Study of different barrel makers over time by NMR metabolomics

Inès Le Mao, Grégory Da Costa and Tristan Richard*

UMR 1366, Univ. Bordeaux, INRAE, Bordeaux INP, Bordeaux Science Agro, UMR OENO, ISVV, 210 chemin de Leysotte, 33882 Villenave d'Ornon, France

**Corresponding author: tristan.richard@u-bordeaux.fr*

Keywords: barrel ageing; ^{1}H-NMR, metabolomics, chemometrics

1. INTRODUCTION

Wine is the product of many human interventions; one of the key stages is the maturation process which takes place between the end of the fermentations and the bottling. This stage can be conducted in different containers: stainless steel tanks, concrete tanks or oak barrels. The maturation in oak barrels is a generally longer process that can take between 6 and 18 months. Aging in oak barrels is a very common technique because it greatly influences the quality and aromas of the wines [1]. It is a technique widely used for Bordeaux wines, which contributes to their reputation. The process favors, among other things, the balance between oxygen and the different components of the wine such as tannins, colors, aromas, proteins or enzymes. During the maturation process, the wine is also subjected to numerous chemical reactions due to the reactions between the wine constituents and the compounds extracted from the oak barrel [2, 3].
Nowadays, ^{1}H-NMR-based metabolomics emerged as a meaningful tool to insure wine traceability [4-7]. Based on quantitative analysis (targeted approach) or metabolomic fingerprinting (untargeted approach), NMR metabolomics allows to control geographical origin, real composition including grape variety for example and vintage. More recently, ^{1}H-NMR-based metabolomics was successfully used to monitor wine quality during winemaking process [8, 9]. In addition, it has been shown that ^{1}H-NMR spectrometry combined with multivariate statistical analysis provides a reliable tool to monitor wine evolution during bottle aging [10]. NMR permits to follow overall wine metabolite changes.
The aim of the present study is to investigate the evolution of wine during aging in oak barrels by NMR-based metabolomics. The wine compound variation during aging could be useful to control the overall wine quality. Red wine (blend of Cabernet Sauvignon, Merlot and Cabernet Franc varieties) was analyzed a month after running into barrels and after twelve months conservation in a cellar controlled environment. Red wines were conserved in new oak barrels provided by three different manufacturers. Wine constituent evolution was monitored by ^{1}H-NMR spectrometry combined to multivariate statistical analysis (MSA).

2. MATERIALS AND METHODS

2.1. Sample preparation

Wine samples from Bordeaux area were used. Following malolactic fermentation, the wine was submitted to clarification and stabilization treatments. After the classical step used for winemaking, wine was transferred into new oak barrels (225 L) from three different manufacturers for 12 months. Six samples were collected in different barrels for each manufacturer after a month and twelve months of aging.
For ^{1}H-NMR analysis, the same protocol than our previous works was employed [9]. Briefly, wine samples underwent a 3 minutes step of centrifugation at 10000 rpm. The supernatant was prepared as follow: wine, phosphate buffer (1 M, pH 2.6) and D_2O containing 0.5 mM trimethylsilylpropanoic acid (TMSP) and 7 mM calcium formate (FCa) (ratio 7:2:1, v/v/v). Finally, the pH was adjusted at 3.1 with HCl and NaOH solutions (1 M) by a BTpH unit (Bruker BioSpin, Germany). Then, 600 µL of the wine solution was introduced into 5 mm tubes for NMR analysis.

2.2. ^{1}H-NMR analysis

^{1}H-NMR spectra were acquired on an Avance III NMR spectrometer (Bruker BioSpin, Germany) operating at 600.27 MHz equipped with a 5mm TXI probe Z-gradient coils. Experiences were performed at 298 K using two

specific sequences. The ZGPR pulse program was used for water signal suppression. NOESYGPPS1D sequence was performed for the suppression of water and ethanol signals. Spectra were processed using Topspin 3.2 (Bruker Biospin, Germany) and analyzed using MestReNova 12 (Mestrelab Research, Spain). Spectra were submitted to a manual phase correction before data extraction. For targeted analysis, 35 compounds were quantified using the plugin Simple Mixture Analysis (SMA) of MestReNova software after an automatic baseline correction. For untargeted analysis, buckets were generated with NMRProcFlow 1.4 online (https://nmrprocflow.org/). The spectra were first calibrated to 0.00 ppm using TMSP and then underwent local baseline correction by selecting the areas upstream and downstream of the water and ethanol signals.

2.3. Chemometrics

Multivariate statistics were performed using SIMCA 16 software (Sartorius, Germany). Unsupervised principal components analysis (PCA) was used to observe de general distribution of sample and supervised orthogonal partial least squares discriminant analysis (OPLS-DA) was used to identify the metabolites impacted by oak barrel maturation process. Reliability of the results was controlled threw several tests: analysis of variance testing of cross-validated predictive residuals (CV-ANOVA), receiver operating characteristic (ROC) curves and misclassification table. Univariate statistics were used to confirm the impact of ageing on the metabolites previously highlighted.

3. RESULTS AND DISCUSSION

3.1. Wine ageing in oak barrels

Red wine ageing in oak barrels was monitored by ^{1}H-NMR-based metabolomics. Red wines were analysed after one and twelve months of ageing (n = 18, each). ^{1}H-NMR spectra were acquired and processed using our previously published protocol based on a targeted strategy [9]. This method allows the identification and the absolute quantification of more than thirty wine metabolites including organic and amino acids, alcohols, sugars, polyphenols, etc. The data extracted from NMR spectra were processed by principal component analysis (PCA) followed by orthogonal partial least squares discriminant analysis (OPLS-DA). PCA score plot (Figure 1) based on NMR targeted analysis shows a very strong impact of the time spent in barrels on the chemical composition of the wines.

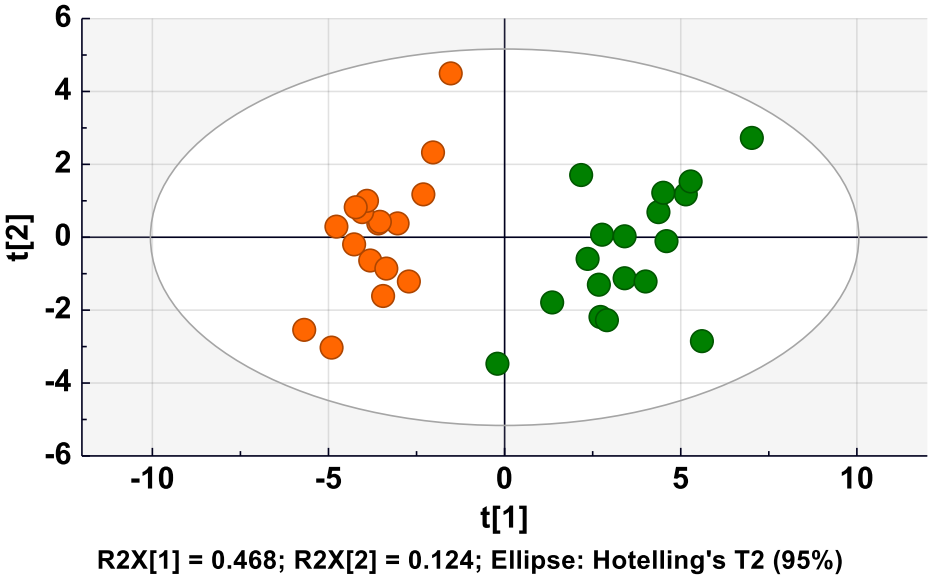

Figure 1. PCA score plot of wine ageing in oak barrels from ^{1}H-NMR targeted data (green: 1 month; orange: 12 months).

Further analyses performed using OPLS-DA model allows to identify the impacted metabolites (Figures 2A and 2B). An increase of acetic acid, ethyl lactate, glucose and arabinose was observed in one part and a decrease of amino acids, choline, acetoin, catechin and epicatechin in other part, after wine ageing in oak barrels. Some of these compounds have already been found after wine ageing in bottle [10]. These results confirm that

NMR-based metabolomics could be a useful tool to monitor and control the wine ageing process from the oak barrel to the final bottle.

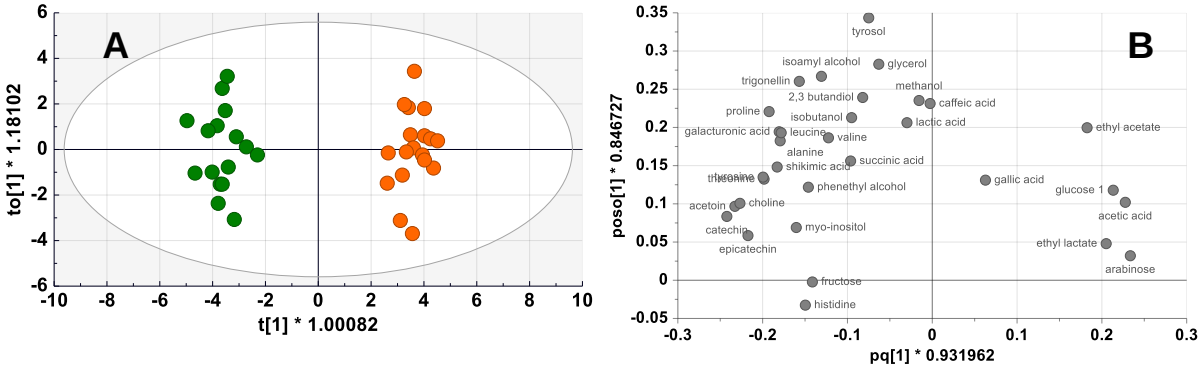

Figure 2. OPLS-DA score plot (A) and loading plot (B) of wine ageing in oak barrels from ^{1}H-NMR targeted data (green: 1 month; orange: 12 months). $R^2X = 0.574$, $R^2Y = 0.975$, $Q^2 = 0.953$, CV-ANOVA p-value<0.05.

3.2. Chemical signature of each barrel maker

Barrel acts as an active vessel depending on its intrinsic qualities. To observe the potential effects of oak barrel properties, wines conserved in barrels of three different manufacturers were compared after one month of ageing. The ^{1}H-NMR spectra of wines were analysed by a non-targeted approach taking into account the overall spectral data [11]. Briefly, NMR spectra were exported into NMRProcFlow for baseline and alignment corrections, followed by intelligent bucketing.

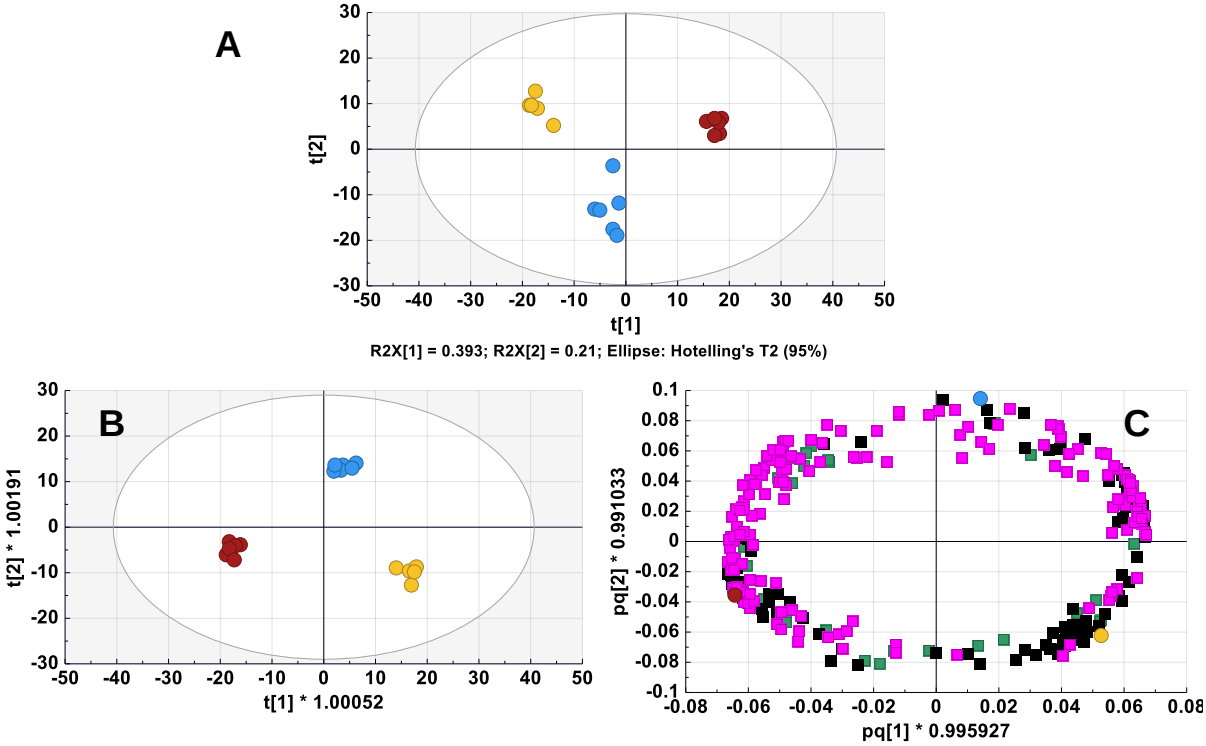

Figure 2. Multivariate statistical analysis of wine ageing in oak barrels for one month from ^{1}H-NMR untargeted data. A) PCA score plot; B) OPLS-DA score plot ($R^2X = 0.698$, $R^2Y = 0.989$, $Q^2 = 0.956$, CV-ANOVA p-value<0.05) (yellow circle: maker 1; blue circle: maker 2; red circle: maker 3); C) loading plot from OPLS-DA (VIP > 1; pink box: aliphatic marker; black box: carbinolic marker; green box: aromatic marker).

The PCA score plot obtained from NMR fingerprinting taking into account the barrel origin from the different manufactures is presented in Fig. 4A. A clear discrimination of the oak barrels of the three manufacturers is

observed. The score plot obtained from the OPLS-DA model is presented in Figure 2B. As observed in this figure, a successful OPLS-DA model was obtained to discriminate the wines maturated in the oak barrels provided by each manufacturer. The loading plot from OPLS-DA model based on variable of importance > 1 (VIP > 1) is shown in Figure 2C. Loadings were ranged into three classes: aromatic (10.0 – 5.5 ppm), cabinolic (5.5 – 3.7 ppm) and aliphatic (3.6 – 0.5 ppm). Markers from the three classes of markers are involved in the discrimination. Further analyses will be necessary to identify these discriminating metabolites by NMR profiling.

4. Conclusion

^{1}H-NMR-based metabolomics is an efficient tool for studying wine ageing in oak barrels. Targeted analysis allows identifying major discriminating compounds involved in the maturation process. Untargeted analysis makes it possible to highlight some specific effect of each barrel maker in wine composition. The combination of these two approaches offers new perspectives for analysing the qualitative evolution of wines during ageing.

Acknowledgments

This work was supported by *Association Nationale de la Recherche et de la Technologie* (Inès Le Mao was the recipient of a CIFRE PhD fellowship from ANRT and Baron Philippe de Rothschild S.A.). We also want to thank Château Mouton Rothschild and Fondation of Bordeaux (donors: Baron Philippe de Rothschild SA, Chateau Cheval Blanc, Château Lafite Rothschild, Le Domaine Clarence Dillon, Château Petrus) for its financial support. The work was supported by the Bordeaux Metabolome Facility and MetaboHUB (ANR-11-INBS-0010 project).

References

1. T. Garde-Cerdán and C. Ancín-Azpilicueta. Trends Food Sci Technol.17 (8) (2006) 438.
2. B. Fernández de Simón, M. Sanz, E. Cadahía, J. Martínez, E. Esteruelas, A.M. Muñoz. Food Chem. 143 (2014) 66.
3. F. Maioli, M. Picchi, L. Guerrini, A. Parenti, P. Domizio, L. Andrenelli, B. Zanoni and V. Canuti. ACS Food Sci. Technol. 2 (2) 2022 221.
4. J. Valls Fonayet, G. Loupit, T. Richard. Adv. Bot. Res. 98 (2021) 297.
5. M. Amargianitaki and A. Spyros. Chem. Biol. Technol. Agric. 4 (1) (2017) 9.
6. M.E. Alañón, M.S. Pérez-Coello and M.L. Marina. TrAC, Trends Anal. Chem. 74 (2015) 1.
7. P.A. Solovyev, C. Fauhl-Hassek, J. Riedl, S. Esslinger, L. Bontempo and F. Camin. Compr. Rev. Food Sci. Food Saf. 20 (2) (2021) 2040.
8. E. López-Rituerto, K.M. Sørensen, F. Savorani, S.B. Engelsen, A. Avenoza, Peregrina JM, J.H. Busto. J. Sci. Food Agric. 102 (9) (2021) 3808.
9. I. Le Mao, J. Martin-Pernier, C. Bautista, S. Lacampagne, T. Richard and G. Da Costa. Molecules 26 (22) (2021) 6771.
10. C. Cassino, C. Tsolakis, F. Bonello, V. Gianotti and D. Osella. Food Res. Int. 116 (2019) 566.
11. D. Jacob, C. Deborde, M. Lefebvre M, M. Maucourt and A. Moing. Metabolomics 13 (4) (2017) 36.