# FROM THE "CLIMATS DE BOURGOGNE" TO THE TERROIR IN BOTTLES

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***Abstract***

From a chemical composition point of view, wine is the result of complex interplays between environmental, genetic and human factors. The notion of terroir in viticulture involves the vine and its environment, including phenology, geography, geology, pedology and local climate of a vineyard, along with human inputs. On that basis, it could be assumed that, if grapes hold chemical fingerprints from a given terroir in their compositions, wines made of these grapes should also reflect related fingerprints. Very few strategies, based on the metabolodiversity of grape and/or wine, have tried to tackle the concept of Terroir in wine so far. Here, we report on the application of ultra-high resolution mass spectrometry, used as an untargeted approach, to the study of complex biochemical fingerprints of Pinot noir grapes and related wines from different plots (climats) in Burgundy, but grown/made by the same vinegrower/winemaker. Over three successive vintages, samples were mostly discriminated according to vintages. However within a given vintage, terroir-related signatures were more pronounced in grapes than in wines. In contrast, the single-run analysis of the same wines after bottle ageing clearly allowed for a significant separation between closely related vineyards from the Côte de Beaune and the Côte de Nuits, regardless of the vintages. For the first time, such results indicate that non-targeted experiments can reveal memories of environmental factors, which have impacted the wine's metabolic baggage at the moment of its elaboration, through terroir-related metabolic signatures on a regional-scale that can potentially be as small as the countless "climats" of Burgundy.

***Keywords: Pinot noir grapes, wine, terroir, FTICR-MS, vintage, "Climats de Bourgogne"***

## 1 INTRODUCTION

According to the RESOLUTION OIV/VITI 333/2010, the notion of terroir is related to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area. Several factors such as soil type, environment, agricultural practices, climatic conditions, vine phenology and microbiology or winemaking processes – all of them considered to contribute to the terroir effect –may indeed change the chemical composition of grapes and wine (Bokulich, 2014; Kumsta et al., 2012; Lund & Bohlmann, 2006; Roullier-Gall et al., 2014a, 2014b). Terroir deals with the quality and the typicality of an agricultural product in relation to its geographical origin, and it wears a major importance in Burgundy, where wines are made with single grape varieties (Pinot noir for red wines and Chardonnay for white wines) planted in a mosaic of vineyards. Burgundy vineyards are indeed characterized by chaotic orientations and variable slopes, where soils can exhibit abruptly changing natures beyond a road or a path.

It appears that a robust methodology for the discrimination of terroirs in wines using a single measurement system would be of great interest. Matching techniques now allow the analytical profile (all of the targeted analytical measurements together) of a wine to be used to predict its region of origin (Capron et al., 2007; Smeyers-Verbeke et al., 2009). Non-targeted analytical tools, especially NMR spectroscopy (López-Rituerto et al., 2012; Pereira et al., 2005, 2006) and FTICR mass spectrometry (Cuadros-Inostroza et al., 2010; Gougeon et al., 2009, 2011) have been shown to be efficient methodologies.

In this study, successive vintages were analysed, using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) for metabolite profiling, combined with metabolomics data analyses. FTICR-MS is considered as a non-targeted metabolomics approach through the semi-quantitative description of all low molecular weight metabolites in a specified wine sample. The objective was to implement strategies to discriminate grapes and related wines from distinct terroirs (vineyards) in neighboring villages in Burgundy managed by a same vine grower/winemaker.

## 2 MATERIALS AND METHODS

This work was carried on distinct vineyards managed by a same winemaker, and considering two distinct estates. For the first estate, four distinct vineyards were selected, located in the “Côte de Nuits” and the “Côte de Beaune", thereafter referred to as CN and CB, respectively. For each vineyard, three vintages (2007, 2008 and 2009) were considered. For the second estate, two neighbouring villages were considered: Flagey-Echezeaux and Vosne Romanée, located in the “Côte de Nuit”, and thereafter referred to as GE and VR. In each village, two distinct locations of one vineyard were considered (VR1, VR2, GE1 and GE2). For these two vineyards, three vintages (2010, 2011 and 2012) were considered.

100 Pinot noir berries were collected (in duplicate from two distinct places) at harvest for each of the vineyards and for both estates. Musts and skins were separated by pressing berries. Skin extracts were obtained by crushing in pure methanol. Must samples were obtained by solid phase extraction. For a given estate, winemaking practices were identical for each vineyards, and corresponding wines were collected immediately after the alcoholic fermentation with three samples corresponding to the free run wine, the pressed wine and the blend, for each vineyard. In the case of the first estate, the same wines (CN and CB) from 2007, 2008 and 2009 vintages were re-analysed in 2013 after 5, 4 and 3 years of bottling, respectively.

High-resolution mass spectra were acquired on a Bruker (Bremen, Germany) APEX Qe Fourier transform ion cyclotron resonance mass spectrometer (FTICR-MS) equipped with a 12 Tesla superconducting magnet and a APOLO II ESI source in the negative ionisation mode. Samples were introduced into the micro electrospray source at a flow rate of 120 µl/h. Spectra were externally calibrated on clusters of arginine (10mg/l in methanol) and further internally calibrated using fatty acids, which allowed to reach accuracy values lower than 0.05 ppm. The spectra were acquired with a time domain of 4 mega-word with a mass range of m/z 100-1000. 500 scans were accumulated for each sample.

## 3 RESULTS AND DISCUSSION

Chemical spaces of wines, which we define as the instantaneous diversity of chemical compounds families possibly present, and which certainly include a majority of yet unknown molecules – can be as diverse as there are steps involved in their elaborations, and even within each of these steps, chemical spaces will be subjected to various environmental influences (Lund & Bohlmann, 2006). Advanced characterization of such chemical spaces are provided by the number of elemental formulas that can be identified in the FTICR mass spectrum acquired under selected experimental electrospray conditions (Gougeon et al., 2009). As an example, Figure 1 shows how complex such chemical spaces are, with spectra and corresponding two-dimensional van Krevelen diagrams for the 2009 CN Must, skin and wine. Such diagrams enable a structural representation of detected masses converted to C,H,O,N,S-containing elemental compositions, and correspond to a plot of H/C versus O/C atomic ratios. Identified elemental compositions can thus be sorted according to chemical families, which provide a visual qualitative description of chemical spaces, which may transiently contribute to the chemical matrix of a wine. As an example, and in the case of GE and VR vineyards, typical specific molecular signatures on the basis of pertinent annotations are aliphatic oxygenated structures for the must, polyphenolic structures for the skin and residual sugars along with polyphenols for the wine (Figure 1).

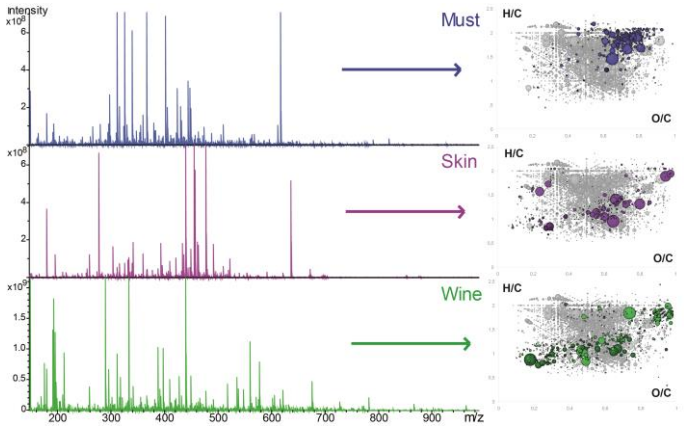


**Figure 1 : Visualization of the ESI(-) FTICR/MS spectrum for the 2009 CN must (blue) and skin (pink) extracts and for the wine (green) in the 100-1000Da mass range, and van Krevelen diagrams (H/C vs. O/C atomic ratios) with grey dots corresponding to common masses to all samples and color dots**

**corresponding to must, skin and wine specific masses. Point sizes in van Krevelen diagrams indicate mass peak intensities.**

Our results do agree with van Leeuwen (van Leeuwen et al., 2004), who showed that vintage climatic conditions, soil types and cultivars can actually influence the berry composition and the corresponding wines, and with Pereira (Pereira et al., 2006), who demonstrated that the vintage has a higher impact on the metabolic pattern of grape berries. Figure 2 shows the hierarchical cluster analysis (HCA) extracted from FTICR-MS mass spectra of all the VR and GE skin extracts from the three vintages (2012, 2011 and 2010), where it can be seen that skins could be rather well separated according to vintages but not according to the terroir (GE in red and VR in orange). Interestingly, there was a better discrimination between the 2010 vintage and the two others, whereas the separation between the 2012 and 2011 vintages was less straightforward. However, such HCA failed to consistently discriminate vineyards, and for some vintages, a GE vineyard place could be more similar to a VR vineyard place as shown for 2010, whereas a consistency could be observed in other vintages, as shown for 2012, where the two places for a given vineyard are more similar than they are to places of the neighbouring village vineyard. Corresponding van Krevelen diagrams representing the specific metabolic signatures for vintages, regardless of the vineyards provide a visual qualitative description of vintages chemical spaces with typical molecular signatures of phenolic glucosides for the 1464 specific masses from 2012 and anthocyanins for the 908 specific masses from 2010.

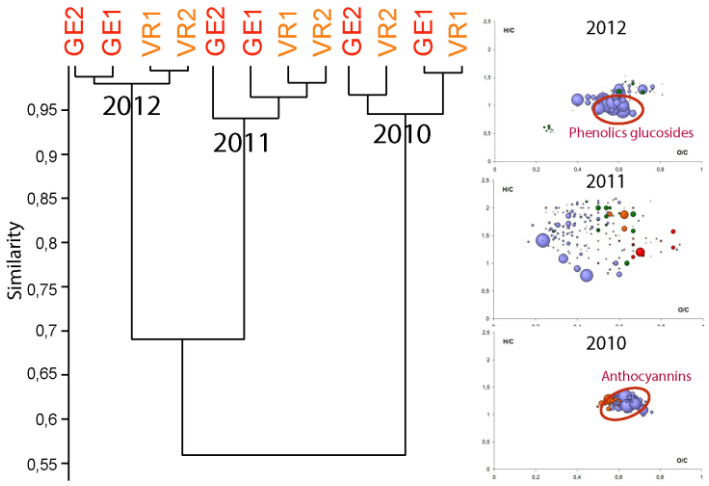


**Figure 2: Hierarchical Cluster Analysis (HCA) of FTICR-MS data for VR and GE Skin extracts and from the three vintages: 2012, 2011 and 2010. van Krevelen diagrams (H/C vs. O/C atomic ratios) corresponding to specifics masses for each vintages. Point sizes in the van Krevelen diagram indicate mass peak intensities.**

When all the samples from both villages (GE and VR) are considered for a given vintage, terroir discriminations are immediately observed, as illustrated for the 2012 vintage (Figure 3). The hierarchical cluster analysis of FTICR-MS data of all the 2012 samples showed the excellent and consistent separation of vineyards within wines, musts and skin extracts, and terroir discriminations were thus visible not only in the wine, but also in the grapes, with a stronger effect seen in musts than in skins and wines (Figure 3A) (Roullier-Gall et al., 2014b). Such results may suggest that winemaking processes could lead to some transient loss of terroir metabolic signatures, at least in young wines. It should be noted, that up to 7850 masses were recorded altogether for the different 2012 samples considered in Figure 3A of which 504 were discriminant for the VR differentiation and 207 for GE. Hypothetical annotations of discriminant masses (VR and GE) could be obtained with the Masstrix translator into pathways (Suhre & Schmitt-Kopplin, 2008; Wägele, Witting, Schmitt-Kopplin, & Suhre, 2012), revealing potential characteristic structures as diverse as sugars, phenolic acids or fatty acids (Figure 3B). Although 504 and 207 specific masses were detected using FTICR-MS for VR and GE respectively, only very few relevant masses could be annotated using MassTRIX. For example, only 21 annotated metabolites from the flavonoid biosynthesis pathway could actually correspond to VR specific masses, and only 7 from the arachidonic acid metabolism pathway for GE masses.

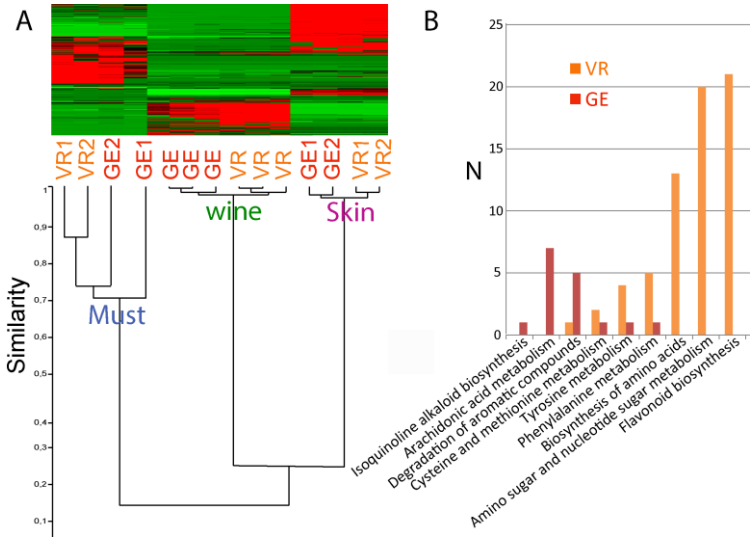


**Figure 3: (A) Hierarchical Cluster Analysis (HCA) of VR (in orange) and GE (in red) Skin extracts, wine and must samples from the 2012 vintage. (B) Histogram plots of the number (N) of annotations in various pathways from the *Vitis vinifera* organism, of VR (orange) and GE (red) specific masses obtained from ICR-FT/MS data, using the Masstrix translator into pathways.**

In order to assess the impact of the ageing in bottle on a possible vineyard-related chemical complexity, CB and CN wines were analyzed after 5, 4 and 3 years of bottling for the 2007, 2008 and 2009 vintages, respectively. FTICR-MS of these wines were thus acquired within the same experimental run. Most interestingly, PLS-DA (Figure 4) treatments of the complex chemical patterns of bottle-aged wines could readily discriminate wines from the Côte de Beaune (purple) from wines from the Côte de Nuits (blue) along the first component, whereas the second component appeared to discriminate vintages within each geographical area, although to a lesser extent for CN wines from the 2007 vintage. This fundamental result highlights the fact that if the terroir impacts the initial chemical complexity of a wine, time might be required to fully reveal it through the in-bottle diagenesis of specific chemical signatures (Roullier-Gall et al., 2014a).

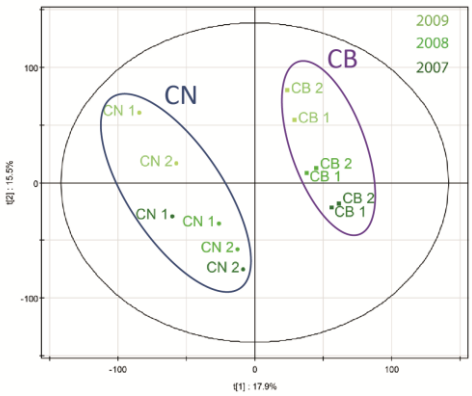


**Figure 3: Scores plot of the PLS-DA depending on the terroir, from FTICR-MS data for wines from the Côte de Beaune (CB) and the Côte de Nuits (CN) vineyards analysed after 5, 4 and 3 years of bottling for the 2007 (darker), 2008 (middle) and 2009 (pale) vintages, respectively.**

## 4 CONCLUSION

This study marks the first implementation of non-targeted analyses of grape extracts and corresponding wines from distinct vineyards managed by a same producer, in order to assess terroir/vintages discriminations among neighboring villages in Burgundy. Our results showed that FTICR-MS spectra of grape extracts and wines can be used to compare terroirs as small as the numerous "Climats de Bourgogne" through their wine and grape chemodiversity. When analyzed immediately after harvest (for the grapes) or after the alcoholic fermentation (for the corresponding wines), the vintage effect is most significantly discriminant within each material (wines, skin extracts or must extracts) taken separately or together. Terroir discrimination could be observed within a given material, only within a given vintage (Roullier-Gall et al., 2014b). In contrast, the single-run analysis of the same wines after bottle aging clearly allowed a perfect separation between closely

related vineyards from the Côte de Beaune and the Côte de Nuits, thus emphasizing a crucial role of the ageing time during which molecular diageneses occur in bottles (Roullier-Gall et al., 2014a). In the particular case of bottled Pinot noir wines from Burgundy, our non-targeted instantaneous analyses were thus able to reveal possible intrinsic terroir-related chemical fingerprints. As such, terroir would not just be the sum of discrete processing steps, but a subtle interplay between a vineyard and its vine grower/wine maker, and this is all the more true in Burgundy. Our approach thus contributes to the representation of how wines – considered as pieces of art in terms of chemical equilibrium –bring messages from their birthplaces to the glass.

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