

**Direct analysis of coffee aroma compounds with Proton Transfer
Reaction-Time of Flight-Mass Spectrometry: traceability, perceived
quality and processing**

A thesis presented to the Faculty of Chemistry and Pharmacy

for the academic degree of Doctor of Natural Sciences

by

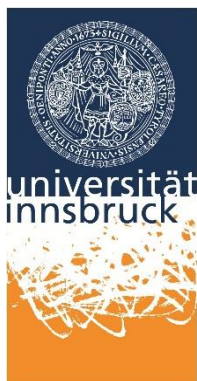
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accomplished at

the Institute of Analytical Chemistry and Radiochemistry

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Leopold-Franzens-University of Innsbruck



Innsbruck, January 2016

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Abstract

This thesis involves developing fast, rapid, and non-invasive headspace and nosespace analysis techniques based on PTR-ToF-MS coupled with an autosampler and tailored data analysis tools. The investigated case studies are related to coffee flavour also in connection with different technological and fundamental aspects as roasting and origin.

In a first study, an automated headspace sampling method was developed by combining a GC autosampler to PTR-ToF-MS to analyse the aroma profiles of three monoorigin (Brazil, Ethiopia and Guatemala) roasted and ground *Coffea arabica* samples from different batches. Unsupervised and supervised multivariate data analysis techniques were applied for data exploration and to classify coffees according to origin. Coffee samples were successfully separated according to origin by unsupervised methods (Principal Component Analysis, PCA). This separation was confirmed with Partial Least Square Regression-Discriminant Analysis (PLS-DA). The samples of one batch could be used as training set to predict geographic origin of the samples of the other batch, suggesting the possibility to predict further batches in coffee production by means of the same approach. As a follow-up study, the developed headspace sampling method was applied to analyse six roasted *Coffea arabica* coffees, both brew and powder, of different geographical origins (Brazil, Ethiopia, Guatemala, Costa Rica, Colombia, and India). For the first time, the volatile compounds released from coffee were analysed with PTR-ToF-MS in Switching Reagent Ion (SRI) mode by using different ionization agents: H_3O^+ , NO^+ and O_2^+ . Significant differences were found among volatile concentrations for the different origins both for powders and brews, in particular high concentrations of terpenes for Ethiopia, sulphur compounds for Colombia and thiazoles for Brazil and India. Effective classification models have been set for the different ionization modes and data fusion of the data obtained by different reagent ions further reduced the classification errors.

The next project was the development and application of an experimental protocol to monitor the volatile compounds released from single coffee beans at different stages of roasting. A laboratory scale oven was used to roast the green coffee beans (*Coffea arabica*) from different geographical origins (Brazil, Guatemala and Ethiopia) by sampling every one min up to 25 min. Two batches of one coffee origin were selected and at each time point, 3 coffee beans were roasted. This resulted in volatile profiling of a large sample-set: 468 coffee beans (3 origins x 2 batches x 3 replicates x 26 time points). The weight losses due to roasting process were

calculated for each coffee bean and time point. The effect of coffee geographical origin was reflected on the final weight losses and therefore volatile compounds formation. We observed a reduction in the amount of terpene fragments and an increase in heat induced volatile compounds. Clear origin signatures, which are in agreement with previous findings, were observed especially in the concentration of the volatiles released. Depending on the phase of roasting, some mass peaks were released earlier than the others and vice versa (e.g. m/z 82.065 around 6th min and m/z 101.060 around 10th min).

Finally, nosespace analysis (NS) was performed via simultaneous combination of PTR-ToF-MS with a dynamic sensory method called “Temporal Dominance of Sensations (TDS)” to gain insight about in-nose volatile release with the perceived aroma during espresso coffee drinking. One goal was to combine real-time instrumental and sensory methods and the second goal was to investigate the impact of roasting degree and sugar addition on aroma release and perception. A set of four coffee samples, two roasting degrees and two sugar levels, has been used for both sensory and instrumental measurements. Eighteen trained judges joined the study and they selected the dominant sensations among a 9-attribute list (sweet, sour, bitter, astringent, roasted, burnt, caramel, nutty and vegetal). The volatile compounds released in the nose of judges were monitored by NS analysis. A significant effect of roasting was observed with both techniques. More compounds and in larger quantity were released when increasing roasting degree, which was described in sensory perception as a greater dominance of the attributes burnt, roasted, astringent and bitter. Sugar addition did not significantly affect the aroma release of volatile compounds as demonstrated by the NS profiles of judges while changing completely the way the coffee was perceived by TDS. As expected, sweet taste became dominant over bitter and sour but it increased global flavour complexity with caramel and nutty notes via reducing the roasted or burnt notes. This result emphasized the presence of taste–smell perceptual interactions, due to congruence effect between sweet taste and some flavours of coffee, and the potential of combining dynamic methods to study the interactions. Besides, the treatment of NS data using clustering methods revealed two different release behaviours, which permitted identifying potential volatile compounds as TDS markers.

The developed methods are of general interest and have been tested also in the case of other food matrixes such as tea. The automated headspace sampling method was applied to measure the volatile compounds emitted from black (n=63, from 12 different countries) and green tea (n=38, from 9 different countries) leaves and their infusions. Black and green teas were

correctly classified by the volatile compounds emitted from tea leaves and their infusions independent from their geographical origins. A fixed time and temperature was applied for preparing black and green tea infusions to reduce the possible variability. Results showed that the release of volatile compounds is higher for tea samples which have smaller leaves or contain broken leaves as compared with the tea samples with bigger leaves, in particular after tea infusion. Depending on the processing method, teas produced in different countries have diverse appearance and flavour. For this reason we built classification models to investigate the possibility to link tea aroma with geographical origin. Results provided a good separation of tea origins, classification errors being mostly between countries geographically close to each other. These findings suggested that a better discrimination of tea samples might have been achieved if teas were classified according to production region rather than just country of origin. The promising outcomes of this thesis suggest PTR-ToF-MS as a successful tool for monitoring the release of volatile compounds from different aspects in coffee flavour science from coffee bean roasting to coffee drinking and as well as from product discrimination to traceability.

Acknowledgements

I wouldn't be able to produce the results presented in this thesis without the contribution of many people that I would like to acknowledge.

First of all, I would like to express my gratitude to my thesis supervisors Prof. Günther K. Bonn and Prof. Tilmann D. Märk for giving me the opportunity to conduct my PhD program under their supervision. I am grateful for their support, encouragement and help, which brought me to the end of my journey.

I would like to give my special thanks and gratitude for my thesis advisor Dr. Franco Biasioli who has always been very helpful and supportive from the first day I started working in his team. Especially, during the last months of my PhD studies, he has been always encouraging whenever I was down and I needed help. His colourful and joyful personality created a friendly working environment, which helped me to handle life much easier.

I would like to thank to my former and present colleagues at Volatile Compound Platform for their guidance, for sharing their knowledge and for their friendship as well. I would like to thank, in particular to Dr. Andrea Romano for his supervision, for teaching me how to operate a PTR-ToF-MS and for helping me whenever I had problems with R; Dr. Luca Cappellin and Dr. José Sánchez del Pulgar, for their help with data analysis and for the fruitful discussions we had during our weekly meetings. I am grateful to Dr. Pablo M. Granitto, for helping me with the data analysis from very far away.

I would like to thank to illycaffè S.p.a for providing the coffee samples for my thesis and I would like to thank, in particular to Dr. Luciano Navarini for his support and for our fruitful discussions on coffee.

I would like to thank to Ass. Prof. Dr. Matthias Rainer from Institute of Analytical Chemistry and Radiochemistry of Leopold-Franzens University of Innsbruck. From the first day, I met him he has always been kind and helpful.

I wish to present my sincere thanks to Dr. Flavia Gasperi, the head of Sensory Quality Research Group at Fondazione Edmund Mach, and her team for their collaboration and help that I will always remember the nice memories. I would like to give my special thanks to Dr. Eugenio Aprea for introducing me into the world of multivariate statistics. I would like to thank to Dr. Mathilde Charles for our collaboration and her amazing cakes. I wish to thank to our lab

technician, Emanuela Betta. She did her best to overcome the English-Italian language barrier between us for helping me in the lab. I should mention my other colleagues, Dr. Isabella Endrizzi, Dr. Maria Luisa Dematte and Dr. Maria Laura Corollaro. I really appreciate their help and home-ware support when I rented my first flat.

I would like to acknowledge the PIMMS (Proton Ionisation Molecular Mass Spectrometry) ITN supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382. I would like to thank to Prof. Christopher Mayhew and Prof. Nigel Mason for keeping the network together, for organizing all the meetings and training courses which were extremely useful for all the ESRs. I am thankful for knowing all the members in this network, all the supervisors and ESRs. I would like to thank, in particular, to Dr. Philipp Sulzer from IONICON team, to Prof. Chahan Yerezian and Dr. Saskia van Ruth, for hosting in their groups and for their collaboration. This network not only built the milestones of a successful career but also strong friendships that I will never forget. José! I will always remember the long hours we have spent in the lab with all the joy and as well as with all the challenges. I am very happy to know Nora and Valentina and of course Xianghui (I will always remember our short Normandy tour with a big smile on my face)!

I would like to thank all my friends and colleagues at FEM who have been a part of my life during the last three years. I am thankful to Salim, for his genuine friendship and support! Maybe the short distance between our hometowns, or the similarity of our cuisines brought us closer but I will always remember him as a dear brother. I would like to thank to Iiulia for her friendship and company. I will always remember our trips to Innsbruck with joy. Besides, I would like to thank Vittorio, Brian, Kajetan, Samia and Valentina. I am thankful to know all the volleyball players at FEM; I always looked forward to Wednesday evenings to train with you! My special thanks to Ben, for always being supportive without getting tired and for all the beautiful places we have seen together. Thank you!

I would like to thank all my best friends. To Fatma, Eda, Demet, Seher, Mızrak, Oğuz and Aslihan, from the bottom of my heart, for knowing them since years and for feeling their existence with me even though we were thousands kilometers away from each other. With them, I shared my life, lots of unforgettable memories besides we shared the challenges of pursuing a PhD and clinging on to life despite everything else.

Last but not least, my greatest thanks to my family, for their continuous support and endless courage.

Abbreviations

ACPI	Atmospheric-pressure chemical ionization
ANOVA	Analysis of Variance
B	Buffer gas
C	Concentration
cps	Counts per second
Da	Dalton
DIMS	Direct Injection Mass Spectrometry
E	Electric field ($\text{V}\cdot\text{cm}^{-1}$)
F	Fragment
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GC-O	Gas Chromatography-Olfactometry
ICP-MS	Inductively coupled plasma mass spectrometry
IMR-MS	Ion-molecule reaction mass spectrometry
IRMS	Isotope ratio mass spectrometry
IT	Ion Trap
IT-MS	Ion Trap- Mass Spectrometer
k_R	Reaction rate coefficient
m/z	Mass to charge ratio
$m/\Delta m$	Mass resolution
mg/kg	milligram/kilogram
N	gas density (cm^{-3})
N	Neutral fragment
PCA	Principal Component Analysis
PDA	Penalized Discriminant Analysis
PLS	Partial Least Squares
PLS-DA	Partial Least Squares-Discriminant Analysis
ppbv	part per billion in volume
pptv	part per trillion in volume

PTR-MS	Proton Transfer Reaction-Mass Spectrometry
PTR-QiTOF	Proton Transfer Reaction-Quadrupole interface Time of Flight-Mass Spectrometry
PTR-ToF-MS	Proton Transfer Reaction-Time of Flight- Mass Spectrometry
QMS	Quadrupole Mass Spectrometer
RF	Random Forests
sccm	Standard cubic centimeter per minute
SIFT-MS	Selected ion-flow-tube mass spectrometry
SVM	Support Vector Machine
Td	Townsend (10^{-7} V. cm^2)
TDS	Temporal Dominance of Sensations
TI	Time Intensity
VOC	Volatile organic compound

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Introduction

Consumers nowadays are starting to engage with what they eat and drink. This prompted the food industry to change so that the information about the quality of the products; where their food comes from, and how they taste and/or smell and possibly the reasons behind these characteristics. For consumer acceptance and preferences, flavour plays a key role in determining the main characteristics of a food product [1], which is strongly linked to the perceived quality.

Volatile organic compounds (VOCs) play a fundamental role in foods science and technology. They are not only responsible for the specific odours of foods but also they interact with human senses involved in flavour perception which is, in the end, associated to food liking and appreciation [2]. When VOCs are released from food they can directly reach human nose via direct sniffing (orthonasal perception, odor, above-the-food aroma) or via exhaled breath (retronasal perception, in-mouth aroma) after swallowing. As a result, consumers' acceptability, judgment and their perception are shaped depending on these processes; the properties and concentrations of volatile compounds in the foods as well as consumer physiology [1, 3-5].

Coffee has been one of the most consumed drinks in the world, both for the stimulatory effects of caffeine and for its unique smell and flavour properties [6]. As a reaction to the rising consumer demand for product diversification, an increasing product differentiation based on geographical origin can also be observed in the coffee market, particularly in the so-called specialty coffee market [7]. Specialty coffees are defined as products that come from a particular geographical origin with higher quality attributes of distinctive flavour and aroma profile than other commodity coffees. For product differentiation and specification, more effort and attention have to be devoted to highlight the main differences behind the unique aroma characteristics of coffee. Moreover, when specific links are established between volatile compounds and sensory attributes, an enhanced understanding of flavour can be achieved [8] to ensure the perceived quality.

Direct injection mass spectrometric (DIMS) techniques [9] broaden the borders of aroma analysis by eliminating the time consuming aroma isolation step and long analysis times those not permitting the real-time monitoring of VOCs, which is crucial for *in-vivo* aroma release studies. In particular, a break-through was recorded when proton transfer reaction-mass

spectrometry (PTR-MS) was introduced for the direct, simultaneous and real-time detection and quantification of volatile compounds at very low levels [10]. High mass resolution and sensitivity provided by combining time-of-flight (ToF) mass analysers to PTR-MS [11] allow the analysis of volatile compounds at very low levels and provide high time resolution. One of the main advantages of direct injection is that it brings a closer approach for the analyst to find connections between VOCs released from a food product and perceived aroma. Another aspect is that, VOCs directly detected in the headspace are expected to give significant information related to orthonasal perception whereas monitoring VOC release in the nose during food consumption, will clarify their role in aroma perception. The advantages of this novel methodology makes it a very valuable tool in flavour science however it has be noted that this technology does not propose an alternative to gas chromatographic methods due to the complications in compound identification but offer a reliable complementary tool for the study of volatile compounds especially when fast, sensitive and real-time measurements are needed.

This thesis covers the potential applications of PTR-ToF-MS for the analysis of coffee volatile compounds for characterization of coffee powder, coffee brew and roasted single coffee beans from different geographical origins and also for *in-vivo* perception of coffee aroma via monitoring the in nose volatile compounds release during coffee drinking for an enhanced understanding of flavour perception in combination with dynamic sensory methods.

This thesis is organized as follows. The first chapter is intended as an introduction into the background of this thesis. The first part of this chapter provides an overview about coffee, coffee volatile compounds and discusses the effect of geographical origin on the aroma profile of coffee. Later, it describes the role of volatile compounds in flavour perception and compares the existing analytical methods for detection of aroma compounds. This chapter gives detailed information of the methodologies applied in this thesis and in particular the analytical technique that has been used: PTR-ToF-MS. The final part of this chapter is dedicated to a brief outline about the scope and the aims of this thesis. Published results are introduced with Chapters 2, 3 and 4. Chapter 2 consists of 2 published articles and focuses on the applications of PTR-ToF-MS for discrimination of aroma profiles of coffees from different geographical origins. This chapter also represents the first examples of the application of an automated headspace sampling system developed for the rapid and high-throughput screening of large sample sets. Chapter 3 is dedicated to monitoring of volatile compounds released from single coffee beans. For this purpose, a large number of green coffee beans (n=468) were roasted and volatile

profiles were analysed with PTR-ToF-MS at different stages of roasting to obtain an insight into volatile formation and their relations with coffee geographical origin. Chapter 4 describes a methodology based on simultaneous combination of dynamic instrumental and sensory methods for monitoring flavour release. The effect of roasting degree and sugar addition on coffee aroma perception and in nose volatile compound release was investigated. Lastly, Chapter 5 demonstrates the interest of the developed methods by describing their application to a different, important matrix: the characterization of black and green teas from different countries. PTR-ToF-MS was used to analyse the volatile profiles of 63 black and 38 green teas from different countries emitted from dry tea leaves and tea infusions. Headspace volatile fingerprints were used to build different classification models for discrimination of black and green teas according to tea type and geographical origins. Two different cross validation methods were applied and their effectiveness for origin discrimination was discussed. Black and green teas were successfully separated and the differences in their volatile profiles were discussed. The classification models showed a separation of black and green teas according to geographical origins the errors being mostly between neighbouring countries.

Finally, this thesis ends with summary and future perspectives.

Chapter 1. Background

The first chapter is intended as an introduction into the background of this thesis. The first part of this chapter provides an overview about coffee, harvesting and the properties of coffee beans. Following sections describe the reactions responsible for formation of aroma compounds during coffee roasting and discuss the specialty coffees and the effect of geographical origin on the final aroma characteristics of coffee. Later, it describes the flavour perception phenomena and the role of volatile compounds in flavour perception. The existing analytical methods for detection of aroma compounds are described and compared nevertheless more attention has been devoted to the analytical methodology that has been applied for this thesis. Therefore, proton transfer reaction-mass spectrometry has been introduced in detail via explaining the main parts of the instrumentation, the ionization mechanisms, recent developments and as well as an overview on the pros and cons. The so-called nosespace analysis for *in-vivo* flavour release monitoring has been described and the potential of combining this technique with dynamic sensory methods has been discussed. The final part of this chapter has been dedicated to the scope and the detailed aims of this thesis.

1.1 Coffee in perspective

1.1.1 Green coffee, harvesting and post processing

The term ‘coffee’ comprises not only the consumable beverage obtained by extracting the roasted coffee with hot water, but also a whole range of intermediate products starting from the freshly harvested coffee cherries [12].

The coffee plant (*Coffea*) grows in tropical and subtropical regions of Central and South America, Africa and South East Asia, preferably in temperate and humid climates at altitudes between 600 and 2500 m. More than 100 species belong to *Coffea* genus; but two main species of commercial interest are *Coffea arabica* and *Coffea canephora* var. *robusta*. They are usually referred to as Arabica and Robusta, respectively [6, 12]. Arabica holds a higher price in the commercial market, is more delicate, demands greater care in its culture, and possesses superior organoleptic characteristics in comparison with Robusta. On the other hand, Robusta is more resistant, has a lower cost of production, but results in an inferior cup quality [6].

The berries (or cherries) of the coffee plant contain the seeds (normally two in one berry), which are usually known as beans. Harvesting should start after a very careful examination of the level of maturation, when most of the cherries are ripe. It is carried out either by non-selective stripping of whole branches or by selective handpicking. Handpicking is more time consuming

but results in higher quality coffee. After harvesting, the pulp of the cherries (skin) is removed via dry processing or wet processing; the latter is more complex but provides a better quality coffee. The green coffees are then subjected to sorting and size grading to eliminate the foreign materials and as well as defective beans. Lastly, the outer silverskin covering the coffee beans are removed with polishing [6, 13]. The dry green coffee beans are then classified based on species and variety of origin, prior to shipping and/or roasting.

The quality of the green beans are highly affected by the agricultural factors related to geographical origin including the chemistry of the soil, the weather conditions and the altitude the coffee plant is grown. The green coffee beans differ in many aspects. For instance, they vary in mass from 100 mg to over 200 mg, with some evidence of variation associated with the bean variety and its origin, degree of maturation, green coffee processing and storage of processed coffee. Depending on the processing method (dry or wet), green coffee beans may contain around 9–13% moisture. The total amount of carbohydrates represents about 50% on dry basis. Arabica contains more lipids (~16%) and trigonelline (~1.0%) than Robusta (~10 and 0.7%, respectively); while Robusta contains more caffeine (2.2%, almost twice as much) and chlorogenic acids (10% compared to 6.5%). Total proteins in Arabica and Robusta coffee is around 10% [6, 13].

1.1.2 Coffee roasting and aroma formation

The unroasted green coffee beans have a greenish and pea-like odour which is not appealing to the consumers. However they contain all the necessary ingredients to form the desired and well-known coffee flavour. Up to date, almost 300 volatile compounds have been detected in the aroma fraction of green coffee beans mainly for evaluating the quality of the raw material and for detecting the off-flavours caused by the defective coffee beans [13].

Roasting is defined as the method that utilizes dry heat (usually hot air) for cooking purposes, which results in enhancing the flavour of the food product. Coffee roasting is induced by the hot air coming from the roaster which brings the coffee beans within a temperature range (170-230°C) in a given period of time. Roasting creates a complex series of reactions influenced by several parameters. The main aspects of coffee roasting are illustrated in **Figure 1.1-1**. As shown in **Figure 1.1-1**, the main physical and chemical changes observed during coffee roasting are the generation of the characteristic flavour, the development of brown to dark brown colour, and the increase of coffee bean volume (by 50-100%). During roasting, the coffee beans

undergo a two-stage transformation; at first the free moisture content is reduced from 10-12% to a few percent. Here, the colour of the beans changes from green to pale brown. When the coffee beans reach around 6% moisture content, pyrolytic reactions start (second stage) and therefore the beans swell, show a rapid darkening and large quantities of gases (mainly CO₂) are generated which cause cracking of the coffee beans. Mainly due to the evaporation of water, the coffee beans lose weight between 14 and 20 %, depending on the green bean quality and the process conditions that affect the final degree of roast (light, medium-light, medium, medium-dark, dark, very dark) [12].

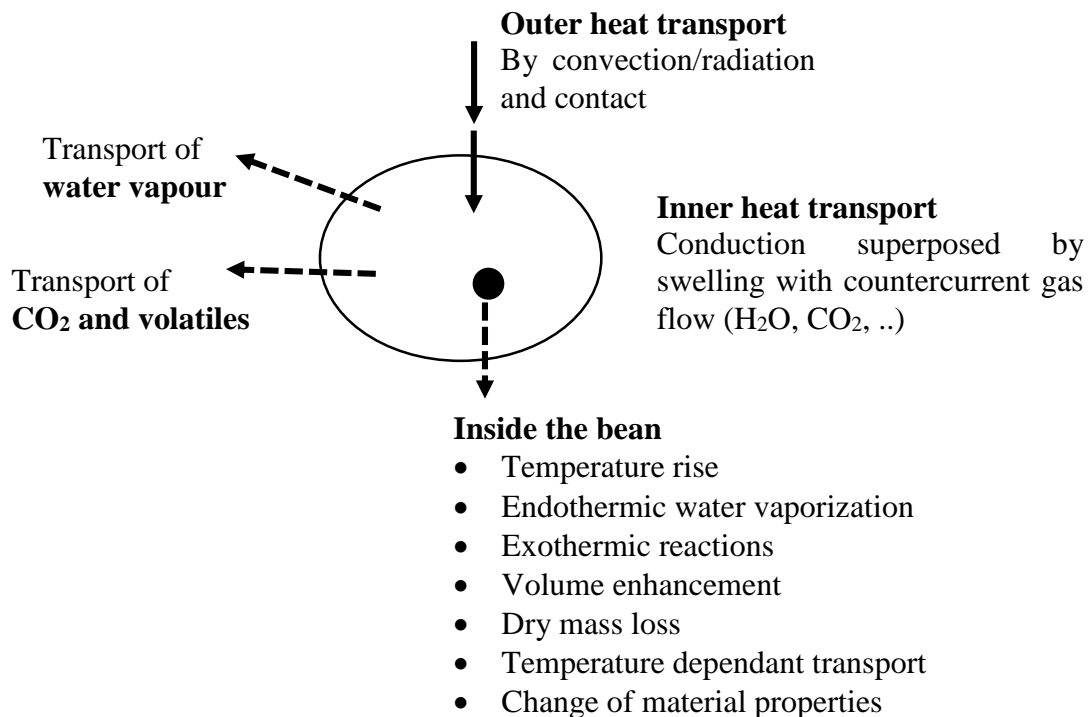


Figure 1.1-1. Main aspects of coffee roasting (Reproduced from [6, 12])

The non-volatile constituents of green coffee evolve differently during roasting which result in formation of different classes of aroma compounds with various odour characteristics. The extensive and complex chemical reactions mostly affect the carbohydrate fraction via Maillard reactions, Strecker degradation, pyrolysis and caramelization. Proteins are denatured and degraded while free amino acids, peptides and proteins with free amino groups react with reducing sugars (Maillard reactions) to form glycosylamines and/or aminoaldoses and/or aminoketones after Amadori and Heynes rearrangements [6, 12]. Several further rearrangement and breakdown reactions lead to the formation of various volatile compounds and dark coloured

compounds (i.e. melanoidins). Other components of green coffee, such as nitrogen containing substances, chlorogenic acids, and lipids, are also involved in reactions that lead to the generation of aroma compounds. A simplified scheme for the formation of coffee flavour compounds was presented by Yeretizian et al., 2002 [14] as shown in **Figure 1.1-2**. Chlorogenic acid contributes to body and astringency, sucrose contributes to colour, aroma, bitterness and sourness. Caffeine does not contribute to the aroma of coffee but to the bitterness [13].

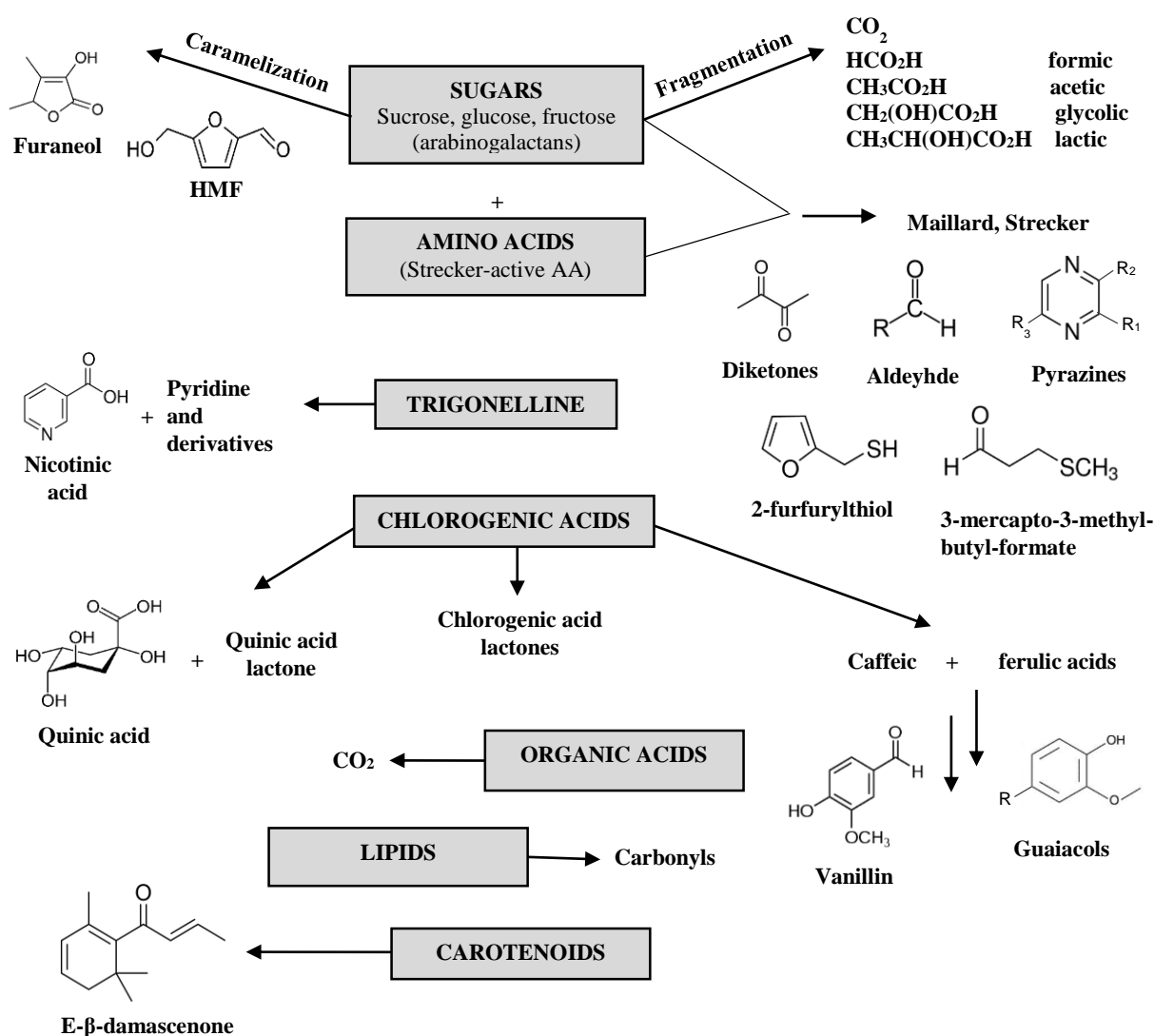


Figure 1.1-2. A simplified scheme showing the main classes of volatile compounds formed from non-volatile precursors in the green coffee beans during roasting

Roasting results in formation of more than 950 volatile compounds depending on the origin, degree of roasting and analytical methods used [15]. The classes of volatiles found in coffee are pyrans and furans, pyrazines, pyrroles, ketones and aldehydes, phenols, oxazoles, alcohols, hydrocarbons, esters and acids, sulphur compounds, thiazoles, pyridines and lactones. Generally, carbohydrates (including soluble polysaccharides) produce furans, aldehydes, ketones, and phenols; proteins, peptide, and amino acids produce ketones, pyrroles, and pyrazines; lipids are responsible for only small amounts of aldehydes and ketones (they show resistance during roasting); chlorogenic acids produce phenolic volatile compounds (e.g., catechols, pyrogallol, and phenol); and trigonelline produces pyrroles, pyridines, and pyrazines [15]. Among the high number of volatile compounds that have been found in coffee aroma, a small part has been considered to be responsible for the characteristic coffee aroma. Indeed, around 40 volatile compounds have been identified in a medium roasted Arabica coffee powder and brew as representative potent odorants of coffee [16]. Among them, 2-furfurylthiol, 4-vinylguaiacol, the alkylpyrazines and furanones, acetaldehyde, propanal, methylpropanal, and 2- and 3-methylbutanal were found to be the odorants with the highest impact on coffee aroma. Some potent coffee volatile compounds with odour properties are presented in **Table 1.1-1**.

Table 1.1-1. Character impact odorants of roasted powder and brew of Arabica coffee

No	Compound	Odor quality
1	3-Methylbutanal	Malty
2	2,3-Butanedione	Buttery
3	2,3-Pentanedione	Buttery
4	3-Methyl-2-buten-1-thiol	Amine-like
5	2-Methyl-3-furanthiol	Meaty, boiled
6	2-Furfurylthiol	Coffee-like
7	3-(Methylthio)propanal (Methional)	Boiled, potato-like
8	3-Mercapto-3-methylbutyl formate	Catty, roasty
9	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	Seasoning-like
10	3-Hydroxy-4,5-dimethyl-2(5H)-furanone	Seasoning-like
11	(E)- β -Damascenone	Honey-like, fruity
12	2-Methoxyphenol (Guaiacol)	Phenolic, burnt
13	2-Methoxy-4-ethylphenol (4-Ethylguaiacol)	Spicy
14	2-Methoxy-4-vinylphenol (4-Vinylguaiacol)	Spicy
15	3-Methoxy-4-hydroxybenzaldehyde (Vanilline)	Vanilla-like
16	2-Ethyl-3,5-dimethylpyrazine	Earthy, roasty
17	2,3-Diethyl-5-methylpyrazine	Earthy, roasty
18	3-Isobutyl-2-methoxypyrazine	Earthy
19	Phenylacetaldehyde	Honey-like
20	2-Hydroxy-3,4-dimethyl-2-cyclo-penten-1-one	Caramel-like
21	<i>p</i> -Anisaldehyde	Sweet, minty
22	Linalool	Flowery

1.1.3 Coffee geographic origin

Standardization of coffee quality starts from the green beans, for which, the information of geographical and botanic origins of the coffee, the harvest year, the moisture content, the total defects and the bean size is required [17]. According to The Specialty Coffee Association of America [18], coffees with attributes of high quality and distinctiveness coming from a particular origin are regarded as “specialty coffees”. These coffees can be also named as ‘monorigin coffees’ or ‘single-origin coffees’. Even though ‘single-origin’ term is not precisely defined, it can originate in one country, one region, or one estate or farm [7]. The main organoleptic characteristics of specialty coffees are their unique qualities (judged by a consumer), distinct taste and aroma different from other commodity coffees.

Coffee beans used in commercial coffee blends may come from a wide range of geographical areas and have different chemical and organoleptic properties. From an economic point of view, data from U.S. online retail stores indicate that single-origin coffees receive significant higher retail prices [7]. Global recognition and market power also considerably increase the practices associated with selling coffees on the basis of their geographical origin. In this regards, declaration and marking of geographical origin is needed for product specification, traceability, and authentication [19].

One of the most commonly utilized strategies in coffee authenticity is to discriminate coffees based on the coffee bean variety (i.e. Arabica or Robusta). Another important aspect of coffee authenticity is geographical origin declaration [20]. There are a number of analytical methods developed for the determination of the geographical origin of foods. Among them, methods based on determination of mineral contents or composition, light- or heavy-element isotope ratios (i.e. Isotope ratio mass spectrometry (IRMS), Inductively coupled plasma mass spectrometry (ICP-MS)) [21] are highly developed and commonly used by the legal authorities. However, they do not measure the properties directly related to sensory perception and do not reflect the organoleptic characteristics of a food sample which is crucial for specification of high quality products such as coffee. The need for the analytical methods capable of verifying the geographical origin of coffee and providing links to highlight the main differences behind their unique aroma characteristics is therefore outstanding.

1.2 Volatile compounds and flavor perception

Volatile organic compounds are the principal components of odour and flavour and literally hundreds of compounds might contribute to characteristic odours we associate with particular foods. Up to date, more than 7000 flavour compounds have been reported in various food products [22]. However, it is estimated that only 5%–10% of them play a significant role in the formation of specific aromas of food products [23]. A continuously updated database on published volatile compounds which have been found in natural (processed) food products can be retrieved online (<http://www.vcf-online.nl>).

Volatile organic compounds go into gas phase by vaporizing at 0.01 kPa at room temperature with boiling points between the ranges from (50°C to 100°C) to (240°C to 260°C) [24]. The majority of the volatile compounds are also relatively nonpolar (hydrophobic) compounds, which favours their partition in aqueous media. Volatile compounds are relatively small molecules (molecular mass <400 Da) and consist of highly diverse chemical classes [25]. Volatile compounds in foods vary in concentration and might be present in foods at extremely low concentrations (mg/kg or even ng/kg). However, the concentration of a specific volatile compound is not the most significant factor determining its contribution to the characteristic aromas. It was proposed that only those odorants might contribute to the aroma whose concentrations in food exceed their odour thresholds (the smallest concentration of a compound that can be perceived by human nose). The importance of a volatile compound for the aroma of a particular food can be estimated via calculating the ratio of its concentration to its odour threshold which can be expressed by different terms such as “aroma value”, “flavour unit”, “odor unit”, “odour value” or “odour activity value” [25]. Volatile compounds with concentrations lower than the odour and/or taste thresholds also contribute to aroma when mixtures of them exceed these thresholds. Briefly, volatile compounds that are attributed to the main contributors any foods should have high odour activity values [26]. Among the aroma substances, special attention is paid to those compounds that provide the characteristic aroma of the food and are, consequently, called key odorants (character impact aroma compounds).

Flavour plays a fundamental role in determining the main organoleptic characteristics of a food product. It affects the overall quality and therefore its assessment by the consumers. It is defined as the combined perception of aroma, taste, and texture at the time of food consumption. While taste is limited to sweet, sour, bitter, salty, and umami sensations; aroma compounds stimulate much more qualities [27]. It is considered that texture is indirectly involved in flavour

perception; however aroma compounds directly affect the overall perception. During eating and drinking, VOCs (stimuli) are released from the food matrix and therefore transported to the odour receptors (olfactory epithelium) in the nose. Flavour integration is then driven by the differences depending on the route followed by the volatile compounds. The volatile compounds can reach the olfactory epithelium via direct sniffing with the nose when the food is not put in the mouth (*orthonasal olfaction*, odour perception) and/or through nasopharynx when the volatile compounds are released in the mouth and then swept by the airflow from the lungs and are exhaled through the nose after swallowing (*retronasal olfaction*, flavor perception). In both ways the volatile compounds reach the same stimuli, however the direction of the volatile flow will determine the order of the receptors to be stimulated and therefore different responses are likely to occur in the brain [27, 28]. A scheme of nasal cavity and aroma perception is shown in **Figure 1.2-1**.

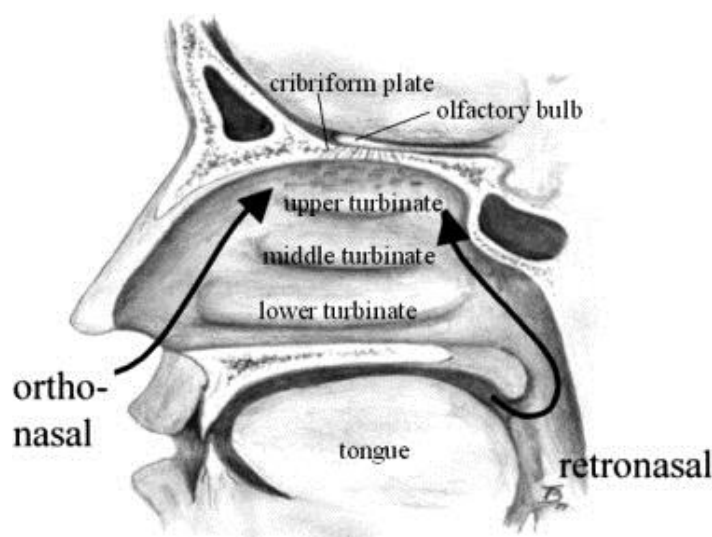


Figure 1.2-1. Schematic drawing of the nasal cavity with the lower, middle, and upper/superior turbinates. The olfactory bulb/peduncle as the first relay station of olfactory processing (it is here where axons from olfactory receptor neurons synapse with mitral cells) is lying in the olfactory sulcus located just above the cribriform plate. The olfactory epithelium is found in the top of the nasal cavity, largely beneath the cribriform plate. Airflow in relation to orthonasal (through the nostrils) or retronasal (from the mouth/pharynx to the nasal cavity) presentation of odours to the olfactory epithelium is indicated by thick arrows [29].

Different volatile compounds may show different concentration-perceived intensity relationships [30] and the interactions of volatile compounds with food matrices may influence their release and subsequently their perception [31]. Moreover, the measurement of these various stimuli can be challenging. The limited sensitivity as well as selectivity of various instruments and analytical techniques may not provide the same information that nasal receptors can detect (nose 10^{-17} g of flavour detection for some compounds). The detection threshold of the analytical instrument can be lower than the odour threshold for a specific volatile compound. Also, as the number of aroma compounds that can be detected in real time increases, the sensitivity of the instrumentation commonly decreases [28].

1.3 Analytical methods for the analysis of aroma compounds

Without aroma analysis, it is very difficult to identify the flavour of any food product. There has been so much focus on the understanding of this crucial component therefore several analytical methods have been developed for the detection of aroma compounds [32-35].

Each analytical method offers different approaches with varying strengths and weaknesses in the isolation and detection of the volatile organic compounds. One of the biggest challenges in aroma analysis is the choice of method which is usually not as sensitive as the human nose. It has been reported that 8 molecules of a potent odorant can trigger 1 olfactory neuron and that only 40 molecules may provide an identifiable sensation [34]. Another difficulty is the distribution of the aroma compound in food matrix, which can complicate the aroma isolation and detection, accordingly. The nature of the aroma compounds (hydrophilic/lipophilic) [34] and interactions between flavor compounds and food ingredients (i.e. proteins, carbohydrates, lipids) significantly affect their release [31]. Another important point is the diverse chemical and physical properties of volatile compounds so that one single method may not be suitable for detection of the volatiles of interest. The concentration and number of the volatile compounds present in foods should be taken into account in aroma analysis. If the volatile compounds are present at trace levels, methods with high sensitivity and precision are needed for a reliable and robust analysis. The analyst should select the analytical method fits best to the objectives of the study [34-36].

Analysis of volatile compounds in foods are performed due to several purposes listed below:

- To obtain a complete aroma profile (volatile fingerprint) of a sample;
- To identify key aroma components responsible for a characteristic food aroma;

- To monitor the volatile release or production over time;
- To detect off-notes in a food product for quality evaluation or detection of food spoilage;
- To find links between sensory attributes and perceived aroma and,
- To check the authenticity of the aroma compounds by detecting if the aroma compounds come from natural sources or produced synthetically [26, 34].

1.3.1 Gas Chromatography- Mass Spectrometry (GC-MS)

In flavour research, gas chromatography coupled with mass spectrometry (GC-MS) is considered as a golden standard for the detection of volatile compounds released from foods. GC-MS is used as the main analytical method for detection and quantification VOCs [37]. GC-MS systems have high precision and can reach detection limits as low as 0.1 pptv but suffer from a relatively low time resolution. One of the most important limiting factors for the analysis is the chromatographic separation on the capillary column which ranges from minutes to tens of minutes.

Preparation of samples for GC-MS analyses starts with VOC isolation which is usually laborious and time consuming. One should consider the polarity and the volatility of the target molecule during aroma isolation and concentration. Nevertheless, no single sample preparation technique is appropriate for every type of analyte or sample matrix [34]. Aroma concentration usually involves the adsorption of VOCs into a suitable fiber and then thermal desorption for injection into the column. Several aroma pre-concentration methods are available in the literature [37] for the detection of food volatiles; in most of the applications methods based on purge-and-trap (*i.e.* Tenax column), distillation methods (*i.e.* vacuum or steam distillation), direct solvent extraction and solid phase micro-extraction (SPME) methods are commonly used [35, 37]. With GC-MS, complex aroma mixtures are separated into individual components and further identified by using available reference libraries (*e.g.* the NIST-98/Wiley library). For improving the resolving power in GC analyses, capillary column GC (high-resolution gas chromatography, HRGC) is preferred as a standard with enhanced column capacities. The resolving power is further improved by multi-dimensional GC such as GCxGC especially when it is interfaced with a time-of-flight mass spectrometer (TOF-MS) [35]. The detailed applications of multi-dimensional gas chromatography in flavor analysis can be found in [38] and [39].

1.3.2 Gas Chromatography-Olfactometry (GC-O)

Gas chromatography-olfactometry (GC-O) methods are used to determine the odor active compounds. Main aim is to sort out the odorant compounds from odorless volatile fraction. The gas chromatographic effluent of a representative aroma isolate is split in two by volume (1:1) where one part is sniffed by a trained person in order to associate odor activity with the eluting compounds; the second half is sent to the detector. A trained person can smell the GC effluent and tell the potency (sensory intensity) of a GC peak, character and often even the chemical identity of a peak. The results help the flavor chemist to decide whether the aroma extract should be concentrated or a different isolation technique should be used to prepare the sample for GC [26, 35, 40].

1.3.3 Direct injection methods

Analytical techniques based on ‘direct injection’ in particular Direct Injection Mass Spectrometry (DIMS) have successfully been applied in various fields for the detection of volatile organic compounds [9]. The main advantages brought by the development of DIMS techniques are the rapid, non-invasive and direct analysis of VOCs with improved mass and time resolution. Continuous sample injection allows online and real-time monitoring of VOC release which is in particular a crucial feature to study *in-vivo* flavor release during food consumption and to monitor online food processes.

The mostly used DIMS techniques for monitoring VOCs and BVOCs in gas samples are Atmospheric-Pressure Chemical Ionization (ACPI), Ion-Molecule Reaction Mass Spectrometry (IMR-MS), Selected Ion-Flow-Tube Mass Spectrometry (SIFT-MS) and Proton Transfer Reaction Mass Spectrometry (PTR-MS) [9, 41]; moreover electronic noses (e-nose) and electronic noses based on mass spectrometry (MS-e-noses) can also be included in direct gas analysis. Among them, IMR-MS has been utilized mostly in medical and environmental applications [41] but not in aroma analysis, therefore further details will not be provided. More attention will be paid on the methods that have been used for volatile compound detection in foods.

ACPI is commonly used as an ionisation source for many mass spectrometry techniques. The ionisation relies on gas phase ion–molecule reactions that take place at or near atmospheric pressure. Generally, multiple ion species are generated from air (e.g., N_2^+ , O_2^+) by using a corona discharge that react with the analytes and as well as the water vapor present in the sample

to produce $[MH^+]$ ions from the neutral molecules $[M]$ [41]. Successful applications of ACPI-MS in flavour release monitoring [42] and in non-targeted foodomics [43] are available in literature.

SIFT-MS and PTR-MS both have a big advantage over the other methods have been mentioned for direct analysis of gaseous samples in real time without chromatographic separation and calibration procedures. Similarly, both techniques allow simultaneous quantification of VOCs. However, SIFT-MS and PTR-MS differ in ion source: a microwave or electron-impact ion source is utilized in SIFT-MS [44]; whereas the ions are generated by a hollow cathode discharge in PTR-MS [10]. SIFT-MS requires pre-selection of main ionization agent (*i.e.* H_3O^+ , O_2^+ and NO^+) via a quadrupole mass filter and is less sensitive when compared to PTR-MS due to the mass analyzer employed. There is no electric field employed in SIFT-MS which makes it possible to carry out ion-molecule reactions under thermal conditions [44]. The ion-molecule reaction rate coefficients can be determined with SIFT-MS under controlled conditions which can be directly used in quantitative analysis. PTR-MS mostly utilizes rate coefficients reported in literature which usually bring a fixed error in quantification [45]. More details on applications of these methods in food science and technology can be found in [41, 46].

Electronic nose (E-nose) instruments consist of an array of electronic chemical sensors with an integrated pattern recognition system [47] which are designed to mimic the human olfactory system. Two types of electronic nose technologies exist: electronic noses based on gas sensors and recently developed electronic nose technology based on MS [9, 33, 48]. Unlike most existing chemical sensors, MS-e-noses cannot identify specific VOCs but with the existing pattern recognition system it is possible to recognize simple or complex odors [41]. Aroma mixtures are directly introduced into the e-nose systems via an aroma delivery system without pre-separation of the sample analyte. Some of the disadvantages are the sensitivity to the operation temperatures and humidity [33]. The output of MS-e-nose is a fingerprint of the sample, however the degree of fragmentation due to the electron impact has to be taken into account for data interpretation. Several applications of e-noses and MS-e-noses are available in literature [48, 49].

This thesis employs PTR-MS with a time-of-flight (ToF) mass spectrometer for the rapid, direct and high throughput analysis of volatile compounds. Therefore, the following section will present a detailed description about the utilized methodology. The applications of PTR-ToF-MS for monitoring *in vivo* flavour release by nosespace analysis and its combination with

dynamic sensory methods will be provided and the advantages and disadvantages of the present technique will be discussed.

1.4 Applied methodology

1.4.1 Proton Transfer Reaction-Mass Spectrometry

Proton transfer reaction-mass spectrometry (PTR-MS) is a technique developed for the detection of trace gaseous volatile organic compounds. PTR-MS is a form of soft chemical ionization mass spectrometry, which was developed in the mid-1990s by Werner Lindinger and co-workers at University of Innsbruck [50-53].

PTR-MS is a direct injection mass spectrometric method which allows the analysis of volatiles without any pre-treatment in real-time and with high sensitivity. It is based on the ionization of VOCs by means of protonated water, hydronium ions (H_3O^+), where the proton transfer is highly dependent on the proton affinities of volatile compounds. For proton transfer to occur, the proton affinities of the target VOCs should be higher than water. There is a big advantage of using H_3O^+ primary ions is that many of their proton transfer processes are non-dissociative, so that only one product ion species occurs for each neutral reactant [53]. The proton transfer from H_3O^+ is very selective to the organic constituents of air which makes it an outstanding proton source for the detection of trace VOCs. The common constituents of air, such as N_2 , O_2 and CO_2 and inorganic species do not undergo proton transfer reaction due to their low proton affinities. Most of the volatile compounds have proton affinities larger than that of water molecules and therefore proton transfer occurs at every collision. Proton transfer from H_3O^+ is exothermic because proton affinities of the volatiles exceed that of water [54]. Some selected compounds with proton affinities and molecular formulae are given in **Table 1.4-1**.

The first PTR-MS instrument was commercialized in 1998 by Ionicon Analytik GmbH which is located in Innsbruck, Austria. Another PTR-MS producing company is KORE Technology Ltd. located in Ely, UK.

Table 1.4-1. Proton affinities of some gaseous compounds [55]

Substance	Molecule	Proton affinity (kJ/mol)
Oxygen	O ₂	421
Nitrogen	N ₂	494
Carbon dioxide	CO ₂	541
Ozone	O ₃	626
Water	H₂O	691
Ammonia	NH ₃	854
Methanol	CH ₃ OH	754
Ethanol	C ₂ H ₅ OH	776
Phenol	C ₆ H ₆ O	817
Ethylene	C ₂ H ₄	680
Acetaldehyde	C ₂ H ₄ O	769
Propanal	C ₃ H ₆ O	786
Acetone	C ₃ H ₆ O	812
Pentan-2-one	C ₅ H ₁₀ O	833
Pentan-3-one	C ₅ H ₁₀ O	837
Acetic acid	C ₂ H ₄ O ₂	784
Propanoic acid	C ₃ H ₆ O ₂	797
Acetonitrile	C ₂ H ₃ N	779
Dimethyl sulphide	C ₂ H ₆ S	831
Isoprene	C ₅ H ₈	826

A typical PTR-MS consists of mainly three parts: (i) ion source, (ii) drift tube and (iii) mass analyser. An exemplificative illustration of PTR-MS instrumentation with a quadrupole mass analyser is shown in **Figure 1.4.1**. The first PTR-MS was introduced with a quadrupole mass analyser [52]; in the recent years PTR-MS with a time-of-flight mass analyser [11, 56] has been developed and applied successfully in various fields with improved mass resolution and sensitivity.

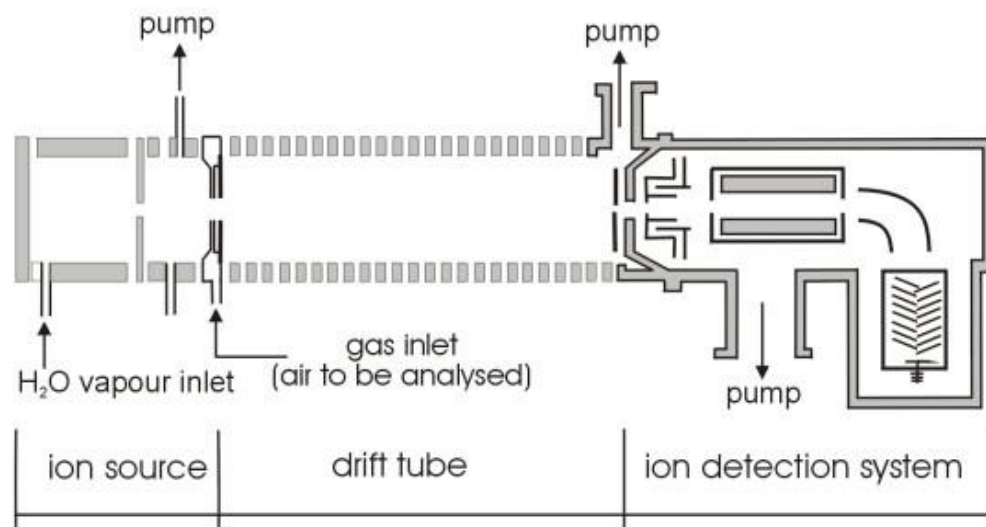
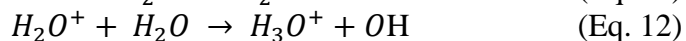
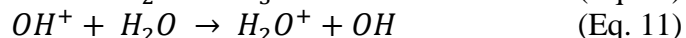
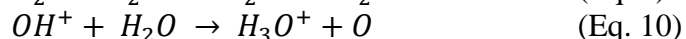
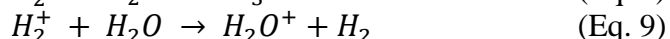
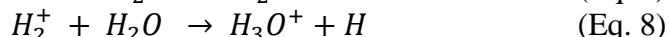
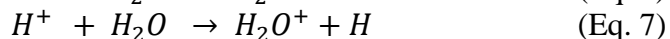
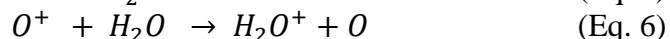
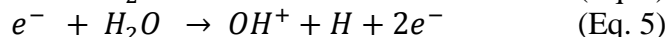
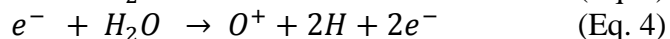
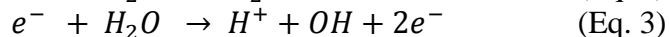
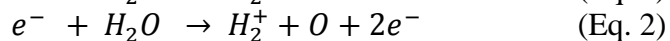
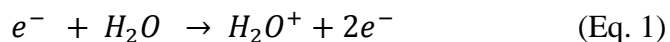


Figure 1.4-1. Schematics of a PTR-MS coupled with a quadrupole mass spectrometer [53]

1.4.1.1 Ion source

In the ion source, H_3O^+ ions are generated. The most commonly employed ion source is a hollow-cathode discharge that generates high purity (>99.5%) ions through water vapor which is injected into the ion source region [57]. The discharge process is fast therefore all the potential contaminant ions generated (O^+ , OH^+ and H_2O^+) in this process lead to the formation of H_3O^+ . Despite the high purity of the primary ion, a small amount of air may enter the ion source (via back flow) as contaminant which leads formation of O_2^+ and NO^+ as impurities.

The reactions that occur in the ion sources are presented in equations 1-12 [57].



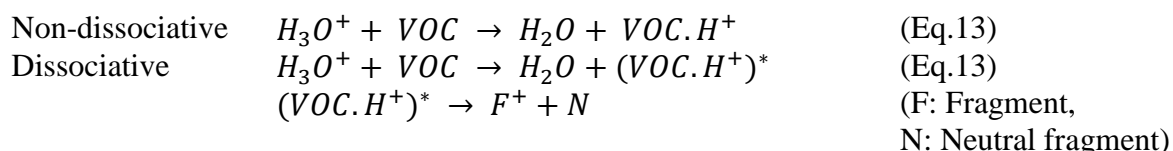
1.4.1.2 Drift tube

Ions produced in the ion source are directed into the drift tube via an electric field. The sample analyte is then directly injected into this region. The drift tube contains a buffer gas (usually air) where the protonation reactions take place via collision. The temperature of the drift tube can be adjusted between 40 and 120 °C and the pressure is usually kept between 2.2 and 2.4 mbar. The proton transfer reaction is controlled by E/N value where E is the electric field (Vcm^{-1}) across the drift tube and N is the number gas density (cm^{-3}). The E/N value is given in Townsend (Td, $10^{-7} \text{ V.cm}^{-2}$) and the E/N value can be adjusted by changing the drift tube voltage by using the instrument software. Higher E/N ratios result in more energetic collisions which may increase fragmentation of the VOCs but also reduce the formation of cluster ions, especially water clusters: $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$. Increased collision energy may also increase the fragmentation of the ions produced by the reactions between H_3O^+ and the analyte gas, which is usually undesirable due to the complications that appear in the mass spectra. For this reason, the E/N ratio should be carefully controlled. The most commonly preferred E/N values change between 100-140 Td [57, 58].

1.4.1.2.1 Reactions with H_3O^+ as main ionization agent

H_3O^+ ions travel through a buffer gas in the drift tube and the reactants (VOCs) are added in small amounts so that the density of the buffer gas [B] stays much larger than the density of the [VOCs]. The VOCs perform many non-reactive collisions with buffer gas atoms and molecules, however when they collide with the reactants, the proton transfer reaction occurs (if energetically allowed). If the analyte molecules are present in trace amounts, the H_3O^+ ion signal does not decline significantly (about one to a few percent) so that $[\text{VOC.H}^+] < [\text{H}_3\text{O}^+]$ remains valid. By creating a high ion count rate $i[\text{VOC.H}^+]$ per unit density of $[\text{H}_3\text{O}^+]$ in the gas, high sensitivity is obtained.

Typical proton transfer reactions between H_3O^+ and VOCs occur as follows [53]:



The reactions of H_3O^+ with the VOCs must occur under well-defined conditions in order to achieve an accurate quantification of the neutral reactants from the data on primary and product ion signals. The concentrations of target volatiles can be calculated without calibration or using standards according to the following formula [10]:

$$C \text{ (ppb}_v\text{)} = \frac{1}{kt} \cdot \frac{[\text{VOC.H}^+]}{[\text{H}_3\text{O}^+]} \cdot \frac{1}{N} \cdot 10^9$$

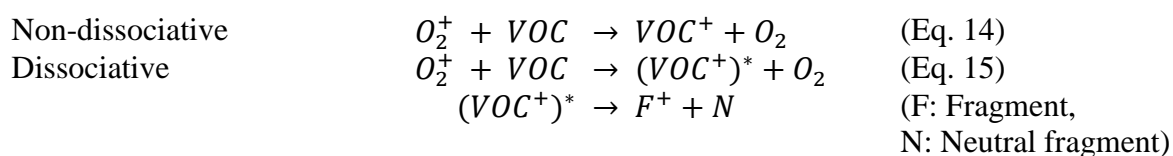
where C is the concentration reported in ppb_v , k is the reaction rate constant for a given molecule ($\text{s}^{-1} \cdot \text{cm}^3$), t is the ion travel time in the drift tube ($\sim 100 \mu\text{s}$), $[\text{VOC.H}^+]$ and $[\text{H}_3\text{O}^+]$ indicate the measured counts per second (cps). N is the gas density in the drift tube (molecules/cm^3). The method has been reversed from measuring the reaction rate coefficients to the usage of known reaction rate coefficients. Several limitations about the accuracy of this formula should be taken into account. For instance, the experimental k values may not be available for many compounds in real applications therefore constant reaction rate coefficients ($k_R = 2 \times 10^{-9} \text{ cm}^3/\text{s}$) can be employed in calculations. However, this can introduce a systematic error for the absolute concentration of each compound which is below 30% in most of the cases and can be accounted for if the actual reaction rate constant is not available [59]. Moreover, the assumption of constant H_3O^+ concentrations and the presence of other ionization mechanisms due to the contaminant ions (explained in the following section) can play significant role in the interpretation of the mass spectra and as well as in calculations of the absolute concentration.

1.4.1.2.2 Ionization agents other than H_3O^+

With the recently developed Switchable Reagent Ion (SRI) system [60], it is possible to produce reagent ions other than H_3O^+ . This system allows easy and fast switching between H_3O^+ , NO^+ , O_2^+ , Xe^+ and Kr^+ primary ions with detection limits in the ppqv range. The reagent ions are produced by using O_2 , charcoal filtered air (or, alternatively, pure N_2 and O_2) and Kr and Xe^+ as the reagent gases in the ion source, respectively. In this case, attention should be paid on the impurities while using different ionization agents: (i) H_3O^+ , NO^+ and NO_2^+ in case of O_2^+ as the reagent ion; (ii) H_3O^+ , O_2^+ and NO_2^+ , in case of NO^+ as the reagent ion; and (iii) H_3O^+ and KrH^+ in case of Kr^+ as the reagent ion.

The PTR-ToF-MS with SRI system allows the analysis of volatile compounds which are not detectable with H_3O^+ due to lower proton affinities than H_2O (i.e. some alkanes) and provides the separation of isobaric compounds such as isobaric aldehyde and ketone pairs.

Different reaction pathways take place when O_2^+ and NO^+ are used as ionization agents where the reaction is not proton transfer anymore. When O_2^+ is used the reactions occur as given in Eq. 14-15 via charge transfer:



When NO^+ is used, the target volatile may undergo charge transfer, hydride (H^-) or hydroxyl (OH^-) ion abstraction or both. The reactions of NO^+ are given in Eq. 16-17. In this equations, X represents the abstracted ion (H^- or OH^-) and B the stabilizing buffer gas.



According to the reaction patterns of NO^+ with VOCs as shown above, aldehydes and ketones appear at their parent masses and H^- subtracted masses, respectively.

PTR/SRI-MS system has been applied for detection of isoprene and 2-methyl-3-buten-2-ol [61], ethylene [62] emissions, picric acid [63], in the detection of drugs [64-66] and in the area of homeland security [67]. However, the applications in food science and technology is rather limited. One study has been recorded for discrimination of dry-cured ham samples which allowed separation of isobaric aldehydes and ketones in the headspace [68]. An example is shown in **Figure 1.4-2** for the reactions of C_6 aldehydes and ketones with different ionization agents.

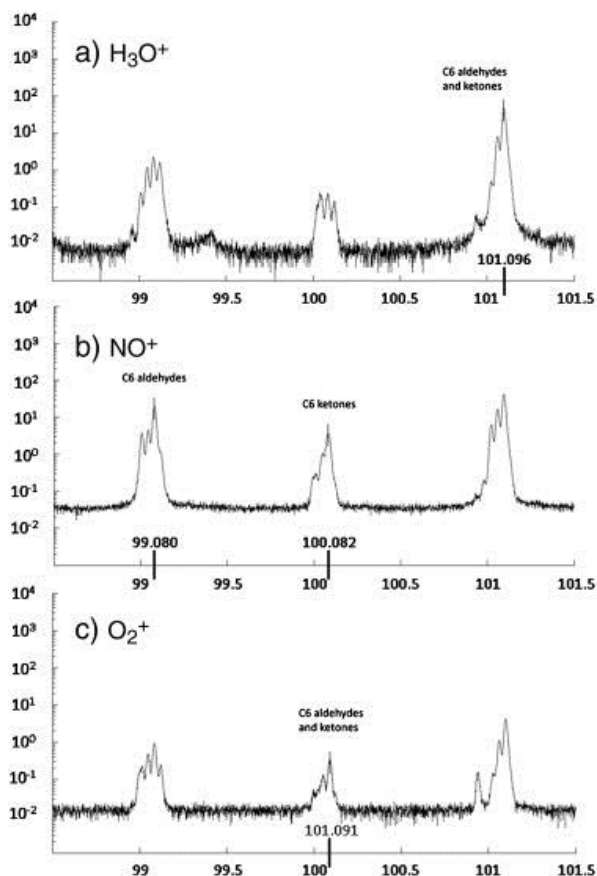


Figure 1.4-2. Example of peaks related to C₆ aldehydes and ketones measured by using different reagent ions: a: H₃O⁺, b: NO⁺, and c: O₂⁺ (reproduced from [68])

1.4.1.3 Mass analysers

Once the gas-phase ions are generated, they need to be separated according to their masses. The positions of peaks in a mass spectrum are determined according to m/z ratio where m is the mass of a specific ion and z is the electrical charge [69]. In PTR-MS instrumentation, the protonated volatiles are transferred to the mass analyser via a lens system. Up to date, three types of mass analysers have been utilized with PTR-MS instrumentation. These are quadrupole, ion trap and time-of-flight analysers. Each mass analyser has its advantages and limitations which are discussed in the related sections below.

1.4.1.3.1 Quadrupole Mass Spectrometer (QMS)

The earliest PTR-MS instruments employed only quadrupole mass spectrometers (QMS) [54]. From a technical point of view, a quadrupole mass spectrometer consists of 4 parallel rods where an electric current is applied on the rods to generate an electric field in xy plane. When

the frequency of an ion with a specific m/z is in resonance with the oscillation frequency of the electric field, the ions pass through the quadrupole and reach the detector [69]. All these properties explain a QMS to act as a mass filter.

The QMS systems are limited to have low mass resolution ($m/\Delta m$) however they are operated in low vacuum levels (10^{-2} - 10^{-3} Pa) and are available in compact size [54]. PTR-MS measures the intensities of the protonated compounds and the nominal masses of the detected ions, thus many volatile compounds having the same nominal mass will not be distinguished therefore an appropriate compound identification cannot be achieved [10].

1.4.1.3.2 Ion trap mass spectrometer (IT-MS)

Ion trap (IT) mass spectrometers utilize an oscillating electric field to store the ions. Ions are trapped by using a radio frequency (RF) quadrupolar field which can trap the ions in two or three dimensions [69]. Several PTR-MS instruments have been constructed with IT mass spectrometers [70-72]. Utilization of an IT mass spectrometer with PTR-MS extends the analytical capabilities of quadrupole mass analysers employed in PTR-MS [72]. IT-MS shows faster data acquisition than QMS so that a full mass spectrum can be obtained in the same time as a QMS [70]. A better compound identification can be obtained by using IT-MS due to the capability to perform collision induced dissociation reactions which provide the possibility to distinguish between isomers and other isobars. Moreover lower E/N values can be reached with IT-MS which increases the sensitivity [71].

1.4.1.3.3 Time of Flight – Mass Spectrometer (ToF-MS)

The essential principle of a time-of-flight mass spectrometer is that a bunch of ions moving in the same direction and having a distribution of masses but a (more-or-less) constant kinetic energy, will have a corresponding distribution of velocities in which velocity is inversely proportional to the square root of m/z [73]. Ions are extracted from the ionization unit in pulses and they are separated according to their flight times under the influence of an electric field. Ions of different mass will arrive at the detector sequentially and in principle it is possible to detect all the ions that were present in the source. Once all the ions are in the flight tube, no further ions are injected till the slowest ion reaches the detector. The reflectron employed in the PTR-ToF-MS systems, acts as energy focusing via the ring electrodes with increasing potential energy allowing the faster ions of the same m/z then slower ones go deeper into the reflectron. The ions are reflected at the bottom of the flight tube therefore they move along a longer path.

By adjusting the appropriate reflector voltages, the slow and fast ions reach the detector at approximately at the same time [11, 54, 58]. A scheme of PTR-ToF-MS is shown in **Figure 1.4-3**.

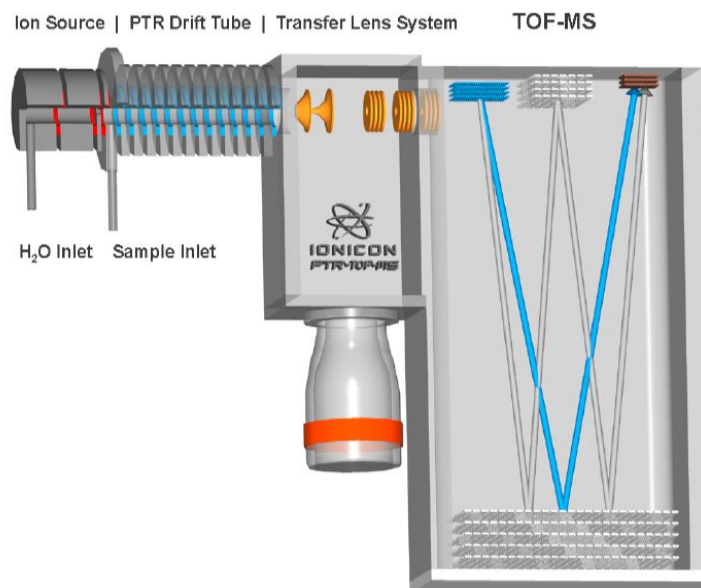


Figure 1.4-3. Schematic of a PTR-MS with time-of-flight mass spectrometer (courtesy of Ionicon Analytik, GmbH)

One of the main and biggest advantages of using a ToF-MS is the simultaneous transmission of all ions which generates a full mass spectra in a very short time (less than a second). This allows the analysis of complex volatile mixtures in real time with high mass resolution ($m/\Delta m=8000$) and at very low detection limits (pptv levels) [60]. The high sensitivity is often in the range of the sensitivity of human nose which allows an easy and reliable comparison between PTR-ToF-MS and the human response.

1.4.2 Recent developments in PTR-MS technology

Thanks to the recent developments in the past two years, a fast gas chromatographic separation can be coupled to PTR-MS that significantly improves the detection capabilities [74]. This recent technique is called as fastGC-PTR-MS and a schematic drawing is shown in **Figure 1.4-4**. fastGC-PTR-MS has much shorter spectral run in contrast to classical GC analyses (less than one minute, depending on the applied temperature ramp and the complexity of the sample). This significantly reduces the long operating time of GC systems. This technique has been

successfully applied on wine matrix [75] which eliminated the saturation of the ethanol and the ethanol-VOC clusters in the mass spectra. With this system, ethanol leaves the column very fast and therefore formation of alcohol-clusters is prevented. fastGC-PTR-MS has been further applied for the separation of monoterpenes with same sum formula [76] and for monitoring isoprenoid emissions from trees [77] which were not available for normal PTR-MS analyses.

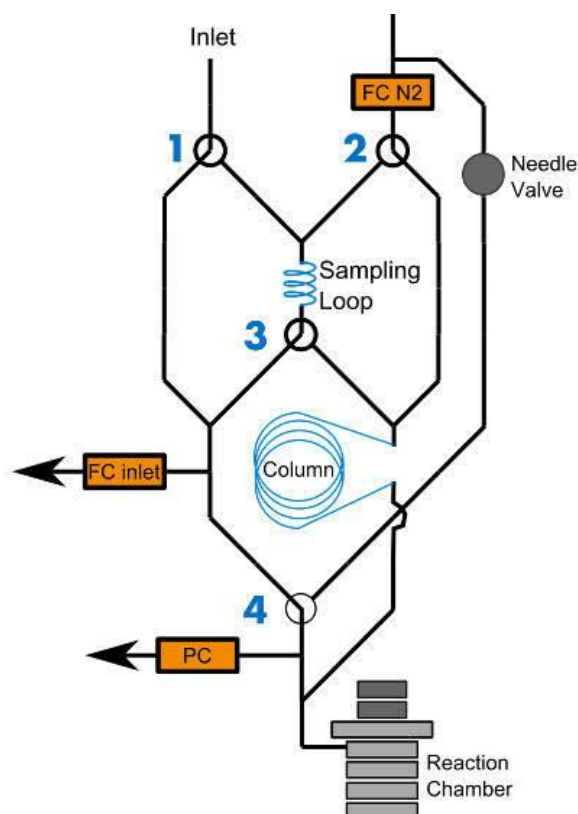


Figure 1.4-4. Schematic drawing of a PTR-ToF-MS inlet system with a FastGC setup. The fastGC module consists of a heated column, a sample loop, several valves (1-4), and an additional flow controller. The valves can configure different operation modes: such as real-time PTR-TOF measurement, sample loop loading and injection, and subsequent fastGC measurement. The valves are depicted in their normally open state, as they are when FastGC is disabled. The column is resistively heated by applying a current, which allows for fast heating rates (>10 °C/s). The low thermal mass of the heating module also ensures fast cooling rates (from 200 °C to 50 °C in less than 20 s) [75].

One of the most recent development is the development of a new prototype of PTR-TOF-MS instrument equipped with a quadrupole ion guide (PTR-QiToF, a scheme is shown in **Figure**

1.4-5 [78] in 2014 (Qi, Quadrupole interface). The main difference of this new system when compared to a PTR-ToF-MS is the transfer region between the drift tube and ToF mass analyser which enables highly effective transfer of ions. PTR-QiTOF can be optimized to a maximum sensitivity of maximum mass resolution depending on the basis of the study, especially for the applications where extremely small concentrations has to be analysed. In addition, PTR-QiTOF can be operated by using SRI system.

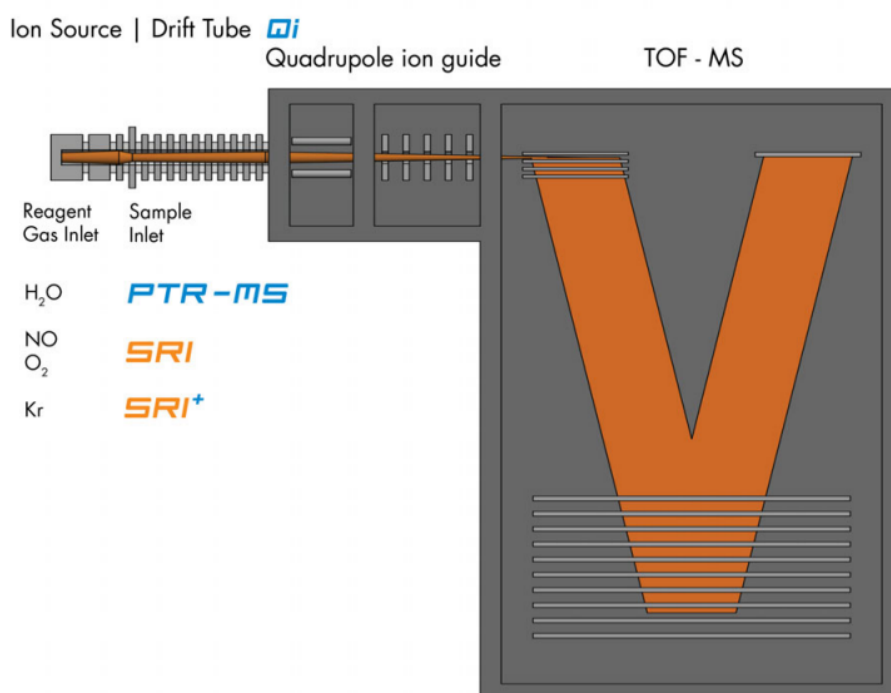


Figure 1.4-5. Scheme of PTR-QiTOF [78]

1.4.3 Nosespace analysis with PTR-ToF-MS

Nosespace analysis is the technique by which the VOC release is measured in the exhaled breath during food mastication or beverage consumption. The so-called “nosespace analysis” via PTR-ToF-MS consists of sampling the exhaled breath through the nose via a nosepiece (usually glass) (**Figure 1.4-6a**) which allows the panelists to maintain their natural eating/drinking habits (**Figure 1.4-6b**) [3, 79].

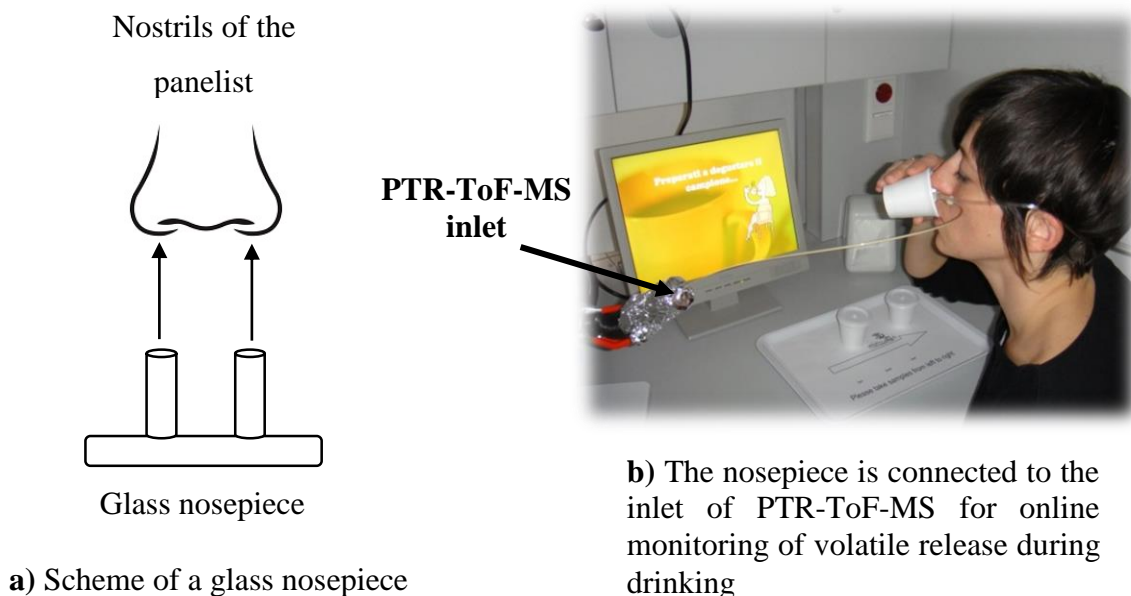


Figure 1.4-6. Experimental set-up for nosespace analysis with PTR-ToF-MS

The main significance of nosespace analysis is that it brings the flavor analyst closer to the real consumption experience. The nosepiece illustrated in **Figure 1.4-6a**, has 4 openings: the two at the upper part are connected to the nostrils of the panelists; one at the lower part is connected to PTR-ToF-MS inlet and the other one is open to the air. The sampling rate (400 sccm) for nosespace analysis is in general faster than the static headspace measurements (40 sccm) which allows monitoring the fast changes happening in real-time during eating/drinking. Since volatile release processes occur on relatively short time, faster sampling rates will extend the analytical capabilities of the instrument.

Aroma perception is a dynamic process where the intensity and profile of VOCs evolve with time. High resolution PTR-ToF-MS allows simultaneous recording of all the VOCs released in the nose. The output is the release curves of all the volatile compounds released in nose, which are described with unique characteristics. An example of a release curve is shown in **Figure 1.4-7** for m/z 80.045, tentatively identified as pyridine during coffee drinking. After the introduction of the coffee sample in the mouth ($t=30$ s), a significant increase is observed in the signal intensity where it reaches a maximum after swallowing the sample (a few seconds after the sample introduction in mouth).

This release curve contains 5 important computable parameters such as (1) *max*, the maximum concentration reached; (2) *tmax* the time necessary to reach the maximum concentration; (3)

median of the release curve; (4) the *slope* of the first descending section of the curve and lastly (5) the *area* under the curve. These parameters characterize the volatile release in time-independent (*area*, *max*, *median*) and time-dependent (*slope*, *tmax*) fashions [80].

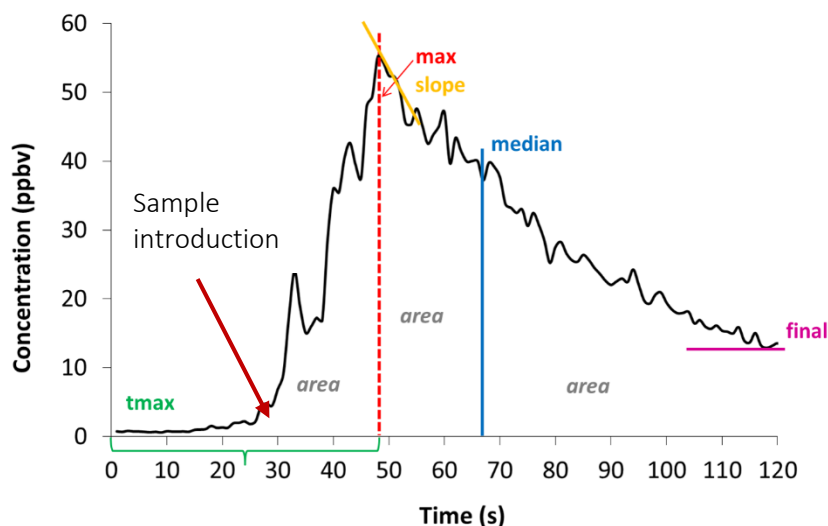


Figure 1.4-7. Release curve parameters for protonated pyridine (m/z 80.045; $C_5H_6N^+$)

One of the major issues in real-time volatile analysis is the handling of the large data sets produced by PTR-ToF-MS [59]. Additionally, the time dimension increases the data size and therefore the complexity of data analysis. Nevertheless, a straightforward methodology was introduced by Romano et al., 2013 [81] for the analysis of complex PTR-ToF-MS mass spectra produced during nosespace analysis of coffee. The same approach was employed in this thesis for analysing the nosespace data. The presented methodology required using tailored data analysis tools which were described in detail from raw data handling to characterization of coffees and coffee tasters.

1.4.4 Combination of PTR-ToF-MS with dynamic sensory methods

Sensory evaluation has been defined as a scientific method that measures, analyses and interprets the responses given to products though using our senses (odour, taste, sight, touch and hearing) [82] or it can be defined as a technique or referred to a technique that highlights and describes organoleptic properties of a product by the sense organs [83]. Different methods exist in literature for sensory analysis and perception [82-84] but the investigation of the

dynamic perception has gained a lot of interest due to the dynamic nature of sensory sensations during food consumption. Since the perception of flavour is a multi-dimensional phenomena, it is necessary to include sensory input in the interpretation of flavour analysis.

Methods that acknowledge the dynamic properties of eating are likely to produce results more valid than static methods. Among the dynamic sensory methods [85], methods based on measuring the intensity of a sensation in relation to the time of its perception are named as ‘time intensity (TI)’. Initial applications of TI were primarily more related to taste sense, which changes during mastication in the mouth when eating, and particularly to the evolution of bitterness and sweetness [83, 85]. The evaluation starts when putting the sample into the mouth. The final point means that the perceived intensity is completely gone. Experiments are performed applying a special device coupled with the computer system which provides an output in the form of a special curve displaying the changes in the intensities with time and the differences between judges [85].

Another dynamic sensory method has been introduced in the recent years. This method is called as Temporal Dominance of Sensations (TDS) which describes the temporal evolution of the different sensations developed during consumption. This method requires trained panellists and they are asked to choose the attribute which dominates their perception (the attribute that catches the attention of the consumer) among a pre-defined list of attributes. TDS is considered as a multi-attribute descriptive method more effective than the other dynamic methods when a food product is described by using a whole range of sensations [86]. This method allows evaluation of several attributes simultaneously (maximum 10) and has demonstrated its utility, through numerous and various applications [87]. TDS results are usually represented as average dominance curves with the estimates of chance and significance levels calculated by using statistical tests [86].

Dynamic sensory methods and in particular TDS, can provide useful information beyond that found using a conventional, ‘static’, descriptive sensory methods [88] for evaluating and understanding the flavour perception. It has also been underlined by several authors that the understanding of flavour perception phenomena could be considerably enriched by adding instrumental measurements performed simultaneously to sensory measurements or combining them particularly in the case of dynamic measurements ([87]; e.g. TDS) and in particular in the context of a coffee matrix [89].

Some combinations of PTR-QMS with TDS were shown to be successful to study the dynamics of aroma release and its relation with food texture [90, 91] and swallowing of liquid samples [92] and the effect of coffee crema on in-nose and in-mouth aroma release [93]. To the best of our knowledge, no studies have been recorded on the combination of TDS to PTR-ToF-MS before establishing this thesis.

1.4.5 Data analysis

PTR-ToF-MS spectra handling and analysis require advanced methodologies [94] for improving the mass accuracy [95] and as well as for quantification of the mass peak concentrations [59, 96]. The high mass resolution provided by PTR-ToF-MS results in production of a very complex mass spectrum in short analysis time, that includes hundreds of mass peaks vary in concentration. The number of the mass peaks and their intensities significantly change depending on the matrix. For some food samples, in particular for coffee, this number may reach around 500. Thus, the resulting mass spectra preserve a lot of analytical information (mass peaks, their concentrations and time dimension when online measurements are performed) [97]. In the experiments where very high number of samples (more than 100) are analysed, the utilization of multivariate and data mining tools becomes crucial to process the PTR-ToF-MS data and to extract the necessary information. Good examples have been reported in the literature for supporting the utility and effectiveness of coupling PTR-ToF-MS with multivariate and data mining methods in fruit metabolomics [98] and for a reliable and fast characterization of agro-industrial products [99].

The data mining methods can be grouped as supervised and unsupervised techniques [100]. As a first step into data exploration, the unsupervised techniques such as Principal Component Analysis (PCA) and clustering methods are employed to visualize the possible patterns and/or grouping in the data. Whereas the supervised methods include advanced classification and prediction models such as Partial Least Squares (PLS), Partial Least Squares-Discriminant Analysis (PLS-DA), Random Forests (RF), Penalized Discriminant Analysis (PDA), Support Vector Machines (SVM) [99]. In this second case, the data analyst is aware of the patterns in the data and usually built a model where the success of the employed method is usually evaluated by classification errors and confusion matrices. In this thesis, softwares created under Matlab and R programming language [101] have been employed for the analysis of PTR-ToF-MS data and as well as for data mining.

1.4.6 An overview on Pros-and-Cons of PTR-ToF-MS

PTR-MS technology provides several advantages and various applications in flavour analysis. On the positive side, the biggest feature of this technology is the simultaneous ionization based on direct injection which allows obtaining a rapid full scan (fingerprint) of the whole mass spectra almost simultaneously [11, 56, 58]. The high time resolution brought by this technology allows real-time monitoring of fast food processes (i.e. food fermentation, formation of volatile compounds during cooking, baking or other thermal processes, volatile compounds release during and/or after food consumption in the case of nosespace analysis). Besides, the sensitivity of PTR-ToF-MS is often in the range of the sensitivity of human nose which allows an easy comparison between the instrument and human response via prediction of a scenario very close to human perception.

On the other side, one weak aspect of this technology is the compound identification. When complex volatile mixtures are considered, it is practically not feasible to provide calibration curves for all the mass peaks for compound identification. For instance, when quadrupole mass analyser is employed with PTR-MS, the volatile compounds are characterized only via their nominal masses (one dimensional), which brings superimposing of several compounds on the same protonated peak. On the other hand, given the higher mass resolution, PTR-ToF-MS separates isobaric compounds and therefore strongly enhances compound identification. Nevertheless, isomers are still not distinguishable, the compound sum formula is in principle determinable [97]. When the sum formula has been identified, the step towards compound identification might not be trivial. A mass accuracy of 5 ppm is usually considered sufficient for the exact determination of the elemental composition of molecules [95]. However fragmentation, complex peak structure and/or the presence of isomeric compounds may still render the challenge unpractical, especially in complex matrices [102]. The ionization based on proton transfer provides a soft ionization where most of the mass peaks appear on their parent ions however, some residual fragmentation that is not always avoidable and negligible.

Overall, it has to be noted that the aforementioned characteristics of PTR-MS technology do not suggest it as an alternative to any gas chromatographic method but as a complementary tool for the study of volatile compounds and a reliable choice when speed, sensitivity and online measurements are required. Still, for accurate compound identification the fragmentation patterns of the molecules has to be taken into account and/or a confirmation by GC-MS has to be performed for the compounds of interest.

1.5 The scope of the thesis and outline

The scope of this thesis is to develop rapid, automatic and high-throughput headspace and nosespace sampling methods based on PTR-ToF-MS for the analysis and characterization of coffee volatile compounds. This thesis covers the development of automated headspace sampling procedures by combining PTR-ToF-MS to a multipurpose autosampler for a more reproducible and straightforward analysis. Coffee is chosen due to the high number of volatile compounds which makes it a very suitable matrix for fingerprinting studies. Additionally, the link between coffee volatile compounds and product quality and the connection between perceived quality and volatile compounds are also taken into account. Moreover, the popularity of this drink among consumers and its value in the food market are some other aspects have to be mentioned.

We can describe the detailed aims of the chapters included in this thesis as given below;

Chapter 2: Discrimination of coffee origin

- To develop an automated headspace sampling system by connecting a multipurpose autosampler to PTR-ToF-MS (Chapter 2.1),
- To develop a methodology for rapid characterization and discrimination of 100 % Arabica coffee powders from different geographical origins (Chapter 2.1),
- To find the possible volatile markers discriminating coffee geographical origins,
- To investigate the effect of different batches in coffee production for ensuring quality control (Chapter 2.1),
- To extend the methodology developed in Chapter 2.1 via increasing the number of coffee origins and analysing the volatile profiles of coffee powders and brews (Chapter 2.2),
- To use different ionization agents (H_3O^+ , NO^+ and O_2^+) via operating the PTR-ToF-MS in Switchable Reagent Ions mode (SRI) for the analysis of coffee volatile compounds (Chapter 2.2),
- To investigate the effect of different ionization agents on discrimination of coffee origin by using different classification algorithms and data fusion method (Chapter 2.2),
- To investigate the effect of coffee brewing on the volatile compound release and its relation with coffee geographical origin (Chapter 2.2)

Chapter 3: Single bean coffee roasting

- To prepare an experimental set-up for monitoring the volatile compounds released from a single coffee bean during roasting,
- To investigate the difference in the aroma profiles of single coffee beans from different geographical origins at different stages of roasting,
- To monitor release behaviors of volatile compounds and their relations with coffee origin,

Chapter 4: Nosespace analysis of coffee

- Simultaneous combination of *in-vivo* nosespace analysis with PTR-ToF-MS (instrumental) and TDS (dynamic sensory method) for a better understanding of coffee flavor perception,
- To investigate the effect of coffee roasting degree (dark and light roasted espresso coffees) and sugar addition on the release of volatile compounds in the nose in comparison with headspace results.
- To find links underlying the mechanisms between volatile compound release in the nose and perceived aroma

Chapter 5: Other projects

- Applying the methodologies described in Chapters 2 and 3 in the case of black and green tea samples,
- Discrimination of the aroma fractions from black and green tea leaves and infusions according to geographical origin based on volatile emissions

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Chapter 2. Discrimination of coffee origin

2.1 PTR-ToF-MS characterisation of roasted coffees (*C. arabica*) from different geographic origins

Published as

Research article

Journal of
MASS
SPECTROMETRY

Received: 23 March 2014

Revised: 15 July 2014

Accepted: 31 July 2014

Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jms.3455

PTR-ToF-MS characterisation of roasted coffees (*C. arabica*) from different geographic origins[†]

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Highlights

- PTR-ToF-MS has been used for the first time for rapid discrimination of ground and roasted 100 % Arabica coffees,
- Three *C. arabica* coffees from different geographical origins (Brazil, Guatemala and Ethiopia) were successfully classified
- Tentative identification of mass peaks highlighted volatile markers of origin discrimination
- Models set on the samples from one batch correctly predicted the second batch

Abstract

Characterisation of coffees according to their origins is of utmost importance for commercial qualification. In this study, the aroma profiles of different batches of three monoorigin roasted Coffea arabica coffees (Brazil, Ethiopia and Guatemala) were analysed by Proton-Transfer-Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS). The measurements were performed with the aid of a multipurpose autosampler. Unsupervised and supervised multivariate data analysis techniques were applied in order to visualize data and classify the coffees according to origin. Significant differences were found in volatile profiles of coffees. Principal component analysis allowed visualising a separation of the three coffees according to geographic origin and further partial least square regression-discriminant analysis classification showed completely correct predictions. Remarkably, the samples of one batch could be used as training set to predict geographic origin of the samples of the other batch, suggesting the possibility to predict further batches in coffee production by means of the same approach. Tentative identification of mass peaks aided characterization of aroma fractions. Classification pinpointed some volatile compounds important for discrimination of coffees.

Keywords: PTR-ToF-MS; geographic origin; coffee; volatile compounds; multivariate analysis

2.1.1 Introduction

Coffee with its distinct aroma is one of the most consumed beverages all over the world [1]. The unroasted green beans are poor in aroma and have no desirable flavour however the roasted and ground coffee is highly aromatic due to the quite complex volatile profile formed during roasting. Hundreds of volatile compounds belonging to different chemical classes are responsible for the pleasant and rich ground and brewed coffee aroma [2]. Several coffee species have been identified worldwide but the two main species extensively cultivated and of commercial interest in the genus *Coffea* are *Coffea arabica* and *Coffea canephora* var. *robusta*. They are referred to as *Arabica* and *Robusta*, respectively. The Arabica beans have higher value than Robusta and the coffee produced has more pleasant aroma and superior cup quality [2-4]. The taste and aroma of high quality coffee can vary considerably among samples from the same species and variety grown in different regions. The differences originate from several factors such as degree of maturation, genetics, soil composition, and climate. These elements cause changes in chemical compositions of the beans and sensory attributes of final product [4]. In addition to the fact that geographic origin is embedded in coffee quality, marking of origin for product differentiation is highly demanded for traceability [5], authentication, and marketability purposes [6].

Coffee is known as a product with added value therefore analysis of coffee aroma has been widely performed by several analytical techniques in order to characterize the potent odorants, differentiate coffee varieties and discriminate the geographical origin. Gas chromatography-mass spectrometry (GC-MS) was widely used for determination of volatile compounds in the headspace of ground coffee. Evaluation of headspace volatile fractions of *Arabica* and *Robusta* coffees by GC-MS enabled characterisation of the two species according to their origins [7]. The separation of coffee species and origin discrimination were obtained also by means of solid-phase microextraction-GC-MS equipped with fibers made of different coatings [8-10] and by electronic aroma sensing arrays [11]. In another study[12] healthy and defective *Arabica* coffee beans were discriminated according to three different roasting degrees (light, medium, dark) based upon their volatiles. In the aforementioned study, Principal Component Analysis (PCA) was used to evaluate the discrimination of the samples. The chromatographic data obtained allowed separation of *Arabica* and *Robusta* varieties according to geographical origin and determination of important volatiles responsible for discrimination.

Besides the techniques mentioned above used for coffee aroma identification and quantification, proton-transfer-reaction mass-spectrometry (PTR-MS) brings a new approach for volatile compound analysis. PTR-MS is a form of soft chemical ionization direct injection mass spectrometry based on proton transfer from a protonated reagent, most commonly H₃O⁺. Compounds with higher proton affinity than H₂O react with H₃O⁺ and accept a proton [13]. PTR-MS, coupled with a time of flight (ToF) mass analyser provides high mass range, fast measuring time (a complete mass spectrum in a split second) and high mass resolution [14]. It is a powerful tool for rapid, direct and highly sensitive on-line monitoring of volatile organic compounds (VOCs) [15]. PTR-MS and, in particular, PTR-ToF-MS have been applied for coffee headspace analysis [16], online monitoring of volatile release during coffee roasting [17, 18] and also for investigating the dynamics of real-time VOC formation of coffees from different origins [19], for the study of release kinetics of coffee aroma compounds [20] and recently the possible differentiation of organic and conventional coffees by PTR-MS headspace analysis [21]. In addition to the studies mentioned here, PTR-MS has been used as a powerful tool for geographic discrimination and characterisation of several food products like olive oils [22], palm oils [23] butters [24], cheese [25] and dry cured ham [26].

Most of the coffee consumed in Italy is home brewed and made with a stove-top coffee maker, known as “moka” [27, 28]. This brewing technique needs a specific coffee grinding to ensure both cup quality and safety. In this study the headspace of 3 moka-type ground coffees from different origins (Brazil, Ethiopia and Guatemala) were analysed by PTR-ToF-MS in order to investigate their VOC profiles and use this information to classify samples according to geographic origin.

2.1.2 Materials and Methods

2.1.2.1 Materials

Commercially available medium roasted (total weight loss: 15-18% w/w) *C. arabica* (Monoarabica™) coarsely ground powder for stove-top coffee maker from Brazil (BRA), Ethiopia (ETH) and Guatemala (GUA), packed in inert atmosphere under pressure in 125 g stainless steel cans, were obtained from illycaffè S.p.A, (Trieste, Italy) and kept at room temperature prior to analysis. Samples included two different batches of one origin coffee and 3 jars per batch. 6 measurements were performed for each jar. 100-mg aliquots of ground coffee were weighed in 22-ml glass vials (Supelco, Bellefonte, PA) and 12 empty vials were measured

together with coffee samples and employed as blanks. The final data set consisted of 3 types of coffee x 2 batches x 3 jars x 6 replicates = 108 measurements.

2.1.2.2 PTR-ToF-MS Analysis

The headspace measurements were performed by using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The instrumental conditions for the proton transfer reaction were the following: drift voltage 550 V, drift temperature 110°C, drift pressure 2.33 mbar affording an E/N value of 140 Townsend ($1Td = 10^{-17} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). Sampling was performed with a flow rate of 40 sscm. The mass resolution ($m/\Delta m$) was at least 3700.

Measurements were performed in an automated way by using a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany). The automatic sampler syringe was connected to the inlet of PTR-ToF-MS together with the clean air generated by a gas calibration unit (GCU, Ionicon Analytik GmbH, Innsbruck, Austria) used as zero air generator. The measurement cycle for each vial consisted of flushing the headspace of the sample with clean air for 1 minute at 200 sccm, then incubation for 30 min at 40°C, and finally measurement. The measurement order was randomized to avoid possible systematic memory effects. The sample head-space was measured by direct injection into the PTR-ToF-MS drift tube via a heated (110°C) peek inlet. Each sample was measured for 30 s, at an acquisition rate of one spectrum per second. Headspace volume mixing ratios were calculated by averaging over the whole measurement time.

2.1.2.3 Data analysis

The autosampler and PTR-ToF-MS were synchronized by means of a binary electrical signal (differential TTL). This allowed initialisation of data acquisition at the beginning of each measurement in an automated way. The data thus generated (a distinct file for each sample) were further addressed to the following steps of data analysis. Dead time correction, internal calibration of mass spectral data and peak extraction were performed according to a procedure described elsewhere [29, 30] and the experimental m/z values were reported up to the third decimal. In this paper, we report the VOCs concentrations in ppbv (part per billion by volume) and the calculations from peak areas according to the formula described by Lindinger et al.[31] using a constant reaction rate coefficient ($k_R=2\times 10^{-9} \text{ cm}^3/\text{s}$). For H_3O^+ as a primary ion, this introduces a systematic error for the absolute concentration for each compound that is in most cases below 30% and can be accounted for if the actual rate constant is available [32].

Principal Component Analysis (PCA) and Partial Least Square Regression – Discriminant Analysis (PLS-DA) were carried out for graphical visualisation of the data and sample discrimination, respectively. To investigate the significant changes in VOC concentrations one-way ANOVA was performed. Tentative identifications of mass peaks were performed by automated matching against an in-house library of coffee aroma compounds. The processing of raw spectral data was performed with the aid of packages running in MATLAB environment (MathWorks, Natick, MA) while all additional data analysis was carried out with scripts employing R programming language (Vienna, Austria).

2.1.3 Results

2.1.3.1 PTR-ToF-MS spectra analysis

The analysis of raw PTR-ToF-MS data resulted in extraction of 476 mass peaks in an m/z range of 15-300 Th. After calculation of estimated concentrations in the headspace, a concentration threshold of 1 ppbv was set and the mass peaks with less concentration were filtered out. The signals related to interfering ions (O_2^+ , NO^+ and, water clusters) at m/z 30, 32, 37 and 55 were also eliminated from the matrix and 270 mass peaks remained in the final data set for further analysis.

2.1.3.2 Discrimination of coffees according to origin

2.1.3.2.1 ANOVA results and characterisation of coffees

With regards to peak assignment 85 of the mass peaks (including the ^{13}C isotopes) were tentatively identified by predicting the sum formula and then comparing the measured m/z values with those reported as aroma compounds in headspace of roasted coffee by previous studies [7, 16, 33-43]. A peak can be tentatively assigned to a specific compound but it is impossible to distinguish isomeric and isobaric compounds (e.g. C6 aldehydes and ketones) [44]. Therefore, a mass peak extracted from the coffee headspace spectra has been presented here by its sum formula can be related to single compound or mixtures of different aroma compounds.

The final data set was subjected to one-way ANOVA in order to evaluate the differences between coffee origins and between two batches. The results are shown in Table 1 for the tentatively identified volatile compounds (excluding ^{13}C isotopologues).

A mixture of main aroma components of roasted ground coffee [3] and their fragments were found in the headspace of coffees. When two different batches of same coffee origin were compared there were no significant differences observed between them ($p < 0.01$). Therefore the one way-ANOVA results indicating batch differences are not presented in **Table 2.1-1**. The significant differences are pointed out according to Tukey's *post-hoc* test. On the contrary, significant differences were found in the aroma profiles of coffees from different origins ($p < 0.01$). The aroma differences between different origins can be considered to be effected by the concentration differences and the odour qualities of the volatiles present in headspace. Among 51 mass peaks presented in **Table 2.1-1**, 5 identified volatile compounds (m/z 57.034, 75.044, 97.028, 111.044, 135.121) showed significant concentration differences for different origins of coffee.

The most abundant volatiles found in the headspace of coffees were methanol, fragments of diverse chemical classes, acetaldehyde, methyl pyrazine and acetic acid/methyl formate. The volatile profiles of the three coffee origins consisted of similar chemical groups however it is noteworthy to emphasize that BRA was richer in N-heterocyclic compounds (e.g. pyrroles, pyrazines, pyridines) and furans; ETH and GUA shared closer VOC concentrations higher for aliphatic hydrocarbons (e.g. aldehydes, ketones), in particular ETH had higher concentrations for terpene fragments [45].

The volatile concentrations are plotted against coffee origins and different batches are indicated together with the blank vials. Some distinct examples of volatile compounds in the headspace of coffees clearly different from the others are shown in **Figure 2.1-1**. It's clearly seen from Figure 1 that blank vials have zero concentration for any of the volatiles measured showing the absence of contamination with other volatiles during the experiments and other impurities coming from the zero air generator.

Table 2.1-1. Tentatively identified mass peaks showing average concentrations in the headspace of coffee samples with *p*-values where significant differences are indicated with Tukey letters (one-way ANOVA, $p < 0.01$) Peaks that are highly influential on coffee discrimination (VIP scores higher than 1.5) are marked by an asterisk

Measured Mass (Th)	Sum. Formula	Tentative identification	Average volatile concentrations (ppbv)			<i>p</i> value	VIPs	Ref.
			BRA	ETH	GUA			
33.032	CH ₅ O ⁺	methanol	18268 ab	20296 b	16666 a	0.009		[33]
41.039	C ₃ H ₅ ⁺	alkylic fragment	946 a	1253 b	1103 ab	0.001		[16]
43.017	C ₂ H ₃ O ⁺	fragment (diverse origin)	6805 a	9303 b	8316 b	< 0.001		[16]
43.055	C ₃ H ₇ ⁺	fragment (diverse origin)	169 a	194 b	184 ab	0.003		[16]
45.033	C ₂ H ₅ O ⁺	acetaldehyde	4652 a	6423 b	5826 b	< 0.001		[34]
57.034	C ₃ H ₅ O ⁺	acrolein/ acetol fragment	646 a	869 c	786 b	< 0.001		[35]
57.071	C ₄ H ₉ ⁺	alcohol fragment	155 a	203 b	180 ab	< 0.001		[36]
61.027	C ₂ H ₃ O ₂ ⁺	acetic acid/methyl-formate	3164 a	4865 b	4296 b	< 0.001		[34]
67.054	C ₅ H ₇ ⁺	terpene fragment	111 a	116 a	107 a	0.521		[37]
68.050	C ₄ H ₆ N ⁺	pyrrole	334 b	194 a	197 a	< 0.001	*	[34]
69.034	C ₄ H ₅ O ⁺	furan	1056 b	969 ab	939 a	0.013		[34]
71.086	C ₅ H ₁₁ ⁺	terpene fragment	11 a	12 a	14 b	< 0.001		[37]
73.065	C ₄ H ₆ O ⁺	isobutanal/butanone	1391 a	1862 b	1637 ab	0.012		[34]
75.044	C ₃ H ₇ O ₂ ⁺	methyl-acetate/acetol	2024 a	2727 c	2345 b	< 0.001		[34]
80.048	C ₅ H ₆ N ⁺	pyridine	6227 b	3501 a	3019 a	< 0.001	*	[16]
81.033	C ₅ H ₅ O ⁺	furan fragment	7887 b	6040 a	6377 a	< 0.001	*	[34]
82.066	C ₅ H ₈ N ⁺	methyl-pyrrole	513 b	387 a	339 a	< 0.001		[34]
83.049	C ₅ H ₇ O ⁺	methyl-furan	1146 a	1571 b	1337 ab	0.005		[34]
85.065	C ₅ H ₆ O ⁺	methyl-butenal	390 a	395 a	379 a	0.827		[38]
86.007	C ₃ H ₄ NS ⁺	thiazol	20 b	15 a	16 a	0.000		[39]
87.044	C ₄ H ₇ O ₂ ⁺	butanedione/butyrolactone	1257 a	1255 a	1155 a	0.155		[34]
87.081	C ₅ H ₁₁ O ⁺	methyl-butanal	628 a	930 b	811 ab	< 0.001		[16]
89.060	C ₄ H ₉ O ₂ ⁺	methyl-propanoate/hydroxy-butanone	381 a	492 b	431 a	< 0.001		[34]
93.070	C ₇ H ₉ ⁺	terpene fragment	26 a	32 b	26 a	0.001		[40]
95.059	C ₅ H ₇ N ₂ ⁺	methyl-pyrazine	3893 b	2653 a	2810 a	< 0.001		[16]
96.084	C ₆ H ₁₀ N ⁺	non identified	103 b	62 a	61 a	< 0.001		
97.028	C ₅ H ₅ O ₂ ⁺	furfural	1040 a	2631 c	1909 b	< 0.001	*	[34]
97.065	C ₆ H ₆ O ⁺	dimethyl-furan	354 a	398 a	381 a	0.283		[34]
99.043	C ₅ H ₇ O ₂ ⁺	furfuryl alcohol/a-angelica lactone	445 a	459 a	436 a	0.451		[16]
100.024	C ₄ H ₆ NS ⁺	methyl-thiazole	54 b	33 a	37 a	< 0.001	*	[39]
101.059	C ₅ H ₉ O ₂ ⁺	pentanedione/methyl-tetrahydrofuranone	1832 a	2959 b	2608 b	< 0.001		[34]
101.098	C ₆ H ₁₃ O ⁺	methyl-pentanone/hexanal	28 a	32 a	28 a	0.191		[34]
103.075	C ₅ H ₁₁ O ₂ ⁺	hydroxy-pentanone/methyl-butanoic acid	201 a	258 b	239 b	< 0.001		[16]

(Table 2.1-1 continues)

Measured Mass (Th)	Sum. Formula	Tentative identification	Average volatile concentrations (ppbv)				VIPs	Ref.
			BRA	ETH	GUA	pvalue		
107.087	C ₈ H ₁₁ ⁺	terpene fragment	11 a	20 b	10 a	< 0.001		[40]
109.074	C ₆ H ₉ N ₂ ⁺	dimethylpyrazine/ethylpyrazine	2971 b	2160 a	2250 a	< 0.001		[16]
110.061	C ₆ H ₈ ON ⁺	formyl-methylpyrrole/acetylpyrrole	91 b	90 b	71 a	< 0.001		[16]
111.044	C ₆ H ₇ O ₂ ⁺	acetyl_furan/methyl-furfural	1202 a	1868 c	1620 b	< 0.001	*	[34]
111.081	C ₇ H ₁₁ O ⁺	2/3-dimethyl-2-cyclopenten-1-one	151 a	142 a	148 a	0.558		[38]
112.080	C ₆ H ₁₀ ON ⁺	trimethyl-oxazole	46 b	33 a	36 a	< 0.001		[38]
113.060	C ₆ H ₉ O ₂ ⁺	methyl-furfuryl-alcohol/dimethyl-furanone/methyl-cyclopentanedione/cyclotene	280 a	329 b	312 ab	0.010		[16]
113.098	C ₇ H ₁₃ O ⁺	heptenone	16 b	12 a	14 ab	< 0.001		[38]
115.076	C ₆ H ₁₁ O ₂ ⁺	4-methyltetrahydro-2H-pyran-2-one	268 a	291 a	291 a	0.279		[38]
117.054	C ₅ H ₉ O ₃ ⁺	2-oxopropyl-acetate	303 a	326 a	304 a	0.257		[16]
117.094	C ₆ H ₁₃ O ₂ ⁺	hexanoic acid	15 a	26 b	14 a	< 0.001		[16]
120.080	C ₈ H ₁₀ N ⁺	non identified	8 b	6 a	5 a	< 0.001		
121.074	C ₇ H ₉ N ₂ ⁺	2-ethenyl-6-methyl-pyrazine/6/7-dihydro-5H-cyclopentapyrazine	71 b	55 a	54 a	< 0.001		[16]
123.059	C ₆ H ₇ ON ₂ ⁺	2-acetyl-pyrazine	53 b	45 a	44 a	< 0.001		[41]
123.091	C ₇ H ₁₁ N ₂ ⁺	ethyl-methyl-pyrazine/trimethylpyrazine	588 b	409 a	439 a	< 0.001	*	[16]
125.061	C ₇ H ₉ O ₂ ⁺	guaiaicol/methyl-benzenediol/furyl acetone	145 a	152 a	147 a	0.546		[16]
127.041	C ₆ H ₇ O ₃ ⁺	maltol/methyl-furoate	82 a	80 a	83 a	0.751		[16]
127.076	C ₇ H ₁₁ O ₂ ⁺	3-ethyl-1/2-cyclopentanedione	120 a	133 a	130 a	0.122		[16]
129.091	C ₇ H ₁₃ O ₂ ⁺	pentenyl-acetate/heptanedione	59 a	57 a	61 a	0.559		[38]
135.091	C ₈ H ₁₁ N ₂ ⁺	2-ethyl-6-vinyl-cyclopentapyrazine/6/7-dihydro-methyl-cyclopentapyrazine	25 b	19 a	19 a	< 0.001		[16]
135.121	C ₁₀ H ₁₅ ⁺	terpene fragment	1 a	7 c	2 b	< 0.001	*	[40]
137.108	C ₈ H ₁₃ N ₂ ⁺	ethyl-dimethyl-pyrazine	79 b	53 a	56 a	< 0.001	*	[16]
138.095	C ₈ H ₁₂ ON ⁺	2-acetyl-1-ethylpyrrole/1-acetyl-2/5-dimethylpyrrole	28 b	21 a	23 a	< 0.001		[49]
139.076	C ₈ H ₁₁ O ₂ ⁺	4-ethyl-1/2-benzenediol	61 a	60 a	62 a	0.762		[16]
139.114	C ₉ H ₁₅ O ⁺	E/E-2/4-nonadienal	8 a	8 a	8 a	0.998		[50]
141.054	C ₇ H ₉ O ₃ ⁺	furfuryl-acetate	79 b	64 a	72 ab	< 0.001		[16]
148.075	C ₉ H ₁₀ ON ⁺	1-furfurylpyrrole	11 a	11 a	10 a	0.196		[16]
149.060	C ₉ H ₉ O ₂ ⁺	furfuryl-furan	14 b	12 a	14 b	< 0.001		[38]
151.123	C ₉ H ₁₅ N ₂ ⁺	diethyl-methyl-pyrazine	7 b	4 a	5 a	< 0.001	*	[38]
153.059	C ₈ H ₉ O ₃ ⁺	vanillin	9 a	11 b	10 b	< 0.001		[16]
153.094	C ₉ H ₁₃ O ₂ ⁺	4-ethyl-guaiaicol	10 a	10 a	9 a	0.365		[16]
153.128	C ₁₀ H ₁₇ O ⁺	E/E-2/4-decadienal	7 a	22 b	9 a	< 0.001	*	[41]
157.086	C ₈ H ₁₃ O ₃ ⁺	4-ethoxy-2/5-dimethyl-3-2H-furanone/ethylfuranol	4 a	5 b	4 ab	0.003		[16]
160.075	C ₁₀ H ₁₀ ON ⁺	methyl-quinolinone	5 b	3 a	3 a	< 0.001	*	[42]
163.076	C ₁₀ H ₁₁ O ₂ ⁺	methyl-furfurylfuran	4 a	3 a	4 a	0.157		[43]
165.096	C ₁₀ H ₁₃ O ₂ ⁺	methyl-cinnamate	3 a	3 a	3 a	0.154		[7]
183.100	C ₁₀ H ₁₅ O ₃ ⁺	ethyl-syringol	1 a	1 b	1 a	0.001		[42]

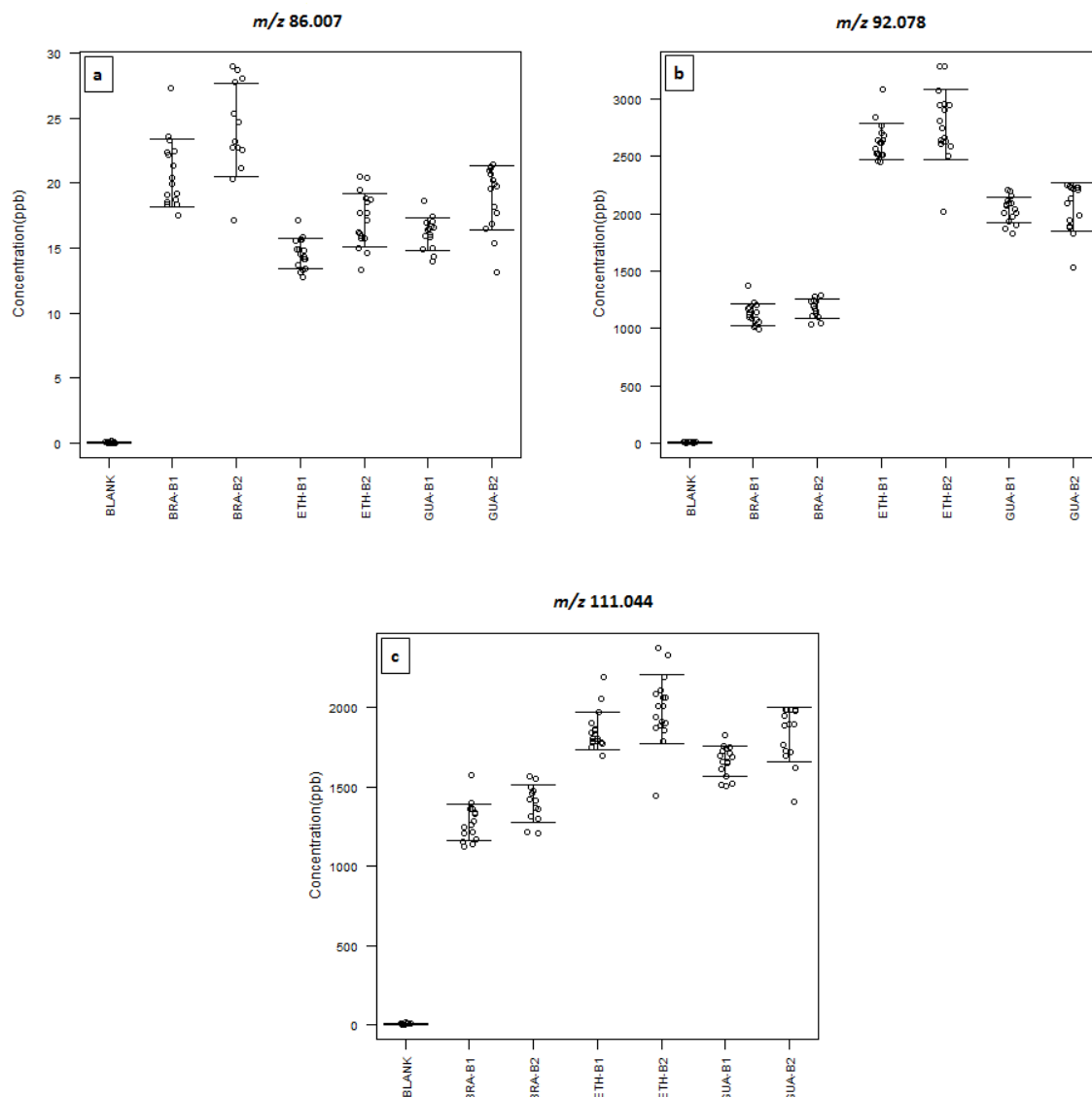


Figure 2.1-1. Strip charts of some selected mass peaks significantly different for the three monoorigin coffees (the mass peaks tentatively identified as; a) m/z 75.044: methyl acetate/acetol; b) m/z 97.028: furfural; c) m/z 111.044: acetyl furan/ methyl furfural).

2.1.3.2.2 Principal Component Analysis (PCA)

For further evaluation of the data, the final data matrix was mean centred, scaled and PCA was performed. The mass peaks in the final data set were chosen as variables and three principal components (PC) were extracted. The score plots corresponding to PC1 vs PC2 and PC2 vs PC3 are shown in **Figure 2.1-2** a and b, respectively.

As presented in **Figure 2.1-2**, the first two principal components which explain 80.88% of the total variability in the data and grouping of them according to geographic origin is clearly visible. PC2 I Separation of coffees seems to be more affected by PC2 than by PC1 due to the variability caused by PC1. The coffee “BRA”, located in the negative scores of PC2, is better discriminated than GUA and ETH which were located in the positive scores of PC2. In Figure 2, different batches belonging to same coffee (indicated by means of different symbols) were placed relatively close to each other showing similar patterns and the separation based on geographical origin was still respected.

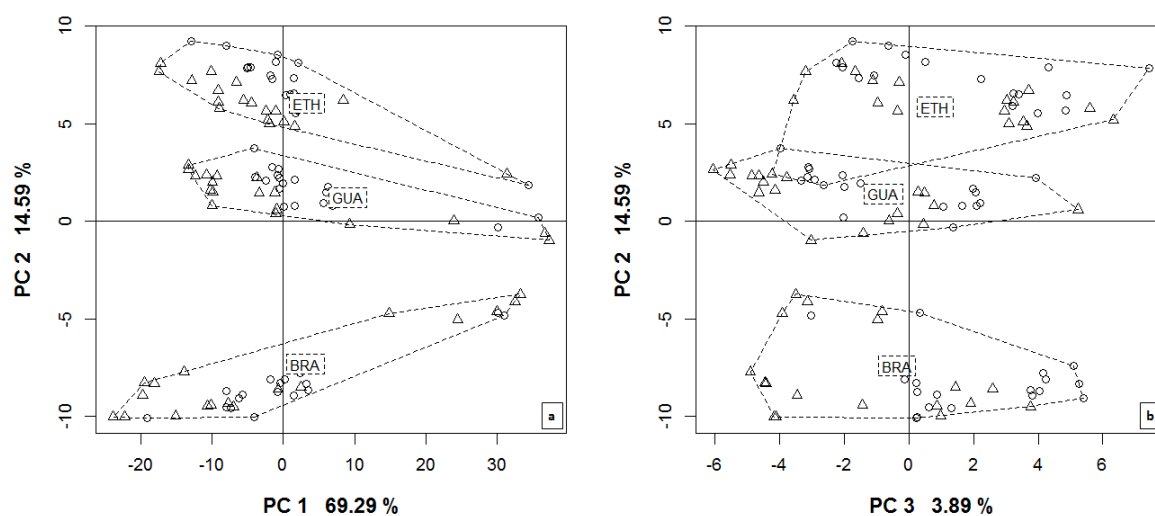


Figure 2.1-2. Scatter plots of PCA scores (a: PC1 vs PC2 and b: PC3 vs PC2, respectively). Different coffee samples are represented by empty polygons. Symbols “ Δ ” and “o” indicate two different production batches.

2.1.3.2.3 Partial Least Squares Linear Discriminant Analysis (PLS-DA)

To evaluate the possibility to predict coffee origin on the basis of the PTR-ToF-MS data a PLS-DA classification model was employed. The model was constructed on the basis of the samples from batch 1 (training set) and cross-validated by using samples from batch 2 (test set). Different models, based on 2 to 10 components, were evaluated on the training set and a model based on three components was finally selected. The selection of the optimal regression model was performed according to Osten [46], by selecting the model that displayed the first local minimum in the prediction error. On the basis of the cross-validated results it was possible to predict the geographic origin of coffees. The confusion matrix (**Table 2.1-2**) shows that the

model was successful to predict the origin of all samples correctly. The model used in the present study was successful to use one batch to predict the other batch showing that this approach can be useful also to predict the following batches in coffee production.

Table 2.1-2. Confusion matrix generated by PLS-DA

		Predicted		
		BRA	ETH	GUA
Original	BRA	18	0	0
	ETH	0	18	0
	GUA	0	0	18

The cross-validated PLS-DA model was also used as basis for the calculation of Variable Importance in Projection (VIP) values. VIP values higher than 1.5 are considered to be highly influential on the prediction model [47]. Hence, this approach was used to pinpoint the variables most influential for discrimination of coffee aroma. The score plot of the first two dimensions of the PLS-DA model are presented in **Figure 2.1-3** and the VIP values higher than 1.5 are indicated in **Table 2.1-1**. The correlation plots show gathering of some mass peaks around different coffee origins where BRA is mostly located in the positive scores than ETH and GUA. Among the variables with VIP values higher than the threshold 12 of them could be associated to tentatively identified mass peaks. The majority of the mass peaks which might be important in discrimination according to geographic origin were found to be in the group of N-heterocycles followed by furans, aldehydes and terpenes. The mass peaks corresponding to N-heterocycles were always at higher concentrations for BRA. The coffee from Ethiopia was richer in aldehydes, terpenes and some furans whereas the Guatemalan coffee had moderate concentration values in the headspace.

As green coffee beans have no pleasant aroma on their own however some aldehydes, ketones, furans, heterocycles and terpenic constituents were identified presumed to be responsible for fruity (coconut, peach, apricot), fatty and soapy notes [42]. Even though, the characteristic coffee aroma and major volatile compounds are formed during roasting by several chemical reactions [3] some compounds like terpenes and aldehydes can resist roasting and be traced from flower to cup contributing the final flavour of the product. Among the terpenic compounds identified in coffee, especially mono-, di- and sesquiterpenes are known to be responsible for

fruity and flowery aroma [45]. Similarly, acetaldehyde found in green and roasted beans and can contribute to fruity notes [3] whereas N-heterocyclic compounds produced during roasting are mainly responsible for chocolate and roast notes [48]. The presence and amount of volatiles in coffee hold great importance in terms of contributing to the originality the flavour. Therefore some of them can be referred as key flavour descriptors for particular *C. arabica* varieties [42].

The findings of this study presented so far were in agreement with some previous studies [7, 8, 11]. Some volatile compounds were identified belonging to similar chemical classes which could be effective on discrimination of coffees. GC-MS analysis of aroma fractions was successful to distinguish Arabica and Robusta varieties according to their geographic origins [7]. Tentative identification of mass peaks after GC-MS analysis has proved that the separation between coffees was influenced by N-heterocycles like pyrazines (e.g. 3-methylpyrazine), pyrroles (methyl pyrrole), pyrazole and furans (3-penthyfuran). In another study [8] ethyldimethylpyrazine, propanoic acid, 2-furfurylthiol and 1*H*-pyrrole-1(2-furanyl-methyl) were found to be important and sufficient for geographic differentiation of coffees from 8 different countries including Brazil and Guatemala as impact odorants.

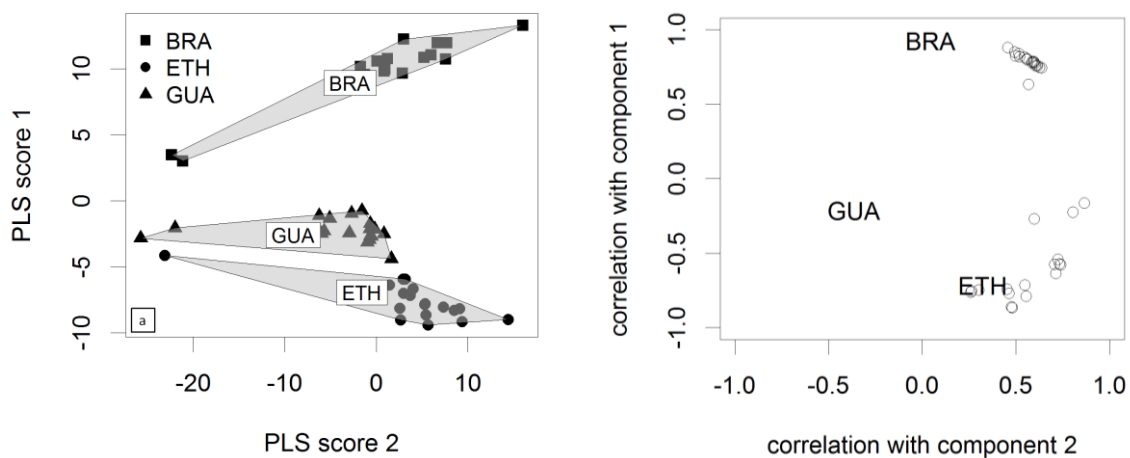


Figure 2.1-3. Score and correlation plots of PLS-DA analysis (a and b, respectively). Variables with VIP scores higher than 1.5 are shown in correlation plots

The data obtained confirm that the volatile composition of roasted and ground coffee is highly influenced by the geographic origin of the coffee beans. As one way-ANOVA results showed the significant differences in volatile concentrations, PLS-DA, in accordance with PCA results,

was able to highlight the differences in the aroma profiles of Brazilian, Guatemalan and Ethiopian *C. arabica* varieties and discriminate them.

2.1.4 Conclusions

PTR-ToF-MS has been used for the first time for the rapid classification of the origin of ground roasted coffee samples. Three *C. Arabica* from different geographical origins (Brazil, Ethiopia and Guatemala) were successfully classified by their volatile profiles. PTR-ToF-MS spectra of the coffees analysed contained almost five hundred mass peaks and the high mass resolution allowed the tentative identification of diverse volatile compounds. Unsupervised principal component analysis indicated the importance of aroma fingerprints enabling origin discrimination which has also been further confirmed by PLS-DA. Models set on samples of one production batch could correctly predict the origin of the samples of the other batch considered. Since geographic origin verification of food products are of great importance, this study shows PTR-ToF-MS as a rapid, direct and non-invasive technique can be used for characterization and geographic origin verification of coffee.

Acknowledgements

PIMMS (Proton Ionisation Molecular Mass Spectrometry) is supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.

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2.2 Tracing coffee origin by direct injection headspace analysis with PTR/SRI-MS

Published as



Highlights

- Brew and powder coffee headspace has been analysed for the first time by PTR/SRI-MS
- An automated set-up allows the rapid measurement of samples (5 min/sample)
- Data mining and data fusion methods efficiently handle the complex datasets
- The volatile profile of coffee is highly affected by geographical origin
- Switching reagent ions improves the classification efficiency of coffees by PTR-MS

Abstract

The headspace of six roasted Coffea arabica coffees, both brew and powder, of different geographical origins (Brazil, Ethiopia, Guatemala, Costa Rica, Colombia, and India) was analysed by Proton Transfer Reaction-Time of Flight-Mass Spectrometry. For the first time, in the case of coffee, a Switching Reagent Ion System has been used to produce different ionisation agents: H_3O^+ , NO^+ and O_2^+ . Significant differences were found among volatile concentrations for the different origins both for powders and brews, in particular high concentrations of terpenes for Ethiopia, sulphur compounds for Colombia and thiazoles for Brazil and India. Effective classification models have been set for the different ionisation modes and data fusion of the data obtained by different reagent ions further reduced the classification errors.

Keywords: Geographic origin, Volatile compounds, Switchable reagent ions, Coffee brew, Coffee powder, Moka

2.2.1 Introduction

Globalization brings a great variety of food products to the market and increases consumers' awareness on food authenticity and product specification. Therefore more information about food products and their geographical and/or botanical origin is required and could support marketing in the positioning of their products. The increasing consumption trend towards single origin food products induces the necessity of to verify and certify origin during quality assurance [1]. Coffee, one of the most important agricultural products traded worldwide, is consumed mostly for its pleasing aroma. Besides its stimulatory effects, the aroma of coffee is one of the most contributory factors for its high acceptability [2, 3]. It is composed of a mixture of more than 800 different volatile compounds (VOCs) [4] which can play a role even if present at trace levels. Therefore coffee flavour research and quality control can take advantage from fast and high sensitivity analytical techniques. Coffee origin is usually determined by elemental composition analysis [5] and stable isotope techniques [6, 7]. These methods are highly developed and used by legal authorities. However, they do not measure properties related to the perceived quality of coffee. A coffee origin signature in its volatile profile would have a direct link to the sensory attributes related to aroma which is significant for the acceptance of coffee.

Proton transfer reaction-mass spectrometry (PTR-MS) enables the analysis of VOCs at very low concentrations by direct injection. A soft chemical ionisation is applied in this system by using H_3O^+ ions as proton donors which can react with a wide variety of volatile compounds [8]. The coupling of PTR-MS to Time-of-Flight (TOF) mass analysers increases the sensitivity of the VOC analysis by detecting the concentrations at parts per billion per volume level (ppbv) [9] with high mass resolution [10]. H_3O^+ is the mostly used ionisation agent in PTR-MS studies, however, recently a Switchable Reagent Ion system (SRI) was developed which allows the usage of NO^+ , O_2^+ , Kr^+ and Xe^+ as precursor ions. The PTR-ToF-MS with SRI system allows the analysis of volatiles which are not detectable with H_3O^+ as alkanes (due to lower proton affinities than H_2O) and moreover the separation of isobaric compounds is possible as in the case of aldehydes and ketones (with NO^+) [11]. PTR-MS have been widely used in food science and technology especially for fingerprinting and profiling of food products [12, 13]. Besides, PTR/SRI-MS system has been recently applied for detection of isoprene [14] and ethylene [15] emissions, in drug research [16, 17] and homeland security [18] and lastly in food science for product discrimination [19] o the best of the authors' knowledge no studies have been published

so far on the application of SRI system for geographical origin discrimination of food products and in particular: coffee.

In a recent paper we demonstrated the possibility of using PTR-ToF-MS for rapid discrimination of coffee origin in the case of coffee powder [20]. In this follow up study we extend the number of coffee origins considered (from 3 to 6), measure both powder and brewed coffee being the latter the product actually consumed, extend the analytical capability of our method by using different precursor ions. Therefore in this study, we aimed to (1) analyse the volatile profiles of six roasted and ground coffees (*Coffea arabica*) from different geographical origins (Brazil, Colombia, Costa Rica, Ethiopia, Guatemala and India) in powder and brew headspace (2) investigate the applicability of SRI system coupled with PTR-ToF-MS on coffee by using H_3O^+ , NO^+ and O_2^+ as precursor ions and compare their classification efficiencies both separately and combined by data fusion methods.

2.2.2 Experimental

2.2.2.1 Sample preparation

2.2.2.1.1 Coffee powder

Six coffees from different geographic origins, Brazil (BRA), Colombia (COL), Costa Rica (CRC), Ethiopia (ETH), Guatemala (GUA) and India (IND), were supplied by Illycaffè S.p.A, (Trieste, Italy) in commercially available forms (Monoarabica™). The coffee beans, *C. arabica*, were medium roasted (total weight loss: 15-18% w/w) at temperatures up to 220°C, coarsely ground to powder for stove-top coffee maker (moka) and packed in inert atmosphere under pressure in 125 g stainless steel cans in the production plant by the producer.

For coffee powder headspace measurements two different jars were taken from one batch. After opening the jars, 100 mg powder coffee was weighed into 22-ml glass vials (Supelco, Bellefonte, PA) and 3 analytical replicates were prepared for each jar. Four empty vials were analysed and considered as blanks.

2.2.2.1.2 Coffee brew

Coffee brewing was performed by steam pressure coffee extraction in a stove-top coffee maker known as “moka” in Italy (two-cup version, Bialetti, Omegna, Italy). For each preparation 100 ml mineral water (bicarbonate 313 mg/l, calcium 50.3 mg/l, magnesium 30.8 mg/l, sodium 6.0 mg/l, potassium 0.9 mg/l, chlorides 2.2 mg/l, as specified by the producer (San Benedetto

S.p.A., Venice, Italy) and 10 g of coffee powder were used. The coffee maker was heated on an electrical plate at 150°C for about 14 min. When almost all coffee was brewed and air started to come out of the nozzle (*i.e.* at the onset of the so-called “Strombolian” phase [21]) the moka was immediately removed from the heating plate. Brewed coffee was poured into a glass vessel, stirred well and 1 ml aliquots were transferred into 22-ml glass vials. Coffee brewing was performed once for each coffee origin and 6 vials were prepared for headspace analysis as analytical replicates. The coffee brews were analysed immediately after the preparation. Four blank vials were also analysed with coffee brews.

2.2.2.2 PTR/SRI-MS Analyses

The headspace measurements were performed by using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The instrument was equipped with a switchable reagent ion system that allowed the operation of PTR-ToF-MS in H_3O^+ , NO^+ or O_2^+ modes as described elsewhere [15, 19]. The instrumental conditions in the drift tube were kept the same for the three ionisation modes as following: drift voltage 550 V, drift temperature 110°C, drift pressure 2.33 mbar affording an E/N value of 140 Townsend ($1 \text{ Td} = 10^{-17} \text{ cm}^2/\text{V.s}$). Sampling was performed with a flow rate of 40 sscm. The mass resolution ($m/\Delta m$) was at least 3900. Measurements were performed in an automated way by using a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) as previously described [20]. The measurement order was randomized to avoid possible systematic memory effects. All the vials were incubated at 40°C for 30 min before PTR-MS analysis. Each sample was measured for 30 s, at an acquisition rate of one spectrum per second. One sample was analysed at every 5 minutes.

2.2.2.3 Data processing and analysis

Data processing of ToF spectra included dead time correction, internal calibration and peak extraction steps performed according to a procedure described elsewhere [22] to reach a mass accuracy ($\geq 0.001 \text{ Th}$) which is sufficient for sum formula determination. The baseline of the mass spectra was removed after averaging the whole measurement and peak detection and peak area extraction was performed by using modified Gaussian to fit the data [23]. Whenever a peak was detected, the volatile concentrations were calculated directly via the amount of detected ions in ppbv (part per billion by volume) levels according to the formulas described by Lindinger, Hansel & Jordan (1998) [8] by assuming a constant reaction rate coefficient ($k_R = 2 \times 10^{-9} \text{ cm}^3/\text{s}$) for H_3O^+ as primary ion. Concentration data in ppbv are called “raw data”

and used directly for most data analysis. Each spectrum was also normalized to unit area, in order to remove sources of systematic variation and produce the “normalized data”, which was used for discriminant analysis. Further tentative peak identification was performed by using an in-house library developed by the authors where the peak annotations were done automatically with the scripts developed under R programming language (Vienna, Austria).

The mass spectrometric data obtained by using H_3O^+ as primary ion were subjected to three different analyses. First, principal component analysis (PCA) was applied on the dataset containing all the mass peaks extracted for the three ionization modes to explore clustering of coffee samples in terms of their volatile compound composition.

Second, supervised classification methods were employed on the data obtained by three precursor ions to assess the separation of the classes. Random Forest (RF), Penalized Discriminant Analysis (PDA), Discriminant Partial Least Squares (dPLS) and Support Vector Machines (SVM) methods were applied following to Granitto et al. (2007) [24]. A six-fold cross-validation procedure was used by dividing the full dataset in six folds, each containing six samples, one from each origin. Then we repeated six times the following procedure: At each time, one of the folds was removed and used as a test set, the rest of the data was used to adjust the discriminant methods, and all methods were used to predict the origins of the samples in the corresponding test set. Results were evaluated using mean classification errors and confusion matrices. In addition to the individual analysis of the three precursor ions, the same data was merged into a multi-precursor dataset, following a data fusion strategy [25]. The same discriminant procedure was applied to the merged dataset.

Third, a one-way ANOVA followed by Tukey’s *post hoc* comparison test (where appropriate) was applied to the three datasets, in order to find the mass peaks that are significantly different between the 6 coffees, both in powder and brew..

2.2.3 Results and discussion

2.2.3.1 Classification of coffees according to geographical origin in switching reagent ion system

2.2.3.1.1 Data exploration by multivariate analysis (PCA)

Analysis of mass spectral data resulted in the extraction of 563, 524 and 563 mass peaks for H_3O^+ , NO^+ and O_2^+ , respectively. The whole data matrix was mean centred and scaled after

eliminating the interfering ions and the mass peaks with a concentration lower than 1 ppbv. The resulting data matrices with 263 (for H_3O^+), 260 (for NO^+) and 233 (for O_2^+) were separately subjected to Principal Component Analysis (PCA) for visualisation of the coffee data. In a first analysis, the powder and brew headspace data were treated together. Two big groups were observed in the score plots located far from each other showing a clear separation of powder and brewed coffee (data not shown). Then data referring to brew and powder coffee were analysed separately.

Figure 2.2-1-Panel A shows the score plots of the first two principal components (PCs) explaining more than 62% of the total variance in H_3O^+ data. A good discrimination of coffees in the powder and brew headspace was observed. Separation of coffees according to origin was clear except for BRA and IND in the powder headspace. However these coffees were well separated when they were brewed. On the contrary COL and GUA were well separated in the powder form but not in the brew headspace. These two plots also point out grouping of “BRA-IND” and “ETH-GUA-COL-CRC” both in powder and brew headspace indicating potential similarities within each group. However this grouping was not related to the geographical location of the coffees.

When PCA was performed on the NO^+ and O_2^+ data separately, the separation of coffees was not clear for the powder headspace. **Figure 2.2-1**-Panel B and **Figure 2.2-1**-Panel C show the score plots of the first two components explaining more than 75% variance in NO^+ data and more than 60% variance in O_2^+ data, respectively. Similarly to the score plots obtained by H_3O^+ data, “BRA-IND” was located close to each other as one group and “ETH-GUA-COL-CRC” as another group in the powder and brew headspace for NO^+ and O_2^+ . Interestingly, data obtained by using O_2^+ as precursor ion suggested a good discrimination of coffees in the brew headspace better than the other two ionisation agents.

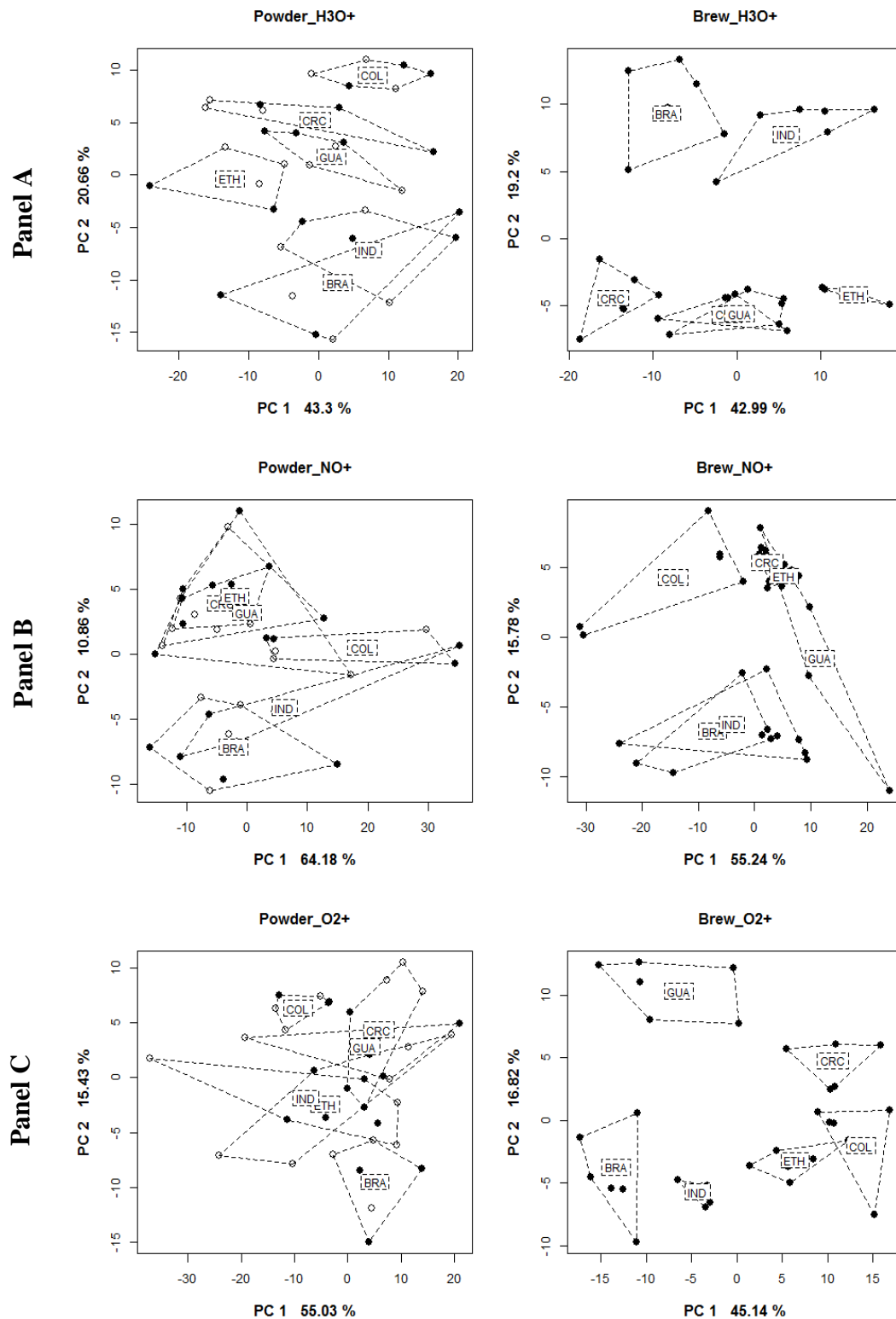


Figure 2.2-1. Score plots of principal components analysis (PCA) for 3 ionisation modes. Panel A: H_3O^+ , Panel B: NO^+ and Panel C: O_2^+ . The first two principal components are shown. Open and solid circles indicate different jars of the same batch.

2.2.3.1.2 Data fusion: classification methods using more than one parent ion

Last column of **Table 2.2-1** shows the results corresponding to the merged dataset, which contains 1650 masses from the 3 reagent ions. For all classification methods and all experimental conditions (brewed/powder, raw/normalized) there is perfect discrimination: all samples are correctly classified. Adding the information gained by 3 ionisation agents significantly increased the performance of classification and provided an optimum discrimination of coffees. As an additional experiment, we developed discriminant methods over all three possible combinations of two diverse reagent ions. Results (not shown) indicate that any combination of reagent ions improves the results of single ions, but cannot reach the complete discrimination of the merged dataset. These results suggest that each ion provides some new information about the volatiles profile of each coffee improving the fingerprinting of the different products.

Table 2.2-1. Classification errors (%) obtained by random forest (RF), penalized discriminant analysis (PDA), discriminant partial least square analysis (dPLS) and support vector machine (SVM) classification models for 3 ionisation modes showing before and after data normalization.

		Classification Method	Primary ion			
			H ₃ O ⁺	NO ⁺	O ₂ ⁺	Aggregate matrix
Powder	<i>Raw data</i>	RF	16.7	19.4	19.4	0.0
		PDA	2.8	8.3	5.6	0.0
		SVM	8.3	16.7	8.3	0.0
		dPLS	5.6	8.3	11.1	0.0
	<i>Normalized data</i>	RF	2.8	8.3	5.6	0.0
		PDA	0.0	0.0	2.8	0.0
		SVM	0.0	2.8	8.3	0.0
		dPLS	5.6	2.8	8.3	0.0
Brew	<i>Raw data</i>	RF	5.6	8.3	11.1	0.0
		PDA	0.0	2.8	5.6	0.0
		SVM	11.1	2.8	5.6	0.0
		dPLS	0.0	5.6	8.3	0.0
	<i>Normalized data</i>	RF	5.6	2.8	8.3	0.0
		PDA	0.0	0.0	0.0	0.0
		SVM	5.6	0.0	2.8	0.0
		dPLS	2.8	0.0	2.8	0.0

Table 2.2-2. Confusion matrices obtained by random forest (RF), penalized discriminant analysis (PDA), discriminant partial least square analysis (dPLS) and support vector machine (SVM) classification models by using raw and normalized H₃O⁺ data.

Classification Method	Coffees	RAW DATA						NORMALIZED DATA														
		Powder			Brew			Powder			Brew											
		BRA	COL	CRC	ETH	GUA	IND	BRA	COL	CRC	ETH	GUA	IND	BRA	COL	CRC	ETH	GUA	IND			
RF	BRA	5	0	0	0	0	1	6	0	0	0	0	0	0	6	0	0	0	0	0	0	
	COL	0	5	1	0	0	0	0	5	1	0	0	0	0	0	6	0	0	0	0	0	
	CRC	0	1	4	0	1	0	0	0	6	0	0	0	0	0	5	0	1	0	0	0	
	ETH	0	0	0	5	1	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	
	GUA	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	6	0
	IND	1	0	0	0	0	5	1	0	0	0	0	5	0	0	0	0	0	6	0	0	5
PDA	BRA	6	0	0	0	0	0	6	0	0	0	0	0	0	6	0	0	0	0	0	0	
	COL	0	6	0	0	0	0	0	6	0	0	0	0	0	0	6	0	0	0	0	0	
	CRC	0	1	5	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	
	ETH	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	
	GUA	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	6	0
	IND	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	6
SVM	BRA	5	0	0	0	0	1	5	0	0	0	0	1	6	0	0	0	0	0	0	0	
	COL	0	6	0	0	0	0	0	4	1	0	1	0	0	6	0	0	0	0	0	0	
	CRC	0	0	4	0	2	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	
	ETH	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	
	GUA	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	6	0
	IND	0	0	0	0	0	6	1	0	0	0	0	5	0	0	0	0	0	6	0	0	5
dPLS	BRA	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	0	
	COL	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	
	CRC	0	0	4	0	1	1	0	0	6	0	0	0	0	0	5	0	1	0	0	0	
	ETH	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	
	GUA	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	1	5	0	0	
	IND	0	0	0	0	0	6	0	0	0	0	0	6	0	0	0	0	0	0	6	0	6

2.2.3.1.3 Monovariate analysis

Coffee powder and brew with H₃O⁺ as precursor ion

The mass peaks extracted from H₃O⁺ data was subjected to one-way ANOVA ($p < 0.001$) separately on powder and brewed coffee. In the powder headspace 151 mass peaks were found to be significant between the different coffee origins whereas 210 mass peaks were at significantly different concentrations in the brew headspace. Among the mass peaks have been extracted sum formulas could be assigned to 102 which leads a possible tentative identification (**Table 2.2-3**).

To better visualize the differences between aroma profiles of coffees, the tentatively identified mass peaks were grouped according to their chemical classes (alcohols, carbonyl compounds, esters/acids, pyrans/furans, oxazoles, phenols, pyrazines, pyridines, pyrroles, sulphur compounds, terpenes and thiazoles). The concentrations of compounds belonging to same group were summed for each coffee type and condition and an average value of each group was calculated including all coffees as the basis of comparison. The percentage difference (%) between a chemical group belonging to one coffee and the averaged value was calculated and presented by column charts for brew and powder, separately (**Figure 2.2-2 a-b**).

In **Figure 2.2-2 a** and **b** the most noticeable difference was found in terpene levels. The coffee from Ethiopia had highest amount of terpenes (more than 3 times) in both powder and brew. High levels of terpenes in Ethiopian coffee powder have been reported by the authors in a recent paper [20] which have been supported by these recent findings once more and also for brewed coffee. Moreover in the coffee powder, ETH was also higher in esters/acids, pyrans/furans and alcohols. Coffees COL, CRC and GUA were rich in sulphur compounds in the powder and brew headspace being COL the richest. BRA and IND had similar volatile profiles in the powder headspace having high levels in pyridines, pyrroles and thiazoles than the average.

Coffee brews showed similar distribution of volatiles to coffee powder for some chemical groups. The alcohols, carbonyl, acids/esters and terpenes for ETH can be given as an example. When the similar powder headspace compositions were compared for IND and BRA, however in the brewed form, the difference in the concentration levels were higher for IND than BRA for the mentioned chemical groups and others like esters/acids, oxazoles and phenols. Overall, the change in the percentage distribution of a chemical group was dependant on coffee origins which will be explained in the following section (2.2.3.2).

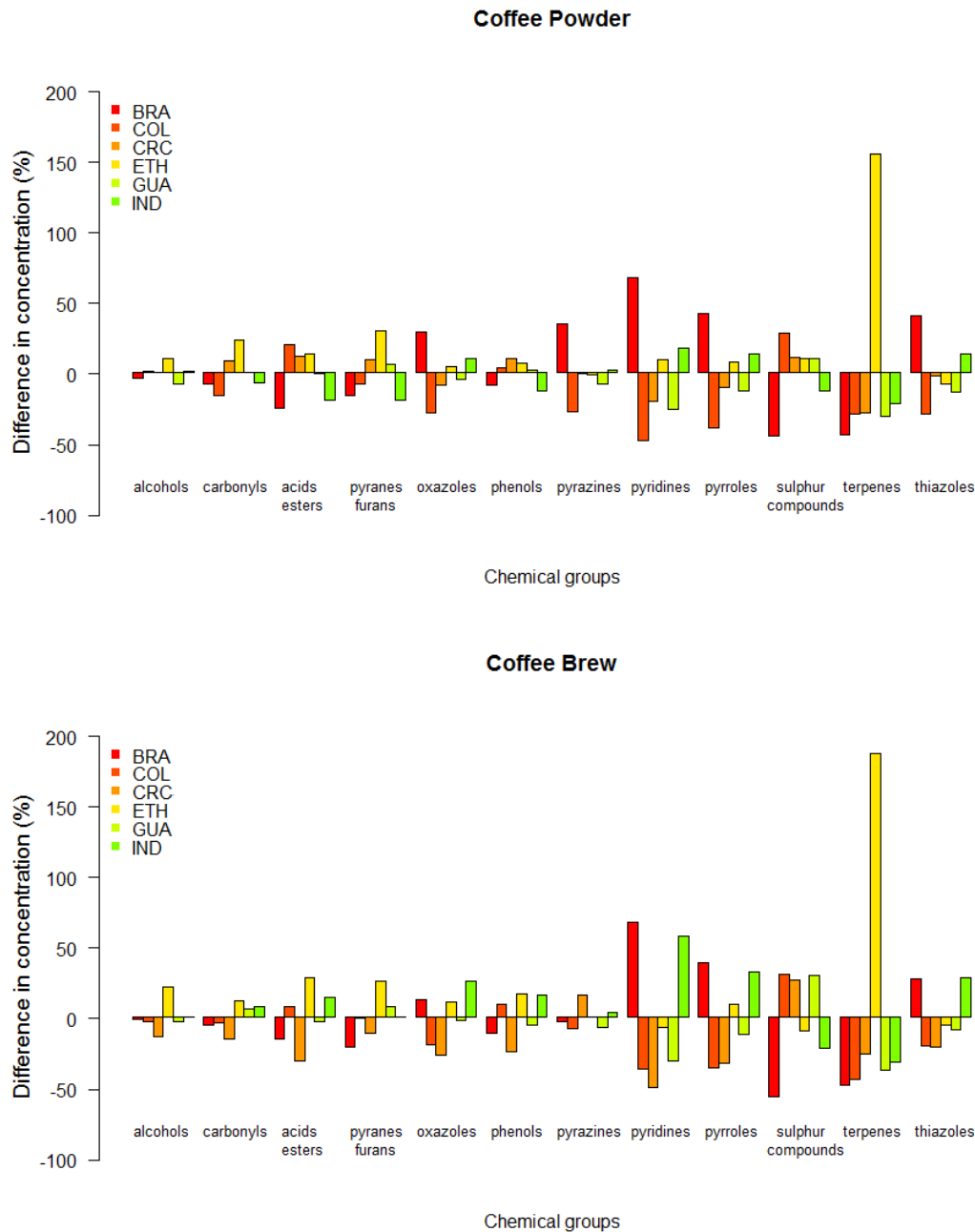


Figure 2.2-2. Column charts showing the differences (%) between chemical groups of the volatile compounds identified for coffee powder (a) and coffee brew (b).

Lastly, the results of the current study were compared with our previous study on coffee powder [20]. All the previously identified ass peaks in the headspace of coffees from Brazil, Ethiopia and Guatemala were found in the same origin samples. The differences among volatile concentrations of the new and former study were found to be very small for most of them and the trend of the volatiles was found to be same for the majority.

2.2.3.2 *The effect of brewing on volatile release*

Brewing of coffees affected the release of diverse chemical compounds in the headspace by a shift in the concentration those led significant changes in the volatile profiles (Figures 2a, 2b and Table 3). The headspace concentrations of brewed coffee were in general lower than that of powder. The total concentrations of some chemical groups tend to increase or decrease: alcohols, esters/acids, furans and pyrans, phenols, pyrazines, pyridines, pyrroles, oxazoles, terpenes and thiazoles were always higher in the powder for all coffee types. However the level of total carbonyls and sulphur compounds were higher in the brew. The biggest losses were recorded for pyrazines, followed by alcohols and pyridines whereas the minimum reduction was observed in pyrrole levels. As previously showed [26] significant increase in methional, 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone (sotolon) and vanillin levels and significant decrease in some thiols and pyrazines was observed when an Arabica coffee was brewed. Our results are in accordance with these findings except the changes for methional and sotolon together with some thiols which might be due to the coffee bean structure and the method used for aroma analysis [27, 28]. The formation of volatile organic compounds depends on several physical and chemical reactions occur during roasting of coffee and highly effected by the non-volatile composition of coffee as being precursors of volatile production [2]. The release of volatiles from coffee is highly dependent on preparation technique that the changes in the concentration of the several chemical groups [2, 21, 29]. Moreover, these changes can be due to the lower solubility of aroma compounds in the brew or degradation of them by hot water [26]. Since the chemical composition of coffee beans is important for their evolution during roasting and the final structure of beans [30] it is expected that the degree of chemical reactions responsible for flavour formation and their texture-dependent release behaviour [31] would be different for coffees from different geographical origins.

To assess whether the release of a mass peak during brewing is dependent on coffee origin a two way-ANOVA was performed. The models with and without interaction were compared by means of an F-test which allowed selection of the interacting variables (Table 3). In most cases, significant interactions were found between coffee origins and brewing. More than 60% of the mass peaks pointed out significant interactions ($p < 0.01$) due to the brewing of coffees from different origins.

Table 2.2-3. Average concentrations (ppbv) of tentatively identified mass peaks in the headspace of coffees detected by PTR-ToF-MS. Mean concentrations are reported for each sample with Tukey letters (p < 0.01) showing the significant differences between coffee origins in the powder and brew forms separately. p-values for powder and brew coffees were obtained on the basis of one-way ANOVA; p-values for interaction were obtained by two-way ANOVA.

Meas. mass (m/z)	Sum formula	Tentative identification	POWDER						p-value powder	BREW						p-value brew	p-value interaction
			BRA	COL	CRC	ETH	GUA	IND		BRA	COL	CRC	ETH	GUA	IND		
49.010	CH ₅ S ⁺	methanethiol ¹⁰	11 ^a	16 ^{bc}	14 ^{ac}	13 ^{ab}	11 ^a	16 ^c	<10 ⁻³	58 ^a	114 ^{ab}	171 ^b	93 ^a	112 ^a	74 ^a	<10 ⁻³	<10 ⁻³
59.048	C ₃ H ₇ O ⁺	propanal/acetone ²	5045 ^{ab}	4834 ^{ab}	5021 ^{ab}	6064 ^b	4745 ^a	5854 ^{ab}	0.036	9149 ^b	8526 ^b	6743 ^a	9237 ^b	9004 ^b	10277 ^c	<10 ⁻³	<10 ⁻³
61.028	C ₂ H ₅ O ₂ ⁺	acetic acid/methyl-formate ³	3945 ^a	7426 ^c	6392 ^c	6182 ^{bc}	5645 ^{ac}	4388 ^{ab}	<10 ⁻³	2001 ^a	3257 ^c	2146 ^a	3358 ^c	2693 ^b	2793 ^b	<10 ⁻³	0.001
63.027	C ₂ H ₇ S ⁺	ethanethiol/dimethylsulfide ¹⁰	19 ^a	86 ^d	68 ^c	66 ^c	71 ^{cd}	46 ^b	<10 ⁻³	43 ^a	212 ^c	144 ^b	128 ^b	211 ^c	114 ^b	<10 ⁻³	<10 ⁻³
68.049	C ₄ H ₆ N ⁺	pyrrole ⁹	298 ^e	140 ^a	199 ^c	185 ^{bc}	160 ^{ab}	265 ^d	<10 ⁻³	346 ^e	146 ^a	166 ^b	205 ^d	183 ^c	331 ^e	<10 ⁻³	<10 ⁻³
69.033	C ₄ H ₅ O ⁺	furan ⁴	572 ^a	637 ^a	652 ^a	809 ^b	630 ^a	582 ^a	0.003	933 ^{ab}	1124 ^{ac}	897 ^a	1321 ^c	1131 ^{ac}	1157 ^{bc}	0.001	0.037
73.064	C ₄ H ₉ O ⁺	isobutanal/butanone ²	3066 ^{ab}	2452 ^a	3612 ^{bc}	4248 ^c	3183 ^{ab}	2837 ^{ab}	0.001	5265 ^{ab}	5687 ^{abc}	5143 ^a	6628 ^d	6299 ^{cd}	6017 ^{bd}	<10 ⁻³	0.002
75.043	C ₃ H ₇ O ₂ ⁺	methyl-acetate/acetol ³	1856 ^a	2490 ^{bc}	2537 ^{bc}	2807 ^c	2180 ^{ab}	1985 ^a	<10 ⁻³	2031 ^b	1860 ^b	1157 ^a	2723 ^c	1913 ^b	2642 ^c	<10 ⁻³	<10 ⁻³
78.967	CH ₃ S ₂ ⁺	dimethyl-disulfide fragment ¹⁰	21 ^a	23 ^{ab}	24 ^{ab}	27 ^b	23 ^{ab}	25 ^{ab}	0.031	47 ^a	56 ^{ab}	53 ^a	52 ^a	46 ^a	70 ^b	0.001	0.002
80.049	C ₅ H ₆ N ⁺	pyridine ⁸	5348 ^d	1649 ^a	2535 ^b	3474 ^c	2340 ^{ab}	3733 ^c	<10 ⁻³	1219 ^c	448 ^a	358 ^a	664 ^b	490 ^a	1138 ^c	<10 ⁻³	<10 ⁻³
81.035	C ₅ H ₅ O ⁺	furan fragment ⁴	2929 ^a	2626 ^a	2843 ^a	2809 ^a	2719 ^a	2750 ^a	0.846	1104 ^{ab}	1201 ^b	841 ^a	1169 ^b	1143 ^b	1312 ^b	0.003	0.191
82.065	C ₅ H ₈ N ⁺	methyl-pyrrole ⁹	887 ^d	303 ^a	492 ^b	657 ^c	498 ^b	611 ^{bc}	<10 ⁻³	881 ^d	380 ^a	405 ^a	723 ^c	558 ^b	803 ^{cd}	<10 ⁻³	0.001
83.049	C ₅ H ₇ O ⁺	methyl-furan ⁴	2522 ^{ab}	1811 ^a	2199 ^{ab}	3685 ^c	2638 ^b	2388 ^{ab}	<10 ⁻³	2428 ^a	2410 ^a	2319 ^a	3603 ^b	2977 ^{ab}	3247 ^b	<10 ⁻³	0.095
85.065	C ₅ H ₉ O ⁺	methyl-butenal ²	548 ^{bc}	370 ^a	517 ^{bc}	590 ^c	528 ^{bc}	468 ^b	<10 ⁻³	247 ^b	195 ^a	173 ^a	282 ^c	258 ^b	262 ^{bc}	<10 ⁻³	<10 ⁻³
86.007	C ₃ H ₄ NS ⁺	thiazol ¹²	26 ^c	16 ^a	22 ^b	22 ^b	19 ^{ab}	23 ^{bc}	<10 ⁻³	20 ^c	14 ^a	14 ^a	18 ^b	16 ^b	22 ^d	<10 ⁻³	<10 ⁻³
87.045	C ₄ H ₇ O ₂ ⁺	butanedione/butyrolactone ²	952 ^a	1039 ^a	1306 ^{bc}	1449 ^c	1172 ^{ab}	1000 ^a	<10 ⁻³	799 ^a	903 ^b	974 ^b	944 ^b	936 ^b	808 ^a	<10 ⁻³	0.002
87.079	C ₅ H ₁₁ O ⁺	methyl-butanal ²	1671 ^{bc}	1127 ^a	2038 ^{cd}	2279 ^d	1858 ^{bd}	1438 ^{ab}	<10 ⁻³	2051 ^a	2126 ^{ab}	2368 ^{ab}	3104 ^d	2801 ^{cd}	2481 ^{bc}	<10 ⁻³	0.003
89.059	C ₄ H ₉ O ₂ ⁺	methyl-propanoate/hydroxy-butanone ³	406 ^a	433 ^{ab}	540 ^c	580 ^c	497 ^{bc}	372 ^a	<10 ⁻³	86 ^b	100 ^b	61 ^a	128 ^c	95 ^b	105 ^b	<10 ⁻³	<10 ⁻³
91.054	C ₄ H ₁₁ S ⁺	diethylsulfide ¹⁰	7 ^{cd}	5 ^a	6 ^{ac}	8 ^d	7 ^{bc}	6 ^{ab}	<10 ⁻³	3 ^a	3 ^a	4 ^a	3 ^a	3 ^a	3 ^a	0.405	<10 ⁻³
91.073	C ₄ H ₁₁ O ₂ ⁺	butandiol ¹	2 ^a	2 ^{ab}	2 ^b	2 ^b	2 ^{ab}	2 ^a	0.003	4 ^a	5 ^{ac}	4 ^{ab}	6 ^d	5 ^{cd}	5 ^{bcd}	<10 ⁻³	0.001
95.059	C ₄ H ₁₁ O ₂ ⁺	methyl-pyrazine	4464 ^c	2253 ^a	3293 ^b	3209 ^b	2996 ^b	3307 ^b	<10 ⁻³	387 ^a	337 ^a	548 ^a	366 ^a	341 ^a	390 ^a	0.337	<10 ⁻³
96.083	C ₅ H ₇ N ₂ ⁺	dimethyl-pyrrole ethyl-pyrrole	129 ^{bc}	70 ^a	102 ^{ab}	118 ^b	97 ^{ab}	160 ^c	<10 ⁻³	77 ^c	33 ^a	39 ^a	62 ^b	54 ^b	83 ^c	<10 ⁻³	0.162
97.028	C ₆ H ₁₀ N ⁺	furfural	890 ^a	2098 ^{bc}	2471 ^d	2263 ^{cd}	1947 ^b	909 ^a	<10 ⁻³	649 ^a	1553 ^e	1315 ^c	1578 ^e	1419 ^d	754 ^b	<10 ⁻³	<10 ⁻³
97.064	C ₅ H ₅ O ₂ ⁺	dimethyl-furan	644 ^b	444 ^a	575 ^b	818 ^c	689 ^b	583 ^b	<10 ⁻³	200 ^a	198 ^a	215 ^{ab}	312 ^d	272 ^{cd}	255 ^{bc}	<10 ⁻³	<10 ⁻³
98.063	C ₅ H ₈ ON ⁺	dimethyl-oxazole ⁵	129 ^d	69 ^a	98 ^b	124 ^{cd}	108 ^{bc}	109 ^{bd}	<10 ⁻³	49 ^b	36 ^a	34 ^a	54 ^c	48 ^b	55 ^c	<10 ⁻³	<10 ⁻³
99.041	C ₅ H ₇ O ₂ ⁺	furfuryl alcohol /a-angelica lactone ⁴	262 ^a	328 ^b	408 ^c	341 ^b	312 ^{ab}	262 ^a	<10 ⁻³	91 ^a	114 ^b	95 ^{ab}	105 ^{ab}	101 ^{ab}	104 ^{ab}	0.105	<10 ⁻³

(Table 2.2-3 continues)

Meas. mass (m/z)	Sum formula	Tentative identification	POWDER							p-value powder	BREW						p- value brew	p- value intera ction
			BRA	COL	CRC	ETH	GUA	IND	BRA		COL	CRC	ETH	GUA	IND			
99.080	C ₆ H ₁₁ O ⁺	hexenal/methyl-pentenone ²	97 ^{bc}	66 ^a	85 ^b	101 ^c	94 ^{bc}	93 ^{bc}	<10 ⁻³	49 ^b	42 ^a	39 ^a	59 ^c	55 ^c	58 ^c	<10 ⁻³	0.001	
100.022	C ₄ H ₆ NS ⁺	methyl-thiazole ¹²	72 ^d	34 ^a	49 ^{bc}	44 ^b	42 ^b	56 ^c	<10 ⁻³	33 ^c	19 ^a	19 ^a	23 ^b	22 ^b	32 ^c	<10 ⁻³	<10 ⁻³	
101.059	C ₅ H ₉ O ₂ ⁺	Pentanedione/methyl-tetrahydrofuranone ²	2828 ^a	3106 ^a	4207 ^b	4398 ^b	3908 ^b	2621 ^a	<10 ⁻³	541 ^a	825 ^d	683 ^c	1009 ^f	915 ^e	612 ^b	<10 ⁻³	<10 ⁻³	
103.075	C ₅ H ₁₁ O ₂ ⁺	hydroxy-pentanone/methyl-butanoic acid ³	213 ^{ab}	217 ^{ac}	257 ^{cd}	264 ^d	239 ^{bcd}	191 ^a	<10 ⁻³	27 ^{ab}	37 ^{ab}	22 ^a	39 ^b	29 ^{ab}	28 ^{ab}	0.037	<10 ⁻³	
105.039	C ₄ H ₉ OS ⁺	methional ¹⁰	10 ^{bc}	7 ^a	10 ^{bc}	11 ^c	10 ^{bc}	9 ^b	<10 ⁻³	5 ^b	4 ^a	5 ^b	5 ^b	4 ^b	5 ^b	<10 ⁻³	<10 ⁻³	
105.069	C ₈ H ₉ ⁺	styrene/phenylethanol fragment ¹³	15 ^c	11 ^a	12 ^b	14 ^c	12 ^b	13 ^b	<10 ⁻³	6 ^a	6 ^a	7 ^{ab}	7 ^{ab}	6 ^{ab}	7 ^b	0.026	<10 ⁻³	
105.093	C ₅ H ₁₃ O ₂ ⁺	methyl-butanol ¹	1 ^{abc}	1 ^a	1 ^d	1 ^{cd}	1 ^{bd}	1 ^{ab}	<10 ⁻³	3 ^a	3 ^a	4 ^{ab}	5 ^c	4 ^b	4 ^{ab}	<10 ⁻³	<10 ⁻³	
107.053	C ₇ H ₇ O ⁺	benzaldehyde ²	44 ^c	28 ^a	35 ^b	35 ^b	33 ^{ab}	36 ^b	<10 ⁻³	23 ^c	18 ^a	18 ^a	21 ^b	19 ^{ab}	23 ^c	<10 ⁻³	<10 ⁻³	
109.074	C ₆ H ₉ N ₂ ⁺	dimethylpyrazine/ethylpyrazine ⁷	2998 ^c	1691 ^a	2174 ^b	2205 ^b	2070 ^b	2317 ^b	<10 ⁻³	197 ^a	205 ^a	177 ^a	221 ^a	207 ^a	220 ^a	0.264	<10 ⁻³	
111.044	C ₆ H ₇ O ₂ ⁺	acetyl_furan/methyl-furfural ⁴	788 ^a	1081 ^b	1247 ^b	1191 ^b	1107 ^b	762 ^a	<10 ⁻³	187 ^a	308 ^b	216 ^{ab}	280 ^{ab}	244 ^{ab}	210 ^{ab}	0.020	<10 ⁻³	
111.079	C ₇ H ₁₁ O ⁺	2/3-dimethyl-2-cyclopenten-1-one ²	221 ^{bc}	176 ^a	204 ^{ab}	243 ^c	227 ^{bc}	212 ^b	<10 ⁻³	54 ^a	54 ^a	52 ^a	70 ^b	64 ^b	67 ^b	<10 ⁻³	<10 ⁻³	
112.078	C ₆ H ₁₀ ON ⁺	trimethyl-oxazole ⁵	75 ^d	41 ^a	52 ^b	61 ^{bc}	55 ^{bc}	64 ^c	<10 ⁻³	22 ^c	15 ^a	14 ^a	21 ^c	19 ^b	24 ^d	<10 ⁻³	<10 ⁻³	
113.019	C ₅ H ₅ O ₃ ⁺	furancarboxylic acid ⁴	7 ^b	6 ^a	7 ^{ab}	7 ^b	7 ^{ab}	7 ^{ab}	0.008	9 ^c	5 ^{ab}	9 ^c	5 ^a	5 ^{ab}	6 ^b	<10 ⁻³	<10 ⁻³	
113.059	C ₆ H ₉ O ₂ ⁺	methyl-furfuryl-alcohol/dimethyl-furanone/methyl-cyclopentanedione/cyclotene ⁴	222 ^a	236 ^{ab}	271 ^b	259 ^{ab}	238 ^{ab}	216 ^a	0.032	34 ^a	41 ^a	31 ^a	40 ^a	35 ^a	40 ^a	0.115	0.014	
113.095	C ₇ H ₁₃ O ⁺	heptenone ²	40 ^c	26 ^a	34 ^b	34 ^b	33 ^b	36 ^{bc}	<10 ⁻³	21 ^{bc}	18 ^a	19 ^{ab}	22 ^c	22 ^c	25 ^d	<10 ⁻³	<10 ⁻³	
114.039	C ₅ H ₈ NS ⁺	ethyl-thiazole/dimethyl-thiazole ¹²	24 ^d	12 ^a	15 ^b	14 ^{ab}	14 ^{ab}	20 ^c	<10 ⁻³	9 ^b	5 ^a	5 ^a	6 ^a	6 ^a	9 ^b	<10 ⁻³	<10 ⁻³	
115.039	C ₅ H ₇ O ₃ ⁺	dihydroxy-cyclopentenone ²	6 ^b	5 ^{ab}	6 ^{ab}	6 ^{ab}	5 ^a	6 ^b	0.008	24 ^b	8 ^a	30 ^b	4 ^a	11 ^a	10 ^a	<10 ⁻³	<10 ⁻³	
115.076	C ₆ H ₁₁ O ₂ ⁺	4-methyltetrahydro-2H-pyran-2-one ⁴	377 ^{abc}	327 ^a	396 ^{bd}	431 ^d	411 ^{cd}	355 ^{ab}	<10 ⁻³	86 ^a	101 ^b	86 ^a	135 ^d	122 ^c	97 ^b	<10 ⁻³	<10 ⁻³	
115.108	C ₇ H ₁₅ O ⁺	heptanal/heptanone ²	19 ^b	14 ^a	18 ^b	20 ^b	19 ^b	18 ^b	<10 ⁻³	9 ^{ab}	9 ^{ab}	8 ^a	12 ^c	10 ^b	12 ^c	<10 ⁻³	<10 ⁻³	
117.053	C ₅ H ₉ O ₃ ⁺	2-oxopropyl-acetate ³	278 ^{abc}	243 ^a	308 ^{cd}	333 ^d	308 ^{bd}	256 ^{ab}	<10 ⁻³	22 ^a	34 ^a	20 ^a	22 ^a	21 ^a	22 ^a	0.570	<10 ⁻³	
117.091	C ₆ H ₁₃ O ₂ ⁺	hexanoic acid/C6 ester ³	39 ^c	25 ^a	31 ^{ab}	72 ^d	35 ^{bc}	40 ^c	<10 ⁻³	19 ^b	16 ^a	15 ^a	50 ^d	21 ^b	29 ^c	<10 ⁻³	<10 ⁻³	
119.052	C ₈ H ₇ O ⁺	benzofuran ⁴	8 ^a	8 ^a	9 ^{ab}	10 ^b	9 ^{ab}	9 ^{ab}	0.018	4 ^a	6 ^d	4 ^{ab}	6 ^{cd}	5 ^{bc}	6 ^d	<10 ⁻³	<10 ⁻³	
121.073	C ₇ H ₉ N ₂ ⁺	2-ethenyl-6-methyl-pyrazine 6/7-dihydro-5H-cyclopentapyrazine ⁷	22 ^a	20 ^a	21 ^a	22 ^a	20 ^a	19 ^a	0.692	9 ^{ab}	12 ^{bc}	8 ^a	11 ^{bc}	10 ^{ab}	12 ^c	<10 ⁻³	0.034	
122.061	C ₇ H ₈ NO ⁺	2-acetylpyridine ⁸	8 ^a	7 ^a	7 ^a	8 ^a	7 ^a	7 ^a	0.594	5 ^{bcd}	4 ^{ab}	3 ^a	5 ^d	4 ^{ac}	5 ^{cd}	<10 ⁻³	0.389	

(Table 2.2-3 continues)

Meas. Mass (m/z)	Sum formula	Tentative identification	POWDER							p-value powder	BREW						p-value brew	p-value interaction
			BRA	COL	CRC	ETH	GUA	IND	BRA		COL	CRC	ETH	GUA	IND			
122.095	C ₈ H ₁₂ N ⁺	propyl-pyridine ⁸	5 ^c	1 ^a	3 ^b	4 ^{bc}	3 ^b	4 ^{bc}	<10 ⁻³	3 ^c	2 ^a	2 ^a	4 ^c	3 ^b	4 ^c	<10 ⁻³	0.002	
123.089	C ₇ H ₁₁ N ₂ ⁺	ethyl-methyl-pyrazine/trimethylpyrazine ⁷	348 ^b	229 ^a	248 ^a	278 ^a	258 ^a	281 ^a	<10 ⁻³	50 ^{ab}	56 ^{ac}	47 ^a	65 ^d	58 ^{bcd}	65 ^{cd}	<10 ⁻³	<10 ⁻³	
124.079	C ₇ H ₁₀ ON ⁺	2-acetyl-1-methylpyrrole ⁹	33 ^a	32 ^a	32 ^a	35 ^a	32 ^a	29 ^a	0.624	21 ^b	22 ^b	15 ^a	23 ^b	20 ^{ab}	25 ^b	0.001	0.032	
124.109	C ₈ H ₁₄ N ⁺	butylpyrrole/methyl-propylpyrrole ⁹	34 ^d	15 ^a	20 ^b	21 ^b	20 ^b	28 ^c	<10 ⁻³	9 ^c	6 ^a	7 ^b	7 ^b	7 ^b	11 ^d	<10 ⁻³	<10 ⁻³	
125.060	C ₇ H ₉ O ₂ ⁺	guaiacol/methyl-benzenediol/furyl acetone ⁶	69 ^{ab}	79 ^{ab}	81 ^{ab}	82 ^b	79 ^{ab}	67 ^a	0.023	38 ^a	45 ^b	34 ^a	48 ^b	41 ^{ab}	46 ^b	<10 ⁻³	0.003	
125.096	C ₈ H ₁₃ O ⁺	butylfuran/methyl-propylfuran ⁴	49 ^b	42 ^a	48 ^{ab}	50 ^b	49 ^b	48 ^{ab}	0.021	17 ^a	17 ^a	19 ^{ab}	20 ^{bc}	18 ^{ab}	22 ^c	<10 ⁻³	0.044	
126.094	C ₇ H ₁₂ NO ⁺	acetyl-dimethylpyrrole/alkyloxazole ⁹	21 ^c	13 ^a	15 ^b	16 ^b	14 ^{ab}	19 ^c	<10 ⁻³	7 ^b	5 ^a	5 ^a	6 ^b	5 ^a	8 ^c	<10 ⁻³	0.000	
127.038	C ₆ H ₇ O ₃ ⁺	maltol/methyl-furoate ⁴	26 ^a	29 ^a	30 ^a	28 ^a	27 ^a	26 ^a	0.778	11 ^a	11 ^a	12 ^a	12 ^a	11 ^a	12 ^a	0.519	0.670	
127.076	C ₇ H ₁₁ O ₂ ⁺	3-ethyl-1-2-cyclopentanedione ²	58 ^a	65 ^a	65 ^a	64 ^a	59 ^a	55 ^a	0.598	17 ^a	24 ^b	17 ^a	24 ^b	20 ^{ab}	23 ^b	<10 ⁻³	0.431	
129.091	C ₇ H ₁₃ O ₂ ⁺	pentenyl-acetate heptanedione ²	67 ^b	58 ^a	62 ^{ab}	69 ^b	67 ^b	68 ^b	0.006	19 ^a	22 ^{ab}	20 ^a	26 ^c	24 ^{bc}	25 ^c	<10 ⁻³	0.028	
131.070	C ₆ H ₁₁ O ₃ ⁺	acetyloxy-butanone/ethanediol diacetate/oxopropoxy-propanone/ethyl-oxobutanoate ³	34 ^{ab}	34 ^{ab}	38 ^{ab}	41 ^b	38 ^{ab}	32 ^a	0.038	3 ^a	5 ^a	4 ^a	4 ^a	4 ^a	3 ^a	0.587	0.041	
131.107	C ₇ H ₁₅ O ₂ ⁺	heptanoic acid/C7 ester ³	4 ^b	3 ^a	4 ^b	5 ^c	4 ^b	4 ^b	<10 ⁻³	3 ^a	3 ^a	3 ^a	4 ^c	4 ^{bc}	4 ^b	<10 ⁻³	1.041	
134.061	C ₈ H ₈ ON ⁺	methyl-benzoxazole ⁵	2 ^a	2 ^a	2 ^a	2 ^a	2 ^a	2 ^a	0.312	2 ^{ab}	2 ^{ac}	2 ^a	2 ^{bc}	2 ^{ac}	3 ^c	0.005	0.002	
135.044	C ₈ H ₇ O ₂ ⁺	bifuran ⁴	3 ^a	4 ^c	4 ^c	4 ^c	4 ^{bc}	3 ^{ab}	<10 ⁻³	4 ^a	5 ^c	5 ^c	5 ^b	4 ^{ab}	5 ^{bc}	<10 ⁻³	0.009	
135.091	C ₈ H ₁₁ N ₂ ⁺	2-ethyl-6-vinyl-cyclopentapyrazine 6/7-dihydro-methyl-cyclopentapyrazine ⁷	15 ^a	13 ^a	16 ^a	16 ^a	14 ^a	12 ^a	0.164	5 ^{ab}	6 ^{ab}	5 ^a	7 ^{ab}	6 ^{ab}	7 ^b	0.025	0.030	
135.121	C ₁₀ H ₁₅ ⁺	terpene fragment ¹³	2 ^a	2 ^{ab}	2 ^a	6 ^d	3 ^{bc}	3 ^c	<10 ⁻³	1 ^a	1 ^{ab}	1 ^{ab}	4 ^d	1 ^{bc}	2 ^c	<10 ⁻³	<10 ⁻³	
136.077	C ₈ H ₁₀ NO ⁺	acetyl-methylpyridine ⁸	8 ^a	7 ^a	8 ^a	8 ^a	7 ^a	7 ^a	0.518	4 ^{ac}	4 ^{ac}	3 ^a	5 ^{bc}	4 ^{ab}	5 ^c	0.001	0.054	
136.112	C ₉ H ₁₄ N ⁺	butyl-pyridine/ethyl-propylpyridine ⁸	3 ^{bc}	2 ^a	2 ^{ab}	4 ^c	2 ^{ab}	3 ^{ab}	<10 ⁻³	2 ^b	1 ^a	1 ^a	2 ^c	1 ^{ab}	2 ^c	<10 ⁻³	0.005	
137.108	C ₈ H ₁₃ N ₂ ⁺	ethyl-dimethyl-pyrazine ⁷	23 ^a	23 ^a	25 ^a	27 ^a	24 ^a	21 ^a	0.542	13 ^{ab}	12 ^{ab}	10 ^a	14 ^{ab}	12 ^{ab}	15 ^b	0.082	0.308	
137.135	C ₁₀ H ₁₇ ⁺	various monoterpenes ¹¹	13 ^a	17 ^a	17 ^a	60 ^b	16 ^a	18 ^a	<10 ⁻³	5 ^a	5 ^a	7 ^b	27 ^c	6 ^{ab}	6 ^{ab}	<10 ⁻³	<10 ⁻³	
138.059	C ₇ H ₈ O ₂ N ⁺	pyridinecarboxylic acid methyl ester ⁸	5 ^a	5 ^a	5 ^a	5 ^a	5 ^a	4 ^a	0.506	5 ^{ac}	5 ^{ac}	4 ^a	5 ^{bc}	4 ^{ab}	5 ^c	0.004	0.022	

(Table 2.2-3 continues)

Meas. Mass (m/z)	Sum formula	Tentative identification	POWDER						p-value powder	BREW						p-value brew	p-value interaction
			BRA	COL	CRC	ETH	GUA	IND		BRA	COL	CRC	ETH	GUA	IND		
138.093	C ₈ H ₁₂ NO ⁺ C ₃ H ₁₄ N ₄ S ⁺	dimethyl-pyrrolylethanone ethyl-pyrrolylethanone/2- acetyl-1-ethylpyrrole/ 1-acetyl-2/5-dimethylpyrrole ⁹	9 ^a	11 ^a	11 ^a	10 ^a	10 ^a	9 ^a	0.695	4 ^{ab}	5 ^b	3 ^a	5 ^b	5 ^{ab}	6 ^b	0.001	0.160
138.131	C ₉ H ₁₆ N ⁺	butyl-pyridine ⁸	7 ^b	5 ^a	6 ^a	13 ^c	6 ^a	8 ^b	<10 ⁻³	3 ^b	2 ^a	3 ^b	5 ^d	3 ^{ab}	4 ^c	<10 ⁻³	<10 ⁻³
139.076	C ₈ H ₁₁ O ₂ ⁺	4-ethyl-1/2-benzenediol ⁶	16 ^a	19 ^a	20 ^a	18 ^a	17 ^a	15 ^a	0.224	13 ^{ab}	17 ^{bc}	12 ^a	18 ^c	15 ^{ac}	18 ^c	<10 ⁻³	0.007
139.113	C ₉ H ₁₅ O ⁺	E/E-2/4-nonadienal ²	12 ^a	12 ^a	12 ^a	14 ^b	13 ^{ab}	13 ^a	<10 ⁻³	5 ^a	5 ^a	6 ^{bc}	6 ^c	5 ^{ab}	6 ^{bc}	<10 ⁻³	0.260
141.053	C ₇ H ₉ O ₃ ⁺	furfuryl-acetate ⁴	35 ^a	35 ^a	35 ^a	34 ^a	35 ^a	35 ^a	1.000	18 ^{ab}	18 ^{ab}	15 ^a	20 ^{bc}	18 ^{ab}	22 ^c	<10 ⁻³	0.335
141.127	C ₉ H ₁₇ O ⁺	nonenal ²	1 ^a	1 ^a	1 ^a	2 ^a	1 ^a	1 ^a	0.232	1 ^a	1 ^{ab}	1 ^a	1 ^b	1 ^{ab}	1 ^b	0.001	0.302
143.107	C ₈ H ₁₅ O ₂ ⁺	methyl-heptanedione/ octenoic acid ²	7 ^a	7 ^a	10 ^b	8 ^a	8 ^a	8 ^a	<10 ⁻³	3 ^a	4 ^b	4 ^{bc}	4 ^{bc}	4 ^b	4 ^c	<10 ⁻³	<10 ⁻³
145.123	C ₈ H ₁₇ O ₂ ⁺	ethyl-hexanoic acid/C8 ester ³	1 ^{ab}	1 ^a	1 ^{bc}	2 ^c	1 ^{ab}	1 ^{ab}	<10 ⁻³	3 ^a	3 ^a	2 ^a	4 ^c	4 ^{bc}	4 ^b	<10 ⁻³	<10 ⁻³
148.076	C ₉ H ₁₀ ON ⁺	1-furfurylpyrrole ⁹	5 ^{ab}	7 ^{ab}	8 ^b	7 ^{ab}	6 ^{ab}	5 ^a	0.085	8 ^{ab}	11 ^{bc}	5 ^a	14 ^c	10 ^{ac}	13 ^{bc}	0.001	<10 ⁻³
149.060	C ₉ H ₉ O ₂ ⁺ C ₆ H ₁₃ O ₂ S ⁺	furfuryl-furan ⁴ mercapto-3- methylbutylformate ¹⁰	4 ^a	4 ^a	5 ^a	4 ^a	4 ^a	4 ^a	0.131	5 ^{ab}	6 ^{cd}	4 ^a	6 ^{bd}	5 ^{abc}	7 ^d	<10 ⁻³	<10 ⁻³
149.106	C ₉ H ₁₃ N ₂ ⁺	ethenyl-ethyl-methylpyrazine dihydrocyclopentapyrazines quinoxalines ⁷	7 ^b	5 ^a	7 ^b	7 ^b	6 ^{ab}	6 ^{ab}	0.002	2 ^a	2 ^{ac}	2 ^a	3 ^c	2 ^{ab}	3 ^{bc}	<10 ⁻³	<10 ⁻³
151.078	C ₉ H ₁₁ O ₂ ⁺	vinylguaiacol ⁶	11 ^{ab}	11 ^{ab}	13 ^b	12 ^{ab}	11 ^{ab}	10 ^a	0.117	9 ^{bc}	10 ^c	4 ^a	12 ^c	8 ^b	11 ^c	<10 ⁻³	<10 ⁻³
151.121	C ₉ H ₁₅ N ₂ ⁺	diethyl-methyl-pyrazine ⁷	5 ^a	6 ^a	6 ^a	7 ^a	6 ^a	5 ^a	0.217	3 ^{ab}	3 ^{ab}	3 ^a	4 ^{ab}	3 ^{ab}	4 ^b	0.027	0.046
153.056	C ₈ H ₉ O ₃ ⁺	vanillin ⁶	7 ^{ab}	8 ^{ab}	9 ^b	7 ^{ab}	7 ^{ab}	6 ^a	0.119	5 ^a	7 ^b	5 ^a	6 ^{ab}	5 ^a	7 ^b	<10 ⁻³	0.001
153.093	C ₉ H ₁₃ O ₂ ⁺	4-ethyl-guaiacol ⁶	6 ^{ab}	7 ^{ab}	8 ^b	7 ^{ab}	7 ^{ab}	6 ^a	0.081	5 ^a	7 ^{ab}	5 ^a	8 ^{bc}	6 ^{ab}	8 ^c	<10 ⁻³	0.000
153.128	C ₁₀ H ₁₇ O ⁺	E/E-2/4-decadienal ²	5 ^a	5 ^a	5 ^a	10 ^b	5 ^a	6 ^a	<10 ⁻³	2 ^a	3 ^{bc}	2 ^{ab}	6 ^d	3 ^{ab}	4 ^c	<10 ⁻³	0.001
157.085	C ₈ H ₁₃ O ₃ ⁺	4-ethoxy-2/5-dimethyl-3-2H- furanone/ethylfuranol ⁴	3 ^{ab}	3 ^{ab}	4 ^b	4 ^{ab}	3 ^{ab}	3 ^a	0.046	1 ^a	1 ^c	1 ^{ab}	1 ^{bc}	1 ^{ac}	1 ^{ac}	0.008	0.033
159.140	C ₉ H ₁₉ O ₂ ⁺	nonanoic acid/C9 ester ³	1 ^{ab}	1 ^a	1 ^{ab}	1 ^b	1 ^{ab}	1 ^{ab}	0.061	2 ^a	2 ^a	2 ^a	3 ^b	3 ^b	2 ^a	<10 ⁻³	<10 ⁻³
161.072	C ₉ H ₉ ON ₂ ⁺	furyl-methylpyrazines ⁷	2 ^a	2 ^a	2 ^a	2 ^a	2 ^a	2 ^a	0.553	1 ^c	1 ^{cd}	0 ^a	1 ^c	1 ^b	1 ^d	<10 ⁻³	<10 ⁻³
163.077	C ₁₀ H ₁₁ O ₂ ⁺	methyl-furfurylfuran ⁴	2 ^a	2 ^{ab}	2 ^b	2 ^{ab}	2 ^{ab}	1 ^a	0.015	2 ^{ab}	3 ^{bc}	1 ^a	3 ^{bc}	2 ^{ac}	3 ^c	<10 ⁻³	<10 ⁻³
165.095	C ₁₀ H ₁₃ O ₂ ⁺	methyl-cinnamate ⁶	2 ^a	2 ^a	2 ^b	2 ^{ab}	2 ^a	2 ^a	0.002	1 ^b	2 ^c	1 ^a	2 ^c	1 ^b	2 ^c	<10 ⁻³	<10 ⁻³
166.088	C ₉ H ₁₂ O ₂ N ⁺	methyl-pyrrolyl-butanedione ⁹	1 ^{ab}	1 ^{ab}	2 ^b	1 ^{ab}	1 ^{ab}	1 ^a	0.034	1 ^a	1 ^{bcd}	1 ^{ab}	1 ^{cd}	1 ^{ac}	2 ^d	<10 ⁻³	<10 ⁻³
167.069	C ₉ H ₁₁ O ₃ ⁺	methylvanillin ⁶	3 ^{ab}	3 ^{bc}	4 ^c	3 ^{bc}	3 ^{ab}	2 ^a	<10 ⁻³	3 ^{ab}	3 ^{bc}	2 ^a	3 ^c	3 ^{bc}	3 ^c	<10 ⁻³	<10 ⁻³
167.112	C ₁₀ H ₁₅ O ₂ ⁺	ethyl-dimethoxy-benzene ⁶	1 ^a	1 ^{ab}	2 ^b	1 ^{ab}	1 ^{ab}	1 ^a	0.029	1 ^a	1 ^{bc}	1 ^{ab}	1 ^{ac}	1 ^{ac}	1 ^c	0.004	0.001

(Table 2.2-3 continues)

Meas. Mass (m/z)	Sum formula	Tentative identification	BRA	COL	CRC	ETH	GUA	IND	p-value powder	BRA	COL	CRC	ETH	GUA	IND	p-value brew	p-value interaction
175.087	C ₁₀ H ₁₁ ON ₂ ⁺	dimethyl-furanyl-pyrazine ⁷	1 ^b	1 ^a	1 ^{ab}	1 ^{ab}	1 ^{ab}	1 ^{ab}	0.082	<1 ^{cd}	<1 ^{cd}	<1 ^a	<1 ^c	<1 ^b	<1 ^d	<10 ⁻³	<10 ⁻³
176.070	C ₁₀ H ₁₀ O ₂ NS ⁺	pyrrole-carboxaldehyde ⁹	1 ^{ab}	1 ^a	1 ^b	1 ^{ab}	1 ^{ab}	1 ^a	0.036	1 ^c	1 ^c	<1 ^a	1 ^c	1 ^b	1 ^c	<10 ⁻³	<10 ⁻³
179.073	C ₁₀ H ₁₁ O ₃ ⁺	vinyl-syringol ⁶	1 ^a	1 ^a	1 ^a	1 ^a	1 ^a	<1 ^a	0.142	1 ^c	1 ^d	<1 ^a	1 ^{cd}	<1 ^b	1 ^e	<10 ⁻³	<10 ⁻³
183.103	C ₁₀ H ₁₅ O ₃ ⁺	ethyl-syringol ⁶	<1 ^a	1 ^{ab}	1 ^b	1 ^{ab}	1 ^{ab}	<1 ^a	0.008	<1 ^a	<1 ^{bc}	<1 ^{ab}	1 ^d	<1 ^{ac}	<1 ^c	<10 ⁻³	<10 ⁻³
191.148	C ₁₃ H ₁₉ O ⁺	beta-damascenone ²	<1 ^a	<1 ^a	<1 ^a	<1 ^a	<1 ^a	<1 ^a	0.176	<1 ^a	<1 ^b	<1 ^a	<1 ^c	<1 ^{bc}	<1 ^{bc}	<10 ⁻³	<10 ⁻³

*Coffee samples in powder and brew form: BRA: Brazil, COL: Colombia, CRC: Costa Rica, ETH: Ethiopia, GUA: Guatemala, IND: India.

**m/z= mass to charge ratio.

***The chemical classes annotated to mass peaks after tentative identification: 1.alcohols, 2.carbonyls, 3.esters/acids, 4.pyrans/furans, 5.oxazoles, 6.phenols, 7.pyrazines, 8.pyridines, 9.pyrroles, 10.sulphur compounds, 11.terpenes, 12.thiazoles, 13.various fragments

2.2.4 Conclusions

The rapid analysis of volatile compounds in the headspace of six Arabica coffees, brew and powder, was performed with PTR-ToF-MS allowing successful separation according to their geographical origins. The high sensitive and high resolution PTR-MS technique enabled quantification of mass peaks at ppb levels and further tentative identification of volatile compounds. The results showed significant differences in the concentrations of volatile compounds in the powder headspace. When the coffees were brewed the release of these volatiles was less in general and significant interactions were recorded between volatile release and coffee origin.

The switching reagent ion system in PTR-MS instrumentation was applied for the first time to coffee by using not only H_3O^+ but also NO^+ and O_2^+ as precursor ions. All methods and precursor ions provide low classification errors. PDA showed the best classification performance in all ionisation modes. The data obtained by all ionisation modes provided valuable information on highlighting the applicability of SRI system on real complex food matrices like coffee. Although data obtained using O_2^+ allow the classification of the samples, the spectra interpretation in this case is more difficult. In general, the data produced showed the possibility and advantages of switching reagent ion system in coffee for successful discrimination of geographic origin by extending the fingerprinting capabilities of PTR-ToF-MS. Merging all the information of the different reagent ions by means of data fusion increased the efficiency of classification.

In conclusion PTR-MS and the SRI system provide a rapid non-invasive method that allows the classification of coffee with different origins. This approach is based on the measuring of volatile compound concentration and is thus expected that the differences observed should be reflected on the sensory characteristics of the investigated samples.

Acknowledgements

Sine Yener is a fellowship holder of PIMMS (Proton Ionisation Molecular Mass Spectrometry) is supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.

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Chapter 3. Single coffee bean roasting

3.1 Analysis of single coffee bean volatile compounds with Proton Transfer Reaction-Time of Flight-Mass Spectrometry

Submitted as

“Sine Yener, Luciano Navarini, Valentina Lonzarich, Luca Cappellin, Tilmann D. Märk, Günther Bonn and Franco Biasioli. Analysis of single coffee bean volatile compounds with Proton Transfer Reaction-Time of Flight-Mass Spectrometry, 2016. J. Mass Spectrometry.”

Highlights

- Volatile compounds released from 468 single coffee beans was measured with PTR-ToF-MS
- Aroma profiles of single coffee beans from different geographical origins were characterized
- Volatile compounds showed different release behaviors during coffee roasting
- Coffee beans from different geographical origins showed different volatile compound release

Abstract

This study applies proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) for the rapid analysis of volatile compounds released by single coffee beans. The headspace volatile profiles of single coffee beans (Coffea arabica) from different geographical origins (Brazil, Guatemala and Ethiopia) were analyzed via offline profiling at different stages of roasting. The effect of coffee geographical origin was reflected on the final weight losses and therefore volatile compounds formation. Clear origin signatures were observed in the volatile release and as well as in the formation of different coffee odorants.

Keywords: direct injection mass spectrometry, geographical origin, volatile compound, coffee roasting, profiling

3.1.1 Introduction

Aroma of coffee is one of the most important attributes to evaluate its quality. It can be considered as a first indication of how your coffee might taste by completing and improving the overall flavour perception. Coffee volatiles are formed during roasting by a series of complex reactions via breaking down the natural components of raw green beans [1]. The non-volatile constituents of green coffee (*i.e.* sugars, acids, alkaloids, free amino acids) evolve differently and contribute to formation of different classes of aroma compounds in coffee with various odour characteristics.

During roasting, heat is transferred from the outer surface through inside the coffee beans. High heat induces evaporation of water (dehydration phase) and temperature increase inside the bean. Outer surface of coffee beans get dry and with the initiation of roasting process, several volatile compounds and roasting gases are formed which are released via bean crack and burst [2-4]. Each individual coffee bean acts as a key unit during roasting and consequently contribute to the final flavour of coffee. When the desired roasting degree is achieved, the process is ended via rapid cooling of the coffee beans. The final flavour profile depends on several factors such as roasting conditions (time-temperature profile) and as well as the variety and origin of coffee beans, growth (*i.e.* climate and soil) and harvest conditions) [1, 4].

PTR-MS is a well-known tool for the direct and online analysis of volatile compounds via soft chemical ionization in various fields [5, 6] and as well as in coffee flavour research [7]. The high mass resolution and sensitivity provided by the recent implementation of ToF mass analysers [8] enables the detection of volatiles at very low levels which is essential for the matrices with low volatile compound emissions such as single coffee beans. Significant amount of literature is available on the detection of coffee volatile compounds however there are a few studies where the volatile compounds released from single coffee beans were measured with PTR-MS or other methods. The earliest study has been recorded on analysing the volatile compounds released from 6 coffee beans [9] during roasting by using PTR-MS in comparison to industrial roasting. Other studies implemented laser-based resonance enhanced multiphoton ionization-time of flight-mass spectrometry [10, 11] and single photon ionization-mass spectrometry [12] for the online and real-time monitoring of aromatic volatile compounds produced inside and outside of an individual coffee bean during coffee roasting with a focus on distinguishing *Arabica* and *Robusta* coffee beans. To the best of our knowledge, PTR-MS with a ToF mass analyser was not utilized for volatile profiling of single coffee beans.

In this study, we used PTR-ToF-MS, for the first time, for simple and rapid profiling of volatile compounds released from a large number of single coffee beans (>450) at different stages of roasting. We selected two different samples of pure green *Arabica* beans from Brazil, Guatemala and Ethiopia and investigated the differences between different coffee samples and coffee origins on volatile formation.

3.1.2 Experimental

3.1.2.1 Green Coffee Samples

In this study, green coffee beans (*Coffea arabica*) from Brasil (2 samples), Guatemala (2 samples) and Ethiopia (2 samples) were used. All samples were wet processed with zero primary and secondary defects and with a clean cup. The samples were received in 200 g bags and stored at room temperature prior to analysis.

3.1.2.2 Roasting

Roasting was performed by using a laboratory oven with ventilation (Binder GmbH, Tuttlingen, Germany) at 190°C. This is in the typical range of coffee roasting (170-230°C) at which the reactions responsible for the characteristic flavour and aroma of roasted coffee are initiated [2]. Single green coffee bean were put in 22 ml-clear open glass vials. The green beans were selected randomly after opening the bags. The open glass vials containing the green coffee beans were placed on the metal oven tray with grid in 3 rows and 6 cloumns. For each time point 18 coffee beans (3 coffee origins x 2 samples x 3 beans) were selected and placed in a randomized order to avoid memory effetcs and to prevent the green bean from the same origin and sample being in the same location. Roasting process was performed by roasting the green beans starting from 1 to 25 min by preparing a new set of beans for each time point. After each roasting step was completed, the vials were cooled down to room temperature and closed. The overall roasting process created a data set consisting of 468 data points (26 time points x 3 origins x 2 samples x 3 replicates). Each coffee bean was weighed before and after the roasting. Weight losses were calculated accordingly: “(green bean weight-roasted bean weight)/green bean weight x 100”.

3.1.2.3 Volatile profiling by PTR-MS

The headspace volatile compounds of roasted coffee beans were analyzed with a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) in an automated

manner by using a multipurpose autosampler (Gerstel GmbH & Co. KG, Mulheim am Ruhr, Germany). The instrumental conditions were as described in [13] and mass spectral data was processed as described in [14-16]. In addition to the vials with coffee beans, 14 vials containing lab air (blanks) were analyzed and their signals were subtracted from the original samples for further data analysis.

3.1.3 Results and Discussion

3.1.3.1 The weight losses during roasting

Total weight loss (or roast loss) is an important parameter for determining the final roasting degree [2]. One-way ANOVA ($p < 0.05$) showed significant changes in weight losses with time showing similar weight loss trends for all coffee origins and different samples (**Figure 3.1-1**). From time 0 to 14 min, the losses were linear (min $R^2 = 0.94$; data not shown) for all coffee origins and samples; after that a plateau region was observed where the weight loss rates were slower. In the first part of the linear region (0-6 min) the weight losses were rapid and slightly decreased in the second part (7-14 min). Coffee beans lost around 5% after 6th min and reached approximately 10% roast loss after 14th min. The weight losses were independent of coffee origin for the linear part; however in the last region, the final weight losses were significantly different for different coffee origins ($p < 0.05$). The first sample of BRA exhibited smaller final weight loss than BRA sample 2 ($p < 0.05$). In addition, there were no significant differences in the final weight losses between the two samples of GUA and ETH.

Table 3.1-1. Average weight losses (%) obtained at 6, 11, 14 and 25th min of single coffee bean roasting

Roasting time (min)	Weight loss (%)					
	BRA		GUA		ETH	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
6	4.9 ± 0.41	4.8 ± 0.80	5.6 ± 0.30	5.2 ± 0.50	5.3 ± 0.51	5.0 ± 0.24
11	7.8 ± 0.15	8.8 ± 0.51	10.4 ± 0.28	9.3 ± 0.58	9.9 ± 0.71	9.2 ± 1.01
14	9.9 ± 0.69	9.8 ± 0.81	12.2 ± 1.28	10.6 ± 0.17	10.7 ± 1.04	10.4 ± 0.42
25	11.4 ± 0.28	12.4 ± 0.63	13.9 ± 0.90	13.0 ± 0.34	13.9 ± 0.67	13.9 ± 0.11

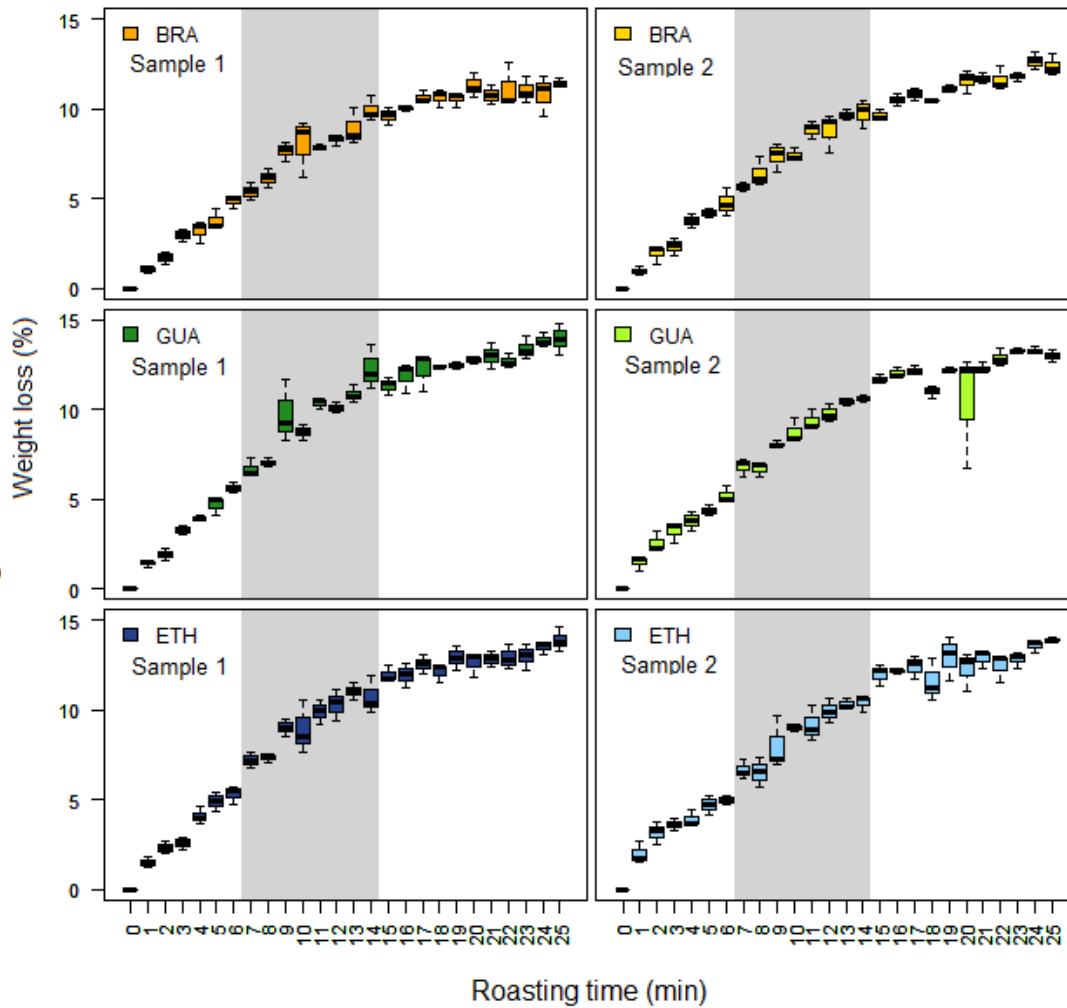


Figure 3.1-1. Weight losses (%) obtained during roasting. Each data point shows the average of 3 coffee beans.

3.1.3.2 Volatile compounds released from single coffee bean

PTR-ToF-MS data processing resulted in extraction of 571 mass peaks. The interfering ions (O_2^+ , NO^+ , water clusters and their isotopologues) were discarded from the whole mass spectra and the average signals of blank vials were subtracted from the samples for noise reduction. The resulting data matrix was used for further data analyses (526 mass peaks x 468 samples).

3.1.3.3 Green coffee beans

The volatile emissions of green coffee beans were compared between the two samples of same coffee origin. Despite the expected biological variability between green coffee beans, no

significant differences were found between the two samples of BRA, ETH and GUA ($p < 0.01$ with Bonferroni correction) while 7 mass peaks were significantly different according to origin being at higher concentrations for ETH, followed by BRA and GUA, respectively. These significant mass peaks are given with measured mass, sum formula and tentative identifications as follows: m/z 78.049 ($C_6H_6^+$; phenyl ring), m/z 79.055 ($C_6H_7^+$; benzene ring), m/z 91.057 ($C_4H_{11}S^+$; buthanethiol); m/z 106.076 ($C_8H_{10}^+$; phenylethenyl), m/z 107.087 ($C_8H_{11}^+$; terpene fragment) and m/z 108.086 (^{13}C isotope of $C_8H_{11}^+$).

3.1.3.4 Roasted coffee beans

Plotting mass peak concentrations against time allowed us to visualize different release behaviours and as well as different evolutions among different coffee origins. For some mass peaks we observed a clear decrease in the headspace (group 1); some mass peaks did not show a clear trend over time (group 2) and mass peaks increased during roasting (group 3). These mass peaks are given with annotated sum formula and possible tentative identifications in Supplementary file 1. Group 1 consist of mainly water clusters, terpenic fragments and a sulphur compound; group 2 compounds contain alcohols, some fragments and a few compounds detected in green and roasted coffee [3, 9]. Most of the peaks belong to group 3 were related to the compounds formed during coffee roasting [3].

Some examples are shown for group 1 and 2 compounds in **Figure 3.1-2a-b** and **Figure 3.1-2c-d**, respectively. As seen in Fig. 2 and b, the concentrations of water cluster and a sulphur compound (m/z 93.073) decrease in the headspace. Up to now, many sulphur compounds have been identified in roasted coffee beans [3] and some of them are also present in the raw beans, but no literature was recorded indicating the presence of this sulphur compound (methylthioethanol) in green coffee beans.

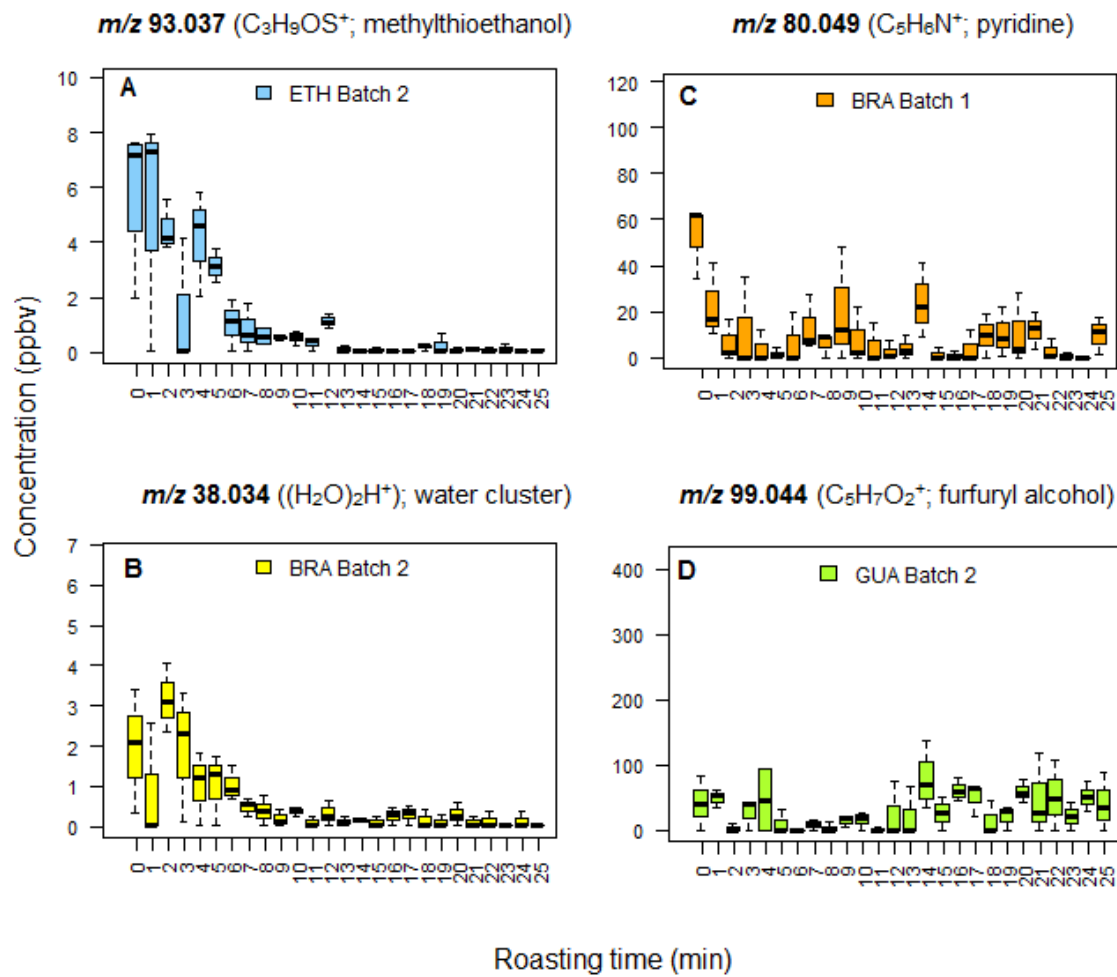


Figure 3.1-2. The release behaviour of different groups of volatile compounds at m/z 93.073 (A), m/z 38.034 (B), m/z 80.049 (C) and m/z 99.041 (D) with annotated sum formula and tentative identifications. Each time point is average emissions of 3 coffee beans.

The abundance of pyridine and furfuryl alcohol do not show clear trends with time **Figure 3.1-3** represents an example for group 3 compounds (m/z 49.010, methanethiol) which highlights some differences between different coffee origins and, to a lesser extent, also between two samples of same coffee origin *e.g.* BRA. Lower volatile intensities for the first batch can be explained by the relatively low roasting temperature to exploit abundant volatile formation. Highest intensities were seen in BRA sample 1 followed by GUA and ETH, respectively. The final weigh losses were lower for BRA than ETH and GUA, however the release of methanethiol shows a higher trend and faster evolution for BRA, which may indicate the effect of coffee geographical origin.

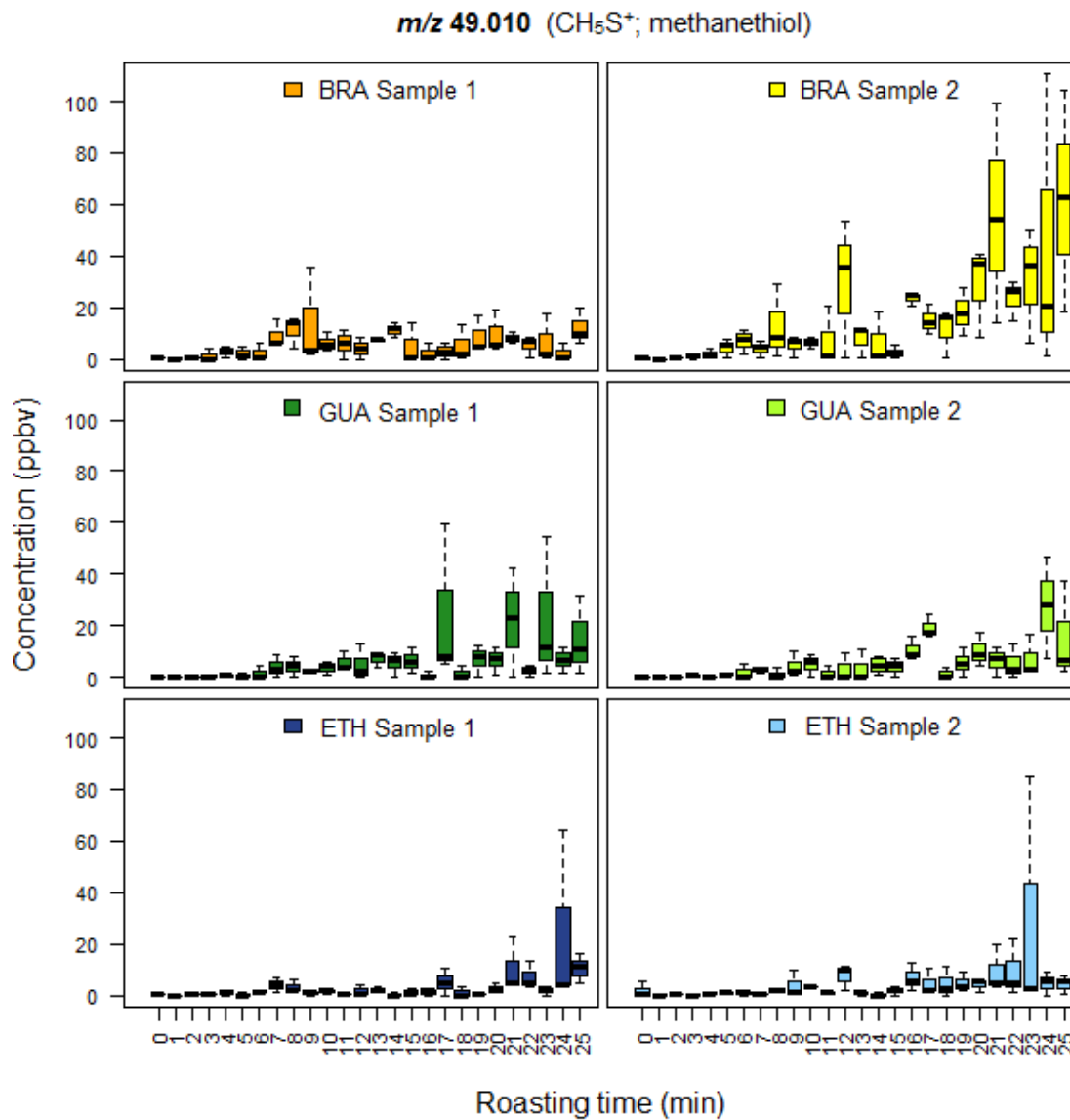


Figure 3.1-3. The release of m/z 49.010 (CH₅S⁺, methanethiol) in the headspace of coffee beans. Each time point is the average volatile emissions of 3 coffee beans.

Cumulative boxplots were created by plotting the summed concentrations up to a given time point (*e.g.* 3rd minute is a sum of 3rd, 2nd, 1st and 0th minutes) to obtain information about the production and/or release rate of the volatile compounds in the headspace and also to reduce the noise. Some examples are given in **Figure 3.1-4a, b and c** for mass peaks m/z 82.065 (C₅H₈N⁺; methylpyrrole), 101.060 (C₅H₉O₂⁺; pentanedione) and 137.135 (C₁₀H₁₇⁺; monoterpenes), respectively.

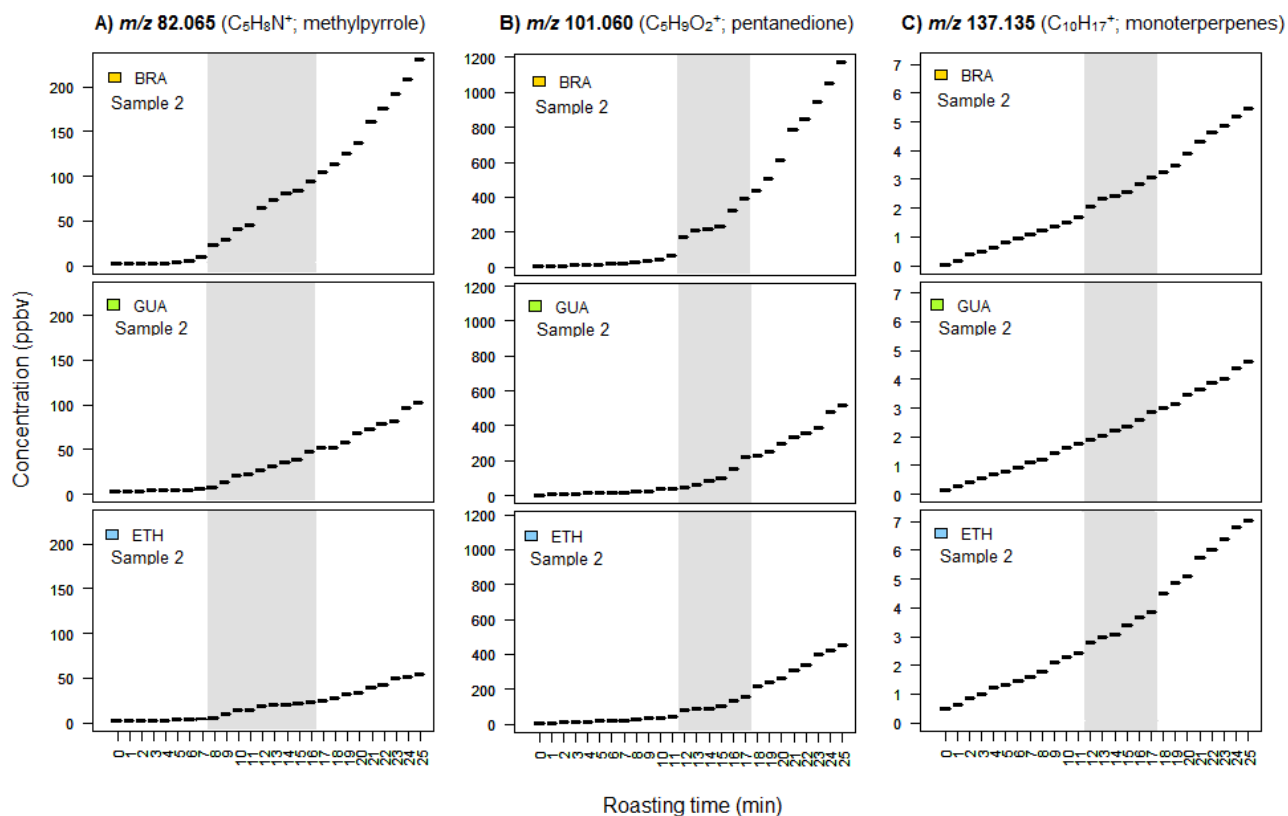


Figure 3.1-4. The cumulative release curves of m/z 82.065 (A), m/z 101.060 (B) and m/z 137.135 (C) for second batches of BRA, ETH and GUA with annotated sum formula and tentative identifications. Each time point is average emissions of 3 coffee beans.

Differences in the amount of the released compounds among the coffee origins were clear and in accordance to our previous findings in which we compared the headspace volatiles of coffee powders from different geographical origins [13, 17]. The release behaviour of methylpyrrole and pentanedione were similar and interestingly, methylpyrrole showed an earlier release (around 6th min) than pentanedione (around 11th min) as highlighted with gray shadows in the **Figure 3.1-4**. Earlier release of methylpyrrole was reported in literature [18] during roasting of 100 g coffee beans which was also supported by our findings in single bean level. The difference in the release times of these compounds can be explained via weight loss release curves (linear part) where most of the water is removed from coffee beans (dehydration stage [19]) and lead formation of volatile compounds and also due to the low volatility of pentanedione [20]. Since the formation pathways of the volatile compounds in coffee are quite diverse [19] and need different activation energies [18]; the highest increase exhibited by volatile compounds may differ depending on the process conditions [3, 9, 21] and the

composition of green beans which is highly affected by the geographical origin [22, 23]. Monoterpenes, associated to flowery and fruity notes [24], exhibited a low level but continuous release being highest for ETH that has been associated with higher amounts of monoterpenes was in accordance with previous findings [13, 17, 25]. This unique group of compounds resist during roasting and their release might be due to the changes happen in bean structure [2] which might allow continuous release of these compounds.

3.1.4 Conclusions

In summary, PTR-ToF-MS has been used for the first time to analyze the volatile compounds released from 468 single coffee beans from different geographical origins. Thanks to the high sensitivity provided by PTR-ToF-MS [8] quantification of volatile compounds at very low levels was possible which allowed visualizing clear differences at single bean level. When same roasting conditions were applied, coffee beans showed different final roast degrees and therefore volatile compound evolutions. Offline aroma profiling coffee beans gave an insight into the volatile formation at different stages of roasting indicating the clear differences in the aroma profiles of coffee beans from different coffee origins. The study pointed out the potential of the experimental approach for the characterization of foods (in particular food origin) and as well as food processes (*e.g.* roasting) in small scales.

Acknowledgements

This research has been funded by PIMMS (Proton Ionisation Molecular Mass Spectrometry) ITN, which is supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.

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Supplementary file 3.1. Classification of mass peaks according to headspace release behaviours. The mass peaks are given with measured mass and sum formula.

Behavior	Measured mass (<i>m/z</i>)	Sum formula	Tentative identification
Group 1: Decreasin g	38.034	(H ₂ O) ₂ H ⁺	Water cluster
	39.032	(H ₂ O) ₂ H ⁺	Water cluster
	55.038	(H ₂ O) ₃ H ⁺	Water cluster
	79.055	C ₆ H ₇ ⁺	Benzene ring
	93.037	C ₃ H ₉ OS ⁺	Methylthioethanol
	106.076	C ₈ H ₁₀ ⁺	Terpene fragment (phenyethenyl)
	107.087	C ₈ H ₁₁ ⁺	Terpene fragment
Group 2: Decrease, increase	33.033	CH ₅ O ⁺	Methanol
	43.018	C ₂ H ₃ O ⁺	Fragment
	47.013	CH ₃ O ₂ ⁺	Formic acid
	51.043	[H ₂ O]CH ₅ O ⁺	Water-methanol cluster
	57.033	C ₃ H ₅ O ⁺	Acrolein/ acetol fragment
	61.028	C ₂ H ₅ O ₂ ⁺	Acetic acid
	69.033	C ₄ H ₅ O ⁺	Furan
	75.044	C ₃ H ₇ O ₂ ⁺	Methyl-acetate/acetol
	80.049	C ₅ H ₆ N ⁺	Pyridine
	81.034	C ₅ H ₅ O ⁺	Furan fragment
	83.085	C ₆ H ₁₁ ⁺	Terpene fragment (cyclohexene)
	87.044	C ₄ H ₇ O ₂ ⁺	Butanedione
	99.044	C ₅ H ₇ O ₂ ⁺	Furfuryl alcohol
	103.076	C ₅ H ₁₁ O ₂ ⁺	Hydroxy-pentanone/methyl-butanoic acid
	123.092	C ₇ H ₁₁ N ₂ ⁺	Ethyl-methyl-pyrazine/trimethylpyrazine
Group 3: Increasing	31.018	CH ₃ O ⁺	Fragment
	39.023	C ₃ H ₃ ⁺	Fragment
	41.038	C ₃ H ₅ ⁺	Alkylic fragment
	45.033	C ₂ H ₅ O ⁺	Acetaldehyde
	49.011	CH ₃ S ⁺	Methanethiol
	55.054	C ₄ H ₇ ⁺	Butadiene
	57.070	C ₄ H ₉ ⁺	Alcohol fragment
	59.045	C ₃ H ₇ O ⁺	Acetone/
	63.027	C ₂ H ₇ S ⁺	Dimethyl disulfide
	68.049	C ₄ H ₆ N ⁺	Pyrrole
	69.070	C ₅ H ₉ ⁺	Isoprene
	73.065	C ₄ H ₉ O ⁺	Isobutanal/butanone
	82.065	C ₈ H ₅ N ⁺	Methylpyrrole
	83.049	C ₅ H ₇ O ⁺	Methylfuran
	85.065	C ₅ H ₉ O ⁺	Methylbutenal
	87.081	C ₅ H ₁₁ O ⁺	Methylbutanal
	89.059	C ₄ H ₉ O ₂ ⁺	Methyl-propanoate/hydroxy-butanone
	95.060	C ₅ H ₇ N ₂ ⁺	Methylpyrazine
	97.028	C ₅ H ₅ O ₂ ⁺	Furfural
	97.065	C ₆ H ₉ O ⁺	Dimethylfuran
	98.065	C ₅ H ₈ ON ⁺	Dimethyl-oxazole
	101.060	C ₅ H ₉ O ₂ ⁺	Pentanedione
	109.076	C ₆ H ₉ N ₂ ⁺	Dimethylpyrazine/ethylpyrazine
	111.045	C ₆ H ₇ O ₂ ⁺	Acetylfuran/methylfurfural
	113.060	C ₆ H ₉ O ₂ ⁺	Methyl-furfuryl-alcohol/dimethyl-furanone/ Methyl-cyclopentanedione/cyclotene

Chapter 4. Nosespace analysis

4.1 Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis

Published as



Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis



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Highlights

- Dynamic sensory and instrumental methods are useful and complementary to study in vivo aroma perception and release
- TDS paired with nosespace can point out the presence cross-modal interactions and is a promising tool to study them
- Nosespace by PTR-ToF-MS allowed to identify some compounds as possible “temporal dominance markers” important for product discrimination

Abstract

The first goal of this work was to gain insight into the mechanism underlying flavour perception and aroma release by coupling two real-time methods: Temporal Dominance of Sensations (TDS) and nosespace (NS) analysis via Proton Transfer Reaction–Time of Flight–Mass Spectrometry (PTR–ToF–MS). The second goal was to investigate the impact of roasting degree and sugar addition on aroma release and perception in espresso coffee. A set of four coffee samples, two roasting degrees and two sugar levels, has been used for both sensory and instrumental measurements. The in-mouth flavour evolution in terms of dominant sensations was measured by mean of TDS carried out by 18 trained judges with a 9-attribute list (Sweet, Sour, Bitter, Astringent, Roasted, Burnt, Caramel, Nutty and Vegetal). The same judges were subjected simultaneously to NS analysis in order to identify and quantify the volatile compounds reaching their olfactory receptors during coffee consumption. A significant effect of roasting was observed with both techniques. More compounds and in larger quantity were released when increasing roasting degree, which was described sensorially as a greater dominance of the attributes Burnt, Roasted, Astringent and Bitter. Sugar addition did not significantly affect the aroma release of volatile compounds as demonstrated by the NS profiles of judges while changing completely the way the coffee was sensorially perceived in mouth. As expected, sweet taste became dominant over bitter and sour but it increased more globally the flavour complexity with Caramel and Nutty notes reducing the Roasted or Burnt ones. This result emphasizes the presence of taste–smell perceptual interactions, due to congruence effect between sweet taste and some flavours of coffee, and the potentialities of this combination of dynamic methods to study them. Besides, the treatment of NS data using clustering methods revealed two different release behaviours, which permitted to identify potential TDS markers.

Keywords: Temporal Dominance of Sensations (TDS), Aroma release, Proton Transfer Reaction Time of Flight Mass Spectroscopy (PTR-ToF-MS), nosespace, multisensory interactions, coffee

4.1.1 Introduction

Product consumption is a complex multisensory experience which changes during the time of consumption itself. It is well known that flavour of food is of greatest importance in the food global sensory experience and in its appreciation. Flavour has been defined by von Sydow [1] as an interaction of the food and the consumer suggesting that to be complete flavour study should consider both sensory and instrumental point of view. Flavour has been studied for many years according to different subtopics such as flavour release, chemoreceptor mechanisms and mathematical modelling (for a review see [2]). It has been demonstrated that flavour release and perception is changing over the time due to different physico-chemical, biochemical and physiological phenomena occurring in-mouth (salivation, changes of temperature, mastication, tongue movements, breathing, swallowing, etc.) [3-6]. Thus, classical sensory methods, which require integration and time-average processes of the perceptions perceived during the whole time of evaluation [7] are not suited to describe the whole perception.

The investigation of the dynamic of perception gained a lot of interest in the recent years and is currently deeply explored. Different sensory descriptive methods allow measuring the temporal aspects of product perception as Time-intensity and Temporal Dominance of Sensations.

Time-intensity (TI) was created a long time ago by Larson-Powers & Pangborn (1978) [8] and permits to follow the intensity of a given attribute over a certain period of time [9]. Temporal Dominance of Sensations (TDS) method on the contrary has been developed in the past few years in order to avoid the time consuming aspect and the “halo dumping” effect of TI [10-12]: this technique measures the in-mouth evolution of dominant sensations during product consumption. The panellist has to select within a list of attributes the one dominant at each moment of the evaluation time. This method allows the evaluation of several attributes simultaneously, with a maximum of 10 [13], and has demonstrated its utility, through numerous and various applications (see [14] for a review). TDS has already been applied on coffee either to investigate the influence of different sweeteners [15] or of the foam/“crema” [16] on the in-mouth perceptions. But it should be reminded that one or the other method - TI or TDS - is recommended depending on the objectives of the study: TI when the interest is focused on the kinetic of one specific sensory attribute while TDS when the concern is to evaluate the sequence of the consecutive perceived sensations, chosen within a multiple sensory attribute list.

It has also been underlined by several authors that the understanding of flavour perception phenomena could be considerably enriched by adding instrumental measurements performed

simultaneously to sensory measurements or combining them particularly in the case of dynamic measurements ([14]; *e.g.* TDS) and in the context of a coffee matrix [17].

Proton Transfer Reaction- Mass Spectrometry (PTR-MS) is a powerful tool for rapid, direct and highly sensitive on-line monitoring of volatile organic compounds (VOCs) [18]. PTR-MS uses a soft chemical ionisation based on proton transfer from a protonated reagent, most commonly H_3O^+ . The compounds with higher proton affinity than H_2O will react with H_3O^+ and the products are further analysed [19]. The addition of a time of flight (ToF) detector to the PTR-MS allows performing *in vivo* aroma release measurement such as nosespace (NS) analysis since it has the advantage to provide high mass range, very fast measurement and high mass resolution [20]. During eating or drinking, VOCs are first released into the oral cavity then reach the olfactory epithelium, via the retronasal route, where a sensory perception occurs. Thus, the *in vivo* measure of aroma by NS analysis is of primary importance to better understand the flavour perception of consumers [21] and NS analysis seems to be a very promising tool to be combined to sensory methods. Among the studies published on NS analysis with PTR-MS, very few have used a ToF mass analyser and have been applied to real food matrices as cereal bars [22] and coffee [23].

Until now and to our knowledge, only four studies [16, 24-26] have used in combination sensory (TDS method) and instrumental (NS analysis) real-time measurements to investigate aroma release and flavour perception: the existing works dealt with the impact of product properties and/or evaluation protocol on flavour. Délérís and colleagues (2011a)[24] and Mesurolle and co-workers (2013) [26] have studied the effect of texture on temporal aroma release and sensory perception respectively on candies and yogurts with fruit pieces. In this first study [24], some relations between the dynamics of release and perception on temporal parameters were evidenced (notably dominance duration, sequence of dominant attributes for sensory data and for instrumental data t_{max} value and intensity ratio for each ion) but it was not clearly the case in the work on yogurts [26] underlying that these links are not so obvious and probably depending on the food matrix. In this second study, they could not distinguish the different influence of sample texture from the natural adaptation of food oral processing when changing the texture on aroma release and perception. Another study explored the impact of swallowing on aroma release and perception of flavoured vodka [25]. They have showed that swallowing implied more complex sensory perceptions than spitting out even if the attribute dominances were weaker. Volatile compounds instrumental analysis corroborated the sensory results as they revealed that swallowing induced a higher and earlier release of larger amounts

of aroma. Barron et al. (2012) [16] have applied these two temporal methods to espresso coffee to measure the impact of foam/“crema” on the aroma release and in-mouth sensory perception. They showed that the presence of foam/“crema” was associated with the dominance of the roasted attribute and also to the release of pleasant high volatile [16]. These four discussed studies indicate the existence of some links between the dynamics of flavour perception and aroma release even though it remains challenging to relate these two types of data (sensory and instrumental data) due to the variety and the convoluted phenomena involved (salivation, breathing, chewing, individual sensitivity to volatile and non-volatile compounds, etc.). This is even more accentuated by the complexity of the coffee matrix [17].

The goal of this study is to get better insight into the flavour perception processes by clarifying the link between the evolution of descriptors of flavour dominant at each moment during the consumption of a complex real product (coffee) and the release kinetics of aroma compounds. In this purpose, we propose and evaluate the pairing of two dynamic methods, one based on the sensory responses by a trained panel (Temporal Dominance of Sensations) and the other one on *in-vivo* instrumental monitoring of volatile compounds present in the nose of judge (NS analysis with PTR-ToF-MS). As a case study, the effect of roasting degree and sugar addition on espresso coffee in terms of dominance of sensations during the consumption and of simultaneous *in-vivo* aroma release is investigated.

4.1.2 Materials and methods

4.1.2.1 Samples

Two 100% Arabica coffees naturally low in caffeine were provided by Illycaffè S.p.a. (Trieste, Italy) in capsules (Iperespresso) suitable to prepare an espresso coffee beverage. They had different roasting degrees according to commercial illycaffè colour standards: light roast (A) and dark roast (B) and were tested at two levels of sugar (**Table 4.1-1**). These two coffee types were selected on results of QDA profile carried out in duplicate by the trained panel of Illycaffè composed of 8 experts (data not shown) in a sensory laboratory designed in accordance with ISO 8589 (2007) [27]. They were described as being significantly different, for 8 attributes out of 11, according to ANOVA results, in terms of taste and flavour: A is sourer and B is bitterer and more aromatic, notably with a stronger chocolate, toasted bread and burnt odour and flavour. The two types of coffee were tested with two level of sugar concentrations: 0 and 100 mg/ml. This sugar dose corresponds to mean value of sugar used in espresso coffee by the panel (from the Fondazione Edmund Mach).

Table 4.1-1. Composition of the tested samples

	Light Roast	Dark Roast
Without sugar	CoffeeA	CoffeeB
With sugar	CoffeeAZ	CoffeeBZ

Coffee was prepared using an espresso coffee machine (Iperespresso X7.1, Illy), coffee capsules and mineral water (San Benedetto S.P.A., Italy, composition: Ca⁺⁺ 50.3 mg/l, Mg⁺⁺ 30.8 mg/l, Na⁺ 6.0 mg/l, K⁺ 0.9 mg/l). The espresso machine was set up to prepare standard volume espresso (30 ml, volume normally served for an Italian espresso). The samples were prepared by pipetting from the bottom of the coffee cup 10 ml of the espresso coffee without “crema”, the foamy surface of espresso, and afterwards eventually by dissolving the sugar. They were prepared one-by-one and served at 55°C in a polystyrene cup with a lid and a straw in order to avoid that the judges smell the sample before putting it in their mouth. Samples were presented in an anonymous manner with random three-digit codes. The four products were analysed in triplicate by each judge. The judges evaluated three products per session: either two coffees without sugar and one with or two coffees with sugar and one without. For each panellist four individual sessions were performed in consecutive days. The presentation order was set up following a Williams Latin square design balancing order and position effects. The complete design for NS/TDS experiment was carried out in 8 days.

4.1.2.2 Subjects

A panel composed of eighteen subjects (10 women and 8 men, aged from 23 to 37) was recruited from the Fondazione Edmund Mach where they were all employed. They were all volunteered and selected for their availabilities during all the duration of the study (3 months). Only three judges had previous experience in sensory analysis. The judges had no history of oral perception disorders. They were all daily coffee consumers except two who consume coffee only several times per week or per month. Espresso and moka coffees are the two types of coffees they were generally drinking. Half of the panel drank usually coffee without sugar. They were asked not to smoke, eat, drink or use persistent products at least one hour before the session.

4.1.2.3 Sensory analysis: Temporal Dominance of Sensations (TDS)

4.1.2.3.1 Training

The training period was set up over a period of two months. The judges were first introduced to sensory analysis principles: 1) “Concept of sequence” which corresponds to the description over the time or in other terms to the succession of the different sensations perceived in-mouth. 2) “Concept of dominance” which is defined as the sensation which triggers the most attention at a given time. 3) “Concept of weak but dominant” which illustrates the fact that a perception can be weak but dominant. Then, the judges were trained to describe and perceive changes over the time of simple aqueous model solutions based on the combination of two or three stimuli (made of varying ingredients sucrose, citric acid, caffeine and potassium alum combined with different simple volatile compounds (linalool, methyl cinnamate and ethyl hexanoate) and thickner (locust bean flour). They were also trained to recognise and describe basic tastes and representative odours/aroma in coffee. Two sessions were dedicated to attribute generation followed by the selection and definition of the most important attributes for coffee evaluation. For these two sessions, light, medium, dark and decaffeinated espresso coffees plus two experimental ones (light and dark roasted coffees both naturally low in caffeine) were used (provided by Illycaffè S.p.a). The judges were also trained to use the data acquisition software (Fizz, Biosystemes, Couternon, France, 2.46A version) and the tasting procedure.

4.1.2.3.2 Evaluation

Nine flavour attributes were selected during the training and used for the TDS evaluation: *Sweet, Sour, Bitter, Astringent, Roasted, Burnt, Caramel, Vegetal* and *Nutty*. They were presented simultaneously on the computer screen and their order was randomised over judges as recommended by Pineau et al. (2012) [13] who showed that judges tend to choose the attributes from the top of the list. The attribute order was identical for all the evaluations of one judge.

The assigned task during TDS evaluation of a product was to choose the attribute corresponding the dominant sensation among the list of attributes. An attribute was considered as dominant until another attribute was chosen. The judges were also told that an attribute can be dominant several times during the evaluation and that it is not necessary that all attributes were selected as dominant for the evaluation of each product.

The evaluation started as soon as the panellist put the whole sample (10 ml) in his/her mouth and click on start button. After five seconds, the panellist was asked to swallow the sample and

continue the evaluation of the dominant sensations while breathing at a normal and regular speed. The TDS evaluation lasted 60 seconds in total. Between two successive samples, judges were asked to clean their mouth with unsalted bread and mineral water and to wait at least 10 minutes. Three samples per session were monadically presented to panellists according to a design balancing order of evaluation over the four session carried out in consecutive days. TDS and nose-space analysis were performed simultaneously and required individual session. Evaluations were conducted in an individual computerised sensory booth under white cold light located in a room with filtered air at constant temperature (20 °C).

4.1.2.3.3 Data treatments

According to the researchers who set up the TDS method [12], it is difficult to evaluate the method in terms of panellists' performance because of the nature the data: the results consist in an evaluation is a sequence of dominant attributes chosen at different times. Besides that, the number and the nature of attributes chosen by each panellist in the different replicates and over the panel were checked.

To build TDS curves, each attribute is considered separately. For each point of time, a dominance rate consisting in the proportion of runs (subjects x replications, here, 18 x 3 so 54) for which the given attribute was assessed as dominant is calculated. The TDS curves are obtained by computing the dominance rate of each point [12]. The TDS curves were represented on one graph per product. To help in curve interpretation, two supplementary lines were drawn on the graphs [12, 28]: 1) the “chance level” which corresponds to the dominance rate that an attribute can obtain by chance. Its value, P_0 , is equal to $1/p$, p being the number of attributes. 2) the “significance level” represents the minimum value that must be reached to consider the dominant rate as significantly higher than P_0 . This value, P_s , is calculated following the equation (1), in other words establishing the confidence interval of a binomial proportion based on a normal approximation [12].

$$(1) P_s = P_0 + 1.645 \sqrt{\frac{P_0(1-P_0)}{n}}$$

n: number of subject x replication.

All significant attributes are also represented on a horizontal bar graph in order to have a more global view per product and better observe the simultaneous significance of different attributes over the time and the differences between products.

4.1.2.4 Instrumental analysis

4.1.2.4.1 Instrumental conditions

All measurements were performed by using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The ionisation conditions were the following: 550 V drift voltage, 110 °C drift temperature, and 2.33 mbar drift pressure, resulting in an E/N ratio of 140 Td ($1 \text{ Td} = 10^{-17} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). Acquisition was set to 1 mass spectrum per second. Sampling was carried out via a heated (110 °C) PEEK tube. Inlet flow was set to 40 and 440 sccm for headspace and NS measurements, respectively. The use of a higher inlet flow rate during NS measurements was found to better comply with the high time resolution required by the technique.

4.1.2.4.2 Headspace analysis of coffee brews

Freshly prepared coffee was briefly stirred and 2-ml aliquots were transferred into 22-ml glass vials (Supelco, Bellefonte, PA). The vials were equilibrated at 40°C for 30 min before the analysis. Each coffee type was prepared and measured eight times, once for every day of NS/TDS experiment, thus encompassing the whole range of experimental variability. Each measurement was the averaged result of 30 seconds of acquisition.

4.1.2.4.3 Nosespace (NS) analysis

NS analysis and TDS evaluation were performed at the same time. Sampling of NS was carried out by applying an ergonomic glass nosepiece to the nose of the judges. The nosepiece was connected to the PTR-ToF-MS by means of a PEEK tube (at room temperature for 10 cm, then heated at 110 °C).

After positioning the nose-piece in the nostrils, judges were asked to breathe normally through nose (mouth closed). Then the panellist received the sample and had to sip it in whole. As soon as he/she put the sample in mouth, he/she had to press a button on the screen to let the experimenters know when he/she put the sample in-mouth and to start the TDS evaluation which lasted 60 s. After the TDS evaluation, the panellist had to continue breathing for 60 s. During all the evaluation, the judges had to keep their mouth closed. In total, the NS measurement lasted around 2.5 minutes.

4.1.2.4.4 PTR-ToF-MS data treatment

Dead time correction, internal calibration of mass spectral data and peak extraction were performed according to a procedure described elsewhere [29, 30]. The analysis of the data generated during NS analysis required additional processing as reported below (**Figure 4.1-1**).

4.1.2.4.5 Peak selection

Following data extraction, a total of 465 mass peaks were obtained, ranging from m/z 15 to m/z 300. An algorithm for peak-like feature selection was applied with the aim to select the release curves related to coffee and discard those that were not associated to NS sessions (e.g. linked to compounds from the judges' breath or interfering ions). The algorithm, which is described in detail elsewhere [23], compares data populations right before and after sample introduction by means of a non-parametric statistical test. A p -value lower than 0.01 (after false discovery rate correction according to Benjamini & Hochberg, 1995 [31]) was deemed to indicate an increase in signal upon sample introduction and the corresponding mass peaks were retained for further analysis. After this selection step, a subset of 168 mass peaks was obtained. This subset, after elimination of redundant peaks related to ^{13}C isotopologues and water clusters, was further reduced to 136 mass peaks.

4.1.2.4.6 Conditional averaging

In order to improve the robustness of parameter extraction (see further), the three curves obtained from the corresponding replicates of individual NS sessions were averaged. The original dataset, containing 216 NS sessions, was thus reduced to 72 aggregate sessions. An additional step of peak-like feature selection was applied before averaging, consisting of the same statistical test applied before (paragraph 4.1.2.4.5), but upon setting a more compliant statistical threshold (p -value < 0.1, no correction for false discovery rate). Whenever a curve was not recognised as peak (not significantly different from the background noise of the instrument, calculated during the 30 first seconds of measurement before sample introduction) this was consequently not employed in constructing the corresponding averaged curve. In the case when none of the three curves, corresponding to each aggregate session, contained peak-like features, the data processing software restituted a "NaN" (Not a Number) type of entry.

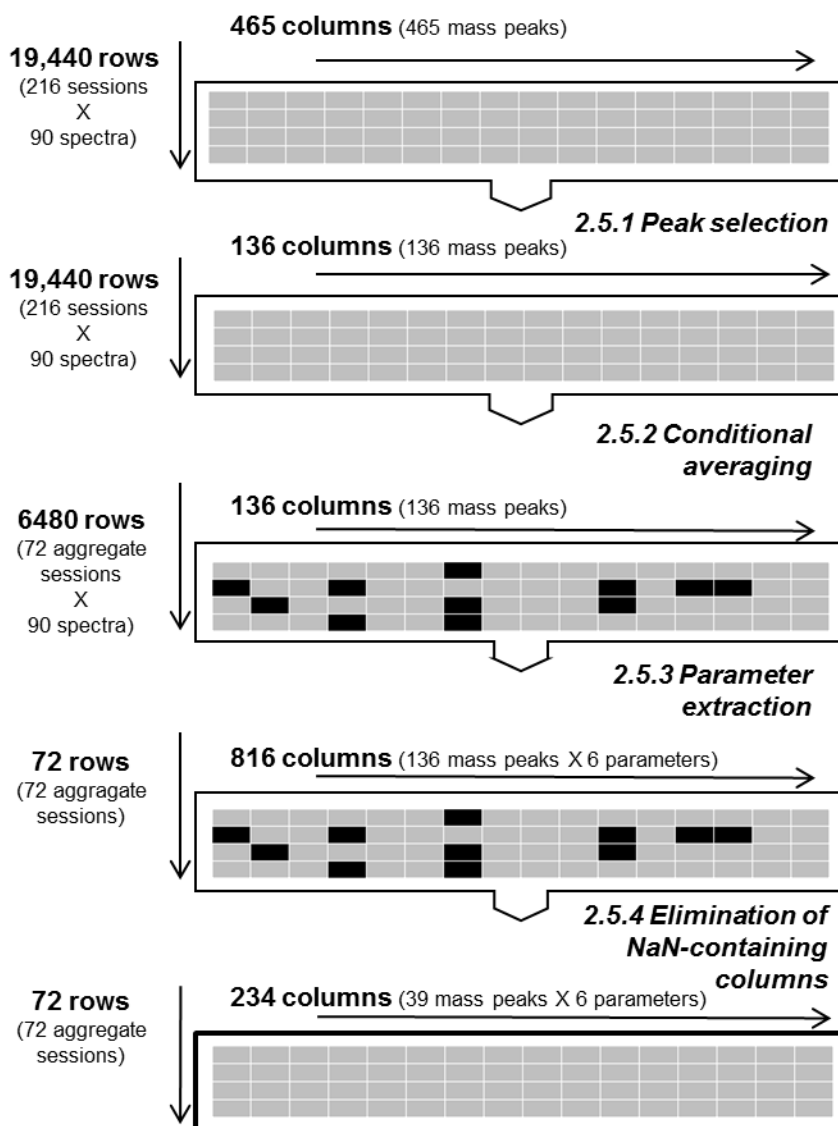


Figure 4.1-1. Schematic representation of the protocol employed for the analysis of nospace data. Representations of the actual datasets are merely schematic (■ = numeric cell; ■ = non numeric cell). Italicized references relate to materials and methods sections where the corresponding steps are explained.

4.1.2.4.7 Parameter extraction

From each of the 136 selected peaks the baseline (obtained by averaging the first 30 cycles) was subtracted and 6 parameters were extracted: the maximum (*maximum*), the area under the curve (*area*), the median (*median*), the time to reach the maximum (*tmax*), the average of the last five seconds of the NS session (*final*), and the slope of the first descending section of the curve (*slope*), assuming a linear relationship between time and the logarithm of peak intensity

[32]. In agreement with the step of data selection described previously (paragraph 4.1.2.4.6.), parameter extraction was not performed on missing NS curves and NaN-containing cells were generated in correspondence to the missing parameters.

4.1.2.4.8 Elimination of NaN-containing columns

As a final step of data processing, all columns having at least one NaN-containing cell were excluded from further analysis. The final data matrix consisted of 234 columns, corresponding to the 6 parameters extracted from 39 mass peaks, and 72 rows corresponding to the aggregate NS sessions.

4.1.2.4.9 Software

Data analysis and statistical analyses (one-way ANOVA with product factor, cluster analysis with “partitioning around medoids” algorithm) were performed using software packages and scripts developed in MATLAB (MathWorks, Natick, MA) and R (R foundation for statistical computing, Vienna, Austria).

4.1.3 Results

4.1.3.1 *A better differentiation of sensory dynamic perceptions of coffees related to sugar concentration than roasting degree*

Figure 4.1-2 and **Figure 4.1-3** show the temporal description in terms of dominance of the four products studied: respectively coffee A/without sugar, coffee AZ/with sugar, coffee B/without sugar and coffee BZ/with sugar. Each curve (**Figure 4.1-2**) represents the evolution of dominance rate over the consumption time (60s). Only attributes with a significant dominance rate are represented. Four attributes for Coffee A and five attributes for the other products (Coffee AZ, Coffee B and Coffee BZ) were significant. P_0 (chance level) and P_s (significance level) were calculated and corresponds respectively to 0.11 and to 0.18. **Figure 4.1-3** shows the significance of the attributes for all 5s time-periods without taking into account the dominance rate and this allows having a more global point of view.

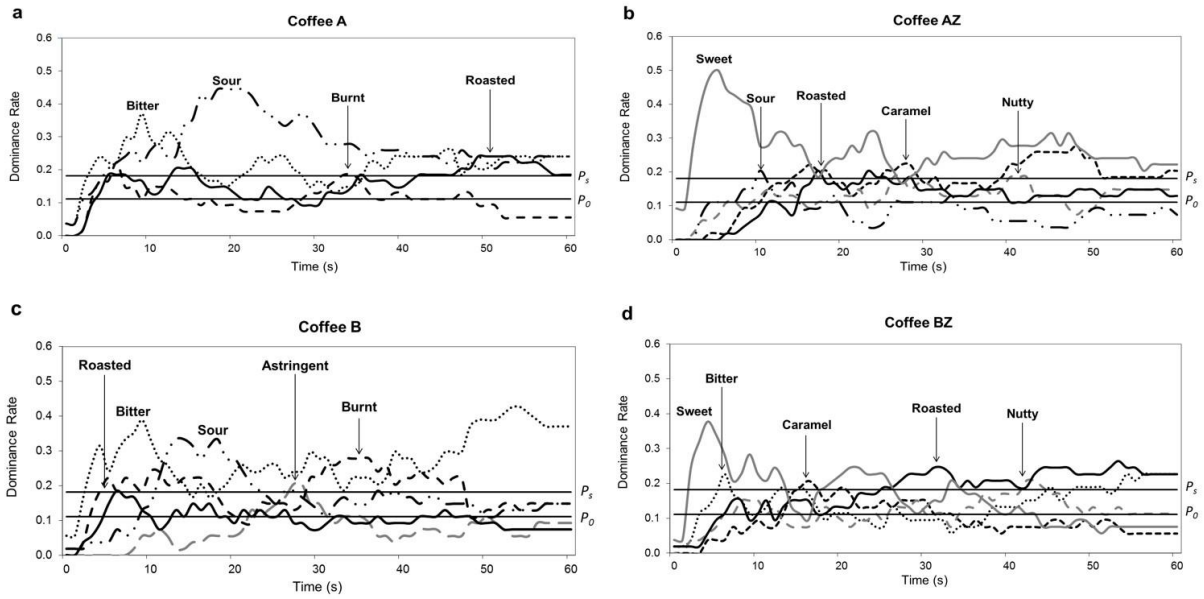


Figure 4.1-2. TDS curves of the different significant attributes for the four coffee samples over a 1 minute time period: Coffee A (a), Coffee AZ (b), Coffee B (c), and Coffee BZ (d). P_0 represents the chance level and P_s is the significance level.

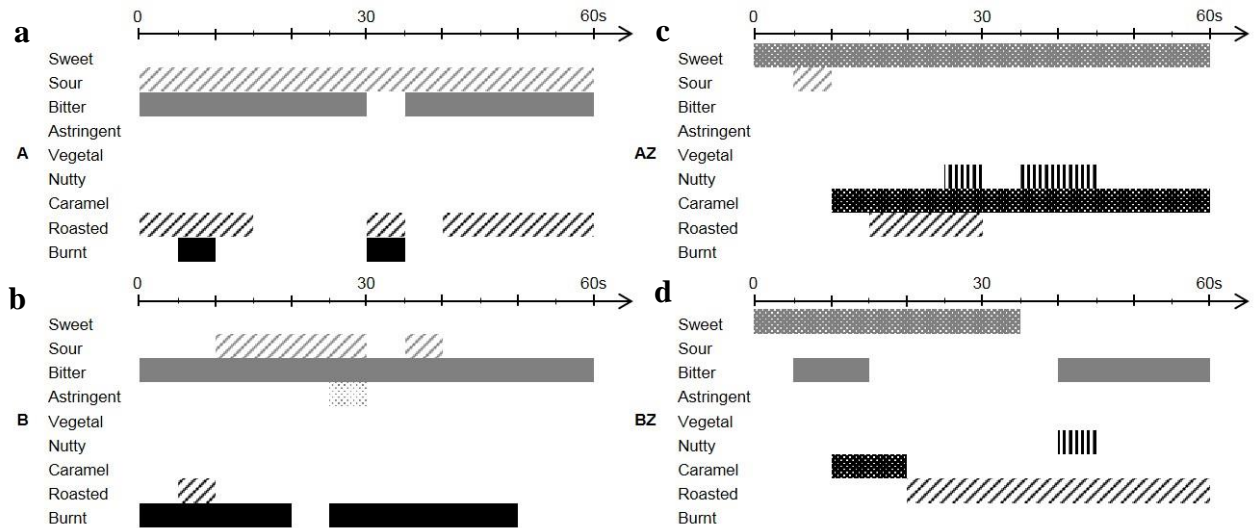


Figure 4.1-3. Simplified representation of significant attribute dominances for each 5s time-period. ■ = sweet; ▨ = sour; ■ = bitter; ▨ = astringent; ▨ = vegetal; ▨ = nutty; ▨ = caramel; ▨ = roasted; ■ = burnt.

For example, **Figure 4.1-2a** shows *Bitter* as a dominant attribute for Coffee A 3s after the beginning of the tasting with a maximum at 9s for 37% of the judges. The temporal dominance description of this product is then *Sour* with a maximum dominance between 18 and 21s for 44% of judges. *Burnt* reaches barely the significant level twice meaning a weaker consensus of the panel on the dominance on this aromatic note and to finish, *Roasted* is significantly dominant at the beginning of the evaluation but it is mainly perceived as dominant at the end from 42 to 60s with a maximum rate of 24%. More globally this coffee is perceived as being *Sour*, *Bitter* and with a *Roasted* flavour (**Figure 4.1-3a**).

Coffee AZ is mainly perceived *Sweet* and with a *Caramel* flavour (**Figure 4.1-3b**). The *Sweet* sensation becomes significant at 2s until the end with a maximum at 5s after the beginning of the evaluation for 50% of the panel (**Figure 4.1-2b**). Other sensations are nevertheless perceived during the one-minute evaluation. The dominance rate of *Sour* becomes significant very briefly at 10s and the *Roasted* flavour at 17s but the judges don't agree perfectly on the dominance of these two attributes as the dominance rate is equal only to 20.4%. The *Caramel* flavour is not significantly dominant at the very beginning of the evaluation (only after 14s) and its dominance is then not continuous. Three peaks can be observed from 14 to 18s (with a max. dominance of 22.2%), from 25 to 29s (with a max. dominance of 22.2%) and from 39 to the end (with a max. dominance of 27.8%). The *Nutty* flavour is also briefly dominant (during 2s at 26s). The temporal description of this sample shows one more significant attribute implying that the addition of sugar seems to add complexity to the perception of espresso coffee. In complexity, we mean the number and the variety of attributes. Another thing to point out, probably due to the addition of sugar, is the change within the nature of the significant attributes over the time: the *Bitter* and *Sour* tastes are erased by the *Sweet* perception. It affects also the flavour perception: from *Burnt* and *Roasted* (Coffee A) the description of dominant attributes switch to *Caramel* mainly (Coffee AZ) and *Nutty* slightly. The changes due to sugar addition can also be observed on **Figure 4.1-3** (a and b) which summarises the information from TDS and allows an easier sample comparison by pair.

Coffee B (**Figure 4.1-2c** and **Figure 4.1-3c**) is described as mostly *Bitter* and with a *Burnt* flavour. Compared to coffee A, it is less *Sour* in the first 30s and more *Bitter* at the end. The differences between the two coffees consist mainly in taste dominance but in the second half of the tasting (from 30 to 60s) a difference in the main dominant flavour is observed: *Roasted* for Coffee A and *Burnt* for Coffee B (**Figure 4.1-2** and **Figure 4.1-3**). The latter is also perceived *Astringent* dominating at 27s and 28s by 20.4% of the judges. The TDS method seems to show

here that two coffees different in nature (different type of roasting) result to be described differently signifying that this sensory dynamic method allows differentiating samples.

When looking at Coffee BZ (**Figure 4.1-2d** and **Figure 4.1-3d**; Coffee B with sugar), the sugar effect is very clear as well but the dominant sensation pattern from Coffee B is maintained: it is more *Bitter* and less *Sour* than Coffee A. The sugar effect is also underlined here by a modification of the dominant attributes of flavour in a quite similar manner than when sugar is added to Coffee A (Coffee AZ). The attributes *Caramel* and *Nutty* become dominant respectively from 15 to 17s (with a max. dom. rate of 20.8%) and from 42s to 44s (with a max. dom. rate of 20.8%). In addition, the attribute *Roasted* is dominant in the evaluation of the sample from 25s to the end with a maximum 26.4%. In both coffees A and B, the effect of adding sugar tends to mask/decrease *Sour* and *Bitter* taste dominance as expected but also to enhance the “empyreumatic flavour” perception described with the attributes *Caramel*, *Nutty* and *Roasted* instead of *Burnt* (**Figure 4.1-2** and **Figure 4.1-3**).

4.1.3.2 NS profiles are greatly influenced by roasting degree, whereas sugar addition plays a minor role

As discussed in a previous work [23], the processing of data generated during NS analysis by PTR-ToF-MS poses multiple challenges to the experimenter, especially when non-modified real food matrices are studied. Most complications originate from the complexity of the data, given by the presence of an additional dimension (*i.e.* time), as well as by its sheer size. The PTR-ToF-MS measurement generated up to 465 mass peaks over a total of 19,440 mass spectra, distributed over 216 sessions overall. In order to isolate the data most relevant to NS itself, the matrix was submitted to a series of steps of reasoned data reduction (see also paragraph 4.1.2.4.4 and **Figure 4.1-1**).

The dataset thus obtained was submitted to one-way ANOVA and *p*-values were corrected taking into account the false discovery rate. Table 2 reports all mass peaks and parameters associated to a *p*-value lower than 0.01. Statistically significant differences were found for 21 mass peaks. Based upon estimation of exact mass (up to three decimal digits) and literature data [33, 34] a tentative identification was proposed for 20 mass peaks. Thirteen of these masses could be related to chemical classes known to be, at least in part, responsible for the olfactory sensory notes employed in TDS; these included pyrroles (*Burnt* sensory attribute), furans (*Caramel*, *Nutty*), pyridines (*Roasted*, *Burnt*), oxazoles (*Nutty*), and thiazoles (*Nutty*) [33].

The parameters that, among the six extracted, showed more often sample-wise variation were those characterising release in time-independent fashion. Statistically significant differences were found for parameters related to overall intensity (*area*, occurring 19 times), maximum intensity (*maximum*, 15 times), mean intensity (*median*, 13 times) and final intensity of the sensory stimulus (*final*, 7 times). The aforementioned parameters were always higher in dark roasted coffees (*i.e.* B and BZ) than in light roasted samples (*i.e.* A and AZ), showing a two- to four-fold increase (Table 2). The impact of sugar addition appeared to be negligible: of all NS mass peaks considered only m/z 99.079 showed a sugar-dependent response, as it was absent in samples A, AZ, and B, being instead detectable in coffee BZ.

Time-related parameters (*i.e.* *slope*, *tmax*) were scarcely represented in **Table 4.1-2**, with slope showing differences for mass m/z 97.027 only (tentatively assigned to furfural). For this mass peak the concentration decrease observed after swallowing was faster in light roasted coffees (A and AZ) than in dark roasted samples (B and BZ).

The results obtained by headspace analysis corroborated well those from NS analysis (results not shown), with a great influence being played by the roasting factor in accordance with the previously cited study on coffee (Romano et al., 2014). One-way ANOVA ($p < 0.01$) resulted into 121 statistically different mass peaks, out of which 15 could also be encountered among the previously described NS mass peaks. The impact of sugar addition appeared once more to be negligible.

Table 4.1-2. Selected nospace mass peaks and corresponding parameters. Means and standard deviations are reported for each sample. *p*-values were obtained on the basis of one-way ANOVA and corrected taking into account false discovery rate. Only parameters whose *p*-values were lower than 0.01 are shown. Superscript annotations are used to display differences between coffees (Tukey's HSD).

meas. mass (Th)	sum formula	tentative identification	parameter	Coffee A	Coffee AZ	Coffee B	Coffee BZ	<i>p</i> -value
68.050	C ₄ H ₆ N ⁺	pyrrole	<i>maximum</i>	3.5±1.8 ^a	4.2±2.6 ^a	11.3±6 ^b	13.2±6.7 ^b	< 10 ⁻³
			<i>area</i>	99.3±45 ^a	102.9±40.3 ^a	274.6±120.5 ^b	320.3±131.9 ^b	< 10 ⁻³
			<i>median</i>	0.7±0.4 ^a	0.8±0.3 ^a	2±0.9 ^b	2.2±0.9 ^b	< 10 ⁻³
			<i>final</i>	0.3±0.2 ^a	0.3±0.1 ^a	0.7±0.3 ^b	0.8±0.4 ^b	< 10 ⁻³
69.033	C ₄ H ₅ O ⁺	furan	<i>area</i>	321.3±120 ^a	404.1±188.4 ^a	718.2±358.8 ^b	908±343.7 ^b	< 10 ⁻³
			<i>median</i>	1±0.7 ^a	1.2±0.7 ^a	2.4±1 ^b	2.6±1.2 ^b	< 10 ⁻³
			<i>final</i>	0.3±0.2 ^a	0.3±0.2 ^a	0.8±0.3 ^b	0.8±0.4 ^b	< 10 ⁻³
73.065	C ₄ H ₉ O ⁺	Isobutanal, butanone	<i>area</i>	1883.3±804.4 ^a	2169.2±882.7 ^{ab}	3269.5±1728.4 ^{bc}	4329.3±1669.8 ^c	< 10 ⁻³
75.044	C ₃ H ₇ O ₂ ⁺	methyl-acetate/acetol	<i>maximum</i>	86.5±52.4 ^a	98.6±47.1 ^a	236±200.5 ^b	333.6±188.2 ^b	< 10 ⁻³
			<i>area</i>	1119.2±417.6 ^a	1310.7±528 ^a	2690.7±1329.3 ^b	3498.9±1355.4 ^b	< 10 ⁻³
			<i>median</i>	6±2.6 ^a	6.4±3.1 ^a	10.5±5.1 ^b	12.2±5.6 ^b	0.009
78.968	n.a.	non identified	<i>maximum</i>	0.7±0.5 ^a	0.7±0.4 ^a	1.3±0.8 ^{ab}	1.9±1.3 ^b	0.003
			<i>area</i>	6.1±2.4 ^a	6.4±2.6 ^a	11.8±6.1 ^b	14.9±5.7 ^b	< 10 ⁻³
80.049	C ₅ H ₆ N ⁺	pyridine	<i>maximum</i>	17.6±8.9 ^a	18.6±9.1 ^a	58.8±30.6 ^b	68.5±41.8 ^b	< 10 ⁻³
			<i>area</i>	868.8±403.4 ^a	859±345.5 ^a	2481.2±1127.4 ^b	2805.1±1253.1 ^b	< 10 ⁻³
			<i>median</i>	8.6±4.2 ^a	8.3±3.2 ^a	22.2±9.8 ^b	25.1±10.5 ^b	< 10 ⁻³
			<i>final</i>	4.5±2.5 ^a	4.2±1.7 ^a	10.6±4.1 ^b	11.9±4.5 ^b	< 10 ⁻³
81.034	C ₅ H ₅ O ⁺	furan fragment	<i>maximum</i>	30±14.8 ^a	40.8±33 ^{ab}	69.2±37.6 ^{bc}	88±47.7 ^c	0.003
			<i>area</i>	856.1±418.3 ^a	895.9±430.9 ^a	1654.8±656.9 ^b	1905.2±828.1 ^b	< 10 ⁻³
			<i>median</i>	6.2±3.4 ^a	6.2±3.3 ^a	11.6±4.9 ^b	12±5.2 ^b	0.002
82.065	C ₅ H ₈ N ⁺	methyl-pyrrole	<i>maximum</i>	12.8±7.9 ^a	14.2±7.8 ^a	34.1±26.8 ^b	48.5±29.1 ^b	< 10 ⁻³
			<i>area</i>	128.1±55.5 ^a	151.7±69.5 ^a	358.2±196.9 ^b	459.4±187 ^b	< 10 ⁻³
			<i>median</i>	0.5±0.3 ^a	0.6±0.3 ^a	1.2±0.7 ^b	1.5±0.8 ^b	< 10 ⁻³
			<i>final</i>	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^b	0.3±0.1 ^b	< 10 ⁻³

(Table 4.1-2 continues)

meas. mass (Th)	sum formula	tentative identification	parameter	Coffee A	Coffee AZ	Coffee B	Coffee BZ	p-value
83.049	C ₅ H ₇ O ⁺	methyl-furan	area	462±188.7 ^a	566.9±282.7 ^a	1110.9±645.6 ^b	1331.5±573 ^b	< 10 ⁻³
			median	1.2±0.7 ^a	1.4±0.7 ^a	2.4±1.2 ^b	2.8±1.5 ^b	0.008
85.064	C ₅ H ₉ O ⁺	methyl-butenal	maximum	5.3±2.7 ^a	6±2.8 ^{ab}	11.1±7.7 ^{bc}	14.9±8.8 ^c	0.004
			area	97.3±40.3 ^a	104.8±38.7 ^a	181.6±82.8 ^b	218.4±84.7 ^b	< 10 ⁻³
			median	0.6±0.3 ^a	0.6±0.2 ^a	1.1±0.4 ^b	1.1±0.4 ^b	0.006
87.043	C ₄ H ₇ O ₂ ⁺	butanedione butyrolactone	area	405.5±161.3 ^a	429.7±156.5 ^{ab}	588.8±215.5 ^{bc}	708.5±237.3 ^c	0.006
			median	2.2±0.9 ^a	2.2±0.9 ^a	3.5±1.3 ^b	3.9±1.3 ^b	0.003
			final	0.9±0.3 ^a	0.8±0.3 ^a	1.5±0.5 ^b	1.4±0.5 ^b	< 10 ⁻³
89.059	C ₄ H ₉ O ₂ ⁺	methyl-propanoate hydroxy-butanone	maximum	3.3±1.6 ^a	3.7±1.5 ^a	7.6±4.2 ^b	11.5±6.7 ^c	< 10 ⁻³
			area	96.8±33.2 ^a	111.1±37.5 ^{ab}	147.5±47.4 ^{bc}	182.4±47.5 ^c	< 10 ⁻³
97.027	C ₅ H ₅ O ₂ ⁺	furfural	slope	0±0 ^b	0±0 ^b	0±0 ^a	0±0 ^a	< 10 ⁻³
98.060	C ₅ H ₈ ON ⁺	dimethyl-oxazole	maximum	0.8±0.5 ^a	0.9±0.5 ^a	2.1±1.5 ^b	2.6±1.5 ^b	0.002
			area	21.2±9.6 ^a	22.9±9.9 ^a	46.5±23.6 ^b	55.8±25.5 ^b	< 10 ⁻³
			median	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^b	0.3±0.1 ^b	< 10 ⁻³
99.079	C ₆ H ₁₁ O ⁺	hexenal methyl-pentenone	maximum	0±0 ^a	0±0 ^a	0±0 ^a	6.3±7.2 ^b	< 10 ⁻³
			slope	0±0 ^a	0±0 ^a	0±0 ^a	0±0 ^b	< 10 ⁻³
100.020	C ₄ H ₆ NS ⁺	methyl-thiazole	area	11.7±5.5 ^a	11±4.8 ^a	19.1±7.8 ^b	21.7±8.7 ^b	0.002
124.072	C ₇ H ₁₀ ON ⁺	2-acetyl-1-methylpyrrole	maximum	0.5±0.3 ^a	0.7±0.5 ^a	1.2±0.6 ^b	1.4±0.7 ^b	0.004
			area	18.1±8.9 ^a	18.4±8.7 ^a	35.7±14.3 ^b	39.5±16.6 ^b	< 10 ⁻³
			median	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^b	0.3±0.1 ^b	< 10 ⁻³
			final	0.1±0 ^a	0.1±0 ^a	0.1±0 ^b	0.1±0.1 ^b	0.001
125.057	C ₇ H ₉ O ₂ ⁺	guaiacol methyl-benzenediol furyl-acetone	maximum	1.5±0.7 ^a	1.9±1.4 ^{ab}	3.3±1.6 ^{bc}	4±2.2 ^c	0.006
			area	46.2±21.2 ^a	47.9±19.4 ^a	87.4±34.3 ^b	99.3±39.8 ^b	< 10 ⁻³
			median	0.4±0.2 ^a	0.4±0.1 ^a	0.6±0.2 ^b	0.7±0.3 ^b	< 10 ⁻³
			final	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^b	0.3±0.1 ^b	< 10 ⁻³
139.072	C ₈ H ₁₁ O ₂ ⁺	4-ethyl-1,2-benzenediol	maximum	0.7±0.3 ^a	0.8±0.5 ^{ab}	1.4±0.6 ^{bc}	1.8±1 ^c	0.007
			area	23.7±10.1 ^a	24±10 ^a	40.9±16.5 ^b	46.2±18.5 ^b	0.001
			median	0.2±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^b	0.3±0.1 ^b	0.006
141.056	C ₇ H ₉ O ₃ ⁺	furfuryl-acetate	maximum	0.9±0.5 ^a	0.9±0.5 ^a	1.8±1.1 ^b	2.3±1.3 ^b	0.003
			area	19.7±7.9 ^a	20.1±7.6 ^a	34.2±13.7 ^b	38.8±15.4 ^b	< 10 ⁻³
149.058	C ₉ H ₉ O ₂ ⁺	furfuryl-furan	maximum	0.5±0.3 ^a	0.5±0.2 ^a	1±0.6 ^b	1.3±0.7 ^b	0.001
			area	10.6±5 ^a	9.9±5.4 ^a	17.7±8.5 ^b	20±8.5 ^b	0.008

4.1.3.3 NS curves fall into two different groups, characterised by distinct release patterns

The results of sensory analysis showed that flavour related TDS curves underwent great variations, both upon roasting and sugar addition. It can be surmised that these differences are due, at least in part, to the fact that the time evolution of coffee NS is different in the different coffees. In the attempt to test this hypothesis, a novel approach was adopted for data analysis. The different mass peaks and corresponding NS curves were considered to be distinct samples, each represented by six variables (*i.e.* the different parameters). In order to even out differences in absolute concentrations all NS curves were transposed to the same scale, ranging between zero and one. Parameters were extracted as described in paragraph 3.2 and for each mass peak the parameters were averaged. This averaging step, aimed at expeditiously reducing the data size, entailed a considerable degree of approximation, as it evened out all inter-individual differences between judges; nevertheless it must be noted this simplification did not undermine the validity of the final conclusions, as it is demonstrated further. A separate analysis was carried out for each coffee and for each sample a matrix was obtained, composed by 39 rows (the different peaks, selected according to the procedure described in paragraph 3.2) and five averaged parameters (*maximum*, which always resulted equal to one after normalisation, was eliminated).

The search for groups within each dataset was conducted by means of cluster analysis, using the “partitioning around medoids” method encoded by the “pam” function of the R “cluster” package. This methodology, which is described in detail elsewhere [35], is based upon an algorithm that divides the dataset into k clusters, where k is defined arbitrarily. A range of k -values between 1 and 10 was tested, with $k=1$ corresponding to no observed clustering. For each k tested, the algorithm carries out the clustering and also yields a silhouette value (S_i), ranging from 0 to 1. This represents a “quality index” of the clustering. Similarly, S_i values can be obtained for each group and for every single variable within a group. For all four datasets, the highest S_i value was obtained for $k=2$, with values for the single groups ranging from 0.55 to 0.71. According to the literature [35], these values were indicative of the presence of either “reasonable” (for $S_i > 0.50$) or “strong” ($S_i > 0.70$) cluster structures.

Table 4.1.3 depicts the clustering of each mass peak for the four different coffees. Whenever a variable was characterised by S_i higher than 0.50, a cluster membership was assigned, otherwise the variable was assigned to no cluster. The overall data showed a clearly discernible pattern, with low m/z compounds being more often assigned to cluster 1 and vice versa. Comparing different samples, mass peaks did not generally shift from one cluster to another. Interestingly, cluster memberships also appeared to be dependent upon chemical structures: cluster 1 comprised esters, acids, and carbonyls whereas cluster 2 was mainly composed by N-heterocycles and phenols. Furans were almost equally represented by both clusters.

The two aforementioned clusters corresponded to NS curves having two distinct time evolutions. **Figure 4.1-4** depicts an example of this difference, as observed in coffee BZ, and relative to mass peaks m/z 73.065 (attributed to isobutanal/butanone) and mass peak m/z 139.072 (tentatively assigned to 4-ethyl-1,2-benzenediol). These peaks were selected as typical representatives of the distinct behaviours depicted by the two clusters. It can be observed that compounds belonging to cluster 1 were associated to NS release curves that increased steeply, reached maximum intensity after approximately 10 s, tailed down relatively fast, and had almost reached zero by the end of the TDS/NS session. As for the release curves of the mass peaks from cluster 2, these increased more slowly, reached maximum intensity at 20 s or later, and, 60 s after sample introduction (*i.e.* at the end of the session), still retained around 20% of maximum intensity. This difference in release patterns between the two clusters, based upon averaged parameters, was also reflected by the parameters extracted from the individual NS curves, with normalised curves from cluster 2 having higher *area*, *median*, *final* and *tmax*, and lower *slope*, than those from cluster 1 (Figure 4). These differences could regularly be observed between release curves belonging to different clusters, and in spite of the well-known inter-individual differences (results not shown).

Table 4.1-3. Mass peaks and corresponding cluster assignments. ■ = cluster 1; ■ = cluster 2 □ = no assignment.

meas. mass (Th)	Coffees				sum formula	chem. class	tentative identification
	A	AZ	B	BZ			
43.018	■	■	■	■	C ₂ H ₃ O ⁺	fragment	fragment (diverse origin)
45.034	■	■	■	■	C ₂ H ₅ O ⁺	aldehydes	acetaldehyde
49.012	■	■	■	■	CH ₅ S ⁺	sulfurs	methanethiol
61.029	■	■	■	■	C ₂ H ₅ O ₂ ⁺	acids/esters	acetic acid/methyl-formate
63.043	■	■	■	■	C ₂ H ₇ O ₂ ⁺	n.a.	non identified
68.050	■	■	■	■	C ₄ H ₆ N ⁺	N-heterocycles	pyrrole
69.033	■	■	■	■	C ₄ H ₅ O ⁺	furans	furan
71.049	□	□	□	■	C ₄ H ₇ O ⁺	fragments/aldehydes/ketones	fragment (methyl-butanol)/butenal/butenone
73.065	■	■	■	■	C ₄ H ₉ O ⁺	aldehydes/ketones	isobutanal/butanone
75.044	■	■	■	■	C ₃ H ₇ O ₂ ⁺	esters/hydroxyketones	methyl-acetate/acetol
78.968	■	■	■	■	n.a.	n.a.	non identified
80.049	□	□	■	■	C ₅ H ₆ N ⁺	N-heterocycles	pyridine
81.034	■	■	■	■	C ₅ H ₅ O ⁺	fragments	furan fragment
82.065	■	■	■	■	C ₅ H ₈ N ⁺	N-heterocycles	methyl-pyrrole
83.049	■	■	■	■	C ₅ H ₇ O ⁺	furans	methyl-furan
85.064	■	■	■	■	C ₅ H ₉ O ⁺	aldehydes	methyl-butenal
87.043	■	■	□	■	C ₄ H ₇ O ₂ ⁺	ketones	butanedione/butyrolactone
87.080	■	■	■	■	C ₅ H ₁₁ O ⁺	aldehydes	methyl-butenal
89.059	□	□	■	■	C ₄ H ₉ O ₂ ⁺	esters/hydroxyketones	methyl-propanoate/hydroxy-butanone
94.039	■	■	■	■	n.a.	n.a.	non identified
95.010	■	■	■	■	n.a.	n.a.	non identified
97.027	■	□	■	■	C ₅ H ₅ O ₂ ⁺	furans	furfural
98.060	■	□	■	■	C ₅ H ₈ ON ⁺	N-heterocycles	dimethyl-oxazole
99.041	■	■	■	■	C ₅ H ₇ O ₂ ⁺	furans/lactones	furfuryl alcohol/angelica lactone
99.079	■	□	■	□	C ₆ H ₁₁ O ⁺	aldehydes/ketones	hexenal/methyl-pentenone
100.020	■	■	■	■	C ₄ H ₆ NS ⁺	N-heterocycles	methyl-thiazole
101.058	■	■	□	■	C ₅ H ₉ O ₂ ⁺	ketones	pentanedione/methyl-tetrahydrofuranone
103.072	■	■	■	■	C ₅ H ₁₁ O ₂ ⁺	esters/hydroxyketones	hydroxy-pentanone/methyl-butanoic acid
105.068	■	■	■	■	C ₈ H ₉ ⁺	aromatic hydrocarbons/fragments	styrene/phenylethanol fragment
109.071	■	■	■	■	C ₆ H ₉ N ₂ ⁺	N-heterocycles	dimethylpyrazine/ethylpyrazine
111.042	■	□	■	■	C ₆ H ₇ O ₂ ⁺	furans	acetyl_furan/methyl-furfural
113.056	■	■	■	■	C ₆ H ₉ O ₂ ⁺	mixed	methyl-furfuryl-alcohol/dimethyl-furanone methyl-cyclopentanedione/cyclotene
115.072	■	■	■	■	C ₆ H ₁₁ O ₂ ⁺	pyrans	4-methyltetrahydro-2H-pyran-2-one
124.072	■	■	■	■	C ₇ H ₁₀ ON ⁺	N-heterocycles	2-acetyl-1-methylpyrrole
125.057	■	■	■	■	C ₇ H ₉ O ₂ ⁺	phenols/furans	guaiacol/methyl-benzenediol/furyl acetone
139.072	■	■	■	■	C ₈ H ₁₁ O ₂ ⁺	phenols	4-ethyl-1/2-benzenediol
141.056	□	□	□	■	C ₇ H ₉ O ₃ ⁺	furans	furfuryl-acetate
148.069	■	■	■	■	n.a.	n.a.	non identified
149.058	□	□	■	■	C ₉ H ₉ O ₂ ⁺	furans	furfuryl-furan

n.a. = non applicable

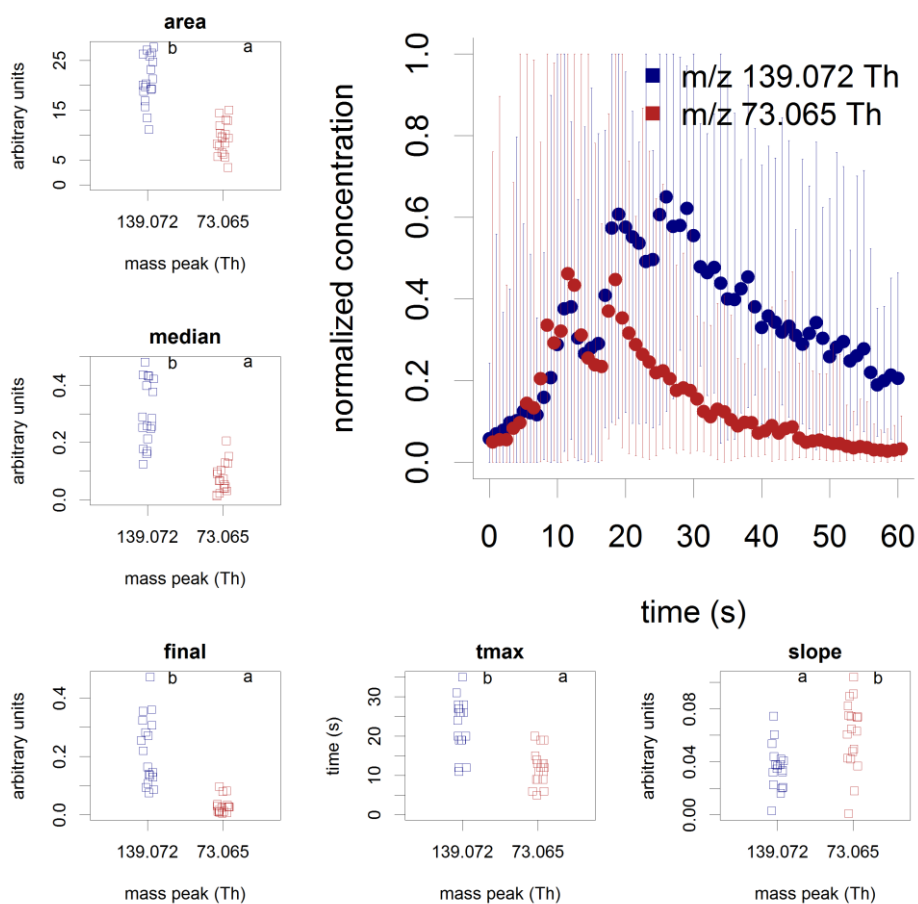


Figure 4.1-4. Comparison between peaks from cluster 1 (blue) and cluster 2 (red), measured in coffee BZ. The normalized release curves show mean, maximum, and minimum values (dots and error bars). Stripcharts display the distribution of single values for the curve parameters, with letter annotations indicating statistically significant differences (one-way ANOVA, $p < 0.01$).

4.1.4 Discussion

As it was already demonstrated on different types of matrices, liquid, semi-liquid or solid (see [14] for a review), TDS allows to differentiate and to describe the different product samples in terms of dominant sensations during their consumption time. In this study, samples were discriminated and characterised according to the nature of selected attributes/sensations, the number of significant attributes and the evolution of sensations in time (sequence of attributes). Recently, some studies have been published on describing the perception of complex products like coffee. Barron and co-workers (2012) [16] studied the impact of “crema”, the foamy

surface of espresso coffee, on the overall perception of coffee including flavour (*Carbony*, *Roasted*, *Cereal*, *Fruity*), taste (*Bitter*, *Acidic*, *Sweet*) and texture (*Liquid*, *Thick*, *Gritty*, *Silky*) attributes. TDS evidenced that the presence of “crema” induced a dominance of the *Roasted* attribute and that this dominance increased with the quantity of foam by pressure. Their results showed also that the presence of “crema” tends to mask bitterness of coffee. Dinnella and colleagues (2013) [15] chose to use a shorter attribute list (5 attributes in total) to describe the evolution of coffee perception: three tastes (*Bitter*, *Sour*, *Sweet*), one tactile sensation (*Astringent*) and one flavour (*Coffee Flavour*). They analysed one type of coffee sweetened with three different kind of sweeteners (sucrose, acesulfame, steviol). The perception of coffee changed depending on the used sweetener considering TDS curves produced when no intensity rating was collected. Coffee sweetened with sucrose was described with *Sweet* then *Coffee flavour* dominant sensations while for the coffee with acesulfame, the main dominant sensation was *Coffee flavour* (after 37s), the *Sweet* sensation was a lot lower (below significance level) and a slight *Bitter* dominant sensation appeared after 20s. Regarding the addition of steviol in coffee, it modified a lot its perception, which was then described as *Sour* and then *Bitter*. One thing, worthy to mention, is that a different attribute list will necessarily generate different temporal descriptions. Indeed, the TDS method consists in selecting an attribute within a list and so consequently TDS results - a sequence of selections of a specific attribute at a specific time - is very dependent on the attributes available in that list. The decision to add or not in the list only taste, flavour, texture attributes or all categories together is not trifling especially when the link between TDS data and instrumental data (e.g. NS data) is investigated afterwards. In this regard, it made more sense to us not to use texture/mouthfeel attributes in our study, as one of the objective was to correlate both sensory and instrumental aroma data and to evaluate their complementarities. Moreover, less attention is usually given to texture characteristics when analysing liquid matrices compared to solid or semi-solid ones. Considering taste attributes, it was not easy to decide whether to include them in the list or not. On one side, as the objective of the study was to investigate the link between flavour perception and aroma release, they would be considered as adding noise in the results. But on the other side, flavour should be considered in its complexity and therefore taste attributes must be included as well to obtain a complete description of the perceptions of the tasting [36]. Thus, we decided to keep taste attributes in the list.

The roasting effect of coffee was well underlined both in TDS curves and NS data. The increase of roasting degree (from light (Coffee A) to dark (Coffee B)) was characterised sensorially

mainly by a change in the taste of coffee, a decrease of sourness and an increase of bitterness (in the first half of the evaluation) corroborating the work of Clifford (1985)[37] saying that roasting is the most efficient way to reduce acid content and perceived acidity in coffee. From a sensory point of view, a change in the flavour perception is also observed with a switch of dominance from *Roasted* to *Burnt* (in the second half of the evaluation; **Figure 4.1-2a** and **b**). The NS data indicated that globally more volatiles and in higher concentrations were released in the NS of the panellist and as well as in the headspace of coffee samples with increasing roasting degree. The literature agrees well with this flavour/aroma modification due to roasting observed at a sensory level [38] and also at an instrumental level both with *in vitro* (headspace) or *in vivo* (NS) [23, 38, 39].

Furthermore, TDS shows that differences in flavour attributes between samples come out at the middle/end of the session and the cluster analysis performed on NS data shows that release of potent odorants of coffee show different characteristics. For instance N-heterocycles and most furans are in cluster 2 (late and persistent release). That being so, these compounds could be related to the flavour attributes used in TDS and NS analysis could be used as a tool to identify some key compounds/markers might be responsible for some sensory attributes due to their odour activities. In terms of sensory perception, when the roasting degree increases (from A to B), a surprising reduction in the dominance of the *Roasted* note is observed. In addition to this, a rise in the *Burnt* note dominance in coffee B is recorded. When considering sensory and NS data together, different compounds seem to be potential markers for *Burnt* sensory note: e.g. methyl-pyrrole (Cluster 1) and pyridine and acetyl-methyl-pyrrole (Cluster 2). The fact that these three possible markers come from different clusters could predict an early and a late onset for a *Burnt* sensory note upon *Roasting*, observed in sensory data. When coffee AZ and BZ (increase of roasting degree but with sugar in both samples) were compared, an increase of *Roasted* flavour note dominance can be seen. Related to this finding, a good temporal dominance marker from NS data, could be pyrazines (peak m/z 109) that expectedly are in Cluster 2 (late and persistent perception). By saying this, we also accept that the flavour of a product is made up of a complex mixture of volatiles [40] and it is difficult to determine the contribution of a single volatile compound responsible for a sensory note.

Considering the analytical aroma measurement in real time called NS analysis, this study describes a novel methodology to analyse data and points out the possibility to describe tasting experience in terms of “temporal dominance markers” as opposed to the classical approach where “static” sensory perception or overall sensory experience is described by means of

“static” chemical markers. The clustering of volatile compounds in two groups can be justified on a physico-chemical basis: “lighter” compounds tend to interact less and are released more quickly, vice versa for “heavier” compounds. Obviously it is more complicated than this because chemical and physiological characteristics (e.g. hydrophilicity/hydrophobicity, interactions with specific salivary constituents, different coefficient of partition into the lipid phase of the espresso coffee) seem to play a role, for example carbonyls (aldehydes, ketones) supposedly interact less with mucosal surfaces and are released faster than N-heterocycles that interact more and are more persistent.

The effect of sugar addition can be observed when comparing results from sample A to AZ and from B to BZ. Only a modification in the sensory results was observed (Fig. 2). In other words, even though it has been not possible to identify a significant effect of sugar addition neither on in-nose aroma release nor on headspace aroma composition, the coffee was very differently perceived in-mouth. For both coffees, A and B, the addition of sugar provoked a dramatic drop of the dominances of *Sour* and *Bitter* attributes which were replaced by a dominance of the *Sweet* taste. Additionally, it caused a modification in the perception of flavour. The description of coffees changed from being *Burnt* and *Roasted* to *Roasted*, *Caramel* and *Nutty*. These changes suggest the presence of taste-smell interactions occurring at the brain level when treating and combining different type of simultaneous sensory stimuli (*i.e.* tastes and odours, [36]).

First, a masking effect of sugar is observed on bitterness and acid taste: the sweet perception overcame and reduced the two others. Some works of the literature are in agreement with the switch of dominance observed by sugar addition both on bitterness [41-43] and/or on sourness [43, 44] in water solutions and in complex matrices (see [45]; for a review).

Second, an interaction between sweet taste and flavour of coffee seemed to occur: when sucrose was added, the perception of the flavour of coffee was modified and particularly the dominance of empyreumatic flavour notes increased (with *Caramel*, *Roasted* and *Nutty* notes). Sugar seems to play the role of a flavour enhancer. This confirms the results of different studies showing that a specific tastant can increase the perceived intensity of a specific flavour when the two stimuli taste and flavour are congruent in other words when they are appropriated to be combined in a food product [46]. For instance, Hort & Hollowood (2004)[47], demonstrated a rise of ‘fruity’ flavour by sweetness and Hewson and colleagues (2008) [48] an increase in ‘lemon’ flavour by sour taste. Furthermore Dalton et al. (2000) [49] demonstrated a taste-smell interaction for congruent pair of stimuli (benzaldehyde-sodium saccharin) and not for

incongruent pair (benzaldehyde-msg) (see also [50]; for a review). Here in our study, we talk about dominance, as TDS was the sensory chosen method, and not intensity of flavour attributes however it is the same perceptual process taking place.

4.1.5 Conclusions

This study proposes to combine two temporal methods, one sensorial and one instrumental, in order to better understand coffee flavour perception. TDS method permits to describe the evolution of in-mouth perception over the time during product consumption. TDS curves allowed differentiating the samples, according to their roasting degree and level of sugar in both terms of taste and flavour. This study confirmed the ability of TDS to be applied and to describe complex products like coffee. NS analysis, as an in vivo dynamic instrumental method, allowed monitoring the aroma release during drinking and also discrimination of the products according to their roasting degrees. The addition of sugar did not affect aroma release of coffee. The novel NS data analysis methodology employed permits to identify compounds with two distinct release behaviours along the time dimension. Coupling TDS with NS analysis underlined the presence of multisensory taste-smell interactions in coffee and demonstrates the potentialities of this method combination to study cognitive interactions with a temporal dimension. It also offers the possibility to describe “tasting experience” by using “temporal dominance markers” as opposed to the classical approach where you describe “static” products by means of “static” chemical markers.

Acknowledgements

This work was supported by the Autonomous Province of Trento (Piazza Dante, 15, 38122 Trento, Italy, AP 2013/2014), Illycaffè S.p.a. (Trieste, Italia) and PIMMS (Proton Ionisation Molecular Mass Spectrometry) project supported by the European Commission’s 7th Framework Programme under Grant Agreement Number 287382. Judges are sincerely acknowledged for their involvement and motivation throughout the duration of the experiment.

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Supplementary File 4.1. Tentative identifications of detected mass peaks.

meas. mass (Th)	theor. mass (Th)	sum formula	tentative identification	ref.^a
31.018	31.018	CH ₃ O ⁺	formaldehyde	1
33.034	33.033	CH ₅ O ⁺	methanol	1
35.038	n.a.	n.a.	non identified	
41.039	41.039	C ₃ H ₅ ⁺	alkylic fragment	2
42.010	42.010	C ₂ H ₂ O ⁺	fragment (diverse origin)	
43.018	43.018	C ₂ H ₃ O ⁺	fragment (diverse origin)	2
43.055	43.054	C ₃ H ₇ ⁺	fragment (diverse origin)	2
45.034	45.033	C ₂ H ₅ O ⁺	acetaldehyde	3
46.999	n.a.	n.a.	non identified	
47.013	47.013	CH ₃ O ₂ ⁺	formic acid	1
49.012	49.011	CH ₅ S ⁺	methanethiol	1
53.003	n.a.	n.a.	non identified	
53.040	53.039	C ₄ H ₅ ⁺	fragment (diverse origin)	
54.038	n.a.	n.a.	non identified	
56.046	n.a.	n.a.	non identified	
57.038	57.033	C ₃ H ₅ O ⁺	acrolein/ acetol fragment	1
57.070	57.070	C ₄ H ₉ ⁺	alcohol fragment	4
59.049	59.049	C ₃ H ₇ O ⁺	propanal/acetone	1
61.029	61.028	C ₂ H ₅ O ₂ ⁺	acetic acid/methyl-formate	3
63.026	63.026	C ₂ H ₇ S ⁺	ethanethiol/dimethylsulfide	1
63.043	63.044	C ₂ H ₇ O ₂ ⁺	non identified	
67.054	67.054	C ₅ H ₇ ⁺	terpene fragment	5
68.026	68.026	C ₄ H ₄ O ⁺	furan fragment	3
68.050	68.049	C ₄ H ₆ N ⁺	pyrrole	3
69.033	69.033	C ₄ H ₅ O ⁺	furan	3
69.070	69.070	C ₅ H ₉ ⁺	isoprene/fragment (diverse origin)	1,2
71.049	71.049	C ₄ H ₇ O ⁺	fragment(methyl-butanol)/butenal/butenone	1,2
71.085	71.086	C ₅ H ₁₁ ⁺	terpene fragment	5
73.065	73.065	C ₄ H ₉ O ⁺	isobutanal/butanone	3
74.035	n.a.	n.a.	non identified	
75.044	75.044	C ₃ H ₇ O ₂ ⁺	methyl-acetate/acetol	3
77.059	77.060	C ₃ H ₉ O ₂ ⁺	propandiol	2
78.968	n.a.	n.a.	non identified	
79.039	n.a.	n.a.	non identified	
79.052	79.054	C ₆ H ₇ ⁺	benzene/benzaldehyde fragment	1
80.049	80.049	C ₅ H ₆ N ⁺	pyridine	2
81.034	81.033	C ₅ H ₅ O ⁺	furan fragment	3
81.066	81.070	C ₆ H ₉ ⁺	methylpentene/terpene fragment	1
82.065	82.065	C ₅ H ₈ N ⁺	methyl-pyrrole	3
83.049	83.049	C ₅ H ₇ O ⁺	methyl-furan	3
83.084	83.086	C ₆ H ₁₁ ⁺	fragment (diverse origin)	
85.023	n.a.	n.a.	non identified	

(Supplementary file 4.1 continues)

meas. mass (Th)	theor. mass (Th)	sum formula	tentative identification	ref. ^a
85.064	85.065	C ₅ H ₉ O ⁺	methyl-butanal	6
85.100	85.101	C ₆ H ₁₃ ⁺	methyl-butene	1
87.043	87.044	C ₄ H ₇ O ₂ ⁺	butanedione/butyrolactone	3
87.080	87.080	C ₅ H ₁₁ O ⁺	methyl-butanal	2
89.059	89.060	C ₄ H ₉ O ₂ ⁺	methyl-propanoate/hydroxy-butanone	3
90.058	n.a.	n.a.	non identified	
90.948	n.a.	n.a.	non identified	
91.033	n.a.	n.a.	non identified	
91.074	91.075	C ₄ H ₁₁ O ₂ ⁺	butandiol	1
93.068	93.070	C ₇ H ₉ ⁺	terpene fragment	7
94.039	n.a.	n.a.	non identified	
94.065	94.065	C ₆ H ₈ N ⁺	methyl-pyridine	1
95.010	n.a.	n.a.	non identified	
95.056	95.060	C ₅ H ₇ N ₂ ⁺	methyl-pyrazine	2
96.075	n.a.	n.a.	non identified	
97.027	97.028	C ₅ H ₅ O ₂ ⁺	furfural	3
97.064	97.065	C ₆ H ₉ O ⁺	dimethyl-furan	3
97.099	97.101	C ₇ H ₁₃ ⁺	fragment (aldehydes)	4
98.060	98.060	C ₅ H ₈ ON ⁺	dimethyl-oxazole	1
99.041	99.044	C ₅ H ₇ O ₂ ⁺	furfuryl alcohol/ α -angelica lactone	2
99.079	99.080	C ₆ H ₁₁ O ⁺	hexenal/methyl-pentenone	1
100.020	100.022	C ₄ H ₆ NS ⁺	methyl-thiazole	1
101.058	101.060	C ₅ H ₉ O ₂ ⁺	pentanedione/methyl-tetrahydrofuranone	3
103.037	n.a.	n.a.	non identified	
103.072	n.a.	n.a.	non identified	
103.072	103.075	C ₅ H ₁₁ O ₂ ⁺	hydroxy-pentanone/methyl-butanoic acid	2
105.032	n.a.	n.a.	non identified	
105.068	105.070	C ₈ H ₉ ⁺	styrene/phenylethanol fragment	1
107.049	107.049	C ₇ H ₇ O ⁺	benzaldehyde	6
107.082	107.086	C ₈ H ₁₁ ⁺	terpene fragment	7
108.049	n.a.	n.a.	non identified	
108.080	108.081	C ₇ H ₁₀ N ⁺	ethyl-pyridine	1
108.956	n.a.	n.a.	non identified	
109.071	109.076	C ₆ H ₉ N ₂ ⁺	dimethylpyrazine/ethylpyrazine	2
110.058	110.060	C ₆ H ₈ ON ⁺	formyl-methylpyrrole/acetylpyrrole	2
110.097	110.096	C ₇ H ₁₂ N ⁺	propyl-pyrrole/methyl-ethylpyrrole	2
111.042	111.044	C ₆ H ₇ O ₂ ⁺	acetyl_furan/methyl-furfural	3
111.084	111.080	C ₇ H ₁₁ O ⁺	2,3-dimethyl-2-cyclopenten-1-one	6
113.017	n.a.	n.a.	non identified	
113.056	113.060	C ₆ H ₉ O ₂ ⁺	methyl-furfuryl-alcohol/dimethyl-furanone/methyl-cyclopentanedione/cyclotene	2
113.094	113.096	C ₇ H ₁₃ O ⁺	heptenone	6

(Supplementary file 4.1 continues)

meas. mass (Th)	theor. mass (Th)	sum formula	tentative identification	ref. ^a
114.041	114.037	C ₅ H ₈ NS ⁺	ethyl-thiazole/dimethyl-thiazole	1
115.072	115.075	C ₆ H ₁₁ O ₂ ⁺	4-methyltetrahydro-2H-pyran-2-one	6
115.110	115.112	C ₇ H ₁₅ O ⁺	hepanal/heptanone	1
116.072	116.071	C ₅ H ₁₀ O ₂ N ⁺	non identified	
117.047	n.a.	n.a.	non identified	
117.086	117.091	C ₆ H ₁₃ O ₂ ⁺	hexanoic acid	2
119.047	119.049	C ₈ H ₇ O ⁺	benzofuran	1
121.061	121.065	C ₈ H ₉ O ⁺	phenylacetaldehyde	1
121.096	n.a.	n.a.	non identified	
122.095	122.096	C ₈ H ₁₂ N ⁺	propyl-pyridine	1
123.040	123.044	C ₇ H ₇ O ₂ ⁺	benzoic acid/hydroxy- benzaldehyde/furanyl-propenal/	1
123.085	123.080	C ₈ H ₁₁ O ⁺	2-phenylethanol/4-ethylphenol	1
124.072	124.076	C ₇ H ₁₀ ON ⁺	2-acetyl-1-methylpyrrole	2
124.109	124.112	C ₈ H ₁₄ N ⁺	butylpyrrole/methyl-propylpyrrole	1
125.057	125.060	C ₇ H ₉ O ₂ ⁺	guaiacol/methyl-benzenediol/furyl acetone	2
125.099	125.096	C ₈ H ₁₃ O ⁺	butylfuran/methyl-propylfuran	1
125.956	n.a.	n.a.	non identified	
126.057	n.a.	n.a.	non identified	
126.092	126.091	C ₇ H ₁₂ ON ⁺	acteyl-dimethylpyrrole/alkyloxazole	1
127.031	n.a.	n.a.	non identified	
127.070	n.a.	n.a.	non identified	
127.106	n.a.	n.a.	non identified	
129.040	n.a.	n.a.	non identified	
129.084	n.a.	n.a.	non identified	
129.122	n.a.	n.a.	non identified	
131.066	131.070	C ₆ H ₁₁ O ₃ ⁺	acetyloxy-butanone/ethanediol diacetate/oxopropoxy-propanone/ethyl- oxobutanoate	1
131.098	n.a.	n.a.	non identified	
135.048	135.044	C ₈ H ₇ O ₂ ⁺	n.a.	
135.102	135.102	C ₆ H ₁₅ O ₃ ⁺	n.a.	
138.050	n.a.	n.a.	non identified	
138.087	138.091	C ₈ H ₁₂ ON ⁺	dimethyl-pyrrolylethanone/ethyl- pyrrolylethanone	1
138.129	138.128	C ₉ H ₁₆ N ⁺	butyl-pyridine	1
139.072	139.075	C ₈ H ₁₁ O ₂ ⁺	4-ethyl-1,2-benzenediol	2
139.116	139.112	C ₉ H ₁₅ O ⁺	E,E-2,4-nonadienal	1
140.074	140.071	C ₇ H ₁₀ O ₂ N ⁺	dimethyl-oxazolyl-ethanone	1
141.056	141.055	C ₇ H ₉ O ₃ ⁺	furfuryl-acetate	2
143.088	n.a.	n.a.	non identified	
145.114	n.a.	n.a.	non identified	
148.069	n.a.	n.a.	non identified	

(Supplementary file 4.1 continues)

meas. mass (Th)	theor. mass (Th)	sum formula	tentative identification	ref. ^a
148.069	n.a.	n.a.	non identified	
149.058	149.060	C ₉ H ₉ O ₂ ⁺	furfuryl-furan	6
149.110	149.107	C ₉ H ₁₃ N ₂ ⁺	ethenyl-ethyl-methylpyrazine dihydrocyclopentapyrazines/quinoxalines	1
152.067	152.071	C ₈ H ₁₀ O ₂ N ⁺	n.a.	
153.046	n.a.	n.a.	non identified	
153.086	153.091	C ₉ H ₁₃ O ₂ ⁺	4-ethyl-guaiacol	2
153.124	153.127	C ₁₀ H ₁₇ O ⁺	E,E-2,4-decadienal	1
155.125	n.a.	n.a.	non identified	
159.125	n.a.	n.a.	non identified	
163.123	163.123	C ₁₀ H ₁₅ N ₂ ⁺	dihydrocyclopentapyrazines/quinoxalines	1
177.149	177.149	C ₉ H ₂₁ O ₃ ⁺	non identified	
201.164	n.a.	n.a.	non identified	
235.174	n.a.	n.a.	non identified	
239.208	n.a.	n.a.	non identified	

(n.a. = non applicable).

a) **references:** **1.**Flament, I. & Bessière-Thomas, Y. *Coffee flavor chemistry* (Wiley, 2002); **2.**Yeretzian, C., Jordan, A. & Lindinger, W. Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. *Int. J. Mass Spectrom.* **223-224**, 115–139 (2003); **3.**Lindinger, C. *et al.* When Machine Tastes Coffee: Instrumental Approach To Predict the Sensory Profile of Espresso Coffee. *Anal. Chem.* **80**, 1574–1581 (2008); **4.**Buhr, K., van Ruth, S. & Delahunty, C. Analysis of volatile flavour compounds by Proton Transfer Reaction-Mass Spectrometry: fragmentation patterns and discrimination between isobaric and isomeric compounds. *Int. J. Mass Spectrom.* **221**, 1–7 (2002); **5.**Tani, A., Hayward, S. & Hewitt, C. . Measurement of monoterpenes and related compounds by proton transfer reaction-mass spectrometry (PTR-MS). *Int. J. Mass Spectrom.* **223-224**, 561–578 (2003); **6.**Chin, S.-T., Eyres, G. T. & Marriott, P. J. Identification of potent odourants in wine and brewed coffee using gas chromatography-olfactometry and comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **1218**, 7487–7498 (2011); **7.**Demarcke, M. *et al.* Laboratory studies in support of the detection of sesquiterpenes by proton-transfer-reaction-mass-spectrometry. *Int. J. Mass Spectrom.* **279**, 156–162 (2009).

Chapter 5. Other projects

5.1 Rapid and direct volatile compound profiling of black and green teas (*Camellia sinensis*) from different countries with PTR-ToF-MS

Submitted as

“Sine Yener, José A. Sánchez-López, Pablo M. Granitto, Luca Cappellin, Tilmann D. Märk, Ralf Zimmerman, Günther Bonn, Chahan Yeretizian, Franco Biasioli. Rapid and direct volatile compound profiling of black and green teas (*Camellia sinensis*) from different countries with PTR-ToF-MS. *Talanta*, 2016.”

Main Highlights

- 101 teas, both leaves and infusions, of different origins have been analysed by PTR-ToF-MS
- Black and green teas were separated based on VOC emissions
- The volatile profiles of teas are highly affected by type and brewing process
- Multivariate data analyses indicate a possible classification according to geographical origin

Abstract

Volatile profiles of 63 black and 38 green teas from different countries were analysed with Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) both for tea leaves and tea infusion. The headspace volatile fingerprints were collected and the tea classes and geographical origins were tracked with pattern recognition techniques. The high mass resolution achieved by ToF mass analyser provided determination of sum formula and tentative identifications of the mass peaks. The results provided successful separation of the black and green teas based on their headspace volatile emissions both from the dry tea leaves and their infusions. The volatile fingerprints were then used to build different classification models for discrimination of black and green teas according to their geographical origins. Two different cross validation methods were applied and their effectiveness for origin discrimination was discussed. The classification models showed a separation of black and green teas according to geographical origins the errors being mostly between neighbouring countries.

Key words: tea aroma, tea leaf, tea infusion, volatile profiling, headspace volatile fingerprinting, geographic origin classification

5.1.1 Introduction

In tea production, the leaves of the tea plant *Camellia sinensis* are used as the same starting material but the differences in the processing techniques result in a wide range of characteristic teas with distinct sensory properties. According to the way of processing, teas are usually classified into three big groups based on their fermentation degrees: non-fermented (green and white), semi-fermented (oolong) and fully fermented (black tea including pu-erh tea) [1]. There are several tea producing countries in the world. The main five tea producing countries are China, India, Kenya, Sri Lanka and Turkey [2]. Each country has different regions with their own climate and tea processing methods which characterize colour, appearance and flavour of the final product. For this reason, most tea products are marketed with the indication of the production region for product authentication and valorisation.

Aroma compounds play an important role for consumer preferences and perception of tea. Starting with the fresh tea leaves, which have a greenish and unripe odour, the characteristic tea aroma is developed during tea leaves processing. The most investigated volatile compounds (VOCs) in tea mainly consist of non-terpenoid and terpenoid components; the former are products of fatty acid degradation and provide the fresh green flavour, the latter are mostly monoterpene alcohols which give a floral sweet aroma [3, 4].

Various studies have been conducted in the field of tea aroma research as recently reviewed by Yang et al. (2013) [5]. In short, gas chromatography-mass spectrometry (GC-MS) is generally used as a reference method in order to identify and quantify VOCs. The odour characteristics of volatiles have been detected with aroma dilution and GC-olfactometry; and recently electronic nose techniques have been used for fast analysis of tea aroma. These methods have allowed analysing the volatile profiles of teas at different fermentation degrees and also to classify green, black and oolong teas according to their geographical origins [6-11]. Among them, GC-MS has turned out to be the most accurate and effective method for identification, separation and quantification of volatile compounds; however it requires capturing volatiles by various extraction methods which are generally time consuming and their efficiency depends on the characteristics and limitations of the analytical approach (e.g. the absorption and desorption of volatiles from a specific material in the case of SPME)[12].

To link sensory perception of tea with instrumental data, direct and non-destructive instrumental analysis of volatiles can be considered to be the most appropriate approach because it provides a direct estimation of the VOCs released of from tea and that reach the

human olfactory system. In this regard, proton transfer reaction-mass spectrometry, PTR-MS, provides an efficient approach as a direct injection, soft chemical ionization method for the analysis of VOCs at trace levels. The direct injection method requires no sample pre-treatment which allows real-time monitoring of VOCs [13, 14] without making any changes in the volatile composition of samples. The technique uses H_3O^+ ions for protonation of VOCs with proton affinities higher than that of water which can be further analysed by a quadrupole or a time-of-flight (ToF) mass analyser [15]. ToF mass analysers provide high sensitivity that leads to detection of volatiles at ppt levels and high mass resolution which allows, in most cases, the identification of the sum formula of the observed peaks [13].

PTR-MS allows collecting the overall mass spectral fingerprints of the samples which can be further processed with advanced data analysis tools for successful discrimination and classification of the food products [16]. To the best of our knowledge, neither a study has been conducted on the analysis of volatile compounds emitted from various tea types by PTR-MS nor was this method applied for discrimination of teas from different geographical origins.

With this study, we aim to apply PTR-ToF-MS, for the first time, for aroma profiling of black and green tea samples, both leaves and brew, from different countries and to investigate the possibility of origin tracing on the basis of their geographical origins with the aid of chemometric tools.

5.1.2 Materials and Methods

5.1.2.1 Tea samples

In total, 101 commercially available pure tea samples, without addition of flavouring agents, from 16 different countries (**Table 5.1-1**) were purchased from the market; 63 black teas and 38 green teas. The samples were stored in their original bags at room temperature before analysis. Trademarks and producers were kept confidential but the commercial names, origins and other characteristics of the tea samples are provided in Supplementary material S1.

Table 5.1-1. Distribution of tea samples according to tea types and countries of origin

Country (code)	Tea Types	
	<i>Black</i>	<i>Green</i>
Argentina (ARG)	1	-
China (CHI)	13	15
India (IND)	25	4
Indonesia (INDO)	3	-
Japan (JAP)	-	9
Kenya (KEN)	2	-
Korea (KOR)	1	4
Nepal (NEP)	4	1
Rwanda (RWA)	-	1
Sri Lanka (SRI)	8	1
Tanzania (TNZ)	1	1
Turkey (TUR)	3	-
Vietnam (VIE)	1	2
Zimbabwe (ZIM)	1	-
(Total)	63	38

5.1.2.2 Analysis of tea volatiles by PTR-ToF-MS

The volatile compounds of dry tea leaves and their infusions were analysed by PTR-ToF-MS by direct injection headspace analysis without destructing the original samples. For the analysis of dry tea leaves, 500 mg tea leaves were weighted into 22-ml glass vials (Supelco, Bellefonte, PA) and 3 replicates were prepared for each tea sample. Tea brewing was performed by applying a 3 min fixed infusion time for all tea samples. Deionized hot water (25 ml, 85°C) was used for brewing of tea leaves (400 mg) in 40 ml amber vials (Supelco, Bellefonte, PA). The liquid infusion was taken right after brewing by a micropipette and 2 ml of aliquots were transferred into 22-ml glass vials. Each tea sample was brewed 3 times and each brew was analysed in duplicate.

The headspace measurements were performed by using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The instrumental conditions in the

drift tube were set as following: drift voltage 550 V, drift temperature 110°C, drift pressure 2.33 mbar affording an E/N value (electric field strength/gas number density) of 140 Townsend (Td, 1 Td = 10⁻¹⁷ V.cm²). All the vials containing samples and blank vials (air for tea leaves and hot water for tea brews) were incubated at 37°C for 30 min before headspace analysis. The headspace mixture was directly injected into PTR-MS drift tube with a flow rate of 40 sscm via a PEEK tube at 110°C. Sample injection was performed with a multipurpose autosampler (Gerstel GmbH, Mulheim am Ruhr, Germany). A different sample was analysed every 5 min. Each sample was measured for 30 s, at an acquisition rate of one spectrum per second. The measurement order was randomized while measuring the volatile emissions of tea leaves and tea brews.

5.1.2.3 Data processing and analysis

5.1.2.3.1 Treatment of mass spectrometric data

Data processing of ToF spectra included dead time correction, internal calibration and peak extraction steps performed according to a procedure described elsewhere [17] to reach a mass accuracy (≥ 0.001 Th) which is sufficient for sum formula determination. The baseline of the mass spectra was removed after averaging the whole measurement and peak detection and peak area extraction was performed by using modified Gaussian to fit the data [18]. Whenever a peak was detected, the volatile concentrations were calculated directly via the amount of detected ions in ppbv (part per billion by volume) levels according to the formulas described by Lindinger et al. (1998) [13] by assuming a constant reaction rate coefficient ($k_R = 2 \times 10^{-9}$ cm³/s). For H₃O⁺ as a primary ion, this introduces a systematic error for the absolute concentration for each compound that is in most cases below 30% and can be accounted for if the actual rate constant is available [19].

5.1.2.3.2 Selection of mass peaks

The direct injection headspace analysis of tea (leaves and infusion) samples resulted in identifying 455 mass peaks in the range 15-300 *m/z*. After eliminating the interfering ions (O₂⁺, NO⁺ and water clusters) and their isotopologues, 438 mass peaks remained for further analysis. The signals belonging to blank vials were subtracted from the whole data set (air from tea leaf emissions, water from infusion emissions). A concentration threshold of 0.1 ppb was set for further reduction of noise in the data matrices. After this step 257 mass peaks x 303 (i.e. 101 samples, three biological replicates) data points were left to build the matrix containing tea leaf

emissions; 162 mass peaks x 606 (i.e. 101 samples, three infusions, two analytical replicates) data points were left for tea infusion data matrix. These final data matrices were used for univariate and multivariate data analysis methods.

After mass peak selection and extraction, tentative peak identification was performed by using an in-house library developed by the authors where the peak annotations were done automatically with the scripts developed under R programming language [20].

5.1.2.4 Statistical analyses

The significant differences between tea types were calculated using ANOVA (99% confidence level) and the pairwise comparison was performed with Tukey's test to highlight these differences with letter annotations.

As a first step, the final data matrices were subjected to principal component analysis (PCA). Secondly, Random Forests (RF), Penalized Discriminant Analysis (PDA), Support Vector Machines (SVM) and Discriminant Partial Least Squares (dPLS) classification methods were applied for sample discrimination [21] and their classification power was compared.

Two types of validation methods were tested for each classification method: a simple 6-fold cross validation and Leave-Group-Out (LGO) cross-validation. The six-fold cross-validation was performed by randomly dividing the whole data set into 6 folds. One of the folds was removed at each time and used as a test set where the rest of the data (the train set) was used to build the discriminant method and predict the origins of samples. Using this cross validation method, analytical or biological replicates of the same tea sample can be at the same time in both the train and test sets. With the highly flexible classification methods used in this work, this can easily leads to overfitting the data and to produce biased estimates of classification errors. This effect was verified in preliminary experiments (not shown) and the method was discarded. In the case of the more elaborated LGO cross-validation, the analytical and biological replicates of each tea sample were considered as a group when discriminating tea types and geographical origin. Each time, one group was removed from the full dataset and used as a test set. Mean classification errors and confusion matrices were used to evaluate the performance of each classification method. All the multivariate data analyses were performed by using the scripts and packages developed under R programming language [20].

5.1.3 Results and Discussion

5.1.3.1 Volatile profiling of black and green teas and discrimination based on tea type

One-way ANOVA of the mass peaks extracted in black and green tea headspace, showed 135 mass peaks significantly different ($p < 0.01$ with Bonferroni correction) between emissions of black and green tea leaves and 125 mass peaks between their infusions. Among the mass peaks extracted, 62 of them were tentatively identified as one or more volatile compounds based on their presence in dry tea leaves and brews reported in literature. The details of the tentatively identified mass peaks are shown in **Table 5.1-2** with their average concentrations in black and green tea leaves and infusions.

The leaves of different tea types showed greater volatile emissions than that of their infusions. Various terpenes and their fragments dominated the volatile emission of tea leaves, followed by esters/acids and aldehydes/ketones. In particular, green tea leaves emitted more terpenes and sulphur compounds than black teas. The most abundant volatile compounds in the headspace of green tea infusions were sulphur compounds, aldehydes/ketones and terpenes. The headspace of black tea infusions contained aldehydes/ketones the highest; sulphur compounds, terpenes and alcohols were other most abundant chemical groups.

Some distinct differences and similarities can be pointed out between black and green teas: the most abundant sulphur compound detected in both tea infusions was tentatively identified as dimethyl sulphide. It has been reported that this sulphur compound improves the flavour of green teas harvested in spring [3]. The information about the season when the green teas were picked was not available for all the tea samples but for some of the black teas. Interestingly, we observed that the black teas that had the highest dimethyl sulphide contents were indeed picked during spring (e.g. sample no 102, 110, and 116 in Supplementary file 1).

Table 5.1-2. The average concentrations (ppb) of tentatively identified mass peaks in the headspace of black and green tea leaves and infusions. Peaks were selected on the basis of one-way ANOVA and the relative *p*-values are listed in the right columns.

Measured mass	The. mass	Sum formula	Chemical class	Tentative identification	Average concentration ± standard deviation (ppbv)		<i>p</i> -value	Average concentration ± standard deviation (ppbv)		<i>p</i> -value	Reference
					Black tea leaves	Green tea leaves		Black tea infusion	Green tea infusion		
33.0336	33.033	CH ₄ OH ⁺	Alcohols	Methanol	11756±3992	9555±3826	<0.001	170±82	120±63	<0.001	[28]
45.0333	45.034	C ₂ H ₄ OH ⁺	Aldehydes	Acetaldehyde	804±608	265±260	<0.001	416±158	108±58	<0.001	[28]
47.0491	47.049	C ₂ H ₅ OH ⁺	Alcohols	Ethanol	138±152	110±360	0.345	6±14	11±18	<0.001	[28]
49.0110	49.011	CH ₄ SH ⁺	Sulphur compounds	Methanethiol	0.9±0.5	0.5±0.3	<0.001	0.4±0.5	1.2±1.5	<0.001	[28]
59.0488	59.049	C ₃ H ₆ OH ⁺	Aldehydes/ketones	Propanal/acetone	340±479	265±784	0.293	115±99	92±85	0.002	[28]
61.0280	61.028	C ₂ H ₄ O ₂ H ⁺	Esters and acids	Acetic acid	804±605	390±848	<0.001	2.3±4.8	6.34±17	<0.001	[29]
63.0260	63.026	C ₂ H ₆ SH ⁺	Sulphur compounds	Dimethylsulfide	10±8	12±12	0.191	264±289	275±299	0.675	[28]
69.0333	69.034	C ₄ H ₄ OH ⁺	Furans	Furan fragment	7.0±7.4	4.7±6.1	0.004	7.2±7.8	2.0±3.0	<0.001	[30]
69.0697	69.070	C ₅ H ₈ H ⁺	Terpene fragment	Isoprene	183±145	220±217	0.067	145±76	35±21	<0.001	n.a.
71.0489	71.049	C ₄ H ₆ OH ⁺	Aldehydes	Butenal	19±17	12±8	<0.001	4.6±4.0	2.2±2.3	<0.001	[31]
73.0646	73.065	C ₄ H ₈ OH ⁺	Aldehydes	Methylpropanal	206±143	195±565	0.811	292±159	43±26	<0.001	[29]
75.0438	75.044	C ₃ H ₆ O ₂ H ⁺	Esters and acids	Propionic acid	96±103	42±42	<0.001	9.7±6.1	4.5±2.6	<0.001	[28]
79.0536	79.054	C ₆ H ₆ H ⁺	Aromatic hydrocarbons	Benzene	40±18	26±22	<0.001	5.3±7.3	4.1±7.7	0.043	[29]
81.0697	81.070	C ₆ H ₈ H ⁺	Terpene fragment	Cyclohexadiene (Terpene fragment)	612±905	423±461	0.035	29±24	9±12	<0.001	[28]
83.0854	83.086	C ₆ H ₁₀ H ⁺	Terpene fragment	Cyclohexene (Terpene fragment)	175±166	60±70	<0.001	71±63	19±23	<0.001	[28]
85.0646	85.065	C ₅ H ₈ OH ⁺	Aldehydes/Ketones	Pentenal/pentenone	32±33	37±37	0.229	12±9	7.5±7.6	<0.001	[24, 29, 32]
87.0431	87.044	C ₄ H ₆ O ₂ H ⁺	Ketones	Butanedione	19±16	6.4±4.6	<0.001	2.7±3.1	1.3±0.7	<0.001	[29]
87.0802	87.080	C ₅ H ₁₀ OH ⁺	Alcohols	Pentenol	55±37	62±157	0.549	165±89	23±14	<0.001	[24, 29]
91.0559	91.058	C ₄ H ₁₀ SH ⁺	Sulphur compounds	Diethylsulfide/butanethiol (fragment)	7.9±3.6	4.4±2.7	<0.001	0.9±0.9	0.3±0.5	<0.001	[33]
93.0365	93.037	C ₃ H ₈ OSH ⁺	Sulphur compounds	Methylsulfanylethanol	8.6±3.7	5.6±2.6	<0.001	1.7±1.2	0.4±0.6	<0.001	n.a.
93.0698	93.070	C ₇ H ₈ H ⁺	Aromatic hydrocarbons	Toluene	55±88	34±34	0.014	5.9±7.5	2.2±2.3	<0.001	[28, 29, 32]
95.0173	95.016	C ₂ H ₆ O ₂ SH ⁺	Sulphur compounds	Dimethyl sulfone (methylsulfonylmethane)	2.4±0.9	1.3±0.7	<0.001	0.2±0.3	n.d.	<0.001	n.a.
95.0478	95.049	C ₆ H ₆ OH ⁺	Phenols	Phenol	6.5±4.8	3.8±2.1	<0.001	0.3±0.4	0.4±0.4	0.188	[34]
95.0854	95.086	C ₇ H ₁₀ H ⁺	Terpenes	Methylcyclohexadiene (α-terpinene fragment)	87±121	64±61	0.051	4.2±3.2	2.6±2.2	<0.001	[33]
96.0814	96.081	C ₆ H ₉ NH ⁺	Heterocyclic compounds	Ethylpyrrole	7.1±8.2	5.9±4.4	0.144	2.1±1.8	2.5±1.6	0.002	[32]
97.0282	97.028	C ₅ H ₄ O ₂ H ⁺	Aldehydes	Furfural	13±12	2.9±1.9	<0.001	1.8±2.7	0.4±0.7	<0.001	[32, 33]
97.0647	97.065	C ₆ H ₈ OH ⁺	Aldehydes/Furans	Hexadienal/ethylfuran	19±23	19±24	0.797	25±26	8.3±8.6	<0.001	[24, 32]
99.0803	99.080	C ₆ H ₁₀ OH ⁺	Aldehydes	Hexenal/methylpentenone	19±16	15±9	0.009	21±22	4.6±2.8	<0.001	[24, 29]
101.0960	101.096	C ₆ H ₁₂ OH ⁺	Alcohols	Hexenol	24±28	10±13	<0.001	13±11	3.7±4.5	<0.001	[32, 33]
103.0755	103.075	C ₅ H ₁₀ O ₂ H ⁺	Esters and acids	Methylbutanoic acid	12±9	11±11	0.414	1.1±1.4	0.7±1.1	0.002	[23]
105.0343	105.037	C ₄ H ₈ OSH ⁺	Sulphur compounds	Methional	2.0±0.8	0.4±0.4	<0.001	0.2±0.2	n.d.	<0.001	[30]

(Table 5.1-2 continues)

Measured mass	The. mass	Sum formula	Chemical class	Tentative identification	Average concentration ± standard deviation (ppbv)		p-value	Average concentration ± standard deviation (ppbv)		p-value	Reference
					Black tea leaves	Green tea leaves		Black tea infusion	Green tea infusion		
105.0689	105.070	C ₈ H ₈ H ⁺	Aromatic hydrocarbons	Styrene/ethylbenzene/vinylbenzene	13±5	1.4±0.8	<0.001	1.0±0.7	0.1±0.3	<0.001	[9]
107.0488	107.049	C ₇ H ₆ OH ⁺	Aldehydes	Benzaldehyde	33±15	13±9	<0.001	9.4±5.9	1.8±1.8	<0.001	[24, 29, 33, 35]
107.0855	107.086	C ₈ H ₁₀ H ⁺	Aromatic hydrocarbons	Xylene/ethylbenzene	58±43	43±37	0.002	9 ±16	8±15	0.398	[24, 35]
109.0658	109.065	C ₇ H ₈ OH ⁺	Phenols	Benzyl alcohol (cresol)	3.2±1.9	1.8±1.3	<0.001	0.5±0.3	0.2±0.2	<0.001	[24, 29, 33]
109.1013	109.101	C ₈ H ₁₂ H ⁺	Hydrocarbons	Cyclooctadiene	25±29	21±17	0.154	4.0±3.5	2.3±2.2	<0.001	[29]
111.0466	111.044	C ₆ H ₆ O ₂ H ⁺	Furans	Acetyl furan	3.8±3.5	2.2±1.6	<0.001	0.3±0.7	0.2±0.4	0.028	[24, 33]
111.0805	111.080	C ₇ H ₁₀ OH ⁺	Aldehydes	Heptadienal	17±20	20±24	0.213	7.3±7.2	6.4±7.4	0.120	[24, 36]
113.0960	113.096	C ₇ H ₁₂ OH ⁺	Aldehydes	Heptenal	3.3±2.5	2.6±2.2	0.010	1.6±1.3	0.7±0.7	<0.001	[24, 29, 32]
115.0738	115.074	C ₆ H ₁₀ O ₂ H ⁺	Ketones	Caprolactone	2.5±1.7	1.2±1.0	<0.001	0.2±0.1	0.1±0.1	<0.001	[29]
115.1119	115.112	C ₇ H ₁₄ OH ⁺	Ketones	Heptanone	10±10	5.5±5.9	<0.001	3.6±2.9	1.2±1.1	<0.001	[28]
121.0291	121.028	C ₇ H ₁₀ O ₂ H ⁺	Terpenes	cyclohexadienone (fragment)	0.8±0.4	n.d.	<0.001	0.2±0.3	n.d.	<0.001	[24, 29, 33]
121.0648	121.065	C ₈ H ₈ OH ⁺	Aldehydes	Methylbenzaldehyde - coumaran	2.5±1.2	1.1±0.6	<0.001	2.3±1.3	0.5±0.6	<0.001	[24, 32, 35]
121.1004	121.101	C ₉ H ₁₂ H ⁺	Aromatic hydrocarbons	Methylethylbenzene	8.7±6.9	7.6±6.1	0.134	0.8±0.4	0.5±0.5	<0.001	[29]
123.1170	123.117	C ₉ H ₁₄ H ⁺	Terpenes	Santene	5.6±2.3	5.9±2.3	0.242	1.2±0.5	0.7±0.4	<0.001	n.a.
127.1116	127.112	C ₈ H ₁₄ OH ⁺	Ketones	Octenone/methylheptenone	9±11	7.3±6.2	0.030	2.6±2.3	1.2±1.1	<0.001	[24, 29]
129.0901	129.091	C ₇ H ₁₂ O ₂ H ⁺	Esters and acids	Hexenyl formate	1.7±1.1	0.8±1.1	<0.001	0.4±0.3	n.d.	<0.001	[32]
129.1276	129.127	C ₈ H ₁₆ OH ⁺	Ketones	Octanone/Dimethylcyclohexanol	2.9±2.7	2.1±1.8	0.002	1.2±1.1	0.4±0.3	<0.001	[32, 33]
131.1069	131.107	C ₇ H ₁₄ O ₂ H ⁺	Esters and acids	Heptanoic acid/hexyl formate	9.9±9.4	8.2±11.0	0.138	2.3±2.1	1.1±1.7	<0.001	[24, 32, 33]
135.1170	135.117	C ₁₀ H ₁₄ H ⁺	Aromatic hydrocarbons	Methylpropylbenzene	12±14	6.2±4.9	<0.001	1.0±1.0	0.4±0.4	<0.001	[29]
136.1212	136.112	C ₉ H ₁₃ NH ⁺	Heterocyclic compounds	Butyl-pyridine/ethyl-propylpyridine	3.7±4.9	2.3±2.3	0.005	0.2±0.2	n.d.	<0.001	n.a.
137.1321	137.133	C ₁₀ H ₁₆ H ⁺	Terpenes	Various monoterpenes	368±548	252±277	0.033	13±14	5.6±7.5	<0.001	[29, 32, 33, 36]
139.1124	139.112	C ₉ H ₁₄ OH ⁺	Aldehydes	Nonadienal	27±16	19±12	<0.001	2.8±1.5	1.3±0.9	<0.001	[29, 33, 36]
141.1271	141.127	C ₉ H ₁₆ OH ⁺	Aldehydes	Nonenal	3.2±1.5	2.5±1.2	<0.001	0.9±0.6	0.5±0.3	<0.001	[29, 36]
143.1435	143.143	C ₉ H ₁₈ OH ⁺	Ketones/Aldehydes	Nonanone/nonanal	0.9±0.9	0.6±0.4	0.002	0.8±0.8	0.3±0.3	<0.001	[24, 29]
151.1114	151.112	C ₁₀ H ₁₄ OH ⁺	Terpenes	Carvacrol/ safranal	1.0±0.4	0.9±0.5	0.365	0.3±0.13	0.2±0.1	<0.001	[24, 33, 36]
153.0550	153.055	C ₈ H ₈ O ₃ H ⁺	Aldehydes	Vanillin, methyl salicylate	6.1±4.0	n.d.	<0.001	4.2±3.4	n.d.	<0.001	[33, 36, 37]
153.1272	153.127	C ₁₀ H ₁₆ OH ⁺	Aldehydes	Decadienal	29±26	4.4±2.9	<0.001	2.9±1.9	0.6±0.5	<0.001	[36]
155.1430	155.143	C ₁₀ H ₁₈ OH ⁺	Alcohols	Linalool/ geraniol	2.1±1.5	1.1±1.5	<0.001	0.6±0.4	0.2±0.3	<0.001	[29, 33, 35, 36]
171.1332	171.138	C ₁₀ H ₁₈ O ₂ H ⁺	Terpenes	Linalool oxide	2.9±2.6	0.2±0.1	<0.001	0.2±0.2	n.d.	<0.001	[24, 29, 36]
193.1587	193.159	C ₁₃ H ₂₀ OH ⁺	Terpenes	B-ionone	0.4±0.2	0.3±0.2	0.141	0.2±0.2	0.2±0.2	0.626	[29, 33, 35, 36]
195.0879	195.088	C ₈ H ₁₀ N ₄ O ₂ H ⁺	Ketones	Caffeine	n.d.	0.2±0.1	<0.001	0.2±0.1	n.d.	<0.001	[29]

n.a.: Not available, n.d. : Not detected

We observed that the percentage of total monoterpenes and their fragments in the headspace of black tea infusions (~20%) was higher than the amount emitted from green tea infusion (~12%). Terpenes, especially monoterpenes, are responsible for the characteristic floral odour of tea [22]. Important aroma compounds derived from breakdown of carotenoids during black tea processing like linalool, geraniol, linalool oxide and ionone [3] were also higher in the headspace of black teas and their infusions than in green teas. Most of the monoterpenes and derived compounds were significantly lost during tea brewing; in particular linalool oxide (m/z 171.133) in green tea infusions.

Vanillin was previously reported to be one of the compounds of highest flavour dilution factor (FD) in black tea infusion [23]. In our study, the peak corresponding to vanillin was negligible in green tea infusions, but clearly observable in black teas with little effect of brewing.

When PCA was performed, the first three principal components provided a separation of black teas from green teas based on the volatile emissions from dry leaves and infusions (**Figure 5.1-1a, b**).

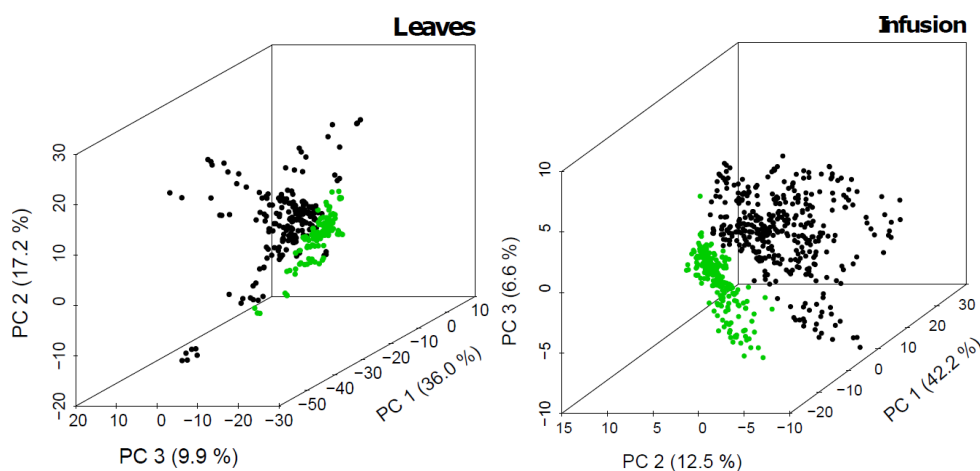


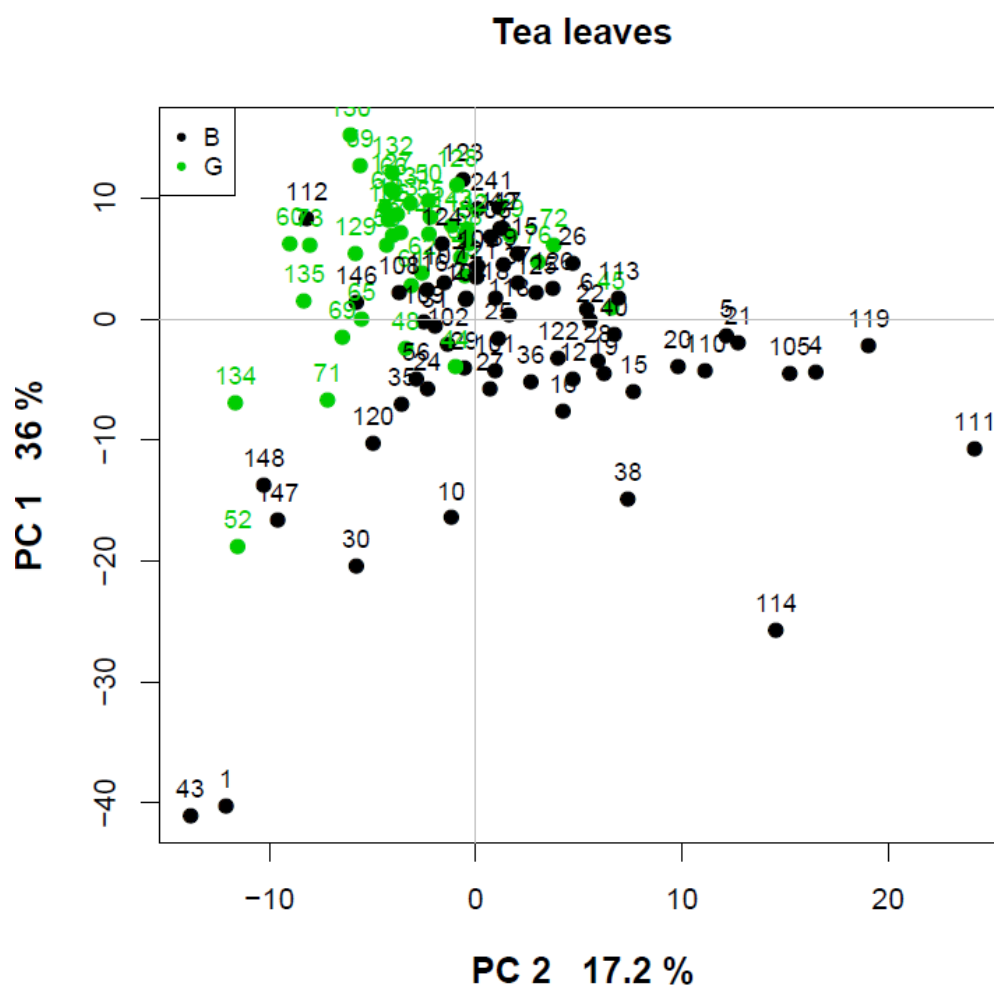
Figure 5.1-1. 3D PCA score plots of black and green tea leaves (a) and tea infusions (b). Black and green colors represent black and green teas, respectively.

The first PCs explain 53.2 and 54.7% variances for the dry tea leaves and infusions, respectively. This reflects the high variance between black and green tea volatile emissions as well as within each tea type (black or green) depending on the different production methods and origins. The release mechanisms of volatiles might be influenced by matrix characteristics (*i.e.* leaf shape and size) as teas can be produced in various shapes. For example, the green

teas can be shaped like needle, twisted, flat, round, compressed shape or even as ground powder as a results of fixing and drying methods. Besides, leaf disruption also occurs in cutting and rolling steps of black tea production that leads to grading of black teas according to leaf size [22].

Figure 5.1-2a and **Figure 5.1-2b** show score plots of the first two PCs of tea leaves and tea infusions (loadings of the first two components of tea leaves and infusions are provided in Supplementary file S2). According to these **Figure 5.1-2a** and **Figure 5.1-2b**, some black tea samples with broken leaves (sample numbers 1, 10, 30, 43, 112, 146-148) were closely located and separated from others. These samples were characterized by the mass peaks at m/z 59.049, 85.065, 97.065, 99.081, 111.081, 113.096, 115.074, 115.112, 139.113, 141.127 and 143.144 which were mostly attributed to aldehydes and ketones; mass peaks at m/z 101.096 and 87.080 to alcohols and mass peak at m/z 169.126 to geranic acid in the headspace of dry tea leaves. In addition, mass peaks; m/z 71.049 (butenal), 77.058 (propandiol), 129.099 (hexenyl formate), 127.112 (methylheptenone) had high loadings in the headspace of tea infusions with broken leaf shape. Broken and smashed tea may release more catechins than firmly pressed tea leaves and they may undergo heavier oxidation [24]. Broken leaves also provide a larger surface area during fermentation favouring enzymatic (*i.e.* glycosidases, fatty acid hydroperoxide lyase) activity for production of volatile aldehydes [25]. These findings indicate the importance of leaf shape on volatile compound generation and their extraction during the infusion process.

PCA showed a relatively good separation of black and green teas by using three principal components (**Figure 5.1-1a-b**, **Figure 5.1-2a-b**). To be able to assess the performance of discrimination, we applied 4 classification models by using two different cross validation methods for discrimination of black and green teas. According to LGO cross validation the average errors for classification black and green tea leaves were 2.6, 3.9, 1.3 and 3.6 %; the average errors for classification black and green tea infusions were 0.6, 0.2, 0.0 and 0.5 % obtained by RF, PDA, SVM and dPLS classification models, respectively. In general, all the classification techniques showed very good classification efficiency with an average error rate less than 4.0% for differentiating black and green tea volatile profiles emitted from leaves and infusions. In all cases, the classification errors were lower for tea infusions than tea leaves.



5.1.3.2 Geographical origin discrimination with supervised classification methods

The results described above highlighted significant differences between black and green tea aroma profiles and successful separation of large number of tea samples according to tea type. However it would more relevant to demonstrate that the volatile composition of tea might be related to its geographical origin, as well. For this reason, we applied supervised classification methods on the black and green tea volatile profiles in order to differentiate them according to their origins.

To get a more representative data set for classification studies, we selected origins (countries) represented by at least 4 different teas. Black teas from China, India, Sri Lanka and Nepal (50 samples) were included for classification of black teas; China, India, Japan and Korea (32 samples) were selected for classification of green teas. Each classification algorithm ended up with an average classification error and a confusion matrix where the original tea origins were compared with the origins assigned by the classification method. The classification methods were applied on normalized volatile concentrations with LGO cross validation tests. The normalized concentrations were obtained by normalizing each mass spectrum to unit are as described in [26].

The classification performances obtained by using emissions of the tea leaves and tea infusions were similar and they provided relatively good separations which were between 30-50%. Due to the fact that, the tea infusions are the final consumed products, in the following discussion, we will focus on the classification models tested for black and green tea infusions.

Table 5.1-3 shows confusion matrices and the performances of the classification models applied on black and green tea infusions. Among the 50 black teas from 4 countries tested, the lowest prediction error was around 32% obtained by RF; the same method also provided the highest classification performance for green tea infusions prepared by 32 samples from 4 countries. Among the black teas, teas from Sri Lanka were classified with lowest errors followed by India, China and Nepal. In the case of green teas, Chinese teas had the lowest error followed by Japan, India and Korea. The confused tea samples were mostly from the neighbouring countries. For instance, Korean green teas were confused with Chinese and Japanese green teas but not with Indian green teas with RF method. This finding is not surprising because political borders are not likely to affect tea quality while climate, growing conditions, picking method and processing traditions [3, 22, 27] are the key factors for

differentiating tea classes and their characteristics. Unfortunately we were not able to find better geographical indications for many samples.

Similar cases have been reported in literature with various classification performances when different tea samples were discriminated according to geographical origin based on their volatile profiles. Togari, Kobayashi and Aishima (1995) [10] performed the first study on the geographical origin determination of different tea categories based on their volatile profiles. The study included GC-MS analysis of 44 tea samples where tea volatiles were extracted by simultaneous dilution and extraction (SDE) method by mixing the tea samples with hot water. Black teas from India (8), Sri Lanka (4) and Japan (1) were successfully classified by supervised pattern recognition techniques, however neither oolong (China (10) and Taiwan (4)) nor green teas (15, from different regions of Japan) could be classified according to origin. Kovács et al., (2010) [8] applied electronic nose technology with electronic tongue and sensory assessment for geographical origin discrimination of five Sri Lankan teas. When electronic tongue responses of tea infusion headspace was treated with linear discriminant analysis, 100% correct classification was obtained for middle and low elevation regions (n=3) however two samples from high elevation showed overlapping. Ye, Zhang and Gu (2011) [11] analysed volatile profiles of 23 green tea samples produced in two different regions of China with SPME/GC-MS via extracting the volatiles from tea powder. They could classify the production areas of tea samples. Lee et al. (2013) [9] analysed 24 green tea samples from 8 different countries (China (7), India (1), Japan (6), Kenya (2), Korea (4), Sri Lanka (2), Tanzania (1) and Vietnam (1)) with GC-SPME method nevertheless no relationship has been found between country of origin and aroma where specific information about the samples other than origin was not known for the tested tea samples. In another study, 38 tea samples from China (2 oolong, 2 green, 3 black), Japan (5 green, 3 black, 2 oolong), India (5 black), Sri-Lanka (5 black), and Chinese Taipei (6 oolong, 2 black) were analysed by GC-MS and they were classified according to their origins by clustering methods [6]. Lastly, four varieties of oolong teas were analysed by olfaction and gustation sensing systems, the samples were classified according to producing regions by using the information each sensing system provided. When all information was merged with data fusion techniques, the discrimination power increased compared to individual classification performances suggesting the possibility to use these systems with multivariate methods for discriminating and classifying tea samples [7].

When our results and the literature were considered together, different tea types from various countries can be discriminated to some extent according to geographical origin based on their volatile emissions from dry tea leaves or tea infusions. Moreover fermented tea products are better classified than non-fermented and semi-fermented teas which was also observed from our results when we compare the classification efficiencies of black and green teas.

When black tea infusions from India (Assam (9) and Darjeeling (12)), China (Anhui (3) and Yunnan (7)), Sri Lanka (all country) and Nepal (all country) were classified according to tea producing regions a significant improvement on the classification performance was observed providing 15% average error rate (confusion matrices not shown). The results indicated 4 classes: China Anhui (class 1); Sri Lanka and India Assam (class 2) Nepal and Darjeeling (class 3) and China Yunnan (class 4) by showing the geographically close regions in the same group.

Overall, these findings point out that the regions might be better differentiators instead of the country and the regions closely located to each other share more similar properties and they are likely to create a group. Besides, there might be other factors affecting the volatile composition of different types of tea in addition to geographical location such as the age of the tea plant, plucking (fine or coarse), plucking season, tea processing, packaging of tea, conditions during storage and storage time, which should be taken into consideration.

Table 5.1-3. Confusion matrices showing the origin separation of black and green tea infusions for leave-group-out cross validation obtained by random forests (RF), penalized discriminant analysis (PDA), support vector machine (SVM) and discriminant partial least squares (dPLS) classification models

Classification method	Black tea					Average Error rate (%)	Green tea					Average Error rate (%)
RF		Chi	Ind	Sri	Nep	32.4		Chi	Ind	Jap	Kor	39.6
	Chi	58	19	0	1		Chi	74	6	4	0	
	Ind	9	146	0	1		Ind	15	0	9	0	
	Sri	0	24	0	0		Jap	10	3	42	5	
	Nep	12	35	0	1		Kor	16	0	8	0	
PDA		Chi	Ind	Sri	Nep	36.5		Chi	Ind	Jap	Kor	45.3
	Chi	55	11	4	8		Chi	69	2	10	3	
	Ind	2	108	30	16		Ind	5	5	14	0	
	Sri	0	19	5	0		Jap	17	0	28	15	
	Nep	0	24	0	24		Kor	5	0	16	3	
SVM		Chi	Ind	Sri	Nep	37.5		Chi	Ind	Jap	Kor	42.2
	Chi	57	16	1	4		Chi	62	5	14	3	
	Ind	4	105	22	35		Ind	7	14	3	0	
	Sri	0	18	6	0		Jap	17	0	31	12	
	Nep	1	26	0	21		Kor	8	0	12	4	
dPLS		Chi	Ind	Sri	Nep	43.3		Chi	Ind	Jap	Kor	48.4
	Chi	44	12	7	15		Chi	67	0	12	5	
	Ind	2	101	29	24		Ind	7	4	13	0	
	Sri	0	19	5	0		Jap	17	0	24	19	
	Nep	0	27	0	21		Kor	6	0	14	4	

5.1.4 Conclusions

In this study, for the first time, the volatile profiles of black and green teas from 12 different geographical origins were analysed by PTR-ToF-MS. The volatile compounds of a large sample set (101 samples, with replicates, both leaves and infusions) were analysed by direct injection of the headspace without altering the original tea components and destructing the original sample. The high mass resolution and sensitivity achieved by the mass analyser enabled annotation of sum formulas to the detected mass peaks. Tentative identifications lead defining important aroma compounds in black and green tea volatile emissions and pointed out the differences among them.

Black and green teas were correctly classified by the volatile compounds emitted from tea leaves and their infusions independent from their geographical origins. Classification models were built to predict the geographical origins of black and green teas. Results provided a good separation of tea origins; however countries geographically close to each other were most likely to be confused. Preliminary analysis indicated that a better discrimination of tea samples might have been achieved if teas were classified according to production region rather than just country of origin. This was not feasible here, since information about production region was available only for a limited number of samples.

Our results showed that PTR-ToF-MS fingerprints combined with multivariate statistical techniques provided successful evaluation of tea products. Considering the very promising results obtained so far, in discriminating for processing and country, it seems highly warranted to collect significantly more detailed information about the individual tea samples, for future studies. This may include e.g. information on production region, producer, harvesting season, post-harvest treatment and age of the product. It may also be significant to investigate the effect of tea leave shape and infusion conditions. Finally, it is also important to direct our interest towards the consumer, by analysing the volatile compounds release from the nospace and analysed by PTR-ToF-MS, when a tea product is being consumed, and conducting sensory profiling as well. Combining such a large spectrum of different data sets might currently seem to be a veritably challenging task; we believe this will need to be approached in steps towards a more complete understanding of the factors affecting tea aroma profiles.

Acknowledgements

This research has been funded by PIMMS (Proton Ionisation Molecular Mass Spectrometry) ITN, which is supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.

5.1.5 References

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Supplementary file 5.1

Sample code	Commercial Tea Name	Country	Region	Tea type	Notes
1	Argentina OP Flor De Oro	Argentina	Unknown	Black	
20	Golden Monkey King	China	Hunan	Black	
21	Golden Spiral	China	Yunnan	Black	
24	Keemun Congou	China	Anhui	Black	
25	Keemun Mao Feng	China	Anhui	Black	
26	Ming Orchidee	China	Yunnan	Black	
35	Sichuan	China	Sichuan	Black	
39	Wuyi Gongfu	China	Unknown	Black	
40	Yunnan	China	Yunnan	Black	
41	Yunnan Celeste	China	Yunnan	Black	
42	Yunnan Golden Bud	China	Yunnan	Black	
114	Keemun Congou	China	Anhui	Black	
125	Yunnan Flowery Orange Pekoe	China	Yunnan	Black	
126	Yunnan Gold	China	Yunnan	Black	
2	Assam Bijleejan	India	Assam	Black	Second flush, with golden tips
3	Assam Birjhora	India	Assam Bongaigoan District	Black	Lower Assam region
4	Assam Borengajuli	India	Assam (Naangaldia)	Black	
5	Assam Bukhial	India	Assam (Golaghat)	Black	
6	Assam Dirial	India	Assam (Dibrugahr)	Black	
15	Darjeeling Gielle	India	Darjeeling Teesta Valley	Black	
16	Darjeeling Lingia	India	Darjeeling (East Valley)	Black	
18	Darjeeling Pussimbing	India	Darjeeling (East Valley)	Black	
19	Darjeeling Tukdah	India	Darjeeling (East Valley)	Black	
30	Nilgiri Korakundah	India	Nilgiri	Black	
36	Sikkim Temi	India	Sikkim	Black	
101	Ambootia Autumnpluck	India	Darjeeling (Ambootia)	Black	
102	Ambootia Springpluck	India	Darjeeling (Ambootia)	Black	
103	Ambootia Rainpluck	India	Darjeeling (Ambootia)	Black	
104	Assam Goudblad Summerpluck	India	Assam	Black	
107	Darjeeling Summerpluck	India	Darjeeling	Black	
108	Dunsandle Jungle	India	Nilgiri (Dunsandle)	Black	
109	Happy Valley Autumnpluck	India	Darjeeling (Happy Valley)	Black	

(Supplementary file 5.1 continues)

Sample code	Commercial Tea Name	Country	Region	Tea type	Notes
110	Happy Valley Springpluck	India	Darjeeling (Happy Valley)	Black	
111	Happy Valley Summerpluck	India	Darjeeling (Happy Valley)	Black	
116	Margareth's Hope Springpluck	India	Darjeeling (Margareth's Hope)	Black	
117	Mokalbari Summerpluck	India	Assam (Mokalbari)	Black	
120	Sewpur Broken Orange Pekoe	India	Assam (Sewpur)	Black	
121	Sewpur Summerpluck	India	Assam (Sewpur)	Black	
122	Sikkim Summerpluck	India	Sikkim	Black	
22	Java Malabar	Indonesia	Java	Black	
23	Java Santosa	Indonesia	Java	Black	
113	Java Kertasari	Indonesia	Java	Black	
56	Kenia Kaimosi	Kenya	Ndanai (Rift Valley)	Black	
115	Kenia Flowery Orange Pekoe	Kenya	Unknown	Black	
112	Jangwon	Korea	Jeju (South Korea)	Black	
27	Nepal Antu Valley	Nepal	Unknown	Black	
28	Nepal Himalaya Tips	Nepal	Unknown	Black	
29	Nepal Maloom	Nepal	Unknown	Black	
118	Nepal Tippy Orange Pekoe	Nepal	Unknown	Black	
10	Ceylon Dimbulla	Sri Lanka	Dimbulla (Western Highlands)	Black	Orange Pekoe, whole leaf
12	Ceylon Kenilworth	Sri Lanka	Dimbulla (Western Highlands)	Black	Orange Pekoe, whole leaf
31	Nuwara Eliya	Sri Lanka	Nuwara Eliya (Central Highlands)	Black	
105	Balangoda Orange Pekoe	Sri Lanka	Ratnapura (Balangoda)	Black	
106	Blackwood Orange Pekoe	Sri Lanka	Uva (Blackwood)	Black	
119	Ruhuna Orange Pekoe	Sri Lanka	Ruhuna	Black	
123	Tippy Orange Pekoe	Sri Lanka	Unknown	Black	
124	Uva Highlands Pekoe	Sri Lanka	Uva	Black	
37	Tanzania Luponde	Tanzania	Njobe	Black	
146	Rize Turist	Turkey	Rize	Black	
147	Filiz	Turkey	Rize	Black	
148	Çay Çiçeği	Turkey	Rize	Black	
38	Vietnam O.P.	Vietnam	Unknown	Black	
43	Zimbabwe Wamba	Zimbabwe	Honde Valley	Black	

(Supplementary file 5.1 continues)

Sample code	Commercial Tea Name	Country	Region	Tea type	Notes
46	Chun Mee	China	Unknown	Green	
50	Green Monkey	China	Fujian (Wuyi Mountains)	Green	
51	Gui Pian	China	Anhui	Green	
52	Gunpowder	China	Zhejiang	Green	
55	Hou Kui	China	Hou Gang City (Yellow Mountains)	Green	
62	Long Ying	China	Zhejiang (Hangzou) (Longying Village)	Green	
63	Lu Ching	China	Zhejiang (Hangzou)	Green	
66	Pi Lo Chun Bio 1	China	Jiangsu (Suzhou)	Green	
79	Yunnan Green	China	Yunnan	Green	
129	Dragonwell Xi Hu Lung Ching	China	Zhejiang	Green	
131	Springdew Mao Feng	China	Anhui (Yellow Mountains)	Green	
134	Sencha	China	Unknown	Green	
135	Sencha Biologic	China	Unknown	Green	bio-organic
136	Snow Bud Lu Xue Ya	China	Fujian	Green	
65	Nilgiri Chamraj	India	Nilgiri	Green	
127	Ambootia Green	India	Darjeeling (Ambootia)	Green	
133	Nilgiri Green	India	Nilgiri	Green	
143	Assan Jamguri	India	Assam	Green	
44	Bancha	Japan	Kyushu	Green	
45	Bancha Arashiyama	Japan	Kyoto	Green	
48	Gabalong	Japan	Unknown	Green	
53	Gyokuro Asahi	Japan	Yame	Green	
61	Kukicha	Japan	Shizuoka	Green	
69	Sencha Makinohara	Japan	Shizuoka	Green	
71	Sencha Shimizu	Japan	Shizuoka	Green	
72	Sencha Tokiwa	Japan	Unknown	Green	
73	Sencha Uji	Japan	Kyoto	Green	
75	Tamaryokucha	Japan	Kyushu	Green	
58	Korea Jeju	Korea	Jeju Island	Green	
59	Korea Jeoncha	Korea	Jeju Island	Green	
60	Korea Sejak	Korea	Jeju Island	Green	

(Supplementary file 5.1 continues)

Sample code	Commercial Tea Name	Country	Region	Tea type	Notes
132	Nepal Green	Nepal	Unknown	Green	
67	Rwanda Green	Rwanda	Unknown	Green	
128	Blackwood Green	Sri Lanka	Uva	Green	
76	Tanzania Luonde Green	Tanzania	Njobe	Green	
77	Vietnam Shan Tran	Vietnam	Unknown	Green	
142	Vietnam Green A42o.P.	Vietnam	Unknown	Green	

Summary and future perspectives

This thesis demonstrates the applicability of PTR-ToF-MS as an efficient method for rapid, non-invasive and high-throughput characterization and discrimination of coffee aroma via headspace and nosespace analyses.

In **Chapter 2**, we address the issues related to the characterization of 100% Arabica coffees according to geographical origin based on rapid, direct and non-destructive headspace volatile compound analysis with proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS). A fingerprinting approach was adapted for discrimination of coffee samples by applying multivariate data exploration methods and advanced classification tools. **Chapter 2.1** shows a methodological approach on discrimination of roasted and ground coffee powders from three geographical origins (Brazil, Guatemala and Ethiopia). This study represents one of the first applications of rapid and automated headspace sampling procedure by combining PTR-ToF-MS to a multipurpose autosampler for a more reproducible and straightforward analysis. By adjusting the autosampler set-up parameters, it was possible to analyse 1 sample every 5 min (when the headspace is flushed before sample incubation), which can be reduced to 1 sample every 2 min if flushing the headspace before sample incubation is not desired. This study involved two different batches of Brazil, Guatemala and Ethiopia, which were roasted under the same conditions to a medium roast degree and ground to same particle size. There were no significant differences observed in the aroma profiles between two batches of same coffee origin according to one-way ANOVA results ($p < 0.01$) and PCA showed successful separation of coffee origins. A PLS-DA classification model was built where the volatile emissions of one batch were used to predict the second batch. Additionally, PLS-DA suggested mass peaks (VIP values > 1.5) significant for separation of coffee origins. For instance, tentative identification of these mass peaks pointed out pyrazines for Brazilian coffee and terpenes for Ethiopian coffee, those could be associated to the chocolate and roasted notes for Brazilian coffee and fruity and flowery notes for Ethiopian coffee. The associated volatile compounds can be referred as key flavour descriptors for particular Arabica coffee origins. The results overall, highlighted the applicability of rapid headspace analysis with PTR-ToF-MS to characterize coffee aroma and to ensure quality control during coffee production. **Chapter 2.2** describes a comprehensive follow-up study by extending the number of coffee origins; and by measuring the headspace volatile compounds of both roasted and ground coffees and their brews prepared by using a stove-top coffee maker (moka pot). For the first time, PTR-ToF-MS in Switchable Reagent

Ions (SRI) mode was used to analyse coffee aroma compounds by using H_3O^+ , NO^+ and O_2^+ as ionisation agents. Six Arabica coffee samples from Brazil, Colombia, Costa Rica, Ethiopia, Guatemala and India were successfully separated according to geographical origins by using not only H_3O^+ but also NO^+ and O_2^+ as precursor ions by advanced classification models. Merging all the volatile profiles obtained by three different ionisation modes via data fusion further improved the classification efficiency. One way-ANOVA showed significant differences between coffee origins and changes during coffee brewing indicating possible effects of coffee geographical origin on volatile compound release.

Chapter 3 covers the development of a methodology based on rapid and simple monitoring of volatile compounds released from a single coffee bean during different stages of roasting. PTR-ToF-MS has been used for the first time to analyse the volatile compounds released from 468 single coffee beans come from different geographical origins. Due to the high sensitivity provided by PTR-ToF-MS, it was possible to detect volatile compounds at very low levels, which allowed visualizing the differences in volatile formations at a single bean level. We observed a reduction in the amount of terpene fragments and increase in heat induced volatile compounds. Clear origin signatures were observed especially in the concentration of the volatiles released. Depending on the phase of roasting, some volatile compounds were released earlier than the others and vice versa.

Chapter 4 includes simultaneous combination of Nosespace analysis and dynamic sensory methods for understanding coffee flavour perception and effect of coffee roasting degree and sugar on VOC release. Dark and light roasted espresso coffees were evaluated by 18 trained panellists in the presence and absence of sugar. Sensory evaluation and in-nose aroma release provided successful discrimination of coffees according to roasting degrees with an increase in overall dominance of attributes (i.e. roasted, burnt) and the quantity of volatiles released in the nose. Sugar addition completely modified the sensory description of coffees however; its effect on retronasal aroma release was rather not significant. Indeed, due to the presence of taste-smell perceptual interactions and the congruence effect between sweet taste and some flavors of coffee, the interpretation of sugar effect remained more visible for TDS evaluation. The volatile compounds showed two different release behaviours analysed by clustering methods signifying the degree of their persistence in nose which were strongly linked to the dominance of selected aroma attributes.

Chapter 5 shows the application of PTR-ToF-MS on discrimination of black and green teas according to tea type and geographical origin. A large sample set was collected (101 teas, without additional flavouring) and the volatile emissions of dry tea leaves and tea infusions were analysed for the first time with PTR-MS technology. The separation of black and green teas were clear and results provided a good separation of tea origins. However countries geographically close to each other were most likely to be confused. Preliminary analyses indicated that a better discrimination of tea samples might have been achieved if teas were classified according to production region rather than just country of origin.

In this work, we have addressed several aspects of PTR-ToF-MS applications in flavour science. Considering the very promising results and the developed methodologies so far, PTR-ToF-MS appears as an effective analytical tool to study the relationships between volatile organic compounds *in vitro* (headspace) and *in vivo* (nosepace) product discrimination and characterization. The results highlighted significant issues in authentication and discrimination of high value food products not only via static headspace measurements but also via real-time monitoring during product consumption. For the first time, we have utilized Switchable Reagent Ion System- SRI (that the primary ion can be switched from H_3O^+ to other ions, such as O_2^+ or NO^+) to measure the volatile compounds released from coffee which improved the discrimination and characterisation capabilities. We believe that, the application of SRI system in food sciences can be extended to other fields such as fruit VOC emissions and bioprocess monitoring.

For future studies, the automated headspace sampling system can be successfully applied for rapid screening of large sample sets and to diverse food matrices. The outcomes also direct our interest towards the consumer, by analysing the volatile compounds released during food consumption and investigate the links between sensory perception and volatile compounds not only in the case of coffee matrix but also for other products. Even though the newly developed fastGC-PTR-ToF-MS system has not been utilized in this thesis, the different applications of this methodology are of interest, in particular for separation of monoterpenes in coffee and/or tea which constitute a significant group volatile compounds responsible for flowery and fruity notes and also might be considered as volatile geographical origin markers.

Further applications of these present methodologies are expected in the near future for other food matrices.

Curriculum vitae

Personal information

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Education

- 2013-2015 PhD student in Analytical Chemistry, Leopold-Franzens University of Innsbruck, Institute of Analytical Chemistry and Radiochemistry, Innsbruck, Austria
- 2008-2011 Master Degree in Food Engineering, Hacettepe University, Ankara, Turkey
Thesis “*The Effects of Washing, Drying and Dehulling on Some Fusarium Toxins in Corn and Wheat*”
- 2004-2008 Bachelor Degree in Food Engineering, Hacettepe University, Ankara, Turkey
Thesis “*Food Allergens*”

Work experience

- 2013-2015 Early Stage Researcher in *Proton Transfer Molecular Mass Spectrometry (PIMMS) Initial Training Network, Grant Agreement Number: 287382*
Volatile Compounds Platform, Department of Food Quality and Nutrition, Fondazione Edmund Mach, San Michele all’Adige (TN), Italy
Personal Project “*The role of aroma and flavor compounds in the perception of food by nosespace analysis*”
- 2009-2013 Research assistant
Food Engineering Department, Hacettepe University, Ankara, Turkey

Analytical skills

PTR-ToF-MS, HPLC, GC, e-tongue, ELISA and dipstick assays, RVA (Rapid Visco Analyzer), DSC (Differential Scanning Calorimeter), Lumisizer, Farinograph, Alveograph, Consistograph, UV-vis spectrometer, PCR

Medium knowledge: R programming language
Basic knowledge: Matlab and Visual Basic

Languages

Turkish (mother tongue), English (B1), Italian (A2), German (A1)

List of publications

Papers published in refereed journals

1. **Sine Yener**, Andrea Romano, Luca Cappellin, Tilmann D. Märk, José Sánchez del Pulgar, Flavia Gasperi, Luciano Navarini, Franco Biasioli. PTR-ToF-MS Characterization of Roasted Coffees (*C. arabica*) from Different Geographic Origins, 2014, *J. Mass Spectrom.* 49: 929-935.
2. Mathilde Charles, Andrea Romano, **Sine Yener**, Massimo Barnabà, Tilmann D. Märk, Luciano Navarini, Franco Biasoli, Flavia Gasperi. Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and *in-vivo* nosespace analysis. *Food Research International* 69: 9-20, 2015.
3. **Sine Yener**, Andrea Romano, Luca Cappellin, Pablo M. Granitto, Luciano Navarini, Tilmann D. Märk, Flavia Gasperi, Franco Biasioli. Tracing coffee origin by direct injection head-space analysis with PTR/SRI-MS. *Food Research International* 69: 235-243, 2015.

Papers submitted or in preparation

1. **Sine Yener**, José A. Sánchez-López, Pablo M. Granitto, Luca Cappellin, Tilmann D. Märk, Ralf Zimmerman, Günther Bonn, Chahan Yeretizian, Franco Biasioli. Rapid and direct volatile compound profiling of black and green teas (*Camellia sinensis*) from different countries with PTR-ToF-MS. *Talanta*, 2016, *Submitted*.
2. **Sine Yener**, Luciano Navarini, Valentina Lonzarich Luca Cappellin, Tilmann D. Märk, Günther Bonn, Franco Biasioli. Insight into single bean coffee roasting with PTR-ToF-MS. *Journal of Mass Spectrometry*, (Application note), *Submitted*.
3. **Sine Yener**, Luciano Navarini, Valentina Lonzarich, Luca Cappellin, Mathilde Charles, Flavia Gasperi, Franco Biasioli, PTR-ToF-MS as a new tool in coffee aroma research for direct and real-time analysis of volatile compounds. *Coffee: Production, Consumption and Health Benefits* (Book Chapter), Nova Publishers, *Submitted*.
4. Vittorio Capozzi, **Sine Yener**, Iulia Khomenko, Brian Farneti, Luca Cappellin, Matteo Scampicchio, Flavia Gasperi, Franco Biasioli, Applications of PTR-ToF-MS coupled with autom and tailored data analysis in the food sector: bioprocess monitoring, massive screening, nosespace analysis. *Journal of Visualized Experiments (JOVE)*, *submitted*.
5. Salim Makhoul, **Sine Yener**, Iulia Khomenko, Vittorio Capozzi, Luca Cappellin, Eugenio Aprea, Matteo Scampicchio, Flavia Gasperi, Franco Biasioli, Rapid non-invasive quality control of semi-finished products for the food industry by direct injection mass spectrometry headspace analysis: the case of milk powder, whey powder, and anhydrous milk fat. *Rapid communications in mass spectrometry*, *To be Submitted*.
6. **Sine Yener**, Valentina Acierno, Luciano Navarini, Saskia van Ruth, Franco Biasioli, Electronic tongue as a tool for discrimination of coffee brews according to geographical origin, *To be Submitted*.

Conference proceedings and posters

Poster Presentations

1. **Sine Yener**, José Antonio Sánchez López, Valentina Acierno, and Xianghui Kong, Soft chemical ionisation mass spectrometry and food science in the framework of PIMMS. 3rd MS Food Day, 12-14 October 2013, Trento, Italy.
2. Mathilde Charles, Andrea Romano, **Sine Yener**, Sofia Miori, Franco Biasoli, Luciano Navarini, Flavia Gasperi, Understanding the dynamics of perception by the combination of sensory and instrumental methods: preliminary results of a study on coffee. 3rd MS Food Day, 12-14 October 2013, Trento, Italy.
3. **Sine Yener**, Andrea Romano, Luca Cappellin, José Sánchez del Pulgar, Tilmann D. Märk, Flavia Gasperi, Luciano Navarini, Franco Biasoli, PTR-ToF-MS for characterization of roasted ground coffees from different origins and secondary shelf life monitoring. 3rd MS Food Day, 12-14 October 2013, Trento, Italy.
4. **Sine Yener**, Mathilde Charles, Andrea Romano, Luca Cappellin, Elisabetta Benozzi, Sofia Miori, Franco Biasoli, Tillman D. Maerk, Luciano Navarini, Flavia Gasperi. Dynamics of flavor perception: combining sensory methods and direct injection mass spectrometry. 3rd International Conference on Food Oral Processing (FOP2014), 29 June-02 July 2014, Wageningen, The Netherlands.
5. Mathilde Charles, Andrea Romano, **Sine Yener**, Franco Biasoli, Luciano Navarini, Flavia Gasperi. Dynamism of flavour perception phenomena: complementarity of sensory and instrumental temporal methods, 6th European Conference on Sensory and Consumer Research, 7-10 September 2014, Copenhagen, Denmark.
6. **Sine Yener**, Andrea Romano, Luca Cappellin, Pablo M. Granitto, Flavia Gasperi, Luciano Navarini, Tilmann D. Märk, Günther K. Bonn, Franco Biasoli. Insight into coffee aroma with PTR-ToF-MS: from the green beans to brewed coffee, ASAC-JunganalytikerInnenforum 2015, 12-13 June 2015, University of Innsbruck, Innsbruck, Austria.
7. **Sine Yener**, José A. Sánchez-López, Pablo M. Granitto, Luca Cappellin, Tilmann D. Märk, Günther Bonn, Ralf Zimmerman, Chahan Yeretian, Franco Biasoli. Classification of Black and Green Teas Based on Volatile Compound Profiles Analyzed with PTR-ToF-MS. 4th MS Food Day, 7-9 October 2015, Foggia, Italy.
8. José Antonio Sánchez-López, **Sine Yener**, Günther Bonn, Tilmann Märk, Ralf Zimmermann, Franco Biasoli, Chahan Yeretian. Exploring extraction dynamics of green tea volatiles by PTR-ToF-MS. 4th MS Food Day, 7-9 October 2015, Foggia, Italy.

Oral Presentations

1. **Sine Yener**, Mathilde Charles, Andrea Romano, Luca Cappellin, Elisabetta Benozzi, Sofia Miori, Franco Biasoli, Tillman D. Maerk, Luciano Navarini, Flavia Gasperi. Dynamics of flavor perception: combining sensory methods and direct injection mass spectrometry. 3rd International Conference on Food Oral Processing (FOP2014), 29 June-02 July 2014, Wageningen, The Netherlands.
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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Alle Stellen, die wörtlich oder inhaltlich den angegebenen Quellen entnommen wurden sind als solche kenntlich gemacht.

Die vorliegende Arbeit wurde bisher in gleicher oder ähnlicher Form noch nicht als Dissertation eingereicht.

Datum

Untherschrift