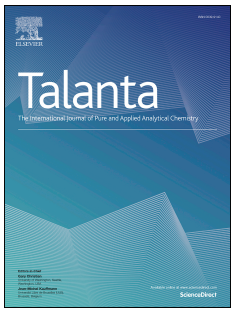

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Maria Mazzucotelli – Methodology; Formal analysis; Investigation; Data Curation; Visualization; Writing - Original Draft; Writing - Review & Editing

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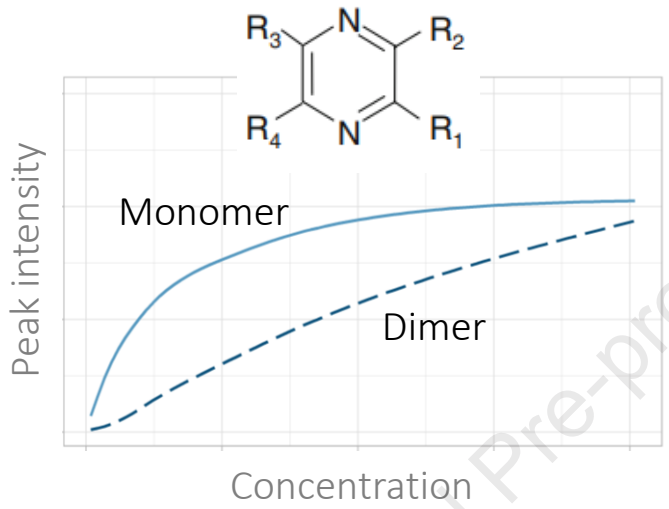
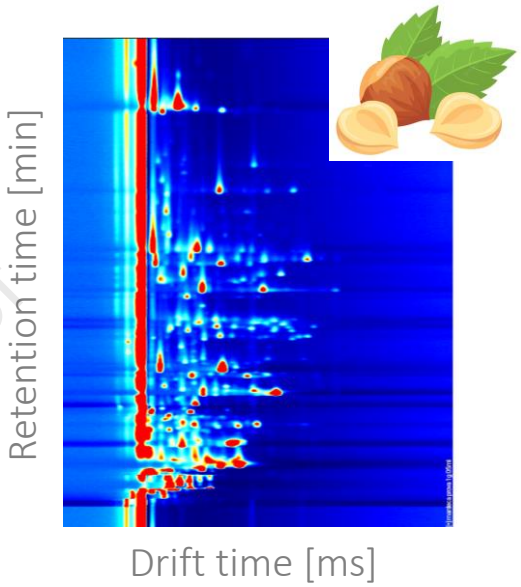
Andrea Cavallero – Conceptualization; Resources; Project administration; Writing - Review & Editing

Eugenio Aprea - Conceptualization; Project administration; Writing - Review & Editing

Franco Biasioli – Conceptualization; Project administration; Writing - Review & Editing

Alkyl pyrazine determination by SHS-GC-IMS

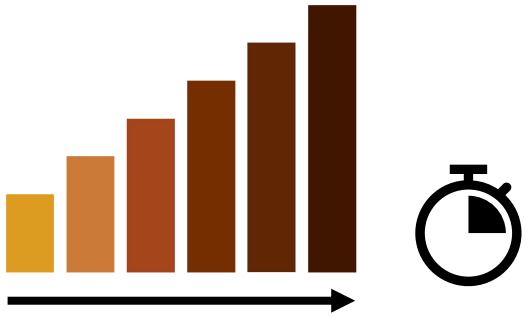




Response assessment and standardization

Identification

Roasting monitoring



Monitoring alkyl pyrazines in roasted hazelnuts by SHS-GC-IMS: IMS response assessment and standardization

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Abstract

Gas chromatography coupled with ion mobility spectrometry (IMS) is an analytical tool which is rapidly becoming widespread in the analysis of food volatiles. Despite this increasing popularity, an assessment of the IMS response for several flavor compound classes is not yet available. This study focuses on alkyl pyrazines and their determination in roasted hazelnut pastes. These Maillard reaction products are crucial to determine the aromatic profile of roasted foods and are suitable markers for industrial roasting monitoring. The instrumental response of 8 alkyl pyrazines was studied using a model matrix and a matrix matching approach. The results showed a relevant effect of the pyrazine ring substitution pattern on the concentration-response curve trends, highlighting that an external standardization of the IMS response is required to make possible relative abundance comparisons between analytes. A response standardization was therefore developed and applied to determine alkyl pyrazines in samples with different roasting intensity and geographical and botanical origin.

Keywords

SHS-GC-IMS; Alkyl pyrazines; Detector response; Standardization; Hazelnut; Roasting

1) Introduction

The development of Ion Mobility Spectrometry (IMS) traces back to the 1960s, and it has been used, for many applications, as a standalone analytical technique to detect compounds in trace amounts [1]. IMS is a gas phase separation technology, operating at atmospheric pressure, and based on two sequential processes i) the ionization of volatile organic compounds (VOCs) in a reaction region (the ion source region); ii) the separation of the ions in a drift region according to their mobility in an electric field through a counterflowing gas (drift gas). Being the mobility of ions inversely proportional to their collisional cross-section, the IMS was defined as an ion separator based on the size/charge [2].

Since the early stages of the IMS development, the potentialities of using it as a detector after a chromatographic separation have been explored [3]. Although the first paper reporting the coupling of IMS detection with gas chromatography separation was published in 1970 [4], only recently we are witnessing a rapid increase in the popularity of GC-IMS commercial instruments, especially for food volatilome analysis, with a big interest in traceability and flavor characterization purposes [5, 6]. The hyphenation of these two techniques results in an analytical platform characterized by high sensitivity, low detection limits, and an orthogonal bidimensional separation of analytes.

The capability to detect VOCs at low concentrations is the main advantage of GC-IMS since it makes it possible to apply static headspace (SHS) sampling and to avoid preconcentration steps. This reflects in minimal sample preparation and relatively short analysis time (less than an hour). Additionally, from a hardware perspective, no vacuum system is needed, resulting in analytical platforms with reduced bench space requirements, simple maintenance, and low energy consumption. The combination among the user friendliness of the hardware, the limited sample preparation required, and the overall rapidity of analytical response makes SHS-GC-IMS a promising analytical tool to be applied both for academic research and industrial food processes monitoring [7], where the implementation of robust and simplified protocols is highly desirable.

On the other hand, the drawbacks of IMS detection must be taken into account when this technique is applied. First, the formation of multiple ionized species (the protonated monomer and the proton bound

dimer) from a single analyte is a peculiarity which significantly complicates the interpretability of the analytical output. As regards the peak identification, the mobility coefficient of the detected ions obtained with IMS detection is a useful information and libraries of mobility coefficients have been made available from instrument manufacturers. However, even their combination with retention indices is not sufficient for a reliable peak identification. In this case, analyte confirmation can only be accomplished by comparison with pure standards or using an additional MS detector [8]. Lasty, IMS has an overall limited dynamic range and shows linear response only for 1-2 orders of magnitude of concentration, due to the radioactive source. These hurdles might explain why only few published studies so far address the challenge of a quantitative approach [9 - 12], while most of the available literature focuses on the characterization of food matrices by SHS-GC-IMS applying untargeted fingerprinting or comparative cross-sample analysis.

In the vast majority of the recently published literature, the IMS is operated in positive ionization mode. Under these operative conditions, volatile compounds with proton affinity higher than water (691 kJ/mol [13]) are ionized and detected, thus providing a non-selective response to several chemical functionalities (alcohols, amines, ketones, aldehydes), similar to a flame ionization detector (FID) [14]. Most of the aroma compounds are, therefore, detectable by SHS-GC-IMS.

Clear explanations of the IMS response were reported for alkyl alcohols, aldehydes and ketones by Jurado-Campos *et al* [15], in particular related to the formation and stability of proton-bound clusters. However, a systematic assessment of the detector response for most of the other aroma compound classes has not yet been fully addressed, neither in terms of sensitivity, nor multiple ionized species formation. Moreover, the underestimation of differences in the IMS responses might lead to misinterpretations of the analytical output, in particular if assumptions are drawn from the relative intensities of peaks.

This study focuses on pyrazines, a class of volatile molecules with a well-established importance as aroma compounds. While pyrazines have been detected in food matrices characterized by SHS-GC-IMS analysis [16, 17], there is no contribution specifically investigating the detector response to this class. They are heterocyclic compounds with a common chemical structure: a monocyclic aromatic ring with two atoms of

nitrogen in *para* position. The positions 2-, 3-, 5-, and 6- of the ring can be substituted with different groups. Alkyl pyrazines are found in thermally treated foods (generated *via* Maillard reaction) and fermented food (produced by microbial metabolism), while alkoxy pyrazines are present in raw vegetables as secondary plant metabolites [18, 19]. Due to their low odor-thresholds, they are crucial to determine the aromatic profile even though their concentrations in food matrices is low (ng/kg), thus providing an interesting case study for a GC-IMS targeted approach.

From an industrial perspective, alkyl pyrazines are particularly important because they are generated during the roasting processes, which greatly contribute to defining the aroma of roasted foods (coffee, cocoa, and roasted nuts – hazelnuts and peanuts) with characteristic aromatic notes (roasted, nutty, earthy, burnt). Heating conditions (temperature and duration) are known to influence not only the level of the formed pyrazines, but also their type [20]. It is therefore clear that the monitoring of these compounds during industrial roasting processes is an effective strategy to control the aroma development of roasted products. For these reasons, alkyl pyrazines have been proposed as roasting markers, and their ratios as roasting indices [21, 22].

The final aim of this study is the assessment of SHS-GC-IMS as a rapid technique to monitor alkyl pyrazines within the context of the industrial roasting of hazelnuts.

The research was structured in two phases: i) the evaluation of the GC-IMS response for a set of target alkyl pyrazines; ii) the development of an external calibration protocol to standardize the GC-IMS response and determine the content of alkyl pyrazines in roasted hazelnut paste samples from different geographical and botanical origins and processed with different roasting intensity.

2) Materials and Methods

2.1 Chemicals and matrices

2-methylpyrazine (2M) (CAS 109-08-0), 2,5-dimethylpyrazine (25DM) (CAS 123-32-0), 2,6-dimethylpyrazine (26DM) (CAS 108-50-9), 2-ethylpyrazine (2E) (CAS 13925-00-3), 2,3,5-trimethylpyrazine (TRI) (CAS 14667-55-1), 2,3,5,6-tetramethylpyrazine (TETRA) (CAS 1124-11-4), and a mixture of isomers of 2-ethyl-3(5or6)-

dimethylpyrazine (2E35DM – 61.4% - and 2E36DM – 38.6%) (CAS 27043-05-6) pure standards were purchased from Merck (Merck KGaA, Darmstadt, Germany). Stock solutions (20.00 μg g^{-1}) were prepared solubilizing pure standards in a mix of medium chain triglycerides (MCT) (chain length ranging from 6 to 10 carbons) provided by Oleon (Ertvelde, Evergem, Belgium). MCT was selected as solvent because, being nonvolatile, minimizes the interferences with the IMS detection. Furthermore, since the roasted hazelnut paste has a relevant fat content (50-60%), this oil-like solvent ensured miscibility and provided a possible suitable model matrix. All the diluted standard mixtures were prepared in the same solvent.

A deodorized roasted hazelnut paste (HZ deo), provided by Soremartec Italia Srl (Alba, Cuneo, Italy), was used as a matrix-matched blank sample. The removal of volatile components from the paste was obtained by a proprietary physical treatment.

2.2 Hazelnut samples

Hazelnut paste samples, supplied by Soremartec Italia Srl (Alba, Cuneo, Italy), were obtained processing raw kernels (*Corylus avellana* L.) from different geographical and botanical origins: Tonda Gentile Romana monocultivar hazelnuts (RO) from Lazio region (Italy), Tonda Gentile delle Langhe monocultivar hazelnuts (TGL) from Piemonte region (Italy), and Akçakoca hazelnuts (AK) from Turkey. The harvesting year was 2021 for all the samples. The raw kernels were stored in sealed bags at 4°C and in controlled humidity conditions for six months prior to roasting.

The roasting process was carried out in a pilot scale infrared roaster at 140°C. Roasted kernels were sampled at three times (15 min, 20 min and 25 min), to determine the content of alkyl pyrazines at different roasting levels and processed to obtain the pastes. For SHS-GC-IMS analysis 1 g of paste was placed in a 20 ml HS vial. Each sample was analyzed in triplicate. All the sample vials were stored at -20°C until the measurement.

2.3 GC-IMS detector response curves and matrix effect assessment

Concentration levels were prepared directly in the headspace vials (20 mL crimp vials), in a range between 0.1 μg g^{-1} and 100 μg g^{-1}. Each level was prepared in triplicate. The desired in-vial concentrations were obtained by adding to 1 g (± 0.0086 g) of tested matrix 0.1 g (± 0.0055 g) of the corresponding standard

mixture (approximately 1:10 dilution). The actual weights of matrix and standard mixture were annotated and used to calculate the in-vial concentrations of the concentration levels.

In order to evaluate the matrix effect of the hazelnut paste, the concentration-response curves were prepared and analyzed in parallel in the two tested matrices: MCT (the model matrix), and the deodorized roasted hazelnut paste (HZ deo) (matrix-matched blank sample) following the same procedure.

All the concentration levels were prepared one day before the HS-GC-IMS measurement, and vortexed for 30 seconds both after preparation and before running the analytical sequence to ensure homogeneity and analyte mixing in the matrix.

2.4. Identification of alkyl pyrazines in roasted hazelnut paste

Peak identification of alkyl pyrazines in the roasted hazelnut paste samples was performed by comparing the retention and drift times of pure standard solutions analyzed under the same analytical conditions. The matching of the experimental drift times (relative to the reactant ion peak - RIP) with those reported in the built-in library of the VOCal software (see 2.6) was also verified. The identification was confirmed by spiking increasing concentrations of the target analytes into a hazelnut paste sample.

2.5. Multiple point external standardization

To standardize the IMS detector response and quantify alkyl pyrazines in hazelnut pastes samples an external calibration approach was applied. A calibration mixture (40 µg g^{-1} in MCT) was prepared from the stock solutions of the target analytes identified in the samples (2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl-, and 2,3,5-trimethylpyrazine). The calibration mixture was diluted in the same solvent to obtain the set of working solutions to prepare the calibration curves. Based on the preliminary results, the deodorized hazelnut paste was used as a matrix for the external standards to ensure the same matrix effect of the samples (matrix-matched calibration). The calibration levels were prepared in triplicate directly in the headspace vials, as described in 2.3, to obtain a set of calibration levels between 0.01 µg g^{-1} and 1 µg g^{-1}. Blanks were prepared by adding 0.1 g of MCT to 1 g of HZ deo.

2.6. Instrumental set-up

Analyses were carried out on a FlavourSpec GC-IMS system (^{3}H-IMS) (G.A.S., Dortmund, Germany) equipped with a HT2000H headspace autosampler (HTA, Brescia, Italy) and a polar column MXT-Wax 30 m, 0.53 mm *dc*, 0.5 µm *df* (Restek Corporation, Bellefonte, US). Nitrogen (99.999% purity grade) was used as carrier gas for the chromatographic separation and as drift gas. The IMS operated in positive ionization mode. Instrument control and data acquisition were performed with the Sequence Designer software (G.A.S., Dortmund, Germany). Data visualization and peak integration were performed with VOCal software (G.A.S., Dortmund, Germany).

2.6.1 HS-GC-IMS parameters

- GC-IMS

Injector and transfer line 1 (inj-oven) temperature 80°C; GC and transfer line 2 (oven-IMS) temperature 60°C, IMS temperature 45°C. GC column flow program: 2 ml/min (constant flow) for 6 min, from min 6 the flow was gradually increased up to 12 ml/min at 16 min, then up to 50 ml/min at 19.5 min, up to 75 ml/min at 22.5 min, up to 124 ml/min at 27 min, up to 150 ml/min at 27 min, ending with 3 min at 150 ml/min constant flow to avoid carryover effects. The total GC runtime was 30 min. IMS drift flow: 150 mL/min.

- SHS sampling

The vials were incubated at 60°C for 20 min under constant agitation. HS syringe temperature was 80°C. For the first set of experiments (phase 1 - detector response curves and matrix effect assessment) the injection volume was 0.5 mL. It was increased to 1.0 mL for the quantification (phase 2 - calibration curves and samples) to improve the detection of alkyl pyrazines present in traces in the samples (2-ethylpyrazine and 2.6-dimethylpyrazine) or with a higher limit of detection (2,3,5-trimethylpyrazine).

2.7. Data processing

The signal intensity of the selected peaks was extracted as raw data with the quantitative module of the VOCal software. Peak height was selected as the intensity measure. Microsoft Excel (version 2210, Redmond, WA, USA) was used to collect data and to perform the calibration and quantification calculations. R.4.2.1. (R

Foundation for Statistical Computing, Vienna, Austria) internal statistical functions and the external packages "ggplot2" were used to generate the detector response curves.

3) Results and discussion

3.1 Evaluation of the GC-IMS response for target alkyl pyrazines

3.1.a Detector response curves

The IMS detector response for the target alkyl pyrazines was evaluated over 2-3 orders of magnitude of concentrations to investigate it both in terms of minimum detectable concentration (low concentration levels), and in terms of proton-bound dimer formation (high concentration levels).

To identify the suitable concentration range for the detector response curves, a preliminary experiment was carried out analyzing solutions of the alkyl pyrazines of interest at 1 and 5 µg g^{-1}. The 2D topographic plot (graphical output of the GC-IMS analysis) (**Figure 1**) shows differences in the GC-IMS response for the investigated analytes.

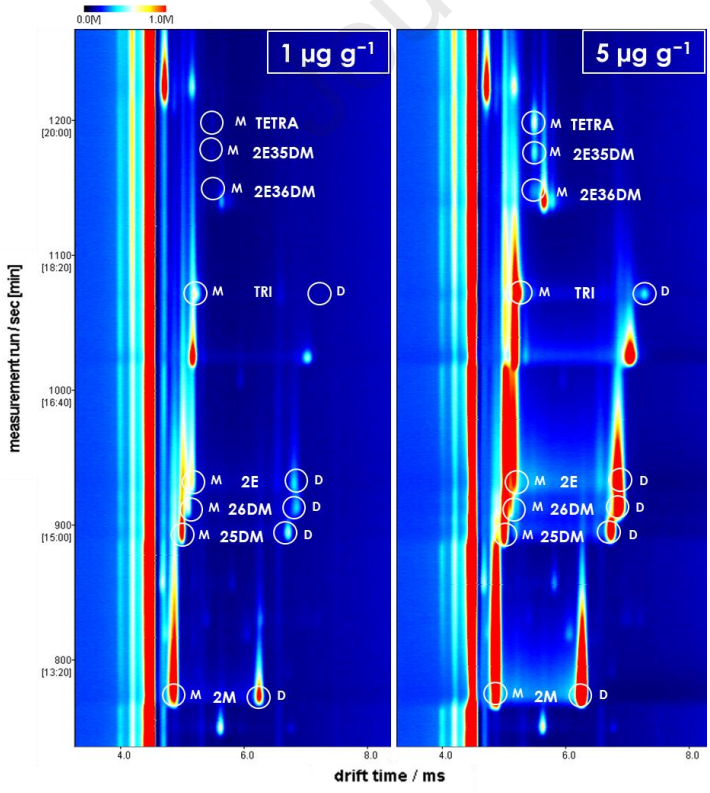

Fig. 1 - *2D topographic plot (chromatogram x mobilogram) of two solutions of alkyl pyrazines (matrix MCT) having concentration of 1 µg g^{-1} (left plot) and 5 µg g^{-1} (right plot). 2M: 2-methylpyrazine, 25DM: 2,5-dimethylpyrazine, 26DM: 2,6-dimethylpyrazine, 2E: 2-ethylpyrazine, TRI: 2,3,5-trimethylpyrazine, TETRA: 2,3,5,6-tetramethylpyrazine, 2E35DM: 2-ethyl-3,5-dimethylpyrazine, 2E36DM: 2-ethyl-3,6-dimethylpyrazine. M: protonated monomer, D: proton bound dimer.*

For 2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, and 2-ethylpyrazine both the protonated monomer and the proton bound dimer were detectable at 1 µg g^{-1}, and the peak signals were saturated at 5 µg g^{-1}. On the opposite, for 2,3,5,6-tetramethyl-, 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine, at 1 µg g^{-1}, only the protonated monomer peak was observed in the 2D topographic plot, with a low signal intensity. For 2,3,5-trimethylpyrazine an intermediate behavior was observed. Based on these first observations, the following concentration ranges were selected to study the detector dynamic range for the target analytes: 0.1 – 10 µg g^{-1} for 2-methyl- , 2,5-dimethyl-, 2,6-dimethyl-, and 2-ethylpyrazine; 1 – 100 µg g^{-1} for 2,3,5,6-tetramethyl-, 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine; 0.1 – 100 µg g^{-1} for 2,3,5-trimethylpyrazine.

Figure 2a reports the concentration response curves of 2-methyl-, 2,5-dimethyl- and 2,3,5-trimethylpyrazine analyzed in the concentration range 0.1 - 10 µg g^{-1}. Despite their common chemical structure, relevant differences in the IMS detector response curves can be observed for the alkyl pyrazine homologues. The most evident difference is the slope of the curves corresponding to the proton bound dimer signals (dark blue), and, as consequence, the ratio of the two ionized species peak signals. Considering the aromatic ring substitution, we hypothesized a less energetically favorable formation of the proton bound dimer for higher homologous, due to the steric hindrance of the methyl groups.

The results obtained by comparing the response curve for 2,3,5-trimethylpyrazine within the higher concentration range (1 - 100 µg g^{-1}) with that of 2,3,5,6-tetramethylpyrazine - **Figure 2b** - were consistent with this hypothesis. While for trimethylpyrazine the signal increase for the proton bound dimer was

observable at high concentration, for tetramethylpyrazine, which is more sterically encumbered, the peak intensity of the dimer remained low along all the concentration range analyzed.

Lastly, as firstly observed in the preliminary experiment, also the lower detectable concentrations, the detector sensitivity (curve slope), and the concentration level leading to monomer peak signal saturation (curve plateau) were different for the alkyl pyrazines studied.

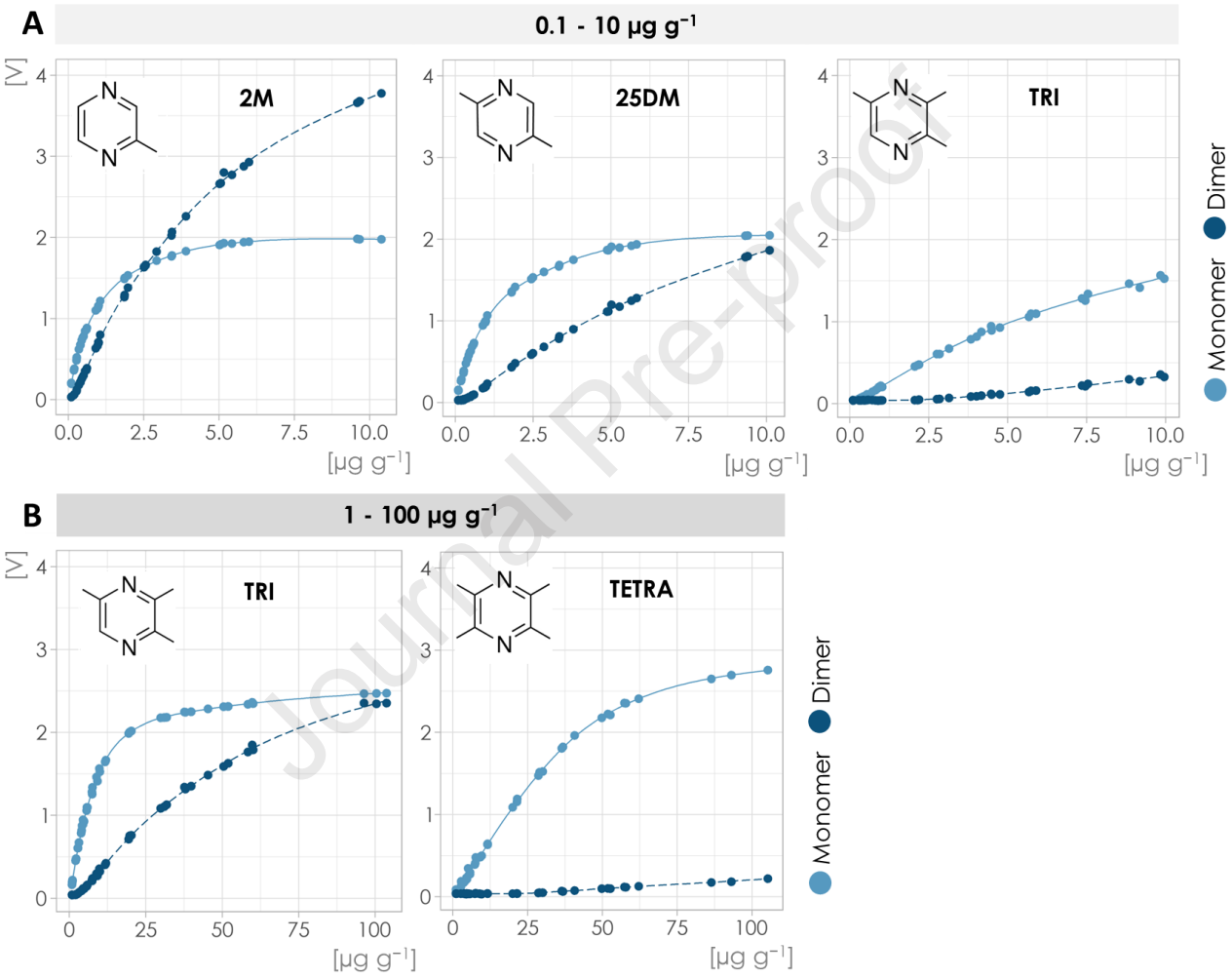

Fig.2 - *SHS-GC-IMS response curves of 2-methylpyrazine (2M), 2,5-dimethylpyrazine (25DM) and 2,3,5-trimethylpyrazine (TRI) with concentration range 0.1 – 10 µg g^{-1} (matrix MCT) (**A**), and 2,3,5-trimethylpyrazine (TRI) and 2,3,5,6-tetramethylpyrazine (TETRA) with concentration range 1 – 100 µg g^{-1} (matrix MCT) (**B**). Detector response extracted from raw data as peak height [V].*

On the one hand, this experimental evidence proved that the relative intensities of the 2D plot peaks could not be used as quantitative measure of analyte abundances, because of the remarkable differences in the GC-IMS response toward different analytes (also for homologous classes having a common chemical structure, such as alkyl pyrazines). On the other hand, they also highlighted the unsuitability of semi-quantification based on an internal standard as standardization strategy, because the difference in response factor of the analytes would lead to inaccurate semi-quantitative results. An external standardization approach is therefore fundamental to obtain quantitative information for the analytes of interest.

3.1.b Matrix effect

Roasted hazelnut paste is a complex matrix in terms of composition, with a high percentage of fat (50-60 %), but also relevant content of proteins (15%), carbohydrates (17%) and fiber (10%) [23, 24]. An oil-like solvent, such as the MCT used in this study, could provide a model system for fat-rich food matrices [25], however, the release of alkyl pyrazines has been reported to be influenced by the protein content in the food matrix [26].

The matrix effect of the hazelnut paste was assessed in the first phase of the research using the deodorized roasted hazelnut paste to prepare the concentration levels with a matrix matching approach. The comparison of the IMS detector response curves in the two tested matrices, reported in **Figure 3** (for 2,5-dimethyl- and 2,3,5-trimethylpyrazine), shows a non-negligible matrix effect. In particular, the lower percentage of fat content of the hazelnut paste, compared to the model matrix MCT, results in higher peak signals for the target alkyl pyrazines. Based on this result, for the second phase of this research, the deodorized roasted hazelnut paste (HZ deo) was used to prepare the external standardization calibration levels, in order to ensure the same matrix effect of the samples.

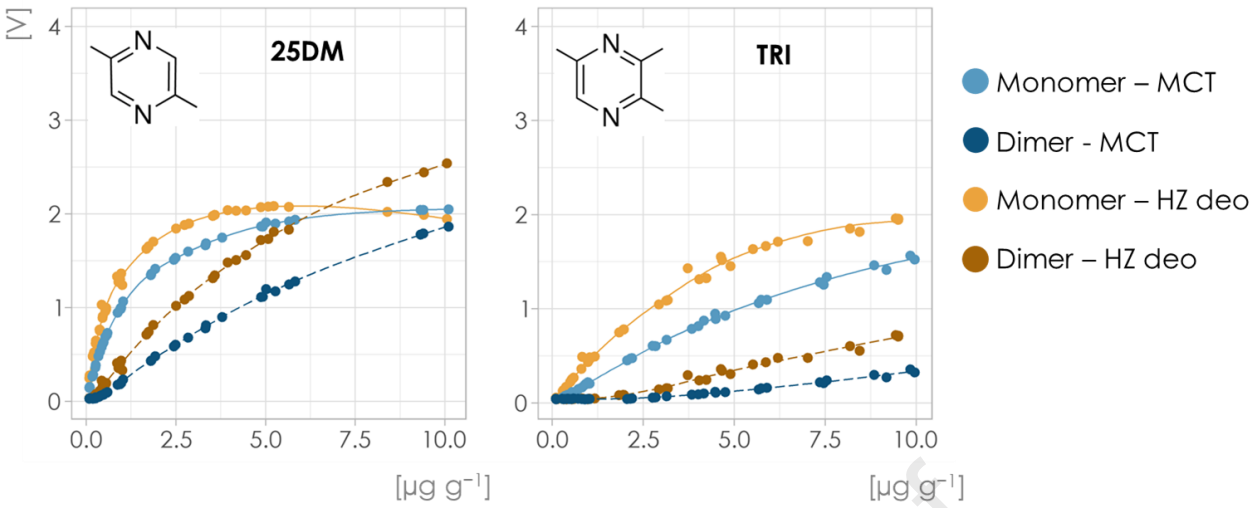

Fig.3 - *Comparison of the SHS-GC-IMS response curves (for 2,5-dimethylpyrazine on the left, and 2,3,5-trimethylpyrazine on the right) obtained with concentration levels prepared in the model matrix (MCT – blue) and with a matrix matching approach (deodorized hazelnut paste - brown). Concentration range 0.1 – 10 µg g^{-1}. Detector response extracted from raw data as peak height [V].*

3.2 Alkyl pyrazines in roasted hazelnut paste samples

The following five alkyl pyrazines were identified in the roasted hazelnut paste samples: 2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl- and 2,3,5-trimethylpyrazine (**Figure 4**). Among them, 2-methylpyrazine and 2,5-dimethylpyrazine showed the most intense signals in the 2D topographic plot, and both the protonated monomer and the proton bound dimer peaks were observed. On the contrary, for 2-ethylpyrazine and 2,3,5-trimethylpyrazine only the protonated monomer peak was detected. Lastly, 2,6-dimethylpyrazine monomer was observed only for samples corresponding to the longer roasting time (25 min). Under the applied analytical conditions, 2,3,5,6-tetramethyl-, 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine were not detected in the roasted hazelnut paste samples.

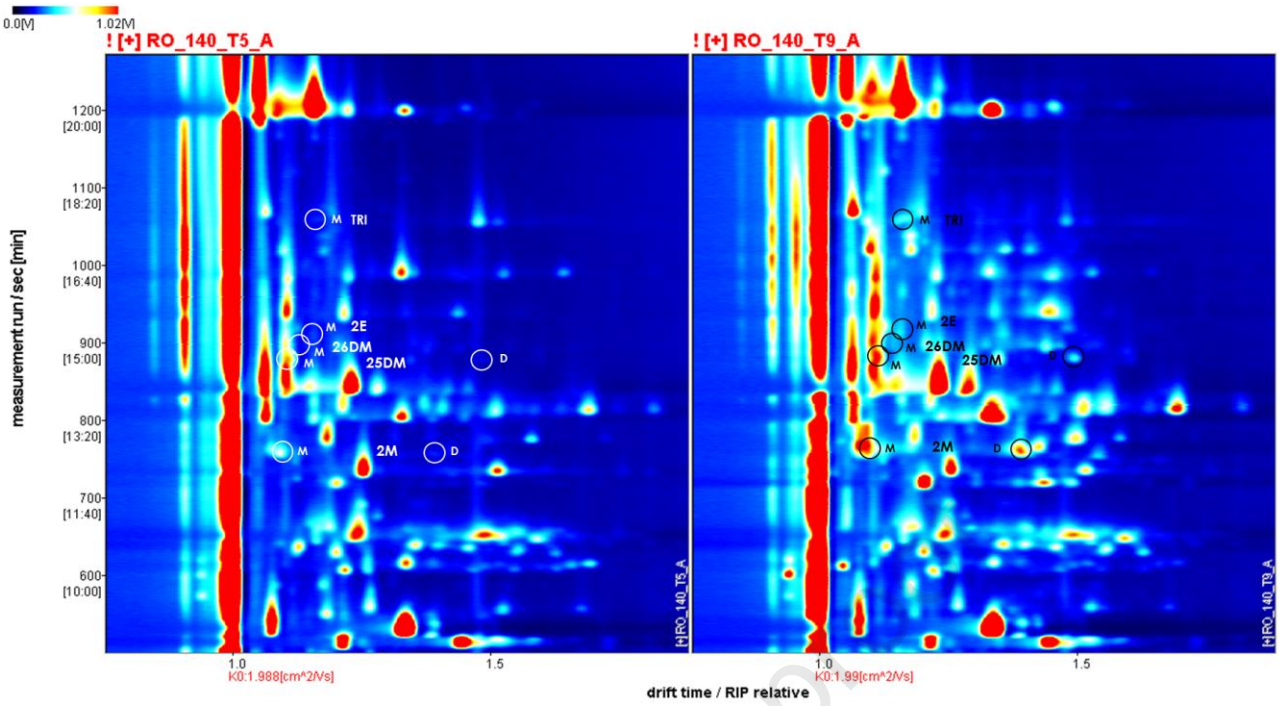

Fig. 4 - *2D topographic plot of two roasted hazelnut paste samples (Tonda Gentile Romana samples - RO). On the left sample obtained with 15 minutes of roasting at 140°C, on the right sample obtained with 25 minutes at the same temperature. 2M: 2-methylpyrazine, 25DM: 2,5-dimethylpyrazine, 26DM: 2,6-dimethylpyrazine, 2E: 2-ethylpyrazine, TRI: 2,3,5-trimethylpyrazine. M: protonated monomer, D: proton bound dimer.*

Based on the results of the first phase of this study, it is clear that the relative intensities of these peaks cannot be used to draw quantitative conclusions about the alkyl pyrazine abundances, but a standardization approach must be applied.

3.3 Multiple point external standardization

The standardization of the GC-IMS responses for the target alkyl pyrazines was carried out developing a quantitative protocol based on multiple point external standardization. Starting from the results of the first phase and from literature data, the concentration range selected for the calibration levels was 0.01 - 1 µg g^{-1}. The ionic species used for the quantification was chosen for each alkyl pyrazine. For 2-methylpyrazine, the quantification was carried out on the proton-bound dimer intensity, due to a coelution of the monomer with an unknown peak in the samples with higher roasting intensity. In the case of 2,5-dimethylpyrazine

279 calibration both ionic species were used separately. The protonated monomer curve was suitable for the low
280 concentration range (0.01-0.6 μg g^{-1}), while the proton bound dimer curve was applied for the high
281 concentration range (0.2-1 μg g^{-1}). For 2,6-dimethyl-, 2-ethyl- and trimethylpyrazine the protonated
282 monomer was used, because it was the only detectable species in the hazelnut samples. The linear dynamic
283 range of GC-IMS is known to be limited to 1-2 order of magnitude [14], and a 2^{nd} degree polynomial function
284 was used to fit the calibration curves of those analytes showing non-linearity of the detector response within
285 the concentration range (2,6-dimethylpyrazine, 2-ethylpyrazine, and the protonated monomer of 2,5-
286 dimethylpyrazine). Limit of detection (LODs) and limit of quantification (LOQs) were calculated from the
287 standard deviation of blanks using the linear approximation of the calibration curves, according to Budzyńska
288 *et al* [10], and confirmed by analyzing decreasing concentrations of standards. Details of the calibration for
289 the target alkyl pyrazines are summarized in **Table 1**.

290

	Ion species	Curve fitting	Concentration range (µg g^{-1})	Equation	R^2	LOD (µg g^{-1})	LOQ (µg g^{-1})
2M	dimer	linear	0.08-1	y = 1.418x + 0.0088	0.9934	0.04	0.08
25DM	monomer	polynomial (2nd)	0.01-0.6	y = -2.9737x^2 + 3.5177x + 0.1063	0.9983	< 0.01	0.01
	dimer	linear	0.2-1	y = 0.647x - 0.0162	0.9985	0.08	0.2
26DM	monomer	polynomial (2nd)	0.06-1	y = -0.8385x^2 + 1.4424x + 0.0115	0.9954	0.03	0.06
2E	monomer	polynomial (2nd)	0.04-1	y = -0.7912x^2 + 1.4628x + 0.0382	0.9957	0.02	0.04
TRI	monomer	linear	0.06-1	y = 0.6693x + 0.0372	0.9925	0.03	0.06

291

292 **Table 1 -** *Summary of the multiple point external standardization. 2M: 2-methylpyrazine, 25DM: 2,5-dimethylpyrazine, 26DM: 2,6-dimethylpyrazine, 2E: 2-*

293 *ethylpyrazine, TRI: 2,3,5-trimethylpyrazine. R^2: regression coefficient; LOD: limit of detection; LOQ: limit of quantitation; reported in µg g^{-1}.*

3.4 Determination of alkyl pyrazines in roasted hazelnut paste samples

The external calibration developed to standardize the GC-IMS response was applied to determine the content of the identified alkyl pyrazines in the roasted hazelnut paste samples.

As mentioned in paragraph 3.2, 2,6-dimethylpyrazine monomer was detectable only for samples corresponding to higher roasting intensity (roasting time 25 min). Even in these analyses, however, the peak intensity was lower than the LOQ. Therefore, it was not possible to determine the content of 2,6-dimethylpyrazine in the samples.

The results obtained for other analytes are reported in **Table 2**. As expected, an increase in the alkyl pyrazines content according to the roasting times was observed. At the same time, TGL samples showed an overall higher amount of all the pyrazines quantified, while lower amounts were observed for AK samples. 2-methylpyrazine and 2,5-dimethylpyrazine are the most abundant alkyl pyrazines in all the samples analyzed, while 2-ethyl pyrazine is the less abundant.

2,3,5-trimethyl pyrazine is detectable in the 2-D plot as low intensity monomer peak, therefore observations based on the raw signal intensities would have led to a considerable underestimation of the amount of this analyte compared to 2-methyl and 2,5-dimethylpyrazine (both monomer and dimer detectable). On the contrary, the results obtained applying the response standardization show that the actual amount of 2,3,5-trimethylpyrazine is comparable to those of 2-methylpyrazine in many samples (low roasting intensity samples), despite the differences in the signal patterns. The standardization enables, therefore, comparative evaluation among analytes within the same sample.

Lastly, after the standardization, a single analytical output is obtained (the content expressed in $\mu g\ g^{-1}$), instead of multiple peak intensity values for the same analyte, making the results more easily interpretable.

	TGL			RO			AK		
	15'	**20'**	**25'**	**15'**	**20'**	**25'**	**15'**	**20'**	**25'**
2M	0.18 ± 0.01	0.36 ± 0.00	0.59 ± 0.01	0.08 ± 0.01	0.28 ± 0.00	0.55 ± 0.01	< LOQ	0.19 ± 0.00	0.42 ± 0.01
25DM	*0.30 ± 0.02*	0.49 ± 0.00	0.65 ± 0.01	*0.26 ± 0.00*	0.36 ± 0.02	0.53 ± 0.00	*0.12 ± 0.00*	*0.24 ± 0.00*	0.40 ± 0.00
2E	< LOQ	0.07 ± 0.01	0.11 ± 0.01	< LOQ	0.06 ± 0.01	0.09 ± 0.01	< LOQ	0.05 ± 0.00	0.09 ± 0.01
TRI	0.17 ± 0.01	0.27 ± 0.01	0.35 ± 0.01	0.09 ± 0.01	0.21 ± 0.01	0.30 ± 0.01	0.07 ± 0.00	0.14 ± 0.01	0.24 ± 0.00

315

316 **Table 2 -** *Quantitative results of the analysis of alkyl pyrazines in roasted hazelnut paste samples (different roasting times: 15 – 20 – 25 minutes) by SHS-GC-IMS.*

317 *Quantitative values, expressed as the mean of three replicates ± standard deviation, reported in µg g^{-1}. For 25DM values obtained from protonated monomer*

318 *calibration are reported in italics. 2M: 2-methylpyrazine, 25DM: 2,5-dimethylpyrazine, 26DM: 2,6-dimethylpyrazine, 2E: 2-ethylpyrazine, TRI: 2,3,5-*

319 *trimethylpyrazine.* ***TGL****: Tonda Gentile delle Langhe,* ***RO****: Tonda Gentile Romana,* ***AK****: Akçakoca*

4) Conclusions

SHS-GC-IMS is becoming widely applied for the analysis of food volatile organic compounds. In this study we evaluated its application within the context of industrial process monitoring, in particular to determine the content of alkyl pyrazines in roasted hazelnut pastes. When IMS is operated in positive ionization mode it works as a non-selective detector and the volatile compounds with proton affinity higher than water are ionized and detected. In the first phase of the study, relevant differences in IMS response were observed for alkyl pyrazine homologous both in terms of minimum detectable concentration and sensitivity, but also concerning the proton-bound dimer formation. These differences highlighted that a response standardization approach is fundamental for IMS detection when comparative evaluations among analyte abundances are performed.

The approach proposed in the second section of this article is based on multiple point external standardization and was carried out applying in-matrix calibration to avoid the matrix effect, which was observed for the hazelnut paste. It allowed to obtain a single standardized analytical output and to compare the abundances of alkyl pyrazines in hazelnut paste samples from different geographical origins and processed with different roasting intensity.

In conclusion, HS-GC-IMS is suitable as a rapid analytical platform to monitor VOCs during industrial processing, however, before any quantitative assumptions are drawn, the IMS response needs to be carefully evaluated and standardized for the analytes of interest, even in the case of homologous compounds.

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Highlights

- The determination of alkyl pyrazines in roasted hazelnut pastes by GC-IMS is presented.
- The SHS-GC-IMS response curves of alkyl pyrazine homologous differ significantly.
- Detectable concentrations, sensitivity, proton-bound dimer formation are different.
- Abundance comparison among analytes requires a response standardization.
- A matrix-matched multiple point external standardization was applied.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: