On-line high-throughput analysis of the volatilome of microorganisms that have agroindustrial relevance

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Abstract

Yeast and bacterial fermentations play a key role in producing not only important technological functions in fermented foods but also the characteristic sensory attributes. While key flavor compounds are generally characterized, the kinetics of formation are poorly understood and poorly controlled. Multipurpose head-space automated sampling (MHSA) together with Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) were investigated as a tool to understand volatile organic compound (VOC) changes during fermentation. Automation of the analytical process as provided by MHSA guaranteed reproducibility over the whole microorganism life cycle, the accurate control of process parameters (temperature and sampling times) while maintaining the rapid sampling rates that PTR-ToF-MS enables. Multivariate data analysis techniques are required to identify important trends in the data.

Introduction

Yeast and bacterial species are widely used for leavening, brewing, wine making or dairy fermentations and play a key role in producing the characteristic sensory profiles and perceived quality of these products through the VOCs they generate [1-3]. These VOCs synthetized by microorganisms as secondary metabolites, not only impart important sensory notes but also have important technological functions [3]. As such, an on-line and non-invasive screening of the microorganism volatilome is of high importance to better understand and control these processes and support innovation in this traditional sector by unlocking the flavor generation process.

Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) coupled with multipurpose head-space automated sampling (MHSA) was investigated to enable the efficient monitoring of agroindustry-relevant microbiological processes: dough leavening, lactic acid fermentation and wine making.

Experimental

The following three experimental datasets are used to illustrate the usefulness of the coupled PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Austria) and multipurpose headspace automated sampler (Gerstel GmbH & Co. Germany):

1. Lactic acid bacteria in low fat milk [4]; 3 cultures from Chr Hansen (A: FD-DVS YF-L812 Yo-Flex; B: FD-DVS YC-380 Yo-Flex; and C: FD-DVS YC-X11 Yo-Flex) and 1 from Danisco (D: YO-MIX 883 D) x 3 replicates x 12 time points (0 – 240 min)
2. Yeast in bread dough [5]; 4 commercial yeast (Y1: Lessaffre, Parma, Italy; Y2: Lessaffre, Parma, Italy; Y3; Pakmaya, Istanbul, Turkey; and Y4: Italmill, Cologne, Italia) x 5 replicates x 3 time points (0 – 2.7 h); headspace dilution of 2:1 inert gas: sample flow

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3. *Sacchromyces cerevisiae* on agar [6]; 6 strains x 12 replicates x 66 time points (0 – 11 d); dilution of 1:3 sample flow to argon was used to overcome the deleterious effect of ethanol on the acquired spectra.

PTR-ToF-MS drift tube temperature and pressure were 110°C and 2.3 mbar, respectively and the drift tube voltage was about 550 V, which resulted in an E/N ratio of about 140 Td (1 Td = 10−17 cm2 V−1 s−1). The inlet flow was 40 sccm for the yogurt and wine yeast fermentations and 120 sccm for the dough.

Data processing of PTR-ToF-MS included dead time correction, external calibration, peak extraction, peak fitting and baseline extraction [4,5] and concentration was calculated as per Lindinger et al. [6].

Results and discussion

The coupling of PTR-ToF-MS with multipurpose headspace automated sampling allows the on-line noninvasive high-throughput screening of microorganism volatilome; and the identification of strain specific features and new metabolic pathways over time frames that are industrially relevant.

PTR-ToF-MS analysis of the VOCs generated during fermentation from doughs fermented with four different commercial yeasts produced complex spectra (Figure 1). After filtering to remove m/z that were unchanged, clusters (water and/or ethanol) and isotopologues (^{13}C and ^{18}O) 46 m/z discriminated the bread dough with respect to time or yeast type. Yeast types were discriminated by 16 VOC. The high mass resolution was advantageous in allowing the discriminate between separate masses within one nominal mass, e.g. m/z 87 where variation in the signal between yeast could be assigned to variation in an aldehyde/ketone rather than diacetyl (Figure 1 inset).

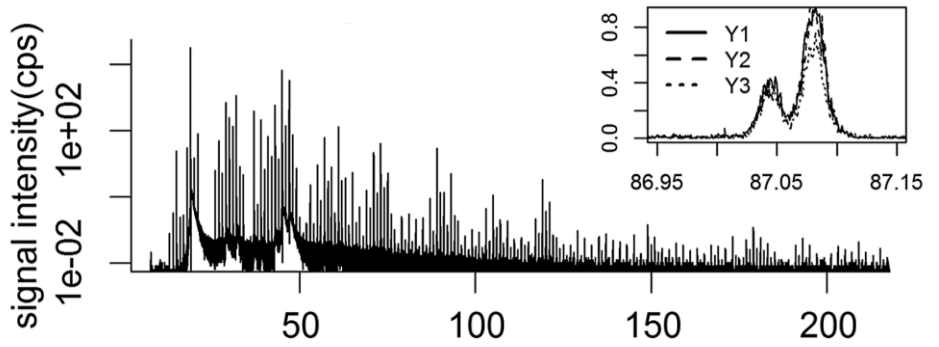

Figure 1: Average PTR-ToF-MS spectrum of fermenting dough; inset shows a double peak for the nominal mass 87 [2] *(Copyright © 2014 John Wiley & Sons, Ltd)*

The four cultures that fermented yogurt were distinguished by 13 m/z including 2 isotopologues. During the conversion of milk into yogurt the MHSA coupled with PTR-ToF-MS allowed sampling times with sufficient temporal resolution to allow depletion/consumption of methanethiol and synthesis/appearance of acetoin to be followed (Figure 2).

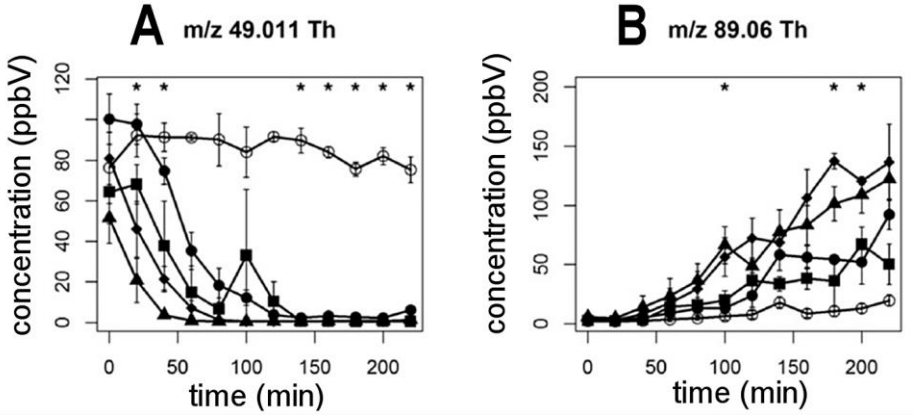

Figure 2: Fermentation kinetics of methanethiol (**A**) and acetoin (**B**) (means of three replicates ± standard deviation). Open circle (○), uninoculated milk; filled square (■), starter A; filled circle (●), starter B; filled triangle (▲), starter C; filled rhombi (♦), starter D. Asterisks indicate statistically significant differences (ANOVA, p b 0.05) among commercial starters. [1] *(Copyright © 2015 Elsevier Ltd*)

The VOC generated by the wine yeast grown on agar were significantly discriminated by 70 m/z based on yeast type and time. Of these 50 could be assigned a chemical formula and 37 were tentatively identified. The principal component analysis (PCA) explained 76.1% of the data variation on 2 principal components (Figure 3). The PCA plot shows from left to right the open circles, which represent the times points for each yeast during the lag phase and growth phase, an increase in fermentation time. Separation is largely due to increases in esters and ethanol. In contrast, during the stationary phase ester and ethanol synthesis cease and due to the sampling conditions stripping occurs, i.e., ester and ethanol concentrations decrease.

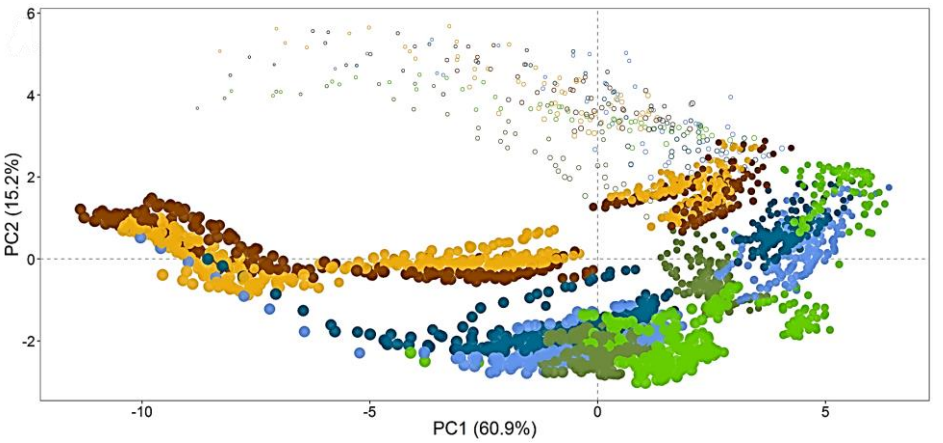

Figure 3: Score plot of principal component analysis showing fermenting wine differentiation due to yeast volatilome evolution during 11 days. Data are logarithmically transformed and centered. Different colors indicate different yeast strains, medium and blank samples. Length of fermentation time is represented by an increase in point size; Open circles represent the lag and growth phase; and closed circles represent the stationary phase [3].

Automation of the analytical process as provided by MHSA guaranteed reproducibility over the whole microorganism life cycle, the accurate control of process parameters (temperature and sampling times). Analysis could be completed as frequently as every second but typically the headspace of each sample was measured for one minute while displacing the headspace with zero air or pure nitrogen. In addition, the fermentation processes can automatically be monitored for several hours in the case of dough leavening and lactic acid fermentations or days for alcoholic fermentations or yeast colonies grown on a solid medium. The set-up allows the monitoring of up to 128 samples at each time point.

To deal with data matrices containing several hundreds of mass peaks for each measurement multivariate data analysis is needed to provide the general overview of biological processes and phenotypic variability among different microbial strains. Observations of single VOC emission curves allow the opportunity to study known metabolic pathways and unravel unknown ones.

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