^{1}H-NMR-based metabolomics to assess the impact of soil type on the chemical composition of Nero d'Avola red wines

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1. INTRODUCTION

The aim of this study was to achieve a better understanding of the role of soil chemistry on the sensorial quality of *Nero d'Avola* red wine. To attain this goal two different approaches of ^{1}H-NMR-based metabolomics were applied, namely the targeted (TA) and the non-targeted (NTA) approaches. The TA provided the wine *profiling* by identifying and quantifying known metabolites. The NTA gave the wine *fingerprinting* by processing the entire spectra by means of chemometrics.

2. MATERIALS AND METHODS

2. 1 Study sites

Four different vineyards with different soil types, located along the southwestern coast of Sicily (Southern Italy), were chosen as study sites (Figure 1). Vineyards were homogeneous with respect to cultivar (*Nero d'Avola L.*), vine age (26 years), vine rootstock (140 Ruggeri), pruning method (Guyot), planting system (2.50 m x 1.00 m), irrigation system (drip irrigation), altitude (100 m a.s.l.), slope (10-20%), sun exposure (south/south-east) and agronomic management (conventional). Different soil parameters were analysed, including texture, pH, total carbonates, organic matter, cation exchange capacity, and electric conductivity. Grapes from each vineyard were separately vinified according to the standard procedure described by Squadrito et al., (2010). The obtained wines were analyzed by means of ^{1}H-NMR spectroscopy.

2. 2 ^{1}H-NMR Spectroscopic Analysis of wines

One milliliter of raw wine was analyzed by means of a Bruker Avance II 400 spectrometer operating at a proton Larmor frequency of 400.15 MHz. The ^{1}H-NMR spectra were acquired by applying the NOESYGPPS1D pulse sequence, to achieve water and ethanol signals suppression. ^{1}H-NMR spectra were processed by using MNova 14.2.3 software (Mestrelab Research, Santiago de Compostela, Spain).

2. 2. 1 Targeted analysis

Metabolites identification was performed by comparing the signal chemical shift, multiplicity and relative coupling constants of pure compounds spectra. The comparison was carried out by means of Simple Mixture Analysis (SMA) plug-in of MNova software. The signals absolute integrals were used as indicative of the concentrations of the identified metabolites. Up to 69 different compounds were identified and quantified. The obtained data matrix was subjected to the one-way analysis of variance (ANOVA) with Tukey's b post hoc test to highlight significant differences among wines.

2. 2. 2 Non-targeted analysis

The generation of the input variables fo NTA was done via bucketing the spectra. The bucketing was performed in the spectral range 0.5-9.5 ppm with a width range of 0.01 ppm. The obtained dataset was imported into MetaboAnalist 5.0 web-based tool suite (Chong et al., 2018) to preprocess the data and to run multivariate statistical analysis.

2. 3 Wine-soil relationship analysis

Data obtained from the TA and the physical-chemical parameters measured for each soil were subjected to the correlation analysis to point out possible relationships between soil and wine. To summarize the information obtained by the correlation analysis the unsupervised principal component analysis (PCA) was applied.

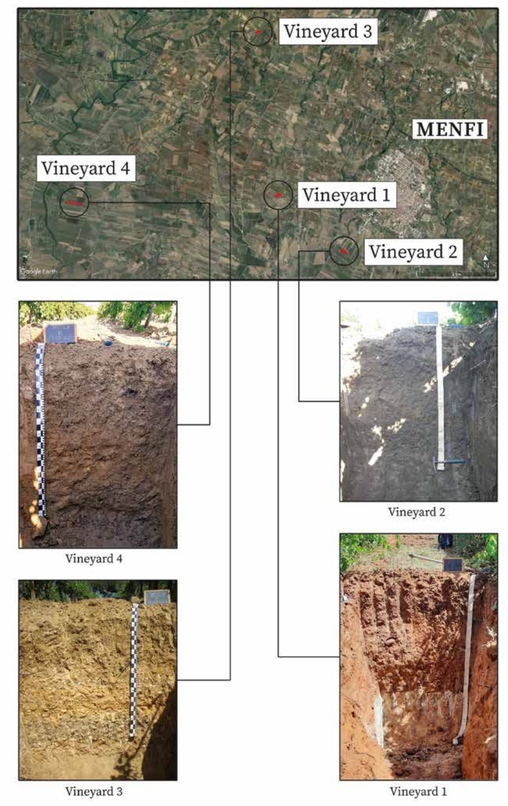

Figure 1 - Location of vineyards and soil profiles pictures

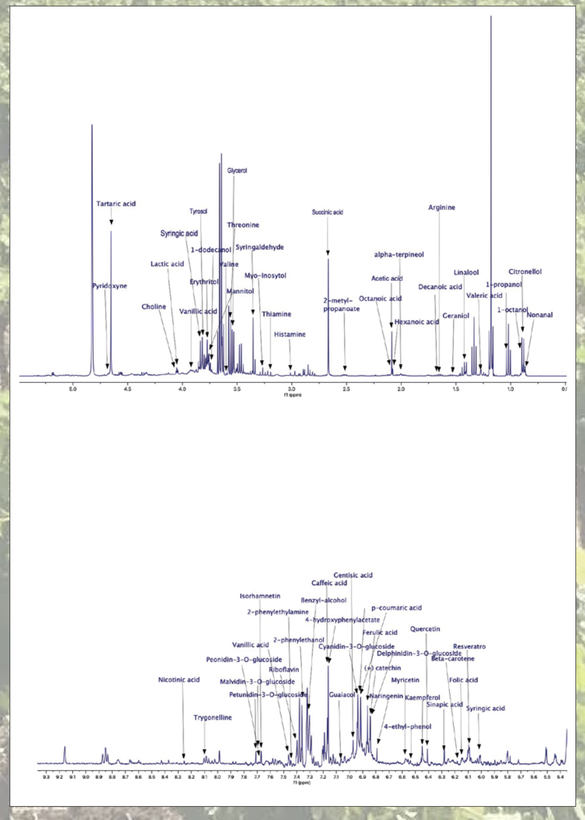

Figure 2 - Representative ^{1}H-NMR spectrum of a Nero d'Avola wine

3. RESULTS AND DISCUSSION

3. 1 Targeted analysis: wine *profiling*

Figure 2 shows a representative ^{1}H-NMR spectrum of one of the analyzed *Nero d'Avola* wines.

All the identified and quantified metabolites are shown in Figure 3. The one-way analysis of variance (ANOVA) applied to the data matrix containing the absolute integrals of the identified compounds highlighted the existence of significant differences ($p < 0.05$) among the investigated wines.

3. 2 Non-targeted analysis: wine *fingerprinting*

Principal component analysis applied on the spectral buckets revealed a great separation among wine samples (Figure 4).

Spectral signals differ not only in terms of peak intensity but also for their chemical shift values (Figure 5).

The chemical shift dispersion depends on the strenght of H-bonds networks in which the compounds are involved (Nose et al., 2004).

3. 3 Wine-soil relationship

The correlation analysis revealed that the soil texture exerts the major role in determining wine chemical composition. Soil texture strongly influences several soil features, including water and nutrient dynamics (Conte et al., 2015). These latter largely affect grapevine development, yield, and berries composition, affecting in turn wine quality.

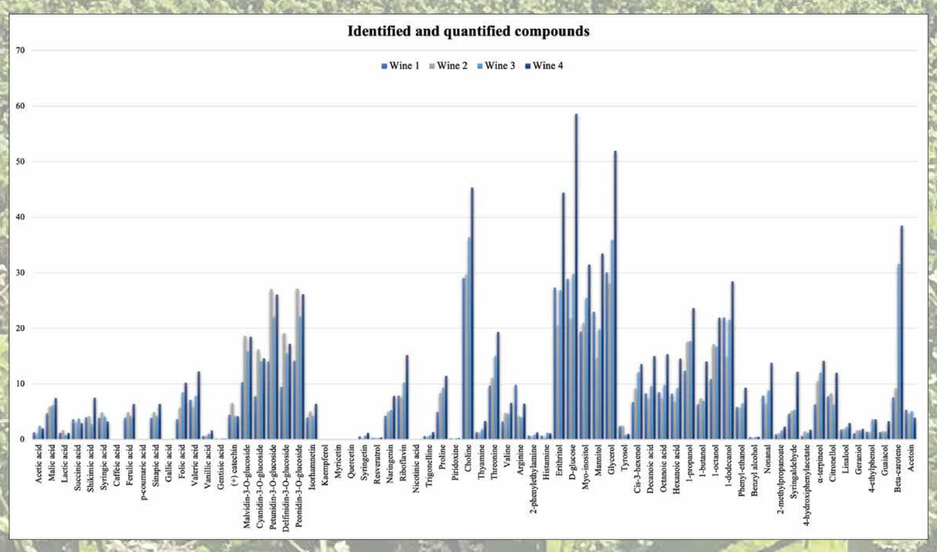

Figure 3 - Identified and quantified compounds

4. CONCLUSIONS

In this study the effects of the soil type on the chemical composition of *Nero d'Avola* wine were highlighted by means of ^{1}H-NMR-based metabolomics. Firstly, TA coupled with ANOVA unveiled significant differences among the concentrations of compounds detected in wines. Then, the NTA together with chemometrics, revealed that the differences among wines concerned not only the concentrations of the detected analytes but also the strength of the hydrogen bonds network in which the different compounds were involved.

The correlation analysis carried out on soil physical-chemical parameters and wine compositional data revealed that the soil texture exerts the major role in determining wine chemical composition. A possible explanation may lay in the effect of soil texture on water and nutrient dynamics in soils.

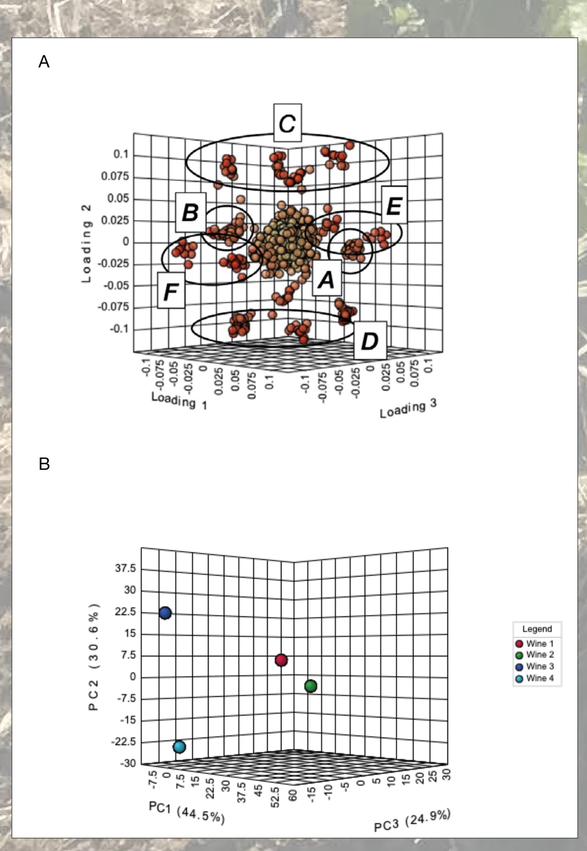

Figure 4 - (A) 3D PCA loadings plot of Nero d'Avola spectral buckets. (B) 3D PCA scores plot for the selected PCs. The explained variances are shown in brackets

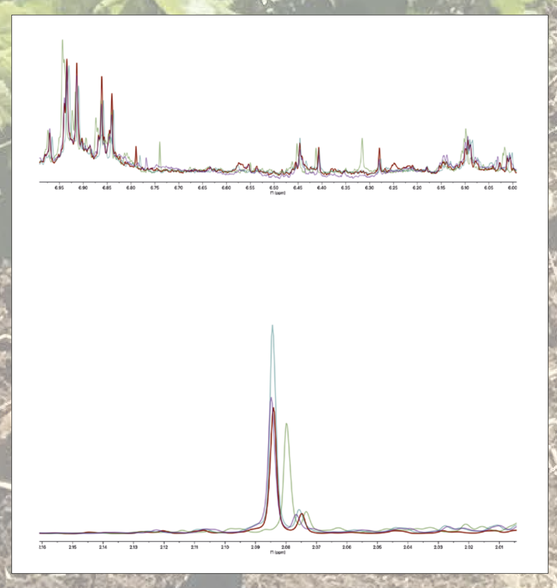

Figure 5 - ^{1}H-NMR chemical shift dispersion in a representative spectral portion of aromatic region (A) and of aliphatic region (B). The red line represents wine 1, the green line represents wine 2, the light blue line represents wine 3, and the purple line represents wine 4.

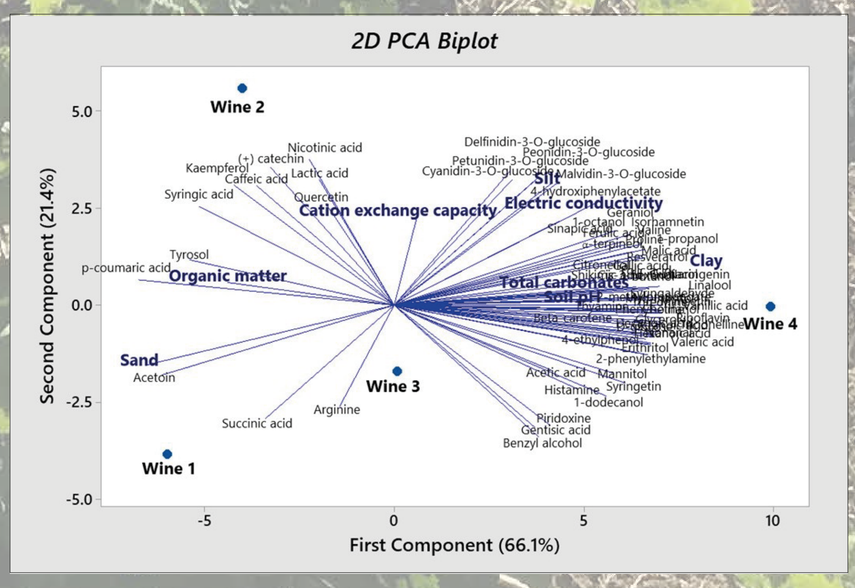

Figure 6 - 2D PCA Biplot. The explained variances are shown in brackets

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