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Selected Ion Flow Tube Mass Spectrometry (SIFT-MS): a promising technology for the high throughput phenotyping of grape berry volatile fingerprints

Thomas Baerenzung dit Baron[1,2], Marie Denat[1], Olivier Yobrégat[3], Valérie Simon[2], Alban Jacques[1], Olivier Geffroy[1]

1 Physiologie, Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-PURPAN, 75 voie du TOEC, BP57611, 31076 Toulouse Cedex 3, France
2 Laboratoire de Chimie Agro-Industrielle (LCA), UMR 1010 INRAe/ Toulouse INP-ENSIACET, University of Toulouse, 31030 Toulouse, France
3 Institut Français de la Vigne et du Vin pôle Sud-Ouest (IFV Sud-Ouest), 81310 Peyrole, France

Over the last decade, there have been renewed efforts into wine grape breeding within the research community. Quick characterisation by phenotyping of quality traits, including aroma composition, remains challenging. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), a high throughput soft ionisation technology first available in 2008, could be particularly useful for this purpose. In light of this, this technical article summarises recent results obtained with SIFT-MS.

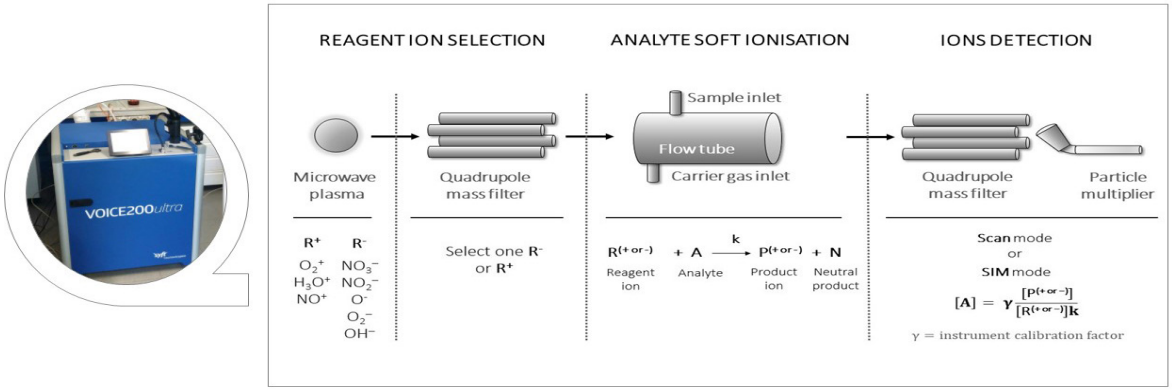


FIGURE 1. Diagram of the selected ion flow tube mass spectrometry (SIFT MS) technique.

A crucial need for high throughput phenotyping of berry quality traits

Vitis vinifera L. is the most widely grown wine grape species in the world. However, it is susceptible to biotic factors, such as pests and fungal diseases. Some of these diseases, including downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*), can be fought by cross breeding with resistant or tolerant species; i.e., mainly American or Asian *Vitis*. As a consequence of the current societal pressure for the reduction of pesticide use, this genetic strategy is nowadays very dynamic throughout the world. Breeding programmes that last between up to 30 years and can generate about 50,000 seedlings rely on controlled sexual reproduction. The use of marker-assisted selection (MAS), notably markers related to mildew resistances, facilitate screening and enable the acceleration of the process by up to 10 years by quickly decreasing the number of vines to characterise[1].

Despite the identification of molecular markers (quantitative trait loci or QTL) for berry and wine over recent decades[1], the assessment of quality traits, known as phenotyping, still comprises one of the most demanding stages. Varietal aroma compounds responsible for wine typicality are some of the most significant molecules driving wine quality and appreciation. In this context, there is a high demand for high throughput technology to quickly assess the aroma composition of a large amount of individuals. Volatiles are frequently found at trace levels and their analysis involves complex sample preparation steps to concentrate the analytes, involving, for example, liquid-liquid extraction (LLE), solid phase extraction (SPE) or solid phase microextraction (SPME)[2]. These time-consuming extractions techniques are associated with gas chromatography (GC) analyses, using mass spectrometry (MS) or pulsed flame photometric detector (PFPD) as detectors and with run times that last between 45 min to 2 hr[3].

How does SIFT-MS work?

Selected ion flow tube mass spectrometry (SIFT-MS) is a technology that has been commercially available since 2008 and has the advantage of allowing real-time headspace analysis in addition to being highly sensitive[4]. This device uses soft ionisation by means of 8 different reagent ions for the most recent equipment (H_3O^+, NO^+, O_2^+, NO_3^-, NO_2^-, O^-, O_2^- and OH^-), and can analyse a sample headspace and determine abundances. It can be operated in scan mode, which involves scanning the mass range from the smallest mass to the highest mass of ions expected, or it can quantify compounds to parts per billion (ppb) level in Selected Ion Monitoring (SIM) mode by only focusing on some particular masses (Figure 1). The reagent ions are generated using a microwave plasma and selection is only a matter of milliseconds.

A discrimination of grape variety berry volatile fingerprint with O_2^+ in a 3 min run

To investigate the ability of SIFT-MS to discriminate varieties on the basis of their volatile composition, 23 different cultivars were sampled in 2020 from a germplasm collection. Fifty grams of berries were gently crushed in 1L-glass bottles, left for 6 hr until equilibrium was reached, re-equilibrated for 30 min at 40°C and directly connected to the SIFT-MS for analysis in scan mode with cations (H_3O^+, NO^+

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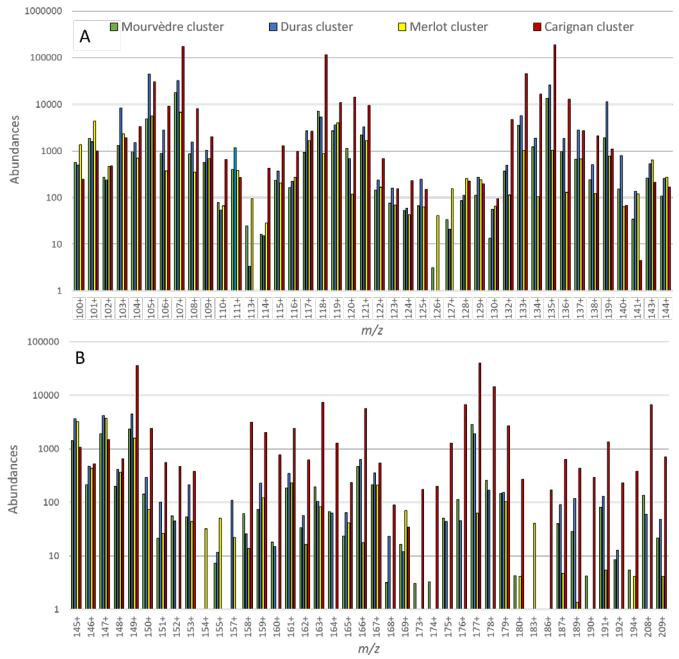


FIGURE 2. Mean SIFT-MS spectrum obtained with O_2^+ reagent ion for the four homogenous clusters of grape varieties identified for masses (A) from 100 to 145 *m/z*, and (B) from 145 to 209 *m/z*.

and O_2^+). These reagent ions were preferred to anions as they are known to ionise most organic compounds[5]. It was possible to easily distinguish the cultivars based on their SIFT-MS volatilome scan[6]. In this former study, four homogenous clusters of cultivars were identified and referred to as Mourvèdre, Duras, Merlot and Carignan clusters (Figure 2). The technology enabled the discrimination of low and high producers of monoterpenols, C13-norisoprenoids, phenols, and small alcohols or aldehydes, as confirmed by the GC-MS analyses. In most cases, the cultivars were also connected depending on their parentage relationship. This was the case for Colombard, Sauvignon and Gewurztraminer, which are all related to Savagnin and exhibit similar volatile compositions[7]. In the same way, Pinot noir, Chardonnay and Gamay, which have parent/offspring relationships, all belonged to the same homogenous cluster. The use of the single reagent ion O_2^+ was particularly relevant for reducing the time of analysis to 3 min[6].

When must berries be sampled during maturation?

With the view to using this methodology for the phenotyping of new cultivars, the sampling procedure cannot rely on destructive methods, such as the measurement of typical maturity parameters (i.e., sugar concentration), due to the high number of individuals and the low quantity of grape material available. To investigate the impact of the sampling time on the SIFT-MS fingerprint, berries from *Vitis vinifera* L. Syrah were collected at 7 time points during maturation in a season characterised by warm climatic conditions in a field trial in 2020. They were analysed by SIFT-MS in scan mode using O_2^+ as a reagent ion. The fingerprint obtained proved to be stable 28 days after mid-veraison (Figure 3)[8]. This finding simplifies the sampling procedure that can only rely on phenological data and a lapse in time after mid-veraison. For most *m/z*, a decrease in abundance, suggesting a loss in volatiles, was observed during maturation, notably between mid-veraison and mid-veraison + 28 days which could be the consequence of their emission or of an increase in non-detectable bound compounds. However, under our experimental conditions, the sensibility of the method remained appropriate 28 days after mid-veraison.

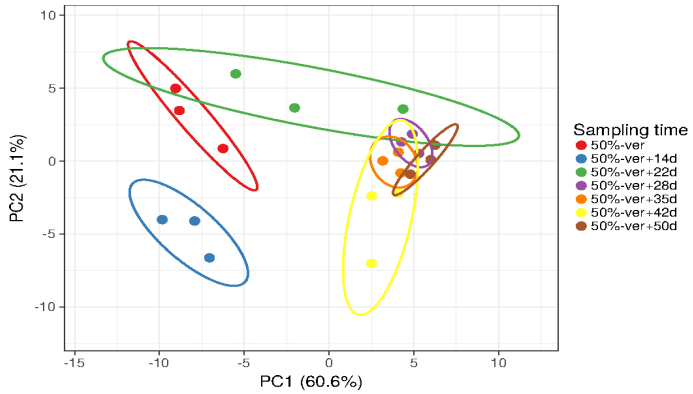


FIGURE 3. Factor scores with 95 % confidence ellipse for a principal component analysis (PCA) performed on the SIFT-MS abundance data using O_2^+ as the reagent ion for berries sampled seven times during maturation, from mid-veraison (50 % ver.) The fact that confidence ellipses tends to overlap from 50% ver+28d indicates that the SIFT-MS fingerprint becomes stable as from that point in time.

What next?

The proposed method is presently suitable for analysing 50 g of gently crushed berries, a choice that was made during preliminary experiments to obtain a good signal intensity without saturation from mid-veraison. Further work, including additional preparation steps, may be necessary to adapt the model dynamically to lower quantities of berries. Indeed, most of the newly-developed vines are grown in greenhouses during the initial years of growth under semi-controlled environmental conditions, and have small clusters composed of a limited number of berries (< 50 g). Other improvements might include additional preparation steps to access the full aroma potential of grapes through either acid or enzymatic hydrolysis of bound compounds[9], and to detect semi-volatile trace aroma compounds by limiting the contribution of major molecules. ■

Sourced from the research article "The SIFT-MS fingerprint of Vitis vinifera L. cv. Syrah berries is stable over the second part of maturation" (OENO One, 2022).

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