Chemical diversity of oak barrels and classification of tannin potential and toasts

In order to improve barrel production, a team of French researchers set out to characterise the extractable wood compounds on a series of oak wood samples with various tannin potentials using high resolution mass spectrometry and different toasts. The team included **Nolwenn Wirgot**, **Maria-Elena Diaz-Rubio**, **Christian Coelho**, **Maria Nikolantonaki** and **Régis Gougeon**, from the Jules Guyot University Institute of Vine and Wine at the University of Burgundy, **Marie-Laure Badet-Murat**, from Œnologie by MLM, and **Jean-Charles Vicard**, from the Vicard Group.

Introduction

Ageing of premium quality wines in oak barrels is an ongoing tradition for enhancing their organoleptic quality. The selection of barrels is therefore of the upmost importance. The large variability in the chemical composition of oak wood, even for the same species from the same forest and within a same tree, can have an impact on the homogeneity and reproducibility of barrels.

However, wood is an extremely complex matrix, mainly due to the large variability in its compounds. Oak wood is made up of macromolecules (cellulose, hemicellulose, lignin), as well as extractable compounds whose proportion varies between 2 and 10% of the dry wood mass. The proportion of macromolecules is 22-50% for cellulose, 17-30% for hemicellulose, and 17-30% for lignin (Le Floch *et al.* 2015). Thermal degradation of lignin during barrel toasting leads to the formation of aromatic compounds, such as guaiacol, 4-methyl guaiacol and syringol, which give spicy aromas and smoky notes (Jordao *et al.* 2006). Extractable wood compounds are elements with lower molecular weight, and are soluble in organic solvents or in water. However, the extractable fraction comprises not only volatile compounds, but also a large number of non-volatile compounds, such as ellagitannins, triterpenes, coumarins, lignins and polysaccharides (Marchal *et al.* 2011, Mosedale *et al.* 1999) whose diversity is still largely unknown (Gougeon 2009). The type of compounds that are extractable from the wood varies with its tannin potential; it also depends to a greater or lesser extent on the intensity and length of the toasting process (Matricardi & Waterhouse 1999).

In order to better control barrel production, this study aims to characterise the overall extractible fraction using high resolution mass spectrometry on a series of oak wood samples with various tannin potential (which were classified according to their total ellagitannin contents as predicted by Near Infrared Spectroscopy on the untoasted wood) and different toasts (high precision toasting by-radiant heat) (Badet-Murat *et al.*, 2016).

Experimental set-up

For this study, a series of oak wood samples with three different tannin potentials (LTP: low tannin potential; MTP: medium tannin potential; HTP: high tannin potential – corresponding respectively to 2000-4000µg, 4001-6000µg and 6001-8000µg ellagic acid equivalents per gram of dry wood), and four different toasting profiles (Blanche: 150°C/1 hour; Gradual toasts with four incremental temperatures, 1h30, the number indicating the initial temperature

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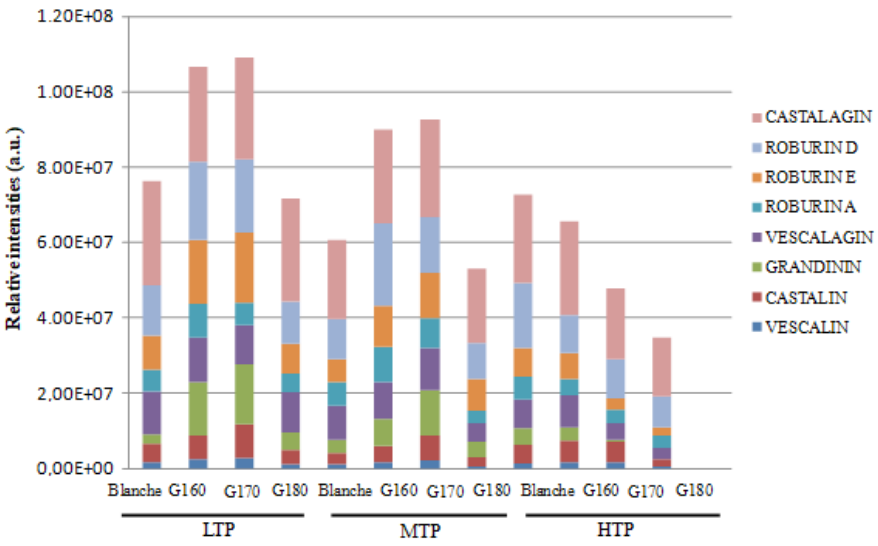
G160; G170; G180) were solubilised in a synthetic wine at room temperature; the extracted fractions were then analysed by high resolution mass spectroscopy (UPLC-MS-QTof).

Analysis of known ellagitannins (targeted approach)

Without any prior knowledge of the dataset, the very first (specific) approach used was to focus on eight ellagitannins, known to be amongst oak wood extractible compounds, found in the wood at non-negligible concentrations. To do this, standard solutions of these reference ellagitannins were analysed using UPLC-Q-ToF-MS under exactly the same analytic conditions as the wood samples, in order to establish their own spectral signature (*m/z* and retention time). The representative masses of these eight ellagitannins were then identified in the wood samples (Figure 1).

Molecular analysis of the ellagitannins shows a strong similarity between the woods'– classification by near infrared spectroscopy (IR) (as is routinely carried out during the manufacturing process on untoasted wood) and the tannin content of the soluble fraction after a low temperature toast (Blanche, 150°C) (Figure 1). Furthermore, we found that the composition of ellagitannins in the extracts varied according to the tannic potential. Namely, grandinin and roburin A,D,E are found in relatively higher concentrations in the HTP modalities,



Ellagitannin profiles of oak extracts, according to tannin potential and toasts.

whereas vescalagin and castalagin are found in relatively higher concentrations in the LTP modalites. Furthermore, the toasting level was found to impact the amount of ellagitannins in the extracts, this being relative to the ellagitannin profiles. Indeed, the highest toasting temperature (G180) resulted in a large decrease in ellagitannins, independent of the wood's tannin potential. These results demonstrate a tannin composition for the woods which is dependent on the toast and the tannin potential, thus undoubtedly having an impact of the wines' sensorial qualities.

Multivariate statistical analyses (principal component analyses, PCA) were carried out on this reduced dataset, taking into consideration only the relative intensities of the eight ellagitannins in each wood sample (Figure 2). For the distribution of the samples in regards to tannin potential (HTP, MTP and LTP), we clearly observed that there is no statistical difference between the three classes. Indeed the three ellipses related to each of the tannin potentials overlap. For the distribution of the samples in regards to the toast (Blanche, G160, G170 and G180), the situation is a little different. Indeed, we observe that the most extreme toasts (Blanche and G180) show separate trends, with no significant difference between the intermediary toasts (G160 and G170). The main conclusion that

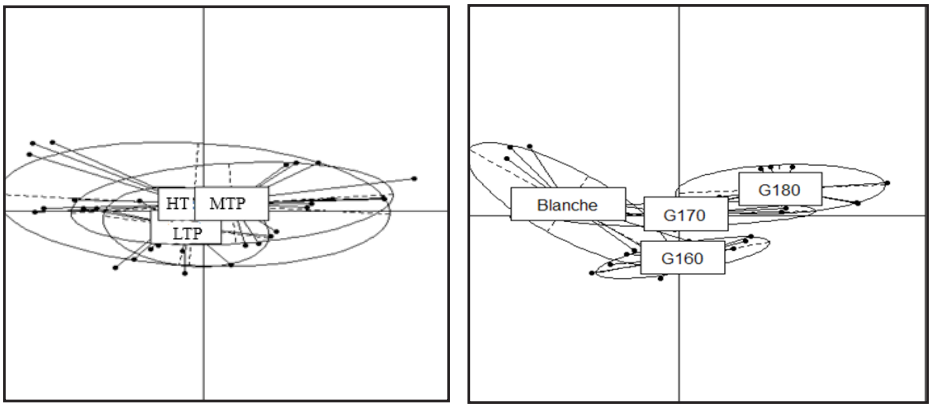


Figure 2. Targetted approach. Principal component analyses (PCA): left, distribution of samples according to tannin potential: component 1 (72.9%) and component 2 (11.0%); right, distribution of samples according to toasting: component 1 (72.9%) and component 2 (11.0%).

we can draw from these initial results is that the chemical diversity of the wood matrix is such that the eight reference ellagitannins used are not sufficient to constitute specific markers for tannin potential and toast. For this reason, we devised a second (non-specific) approach which aims to take into account the entire oak wood metabolome (all of the small molecules that it contains).

Analysis of overall content of extracts (non-targeted approach)

A similar approach was taken to the above, this time also taking into account the full complexity of the dataset that describes all the ionisable compounds in the extracts detected by UPLC-QToF-MS. Principal component analyses were carried out (Figure 3). For the distribution of the samples according to tannin potential (HTP, MTP and LTP), this time we observed a clear separation of the three tannin potential modalities with a maximum effect between the high and low tannin potential modalities. For the distribution of the samples according to toast (Blanche, G160, G170 and G180), the general trend was the same as for the specific approach but the separation between the most extreme toasts (G180 and Blanche) was drastically improved. The results obtained thus reveal new and fundamental information for cooperage, which indicates that precise classification of woods according to tannin potential can not be based only on the measurement of known ellagitannins, but must take into account wider inherent chemical diversity, involving the entire metabolome of the wood.

Marker identification

In order to identify the compounds responsible for the statistical differences between tannin potentials and toasts, multivariate 'Partial Least Squares Regression' (PLS-DA) analyses allowed for optimisation of the groups previously

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defined using principal component analysis (Figure 4).

The combination of these statistical methods helps to accentuate the separation between defined classes of samples (tannin potential and toast), and to gain a clear idea of the robustness of the model(s) studied. At the time of writing, 35 markers specific to the three tannin potential classes have thus been identified (elementary formulae).

Conclusions

We have demonstrated that whilst specific molecular analysis of ellagitannins on toasted wood extracts is relatively concordant with stave classification by IR, non-specific analysis of the overall metabolomic profile allows for more detailed discrimination of woods based on their tannin potential, independent of the toast. Similarly, it is possible to classify woods according to toast levels, independent of tannin potential. Molecular markers responsible for these classifications were thus identified. These compounds could be the basis for detailed classification of staves, allowing for greater homogeneity and reproducibility of barrels

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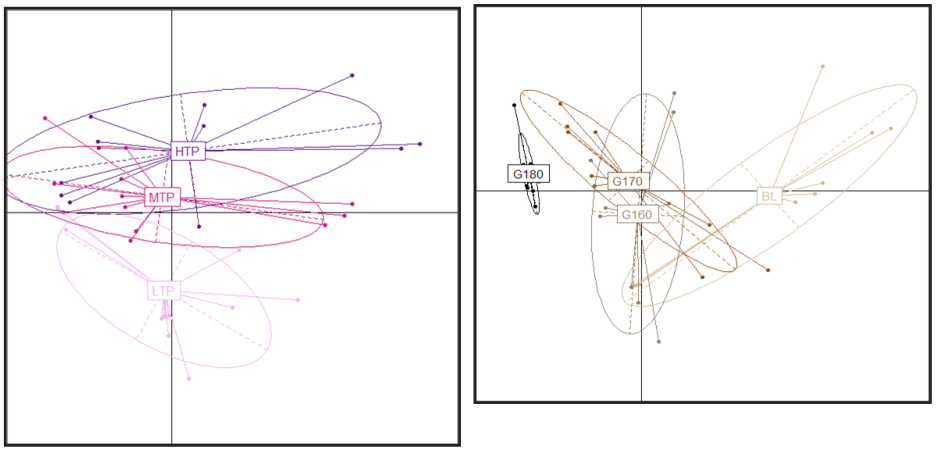

Figure 3. Non-targeted approach. Principal component analyses (PCA): left, distribution of samples according to tannin potential: component 1 (19.0%) and component 2 (12.0%); right, distribution of samples according to toasting: component 1 (19.0%) and component 2 (12.0%).

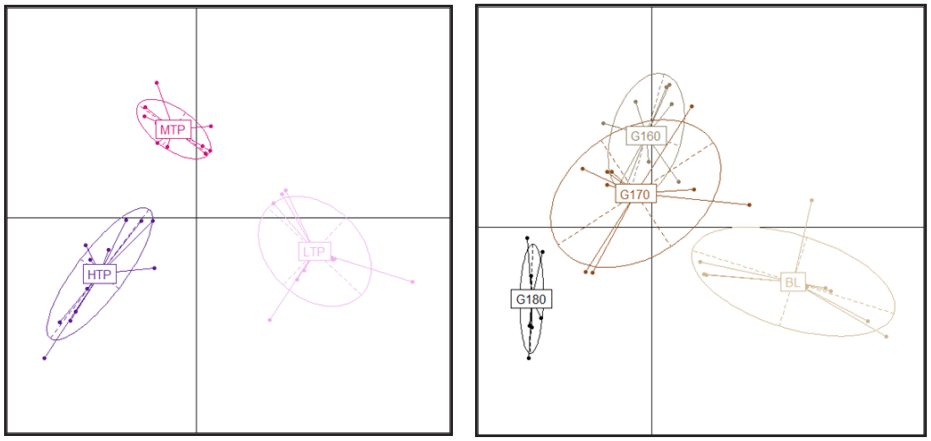

Figure 4. Non-targeted approach. PLS-DA: left, distribution of samples according to tannin potential; right, distribution of samples according to toasting.

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