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HIGH-THROUGHPUT ANALYSIS OF FOOD VOLATILOME BY DIRECT INJECTION MASS SPECTROMETRY

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Abstract–Rapid, non-invasive and real-time analysis of food volatilome by direct injection mass spectrometry (DIMS) techniques contributes to the evaluation of food quality. Among DIMS application, we report three case studies show the potential of the PTR-ToF-MS technique in product characterization and process monitoring.

Keywords: *PTR-ToF-MS, volatile organic compounds (VOCs), food, profiling, screening, online monitoring*

1. INTRODUCTION

Volatile organic compounds (VOCs) are key components of food aroma and flavour. Even at very low concentrations, they can influence the perceived quality of food. Moreover they do not act separately but as a whole. Thus, they play a relevant role in agro-industrial processes and in food science and technology. For these reasons, food VOC profiling is of outmost importance and requires the high-sensitivity and high-throughput screening of large samples sets and/or the rapid and continuous monitoring of processes [1]. Among available analytical methods, direct injection mass spectrometry (DIMS) techniques show advantages in capacity to carry out rapid, high-throughput measurements of large sample sets and to monitor VOC evolution in real time [2]. However, due to the lack of chromatographic separation, DIMS techniques can provide, in association with a given peak, only the chemical formula with a tentative identification of a compound. Nowadays, DIMS techniques are presented by E-noses, atmospheric pressure chemical ionization mass spectrometry (APCI-MS), selected ion flow tube mass spectrometry (SIFT-MS), and proton transfer reaction mass spectrometry (PTR-MS). This work is based on case studies of food volatilome by means of PTR-ToF-MS, which founds a wide range of applications in environmental monitoring, health care, agriculture and food science. In this manuscript, to testify the potential of PTR-ToF-MS in the field, three case studies are presented: characterisation of roasted coffees from different geographical origins [3], volatilome profiling of commercial aromatic bakery yeast of wine and beer origin [4], and screening of semi-finished dairy products [5].

2. EXPERIMENTAL

2.1. Sample preparation

PTR-ToF-MS is a "green" chemistry technique which does not require usage of any solvent, preconcentration or other particular sample preparation. For screenings of large sample sets an equilibration of VOC release in a vial is only needed in order to guarantee standardization and repeatability.

2.1. PTR-ToF-MS analysis

For all three case studies the setup for VOC analysis was the same. 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 550V, drift temperature 110°C and drift pressure 2.33 mbar affording an E/N value of 140 Td. Sampling was performed with a flow rate of 40 sccm. The mass resolution (m/Δm) 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 min at 200 sccm, then incubation for 30 min at 40 °C and finally measurement. The measurement order was randomised to avoid possible systematic memory effects. The sample headspace was measured by direct injection into the PTR-ToF-MS drift tube via a heated (110 °C) peek inlet. Each sample was measured for 30 s, at an acquisition rate of one spectrum per second. Headspace volume mixing ratios were calculated by averaging over the whole measurement time.

In the case of studies of dough volatilome during leavening [4] the whole scan of samples was repeated 16 to 20 times, in order to monitor the fermentation process. Moreover, due to the presence of relevant amount of ethanol in the headspace of the samples an inert gas dilution was applied in an inert gas to sample ration of 2:1.

2.2. Data analysis

The multipurpose GC automatic sampler and PTR-ToF-MS were synchronised 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 [6,7], and the experimental m/z values were reported up to the third decimal. To determine the concentrations of volatile compounds in part per billion by volume, the formulas described by Lindinger et al. were used by assuming a constant reaction rate coefficient (k_R=2×$10^{-9}$$cm^3$/s) for H_3O^+ as primary ion [8]. This approximation 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 coefficient is available [9].

3. RESULTS AND DISCUSSION

PTR-ToF-MS was used as an analytical fingerprinting tool to discriminate food samples in the case studies. Fig. 1 represents an example of an average mass spectrum of a dough sample acquired by PTR-ToF-MS. Thanks to the Time-of-Flight mass analyser it is possible to distinguish isobaric peaks.

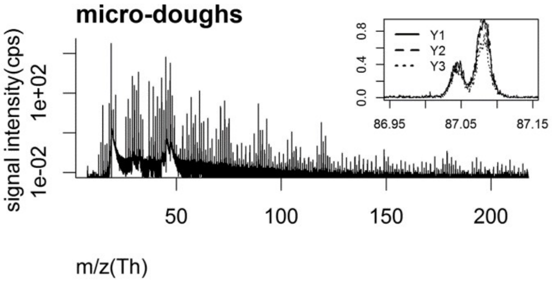

Figure 1. Average mass spectrum of dough. The top right inset shows a double peak in details [10].

In the case of coffee screening, 108 measurements were performed and 270 mass peaks out of the 476 extracted ones were selected for the further analysis. Unsupervised Principal Component Analysis (PCA, Fig. 2) indicated the importance of aroma fingerprints enabling origin discrimination which has also been further confirmed by PLS-DA model [3].

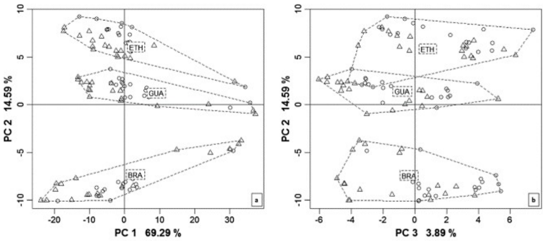

Figure 2. Scatter plots of PCA scores (a: PC1 vs PC2 and b: PC3 vs PC2, respectively). Coffee samples of the same geographical origin (ETH – Ethiopia, GUA – Guatemala, BRA - Brazil) are united in the same empty polygons. Different point shapes indicate two different production batches [3].

As presented in Fig. 2, the first two principal components explain 80.88% of the total variability in the data and group the samples according to geographic origin. Separation of coffees by origin is particularly visible on the second principal component (PC2). Models set on samples of one production batch could correctly predict the origin of the samples of the other batch considered. The data obtained by coffee screening confirm that the volatilome of roasted and ground coffee is highly influenced by the geographic origin of coffee beans.

In the case of the bread leavening, less than one hour was needed for the headspace sampling of one time point of all 32 samples of dough. All samples were monitored for 16 hours. More than 460 peaks were detected during the leavening process and 63 of them were selected for the further analysis. In Fig. 3 volatilome evolution clearly indicates the differences in a fermentative behaviour of the studied strain.

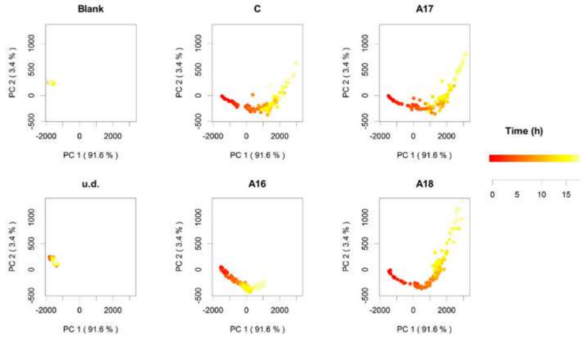

Figure 3. Volatile emission during leavening: principal component analysis of the autoscaled data for five intra-day repetitions for each experimental mode. Score plots for the first two principal components for blank, un-inoculated dough (u.d.), C, and for the three different yeasts, A16, A17, and A18, are depicted. Different color scale, from red to yellow, represents time evolution [4].

In the third case, study PTR-ToF-MS was applied for a rapid and non-invasive VOC analysis of the head-space of different dairy semi-finished products, including milk powder, anhydrous milk fat, and whey [5]. The technique was successful in characterizing each ingredients and separate different samples of the same product type, previously assigned different sensorial characteristics.

These case studies highlight the set-up potential for high-throughput analysis of food volatilome. Among further improvements, the increase in the separation of isobars, the increase in sensitivity and the improvements in the analysis of alcoholic matrixes are the most important ones. A very fast chromatographic separation by means a fast GC add-on should further help with isobar separation and ethanol separation from other VOCs. The increase of sensitivity can be achieved by installation of an ion funnel or an ion guide.

4. CONCLUSIONS

PTR-ToF-MS, as a part of DIMS technologies, provides an optimum tool for the high-throughput screening of large sample sets and for bioprocess monitoring in relation with important food features such as sensory quality, origin, genetic characteristics, and production process.

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