A COMPREHENSIVE METABOLOMIC STUDY OF WINE FROM THE VAUD SWITZERLAND VINEYARD

Lise Bréant[1], Guillaume Marti[1], Vivian Zufferey[2], Katia Gindro[2], Olivier Viret[2] and Jean-Luc Wolfender[1].

1 School of pharmaceutical sciences, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
2 Swiss Federal Research Station Agroscope Changins-Wädenswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland

1. Introduction

The so called "terroir effect" is one of the most important and tricky parameter that influence the vine-fruit-wine continuum. In addition to climate, soil also marks a major impact to the terroir effect but little is known about its main contributing factors. A recent study conducted in the Vaud viticultural area has shown that vine nitrogen content appeared to be one of the most important parameter that influence this continuum [1]. The nitrogen content seems positively correlated with well appreciated organoleptic attributes.
In order to confirm this result a large scale metabolomic investigation on white and red wines around the Geneva lake has been undertaken. Three grape varieties, ten vaud vineyards (switzerland) and five vintages (2006-2010) has been investigated in this study (**Fig. 1**).
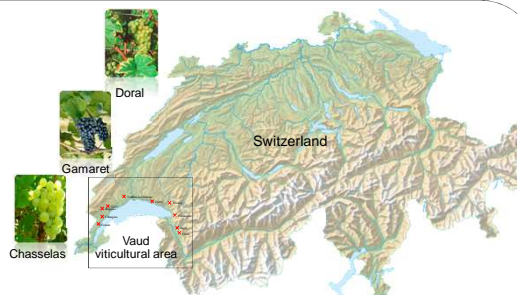

Fig. 1: The ten vineyards and grape varieties investigated in this study

2. Nitrogen influences on vine-fruit-wine continuum

In order to understand this continuum a concentration of assimilable nitrogen has been supplied *in vivo* on vine leaves during the grape maturation.

A metabolomic strategy based on reverse phase and hydrophilic interaction liquid chromatography TOF-MS along with proton NMR fingerprints has been apply to unravel the subtle biochemical changes [2].

Several putative biomarkers in close relation to nitrogen supply could be highlighted by supervised data mining and identified by means of their accurate mass, fragmentation pattern and proton NMR spectra (**Fig. 2**).
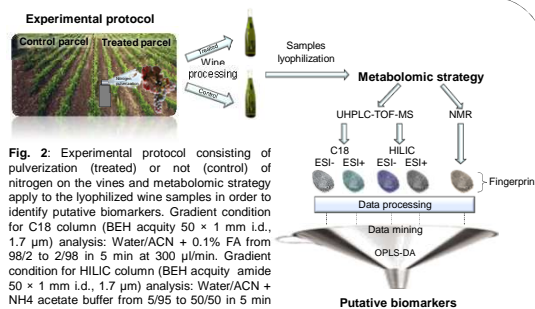

Fig. 2: Experimental protocol consisting of pulverization (treated) or not (control) of nitrogen on the vines and metabolomic strategy apply to the lyophilized wine samples in order to identify putative biomarkers. Gradient condition for C18 column (BEH acquity 50 × 1 mm i.d., 1.7 µm) analysis: Water/ACN + 0.1% FA from 98/2 to 2/98 in 5 min at 300 µl/min. Gradient condition for HILIC column (BEH acquity amide 50 × 1 mm i.d., 1.7 µm) analysis: Water/ACN + NH4 acetate buffer from 5/95 to 50/50 in 5 min at 300 µl/min.

3. Fingerprint profiles of lyophilized wines

Three different fingerprint technics have been used to obtain data for the chemometric analysis. UHPLC-TOF-MS profiles (~ 5 min) enabled us to detect 260 and 400 features in ESI- with reverse phase (C18) or hydrophilic interaction (HILIC) columns, respectively (**Fig. 4**). To complete wine metabolome analysis, NMR fingerprints have been acquired (**Fig. 5**).
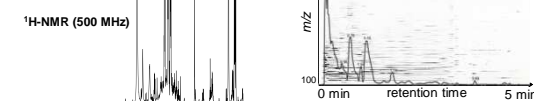

Fig. 5: proton NMR fingerprint of Gamaret red wine.

Fig. 4: UHPLC-TOF-MS chromatogram (BPI-2D map) of lyophilized white wine of Doral grape variety using a C18 (left) or HILIC (right) columns.

4. Chemometric analysis of UHPLC-TOFMS data

The hierarchical cluster analysis (HCA) of the LC-MS dataset for both column used displayed two levels of classification. The first one is linked to the vintage and the second one to the nitrogen treatment. An orthogonal partial least squares discriminant analysis (OPLS-DA) allowed the discrimination of wine samples according to the nitrogen supply with a significant level of confidence (**Fig. 6**). Biomarkers highlighted by the OPLS-DA are positively correlated with nitrogen supply (**Fig. 7**).
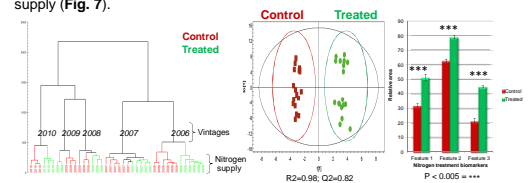

Fig. 6: HCA (left) and OPLS-DA (right) of the 5 vintages of white wine of Doral grape variety obtained with C18 column in ESI-

Fig. 7: Positively correlated biomarkers

5. Conclusion

The used metabolomic strategy allowed us to compute a detailed fingerprint of the chose wine samples and to discriminate them depending on the nitrogen treatment. Thanks to this we could highlight biomarkers correlated to vine nitrogen content and hence determine terroir characteristics that favorize well appreciated organoleptic quality. A large scale study with the same procedure will focus on Vaud vineyards which possess vine nitrogen content variations due to climatic conditions and pedology attributes.

6. References

1. J-S. Reynard *et al. J. Int. Sci. Vigne Vin.* **2011**, *45*, 211-221.
2. G. Glauser, *et al. J. Chromatogr. A.* **2008**, 1180, 90-98.

7. Acknowledgements