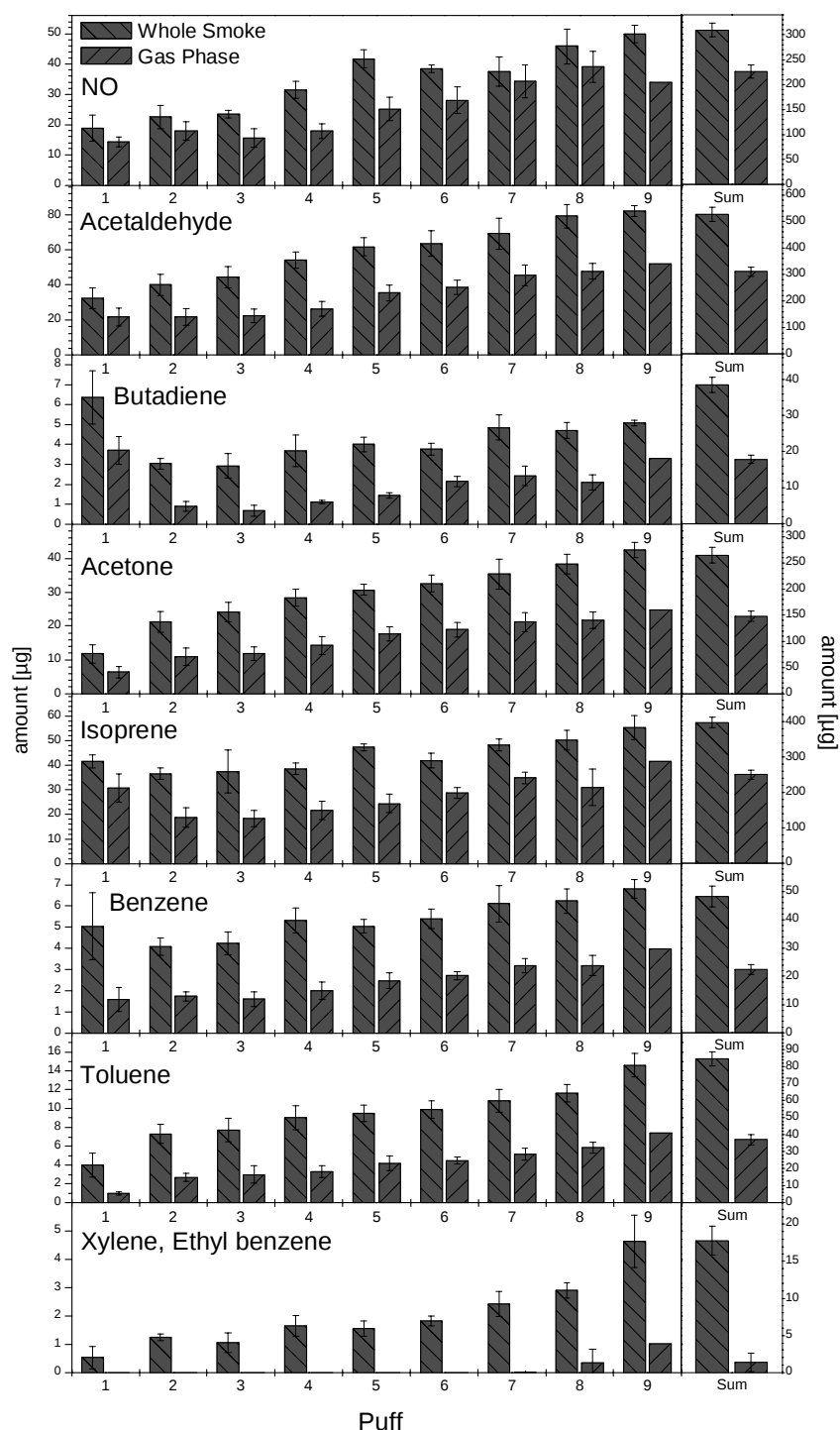


Figure 14. Quantitative, puff-by-puff resolved and total yields of selected substances analysed in the gas phase and whole smoke of the 2R4F research cigarette with cleaning puffs added to the smoking puffs

It is also possible that institutions dealing with the definition of standard smoking conditions are not aware of these facts. One reason is probably the lower relevance for the total yields. However, total yield investigations can be influenced but to a smaller extent. If smoke sampling is immediately stopped after the final smoking puff, the contamination and desorption yields of this final puff (here: 23 µg and 26 µg acetaldehyde for the whole smoke and the vapour phase respectively) remain in the smoking machine and/or filter. As a consequence, approach A was used regarding total smoke analysis.

After all, recently published total yields of some smoke constituents of the 2R4F (52, 55) are in good agreement with the values achieved in the present study when the cleaning puffs are considered. Without including the cleaning puffs the total yields would be lower.

Quantitative evaluation of 2R4F cigarette

Figure 14 illustrates the puff profile of the quantitatively analysed substances in the smoke of 2R4F cigarette for the whole smoke and the gas phase. The cleaning puffs have

Table 4A Averaged yield ($\bar{m}$) and standard deviation (std) of the quantitative, puff-by-puff resolved compounds in μg , analysed in the whole smoke of the 2R4F research cigarette by adding the cleaning puffs to the smoking puffs

Compound	Puff	1	2	3	4	5	6	7	8	9	Σ
NO	$\bar{m}$	18.9	22.5	23.4	31.4	41.7	38.3	37.6	45.9	50	309.6
	std	4.3	3.8	1.2	2.8	2.9	1.2	4.9	5.7	2.9	13.9
Acetaldehyde	$\bar{m}$	32.4	40.1	44.4	54.2	61.7	63.6	69.3	79.2	82.2	527.1
	std	1.3	0.3	0.6	0.8	0.4	0.3	0.6	0.4	0.1	2.2
Acetone	$\bar{m}$	11.9	21.3	24.1	28.4	30.7	32.5	35.3	38.3	42.5	265.1
	std	2.7	3	2.9	2.5	1.6	2.5	4.4	2.9	2.3	15.1
Isoprene	$\bar{m}$	41.6	36.6	37.4	38.6	47.2	41.9	48.3	50.2	55.3	397.2
	std	2.7	2.3	8.8	2.3	1.5	3.1	2.3	4	5	15.3
Benzene	$\bar{m}$	5	4.1	4.2	5.3	5	5.4	6.1	6.2	6.8	48.2
	std	1.6	0.4	0.5	0.6	0.3	0.5	0.9	0.6	0.4	3.6
Toluene	$\bar{m}$	4	7.3	7.7	9	9.5	9.9	10.8	11.6	14.6	84.5
	std	1.3	1	1.2	1.3	0.9	0.9	1.2	0.9	1.2	4.3
Xylene/Ethyl Benzene	$\bar{m}$	0.5	1.2	1.1	1.6	1.6	1.8	2.4	2.9	4.6	17.8
	std	0.4	0.1	0.3	0.4	0.3	0.2	0.4	0.3	0.9	1.9

Table 4B. Averaged yield ($\bar{m}$) and standard deviation (std) of the quantitative, puff-by-puff resolved compounds in μg , analysed in the gas phase of the 2R4F research cigarette by adding the cleaning puffs to the smoking puffs

Compound	Puff	1	2	3	4	5	6	7	8	9	Σ
NO	$\bar{m}$	14.3	17.9	15.6	17.9	25.1	28.1	34.3	39.1	34	226.2
	std	1.6	3	3	2.4	3.9	4.5	5.4	5.1		12.6
Acetaldehyde	$\bar{m}$	21.6	21.7	22.2	26.3	35.2	38.6	45.4	47.8	52	310.7
	std	5.2	4.9	3.9	4.3	4.5	4.2	6.1	4.7		17.6
Butadiene	$\bar{m}$	3.7	0.9	0.7	1.1	1.5	2.1	2.4	2.1	3.3	17.9
	std	0.7	0.2	0.3	0.1	0.1	0.3	0.5	0.4		1.1
Acetone	$\bar{m}$	6.5	10.9	11.9	14.3	17.8	19	21.2	21.8	24.9	148.1
	std	1.6	2.6	2	2.7	2.1	2.1	2.8	2.4		9.6
Isoprene	$\bar{m}$	30.7	18.8	18.4	21.6	24.5	28.8	34.8	31.1	41.5	250.2
	std	5.7	4	3.3	3.8	3.9	2.2	2.3	7.3		13.6
Benzene	$\bar{m}$	1.6	1.7	1.6	2	2.5	2.7	3.2	3.2	4	22.5
	std	0.6	0.2	0.3	0.4	0.4	0.2	0.3	0.5		1.7
Toluene	$\bar{m}$	1	2.7	3	3.3	4.2	4.5	5.2	5.8	7.4	37.1
	std	0.2	0.5	0.9	0.6	0.8	0.4	0.6	0.6		3.1
Xylene/Ethyl benzene	$\bar{m}$	0	0	0	0	0	0	0	0.3	1	1.3
	std	0	0	0	0	0	0	0	0.3		0.3

been added to the corresponding preceding puffs as discussed earlier. The mean values including standard deviation are also listed in Tables 4A and B. The same yields for the smoking puffs only, i.e. without adding the cleaning puffs to the smoking puffs can be found in (1).

The presented results of NO reveal a further flaw of the Cambridge filter separation. NO is a small molecule and should be entirely in the gas phase of cigarette smoke. However, some NO seems to be trapped on the Cambridge pad since the yield is lower in the gas phase which indicates that the Cambridge pad is not an ideal filter for separating all the gas and particulate phase constituents of cigarette smoke. This effect is even more pronounced for the smoking puffs only, which supports the trapping theory. The observed results for NO must reflect a reaction that removes NO, which is enhanced by the presence of the Cambridge pad. Although such an effect of the Cambridge pad has not been reported previously for NO, WILLIAMS (56) has observed recoveries of only 87–91% when NO was added to whole smoke using a non-dispersive infrared analytical technique together with a Cambridge filter pad. He postulated that NO was being lost by its reaction with other smoke constituents such as amines and alcohols to form nitrosamines and methyl nitrite respectively. Nitric oxide is known to oxidise

within a few seconds in smoke to generate nitrogen dioxide, and the nitrogen dioxide subsequently reacts within a further few seconds with smoke constituents such as methyl alcohol, isoprene, butadiene and acrolein (57, 58). The rate of oxidation of nitric oxide to nitrogen dioxide in mainstream smoke has been determined in several studies (56, 58–60). The rate constant in smoke is almost a magnitude higher than for the pure gas phase oxidation, possibly due to catalysis of the reaction by free radicals present in smoke. In the present study the transit time of the smoke to the analyser will be greater in the presence of the Cambridge pad because of the tortuous path of the smoke through the filter pad. This allows more time for reaction of NO and will result in the measurement of lower quantities of NO when the Cambridge filter pad is present. It is experimentally convenient to use a Cambridge pad in smoke analysis but it does not give an absolute separation of the two phases. The materials collected on the pad are affected by a host of factors, including moisture content, temperature, flow rate and specific chemical interaction between aerosol constituents and the fibre glass. Different separation techniques, such as electrostatic precipitation and jet impaction traps give different values for the vapour/aerosol composition (14).

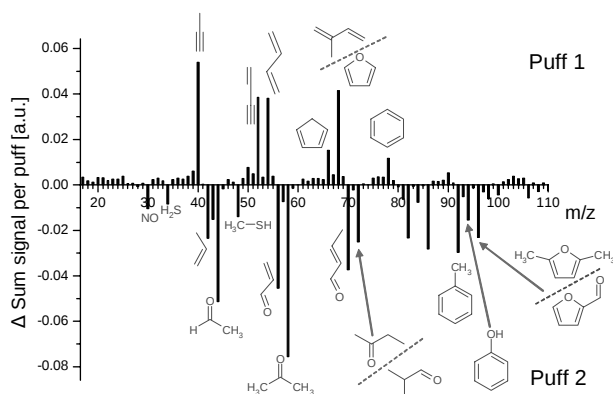


Figure 15. Difference spectrum of the first and second puff of the 2R4F research cigarette

The yields of most of the compounds present in cigarette smoke (whole smoke and gas phase) feature a continuous increase from puff to puff which can be clearly seen for e.g. NO, acetaldehyde, acetone, and toluene. This is due to a gradual reduction in tobacco rod length as the cigarette is consumed, which results in a decrease in filtration by the tobacco rod for products in the particulate phase and their components, and a decrease in air dilution and outward gaseous diffusion for products in the gas phase (61, 62). However, a second puff-by-puff behaviour was also observed in which the first puff exhibits the highest yield, followed by smaller yields in the second puff and then the usual increase with puff number mentioned before. Species observed following this behaviour are mainly unsaturated hydrocarbons. Here (Figure 14) butadiene and, less distinct, isoprene show this puff profile.

The reason for this behaviour must be related to the lighting of the cigarette before the first puff is taken. During lighting the tobacco is heated from ambient temperature, resulting in a much higher temperature gradient than is the case for the subsequent puffs, since the inter-puff smoulder temperature is around 600 °C to 700 °C. Thus, tobacco closest to the burning zone is preheated for all puffs except the first one. This trend attracts special interest, especially regarding the highly toxic smoke constituents butadiene and isoprene, when assuming that human smokers inhale differently during the first puff. For example, it is possible that a smoker may inhale the first puff longer and deeper in order to light the cigarette properly. It is also possible that some human smokers do not inhale the first puff at all. The detailed analysis on the chemical composition of the individual puff as shown in this work will be interesting from human smoking behaviour and smoke retention studies point of view.

Similar high levels in the first puff have also been reported for formaldehyde and ethylene (41, 47–49) as well as benzo[a]pyrene (51, 63). Formaldehyde and benzo[a]pyrene are considered as some of the most hazardous species in smoke (72–75). Therefore, formaldehyde seems to feature a unique smoking profile since it is the only oxygen-containing compound following this behaviour. All the other species having high yields in the first puff are unsaturated or aromatic hydrocarbons. VILCINS (41) has reported that the manner of lighting a cigarette can sometimes cause the yields in the first puff to be higher or lower than expected. LI *et al.* (63) showed that the high levels of benzo[a]pyrene in the first puff were dependent on the method of lighting

the cigarette. They showed that lighting the cigarette with a butane lighter (yellow flame) or match resulted in very high concentrations in the first puff whereas a torch lighter (blue flame) and electric lighter resulted in quite low levels of benzo[a]pyrene in the first puff. They deduced that the excess benzo[a]pyrene in the first puff with a yellow flame or match was from soot particles covered with polycyclic aromatic hydrocarbons, produced in the incomplete combustion of the yellow lighting flame. Furthermore, PARRISH and HARWARD (46) compared butane lighters, electric lighters, and matches and did not find significant changes with respect to the high levels of formaldehyde in the first puff. As a consequence a further study was carried out in order to evaluate the influence of different cigarette lighting devices on the chemical composition of the first puff. These results can be found in the section “Influence of different lighting devices”.

Qualitative evaluation of 2R4F cigarette

The different characters of the first and second puff can also be examined by a difference spectrum in which the summed and normalised (to total ion signal) spectrum of the second puff is subtracted from the summed and normalised spectrum of the first puff as demonstrated for 2R4F research cigarette in Figure 15.

In so doing, positive signal intensities account for higher amounts in the first puff and *vice versa*. The spectrum illustrates that the first puff is dominated by unsaturated species, mainly 40 *m/z* (propyne), 52 *m/z* (1-buten-3-yne), 54 *m/z* (mainly butadiene), and 66 *m/z* (cyclopentadiene). In contrast to this, the second puff is dominated by oxygen-containing species such as carbonyls, e.g. 44 *m/z* (acetaldehyde), 56 *m/z* (mainly acrolein), 58 *m/z* (acetone), and 70 *m/z* (mainly crotonaldehyde) as well as 94 *m/z* (phenol), 30 *m/z* (NO), and the sulphurous components 34 *m/z* (H₂S) and 48 *m/z* (methanethiol). The only obvious exceptions are 92 *m/z* (toluene) and 42 *m/z* (propene).

The origin of mass 68 *m/z* usually can be attributed to two isobaric compounds, isoprene and furan, both identified in cigarette smoke (23, 30). BAKER (9) and HOFFMANN (64, 65) reported that the yield of isoprene in plain non-filter cigarettes is approximately five to ten times higher than for furan. In Figure 15 it can be seen that 68 *m/z* is much more abundant in the first puff. Therefore it is assumed that the main proportion of 68 *m/z* in fact originates from the unsaturated hydrocarbon isoprene, rather than the oxygen-containing furan. Variations in composition between other successive puffs (2→3; 3→4; etc.) could not be observed by this approach. Therefore, the application of statistical methods, similar to the pyrolysis study, was pursued.

In so doing, a further measurement series was carried out, whereby both approaches of data evaluation were carried out. Firstly, the two cleaning puffs were added to the corresponding smoking puff, and secondly, the cleaning puffs were neglected and only the smoking puffs without cleaning puffs were considered. The results of both approaches were incorporated into a PCA and the outcome of the first approach (cleaning puffs added to smoking puffs) is illustrated in the Figures 16A and B.

It can be seen that the first puff is clearly separated on the first principal component (variance 50.06%) confirming the very unique chemical composition mentioned earlier. In

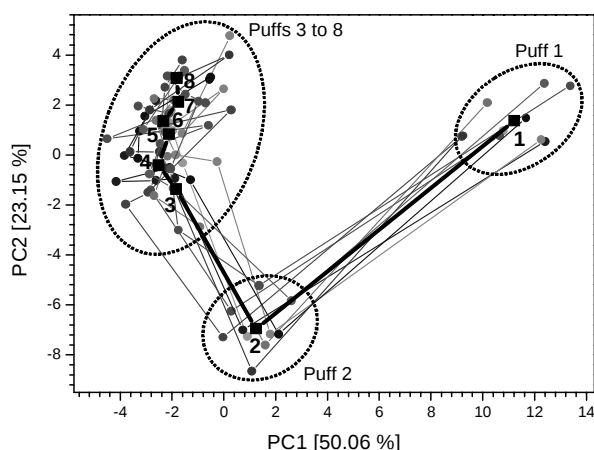


Figure 16A. Score-plot of the first and second principal components of the mean and ten individual puff-resolved cigarette smoke measurements (smoking puffs and cleaning puffs)

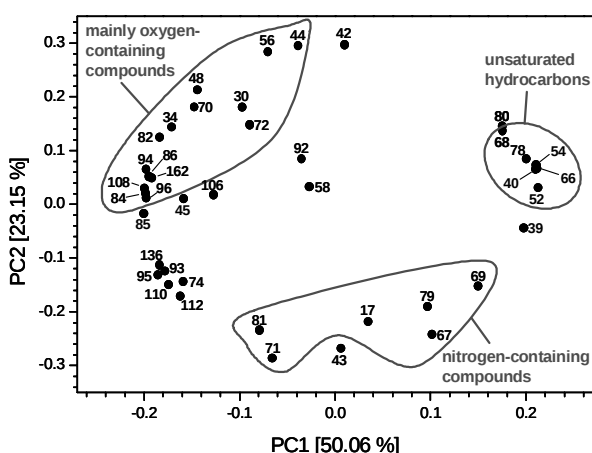


Figure 16B. Loading-plot of the first and second principal component of the ten puff-resolved cigarette smoke measurements (smoking puffs and cleaning puffs)

addition the second puff is also separated but on the second principal component (variance 23.15%). This means that the chemical pattern of the second puff is not just different from the first puff but also from the following puffs three to eight. In contrast the data points of these six puffs (three to eight) seem to form an undefined cluster. However, when incorporating the calculated mean (black dots and line) of the individual score values even puffs three to eight show a trend and are separated on the second principal component. The black figures refer to the puff numbers of the averaged score values. Plotting the first versus the third principal component confirms the described trends but does not give any additional information, subsequently, it is not shown here. According to these findings all cigarette puffs have an individual chemical pattern. The first and the second puff are very unique whereas the variations within the puffs three to eight are small. The corresponding loading-plot visualises the influence of the 41 masses on the respective principal components illustrated in Figure 16B.

The loading-plot shows that the first puff is highly influenced by the masses 39 *m/z*, 40 *m/z*, 52 *m/z*, 54 *m/z*, 66 *m/z*, 68 *m/z*, 78 *m/z*, and 80 *m/z*. Almost all of these are unsaturated hydrocarbons, 80 *m/z* (pyrazine) is a nitrogen-containing aromatic compound. As mentioned earlier, lighting the cigarette and heating the tobacco from ambient

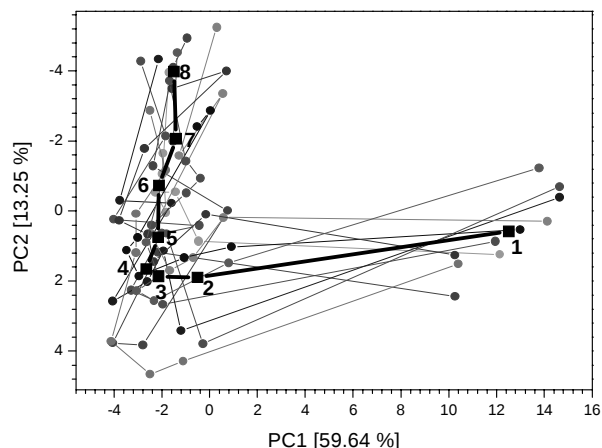


Figure 17A. Score-plot of the first and second principal components of the mean and ten individual puff-resolved cigarette smoke measurements (smoking puffs only)

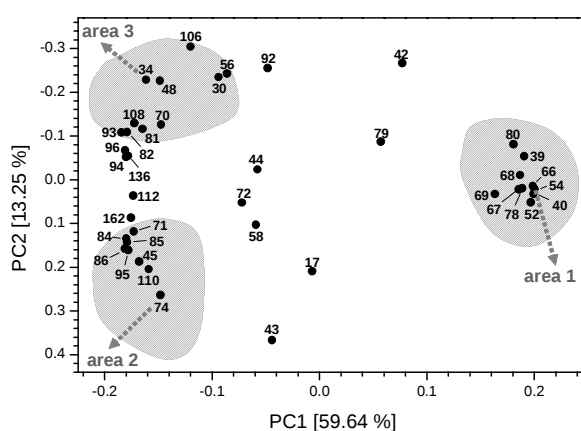


Figure 17B. Loading-plot of the first and second principal component of the ten puff-resolved cigarette smoke measurements (smoking puffs only)

temperature to more than 900 °C (66) seems to enhance the formation of unsaturated species. Contamination by the lighting device can be excluded since an electric lighter was used. In contrast the second cigarette puff is more influenced by nitrogen-containing species such as 17 *m/z* (ammonia), 67 *m/z* (pyrrole), 69 *m/z* (pyrroline), 71 *m/z* (pyrrolidine), 79 *m/z* (pyridine), and 81 *m/z* (methyl pyrrole). In addition, the clustered species in the bottom left corner have an effect on the separation of the second puff on the second principal component, two of them (93 *m/z* and 95 *m/z*) also being nitrogen-containing smoke constituents. From the third puff onward oxygen-containing compounds such as 30 *m/z* (nitrogen oxide), 44 *m/z* (acetaldehyde), 56 *m/z* (mainly acrolein), 70 *m/z* (mainly crotonaldehyde), and 94 *m/z* (phenol) play a greater role but sulphurous species (34 *m/z* and 48 *m/z*) and nicotine peaks (162 *m/z* and 84 *m/z*) can also be found.

The score and loading plots of the PCA when the cleaning puffs are not added to the smoking puffs (Figures 17A and B) look very similar regarding the clear separation of the first puff on the first principal component (variance 59.64 %). However, two nitrogen-containing unsaturated species, 67 *m/z* (pyrrole) and 69 *m/z* (pyrroline), enlarge the cluster of compounds which is responsible for the separation. Thus, the group of smoke constituents which separated the second

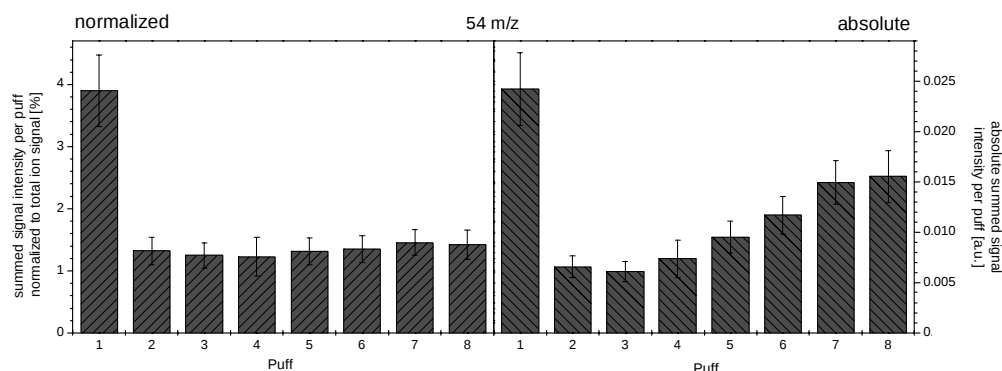


Figure 18A. Puff-by-puff resolved illustration of the first group of clustered compounds in the loading plot by means of 54 m/z (butadiene)

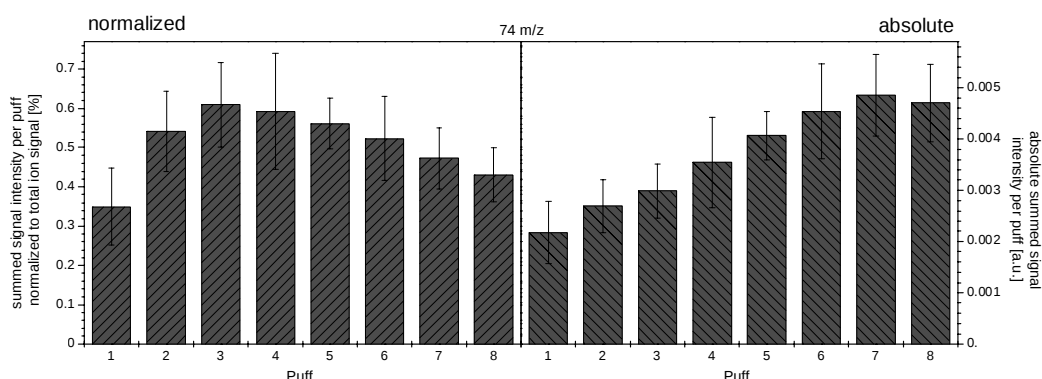


Figure 18B. Puff-by-puff resolved illustration of the second group of clustered compounds in the loading plot by means of 74 m/z (3-hydroxy propanal)

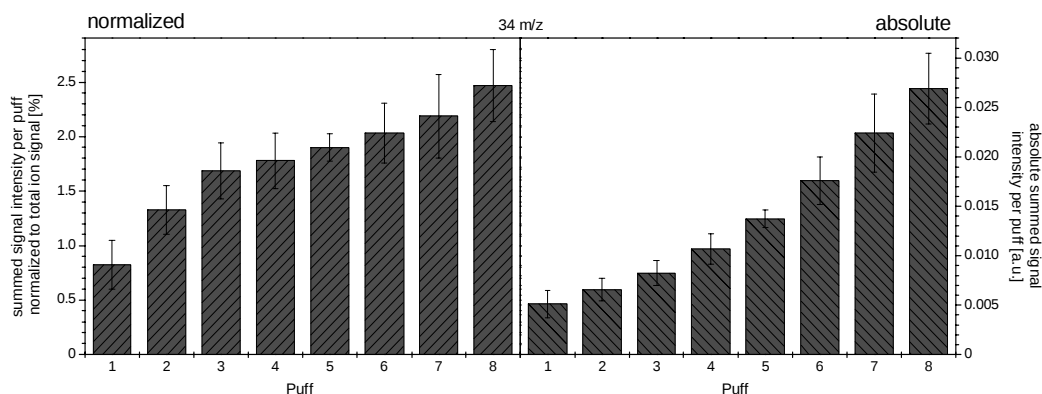
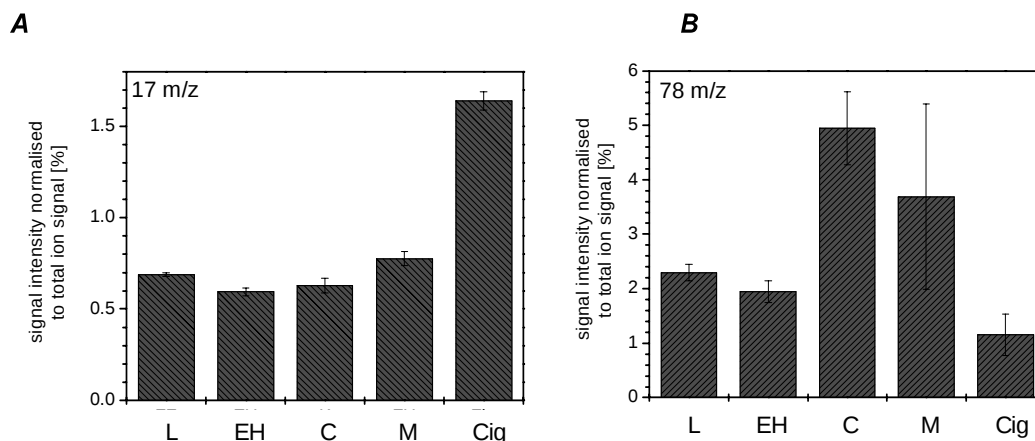


Figure 18C. Puff-by-puff resolved illustration of the third group of clustered compounds in the loading plot by means of 34 m/z (hydrogen sulphide)

puff from the first and the third puff when the cleaning puffs are considered (Figures 16A and 16B) have almost vanished. Only 43 m/z (carbohydrate pyrolysis fragment) and 17 m/z (ammonia) remain. A separation to the following puffs is visible but with approximately the same magnitude as for puffs three to eight (variance of the second principal component 13.25%). Thus when the cleaning puffs are not added to the smoking puffs the clear separation of the second puff is no longer observed in the score plot. Looking at the puff-resolved data of the cleaning puffs revealed (not shown here) that these masses, which separated the second puff from the other puffs in Figures 16A and B, feature rather high signal intensities in the first cleaning puff of the second smoking puff. In all the other cleaning puffs the

signal intensities are not exceptionally high. The reasons for this are unclear. However, it has to be stressed that the modified smoking machine has a much lower dead volume than the most commercial smoking machines. For commercial machines these effects might be even stronger. In the following discussion the puff-by-puff behaviour of the clustered masses is considered by focussing on three different areas on the loading plot of Figure 17B (greyish areas). In so doing the averaged normalised and the averaged absolute puff yields of one selected compound including standard deviations are illustrated in Figures 18A to C. It should be borne in mind that the PCA was carried out by the individual measurements. The standard deviations of all ten measurements are influenced by the fact that



Figures 19A and B. Comparison of the normalised yields for ammonia, 17 *m/z* (Fig. 19 A) and benzene, 78 *m/z* (Fig. 19 B) of all five different lighting methods (L = gas lighter; EH = electric heater; C = candle; M = match; Cig = cigarette)

the same puff number of different measurements can originate by a slightly different length of the cigarette rod depending on the lighting behaviour and burning characteristics of the cigarettes. This variation of the cigarette rod will influence the chemical smoke composition, and thus increases the corresponding standard deviation although all individual measurements exhibit almost exclusively the same trends.

Figure 18A (area 1) represents the unsaturated hydrocarbons located on the right hand side of the loading-plots using 54 *m/z* (butadiene) as an example. This group of compounds is mainly responsible for the separation of the first puff on the first principal component. The normalised illustration on the left clearly shows that the signal intensity of the first puff is much higher than for the following puffs. Puffs two to eight feature about the same normalised yields. The absolute values on the right also exhibit the very unique behaviour of the first puff. The yields of the following puffs are much lower but steadily increase from the second puff onward. The fact that the normalised yields of the second to the eighth puff do not change reveals that the influence of these species on the chemical pattern of all accessible compounds is always similar except for the first puff. Subsequently the rising yields of the absolute values mainly originate by physical reasons i.e. the decreasing ventilation by the reduced cigarette rod and condensation/desorption effects, which were mentioned earlier. A chemical influence of these compounds on the changing smoke pattern from the second puff onward is not observable.

The second class, shown in Figure 18B (area 2) using 74 *m/z* (probably 3-hydroxy propanal) as an example, has a different behaviour. Although the absolute values exhibit a rather steady increase in puff yields, the normalised signal intensities feature an increase in intensity from the first to the third puff followed by a decrease to the last puff. Therefore, this puff behaviour is rather different compared to the former one. The influence of these compounds on the chemical pattern seems to change during the smoking process with respect to the other accessible species.

Figure 18C (area 3) illustrates another puff behaviour demonstrated by 34 *m/z* (hydrogen sulphide). This class of substances shows a more or less steady increase in normalised as well as absolute signal intensities from the first to the eighth puff. Subsequently, these compounds gain importance with respect to the overall chemical pattern of observed species and influence the later puffs more than the earlier ones.

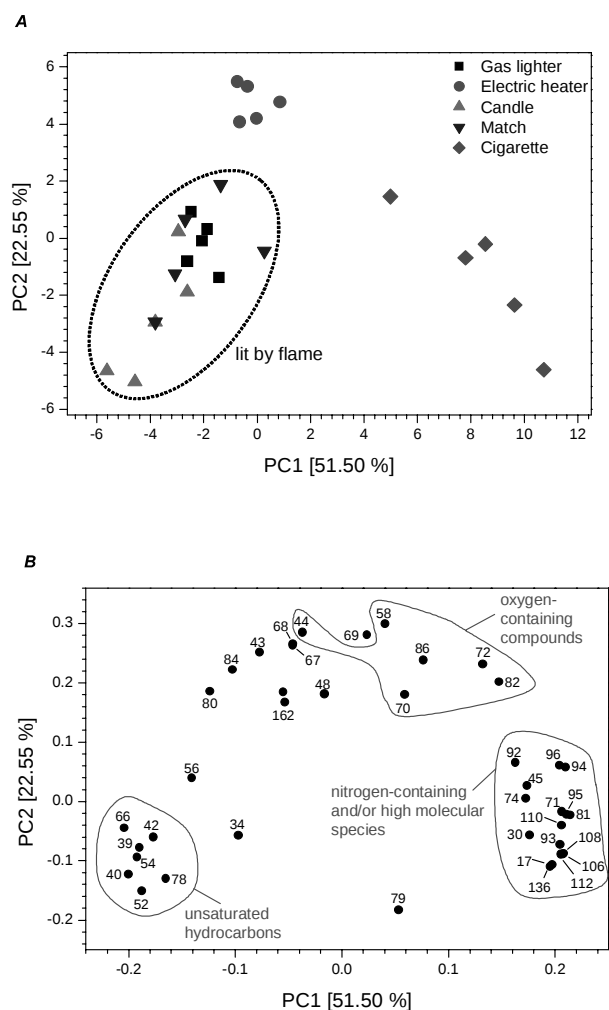
It can be said that the chemical pattern of the observed smoke constituents changes significantly during the cigarette smoking process from puff to puff. Almost every puff features a characteristic chemical composition. In this way the varying influence of certain compound classes can be clearly seen. In this context, the composition of the first puff is unique. This is most likely to be related to the different combustion conditions when the cigarette is lit. The separation of the second puff is also very clear but only if the cleaning puffs are included. For puffs 3 to 8 a change in the chemical pattern is observable but to a much lower extent. Interrelated reactions between involved species are probably the reason for all these changes.

Influence of different cigarette lighting devices on 2R4F cigarette

In previous sections it was demonstrated that a few smoke constituents feature exceptionally high yields in the first puff compared to the following puffs. Among these substances are some carcinogenic and toxic compounds such as butadiene and isoprene. These high yields are probably caused by the unique combustion and pyrolysis conditions when the cigarette is lit. Therefore, the device used for lighting the cigarette may also play an important role.

Figure 19A illustrates a comparison of the normalised yields for ammonia, 17 *m/z*. It can be observed that the gas lighter, electric heater, candle, and match result in similar values whereas another cigarette as the lighter causes much higher signals.

This is also the case for other substances such as 30 *m/z* (nitric oxide), 45 *m/z* (dimethyl amine, ethyl amine), 71 *m/z* (pyrrolidine), 81 *m/z* (methyl pyrrole), 93 *m/z* (aniline, methyl pyridine), 94 *m/z* (phenol), and 95 *m/z* (pyridinol, ethyl pyrrole, dimethyl pyrrole), 96 *m/z* (dimethylfuran, furfural), 106 *m/z* (xylene, ethyl benzene, benzaldehyde), 108 *m/z* (anisole, dimethyl pyridine, methyl phenol), 110 *m/z* (dihydroxybenzene, methyl furfural), 136 *m/z* (mainly limonene) etc. Most of these substances are nitrogen-containing compounds. This can be explained by the fact that part of the sidestream smoke from the lighting cigarette is drawn into the mainstream smoke of the cigarette to be analysed. Sidestream smoke is known to contain higher concentrations of nitrogen-containing compounds than mainstream smoke (9). In contrast, values for 40 *m/z* (propyne), 42 *m/z* (propene), 52 *m/z* (1-butene-3-yne), 54 *m/z*



Figures 20A and B. Score-plot (A) and loading-plot (B) of the first and second principal components of the PCA for the discrimination of the chemical patterns of the five different lighting methods

(butadiene), 66 *m/z* (cyclopentadiene), and 78 *m/z* (benzene), all of them being unsaturated hydrocarbons, are lower in intensity when another cigarette is used as the lighting device (1) (not shown here).

In Figure 19B the normalised yields of 78 *m/z* (benzene) are given. Benzene is a carcinogenic smoke constituent which is believed to be relevant for smoking-related diseases (71–74).

Normalised yields generated by the cigarettes lit with the gas lighter, the electric heater, and the other cigarette are similar whereas values for the match seem to be higher but the relatively high standard deviation does not allow a more detailed interpretation. However, the signals generated by the candle as the lighting device are much higher. In this context it should be noted that even the absolute benzene values were about twice as high as with the four other lighting methods (not shown here). A similar behaviour but to a less extent than for benzene can be observed for 52 *m/z* (1-butene-3-yne), 54 *m/z* (butadiene), and 66 *m/z* (cyclopentadiene), all of them being unsaturated hydrocarbons. It should be noted that butadiene is believed to be the substance with the highest carcinogenic potential in cigarette smoke (72–75). These high yields most likely have their origin in the thermal degradation of the candle's wax material (e.g. paraffin, stearin, beeswax etc.). In contrast to

lighting by the gas lighter, electric lighter, candle, and another cigarette, the information obtained on lighting by a match is lower because the standard deviations of almost all recorded substances are higher. One reason is the fact that to precisely position the lighting device at the cigarette is much easier with the other four devices because of the limited time a match burns. Therefore, the results of the match should not be over-interpreted.

Besides the comparison of the normalised yields, a principal component analysis was performed in order to find other features of the lighting technique.

Figure 20A shows the score-plot of the first and the second principal components of the PCA. It can be observed that the measurements where the cigarette was used as the lighting device are mainly separated on the first principal component with a variance of 51.50%. On the second principal component the measurements related to the electric heater are clearly separated (variance 22.55%), which form a smaller cluster. The three lighting methods where an open flame was used (candle, gas lighter, and match) also cluster together. It should be noted that the fuel of the gas lighter (propane/butane) is not influencing the results because the ionisation potential of both compounds is above the threshold of 10.49 eV for SPI. The corresponding loading-plot of Figure 20B points to the mass values, which are responsible for the separation. In so doing the formation of the cluster of the cigarette as lighting device is almost entirely originated by nitrogen-containing and higher molecular species. These compounds are abundant in sidestream smoke, as explained earlier. The separation of the electric heater from the rest is partially achieved by oxygen-containing compounds. A reason for this is not clear. In contrast, the cluster containing the three open flame devices is dominated by unsaturated hydrocarbons. Thus these three methods seem to have similar properties. The discrimination between the three open flame devices is also possible but requires some pre-selection of most characteristic mass signals according to the Fisher criterion. However, details on this can be found in (7). It should be noted that these three lighting methods (and also another cigarette as lighting device) are used by most human smokers whereas the electric lighter is only used in laboratory experiments with few exceptions such as cigarette lighters in cars etc. The clear separation of electric lighter and other lighting devices with open flames shows that laboratory experiments might have some differences in the results compared to human smoking results.

CONCLUSION – SMOKING EXPERIMENTS

Application of SPI-TOFMS to the analysis of cigarette smoke enabled a detailed characterisation of the smoking process (gas phase and whole smoke) on a puff-by-puff basis. In so doing, the very unique chemical composition of the first cigarette puff was discovered and described. In addition, evidence was found for a continuous change of the smoke composition from the second to the last puff. Puff-resolved quantification of various health-related smoke constituents was carried out, demonstrating the potential varying hazardous burden of each single puff for the human smoker. Thereby, drawbacks and limitations of the existing standardised smoking regime were discovered

when applied to puff-by-puff analysis. Furthermore, the influence of different cigarette lighting devices (gas lighter, electric lighter, match, candle, burning zone of other cigarette) on the smoke composition was investigated.

OUTLOOK

In this work the coupling of SPI-TOFMS to a smoking machine has proven to be a sophisticated and powerful approach for the investigation and characterisation of the cigarette smoking process (whole smoke and gas phase) on a puff-by-puff basis. Current activities focus on the combination of gas phase and particulate phase on-line instrumentation for a comprehensive analysis of both phases in real-time. In so doing, SPI is complemented by a second soft photoionisation technique, the resonance-enhanced multiphoton ionisation REMPI, which is highly sensitive for aromatic species, both coupled to a TOFMS. SPI/REMPI-TOFMS for on-line gas phase analysis is coupled to an electrical mobility spectrometer for on-line particle analysis, working at 10 Hz resolution in the range 5 – 1000 nm (Model DMS-500, Cambustion, Cambridge, UK) in order to gain additional information on several particle properties, such as particle nature, number, size distribution, and surface area. In addition on-line investigations of sidestream smoke have been carried out by using both photoionisation techniques, SPI and REMPI.

Furthermore, to examine the influence of the human puff behaviour and to determine the retention of hazardous smoke constituents in human smokers an experimental set-up has been developed where the SPI/REMPI-TOFMS is coupled to a flow box which measures the stream of smoke inhalation and exhalation of voluntary test smokers. In this way the effects of cigarette design parameters, vent-blocking, and personal smoking habits of the carefully recruited test smokers will be evaluated. The results might give new insights on the exposure of human smokers to the cigarette smoke.

Latest improvements of the ionisation technique are the development of electron-beam pumped rare-gas excimer VUV-lamp systems, which replace costly laser instruments (17, 18). Based on this enhancement a combined system of SPI-TOFMS and smoking machine is under development, which will be easy to handle and suited for large-scale routine analysis.

ACKNOWLEDGMENT

Dr Richard R. Baker was very much involved in setting up this research project. He also reviewed this manuscript shortly before his untimely death. With this paper, the authors would like to pay tributes to Dr Baker for his many contributions to this work and tobacco science.

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