

Tracking of hop-derived compounds in beer during fermentation with PTR-ToF-MS

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Abstract

Proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) was used as a novel, direct and real-time analytical method to monitor small-scale fermentations carried out in 20 mL vials (3 mL sample volume) at 20 °C with repeated measurements of the headspace volatile organic compounds (VOCs) for four days. A design matrix of two yeast biotypes (California Ale and Edinburgh Scottish Ale) and two New Zealand aroma hop cultivars (Motueka and Nelson Sauvín), together with their respective no addition controls, were used to investigate yeast-hop interactions. The results highlighted the advantages of using online analytical measurements, such as PTR-ToF-MS, to understand temporal changes that occur in VOCs during fermentation. Distinct differences were observed in the VOCs profile of the different beers based on combinations of yeast biotype and hop cultivar; e.g. samples with Motueka and Scottish Ale had higher concentrations of m/z 89.057 (3-methyl-1-butanol). Complex dynamics were observed for VOC development during the fermentation; e.g. production maxima for masses such as m/z 145.121 (2-nonanol or ethyl hexanoate) and m/z 173.153 (isoamyl isovalerate or ethyl octanoate).

Introduction

The craft beer market is experiencing a rapid increase in growth. To help brewers optimise hop character, and to make beer with distinctive hop profiles, a better understanding of the role that yeast play in the development of hop character is required. Anecdotally, brewers report that some yeasts “promote” hop flavour or accentuate one hop character over another, while other yeast biotypes are considered to be “hop neutral” or known to reduce hop intensity. However, to date very little scientific research has been published on the impact of different yeast biotypes and fermentation parameters on hop flavour in beer or on the mechanisms responsible for differing aroma and flavour development [1].

Traditionally, hop flavour development has been assessed using GC-MS based approaches, where hop derived compounds in the final beer are identified and measured. The drawback with this approach is that it is time consuming and provides little information on changes that occur during brewing and fermentation. Furthermore, the sensitivity of GC-MS to detect volatile organic compounds (VOCs) is dependent on the extraction method employed and the volatility and polarity of the target analytes and their affinities towards the chosen solvent or solid phase material [2,3].

In this paper PTR-ToF-MS was used to measure and compare the dynamic changes in VOCs during the production of beer containing one of two aroma hops (Motueka and Nelson Sauvín) in combination with one of two yeast biotypes (California Ale and Scottish Ale) and their respective no addition controls.

Experimental

Laboratory scale beer samples were produced with wort standardised to 10.2°P [degree Plato] (1044 OG [Original Gravity]) and 20 IBU [International Bittering Units]. Aroma hops (5 g/L) (Table 1) were added at 90°C for 5 min before being cooled to 20°C. Wort was inoculated with yeast (Table 1) (pitching rate: $\sim 1.0 \times 10^7$ cells/mL) and divided into 6 aliquots (3 mL) of each treatment. Fermentation was carried out in HS-vials at 20°C with consecutive headspace sampling every 6 hours using PTR-ToF-MS. Compounds were identified through an elemental composition calculator and preceding GC-MS measurements [4]. Microfermentations (3 mL) may be regarded to not closely represent industrial fermentations due to differences in convection and pressure in large scale ferments, which might impact on the magnitude of concentrations of some VOCs (e.g. ester formation might be altered in micro ferments). However, the production pathways and the sequence of changed in the volatile profile are expected to stay the same.

Table 1: Experimental design with yeast and hop combinations (treatments)

<i>Treatment</i>	<i>No yeast (NY)</i>	<i>California Ale yeast (CA)</i>	<i>Scottish Ale yeast (SA)</i>
No aroma hop (NH)	Blank	California Ale control	Scottish Ale control
Motueka hop (MT)	Motueka control	California Ale with Motueka	Scottish Ale with Motueka
Nelson Sauvín hop (NS)	Nelson Sauvín control	California Ale with Nelson Sauvín	Scottish Ale with Nelson Sauvín

Results and discussion

The fermentation was monitored for four days and information from 672 mass ions (m/z) over 14 time points were collected. Two-way ANOVA was carried out to select m/z with a significant change during the fermentation; overall, 182 m/z were found to have a significant ($p < 0.01$) change during the fermentation. A principal component analysis (PCA) was carried out on all significant ($p < 0.01$) ions for all treatments (except controls). A score plot coded to highlight treatment effects of hop cultivar is shown in Figure 1, where each point represents the VOC profile of the selected m/z of each sample at each time point. Reproducibility of the replicates ($n=6$) was found to be very good. Separation along PC-1 (39% explained variance) and PC-2 (10% explained variance, data not shown) were mainly due to changes during fermentation. As illustrated on PC-3 (4% explained variance), hop cultivar had a major impact on the VOC profile at the beginning of the fermentation (black circles), with this impact disappearing over time due to either modification by the yeast cells or stripping due to CO_2 production during the fermentation. Towards the end of fermentation yeast biotypes dominated the VOC profile differentiation owing to differences in the metabolites they were producing (red circles).

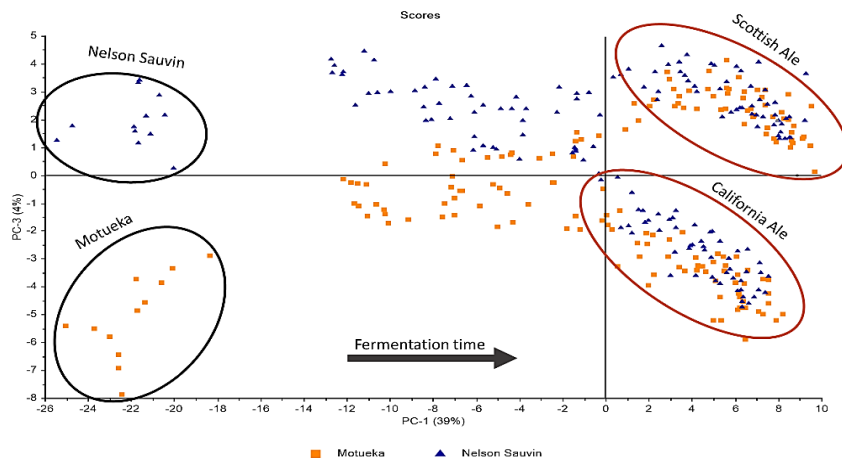


Figure 1: PCA of the samples with the significant m/z VOC profile over the fermentation time for the treatments

Selected m/z were tracked over time to observe VOC development throughout fermentation. Three different dynamics were observed during the fermentation: reduction (through stripping, yeast uptake, or metabolic conversion), production, and increase with a subsequent decrease. In some cases, multiple VOCs shared the same m/z .

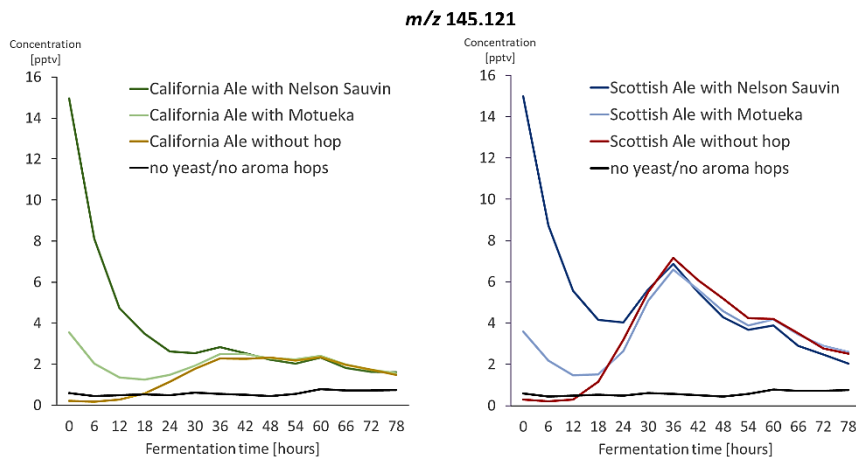


Figure 2: Changes in generation of m/z 145.121 during the fermentation of each treatment

An example of the varying dynamics present during the fermentation is illustrated by m/z 145.121 (Figure 2). This compound was tentatively identified at the beginning of the fermentation as octanoic acid and/or methyl heptanoate, which were determined to be hop-derived compounds. Towards the end of the fermentation m/z 145.121 was more likely to be either ethyl hexanoate and/or 2-nonanol, which were fermentation-derived compounds. Scottish Ale yeast with Nelson Sauvin hop demonstrated a greater reduction in m/z 145.121 towards the end of fermentation indicating an interaction between yeast biotype and hop cultivar. It is believed that differences observed in fermentation-derived VOC production could be due to differential gene expression.

The impact of the hop cultivar is evident for some fermentation-derived compounds, e.g. m/z 89.057 (Figure 3A), which was tentatively identified as ethyl acetate and/or 3-methyl-1-butanol, and m/z 173.153 (Figure 3B) was tentatively identified as isoamyl isovalerate, pentyl pentanoate, 2-methylbutyl-2-methylbutyrate, and/or ethyl octanoate. For both masses, Motueka hops resulted in higher production compared to Nelson Sauvignon. Two production maxima (~ 34 hours and ~ 60 hours) were congruent with the rate of change in ethanol production during the fermentation, possibly related to yeast metabolism and ester production.

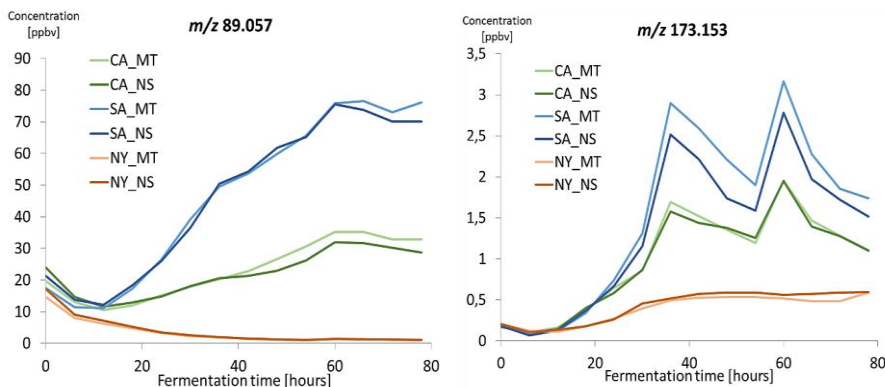


Figure 3: Changes in generation of m/z 89.057 (A) and m/z 173.153 (B) during the fermentation of each treatment

In conclusion, PTR-ToF-MS can successfully differentiate and monitor the change in VOCs during fermentation in real time and demonstrate how interactions between hop cultivars and yeast biotypes result in unique VOC profiles. Dynamic monitoring has the capability to enhance understanding of how metabolic pathways and stress factors influence the production of VOCs and this knowledge will facilitate a better understanding of beer flavour. A better understanding of how yeast biotypes influence hop-derived compounds during fermentation will improve our understanding of hop aroma generation in beer and will give insight on how to accentuate a desired hop character by selecting yeast biotypes and modifying fermentation parameters.

References

1. King, A. J., & Dickinson, J. R. (2003). *FEMS Yeast Research*, 3, 53-62.
2. Rodrigues, F.; Caldeira, M.; Câmara, J. S. (2008). *Analytica Chimica Acta*, 609 (1), 82-104.
3. Richter, T. M., Eyres, G. T., Silcock, P., Bremer, P. J. (2017). *Journal of Separation Science*, DOI:10.1002/jssc.201700676
4. Cappellin, L., Biasioli, F., Granitto, P. M., Schuhfried, E., Soukoulis, C., Costa, F., Märk, T. D., & Gasperi, F. (2011). *Sensors and Actuators B: Chemical*, 155(1), 183-190.